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Serum Level of Naphthalene and 1,2 Benz-anthracene and Their Effect on the Immunologic Markers of Asthma and Asthma Severity in Children-Egypt

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Abstract **Background**: The rate of asthma increases as communities adopt western lifestyles and become urbanized. **Objective**: Was to assess the serum level of polycyclic aromatic hydrocarbons (PAHs) naphthalene, 1,2 benz-anthracene and detect their effects on some of asthma immunologic markers in children with bronchial asthma and their relation to asthma severity. **Methods**: The study was carried out on 60 children with bronchial asthma. They were selected from the inpatient and outpatient clinic of Al–Zahraa hospital, Al-Azhar University. Also the study included 60 children age and sex matched served as a control group. Serum levels of naphthalene and 1,2 benz-anthracene as well as (IgE, IL13 and IL5,) were assessed in the studied groups. **Results**: Serum levels of naphthalene and 1,2 benz-anthracene were significantly increased in children with bronchial asthma than in controls, as their levels were (24.04±13.03ng/ml; 16.07 ±8.96 ng/ml) and (5.23 ± 3.21 ng/ml; 6.99 ± 3.90 ng/ml), respectively with (p=0.000 and 0.006 respectively)with significant positive correlation to bronchial asthma severity and significant relation to urban residence and second hand smoking . There was a significant decrease in IL5 and IL13 serum levels while there was a significant increase in eosinophils counts and serum IgE levels in children with asthma than the controls, with significant positive correlation to bronchial asthma severity. **Conclusions**: Exposure to PAHs (naphthalene, 1,2 benz-anthracene) appears to be associated with asthma in children with significant relation to bronchial asthma severity and significant interaction with asthma markers.

Keywords PAHs, Environmental pollution, Childhood asthma, IL5, IL13

1. Background

Asthma is the commonest of all chronic diseases of childhood and estimates from developed countries suggest that it affects between 11 and 20% of all school age children. The prevalence of asthma among Egyptian children aged 3 - 15 years was estimated to be 8.2%. Of major concern is the annual increase in mortality [1].

The rate of asthma increases as communities adopt western lifestyles and become urbanized. With the projected increase in the proportion of the world's urban population from 45% to 59% in 2005, there will likely be a marked increase in the number of asthmatics worldwide over the next two decades. It is estimated that there may be an additional 100 million persons with asthma by 2025 [2].

The airways of asthmatics are hypersensitive to certain triggers, also known as stimuli such as environmental stimulant allergen, tobacco smoke, cold or warm air, perfume, pet dander, moist air, exercise, exertion and emotional stress. In response to exposure to these triggers, the bronchi contract into spasm "an asthma attack". Inflammation soon follows, leading to a further narrowing of the airways and excessive mucus production; which leads to coughing and other breathing difficulties [3].

Exposure to high levels of air pollution has been associated with decreased lung function, asthma, nasal symptoms, bronchitis and sensitization to inhalant allergens in both children and adults [4].

Polycyclic aromatic hydrocarbons (PAHs) describe chemicals that are often found together in groups of two or more, of these naphthalene and 1,2 benz-anthracene . PAHs are created when products like coal, oil, gas, and garbage are burned but the burning process is not complete [5]. PAHs belong to the group of persistent organic pollutants (POPs). These are organic contaminants that are resistant to degradation, can remain in the environment for long periods,

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and have the potential to cause adverse environmental effects [6].

The major sources of PAHs emissions maybe divided into four classes: stationary sources (including domestic and industrial sources), motor vehicle emissions, agriculture activities, and natural sources [7]. The burning and pyrolysis of coal, oil, gas, garbage, wood, or other organic substances are the main domestic sources. Agriculture activities as open burning of brushwood, straw, moorland heather, and stubble. Natural sources as accidental burning of forests, woodland and volcanic eruptions [8-9].

Vehicles reported to have the highest PAH emission rates are diesel engines, and gasoline engines operated without catalytic converters [10-12]. In urban environments, industrial operations, waste incinerators, and residential boilers are also important sources of ambient PAH [13].

Some studies implicate traffic-related emissions, largely composed of diesel exhaust particles, trace metals, volatile organic compounds, nitrogen dioxide, particulate matter, and polycyclic aromatic hydrocarbons (PAH) in the development of respiratory symptoms, asthma or the onset of allergies [14-16].

Some studies reported that a significant increase in immunoglobulin E (IgE) production due to exposure to PAH from diesel exhaust particles in response to common inhaled allergens [17]. The primary inflammatory lesion of asthma consists of accumulation of CD4+ T helper type 2 (TH2) lymphocytes and eosinophils in the airway mucosa. TH2 cells orchestrate the asthmatic inflammation through the secretion of a series of cytokines, particularly IL-4, IL‑13, IL-5, and IL-9. IL-4 is the major factor regulating IgE production by B cells, and is required for optimal TH2 differentiation [18]. We hypothesized increased serum level of PAHs is associated with asthma severity and had an interaction with some of asthma immunological markers.

We aimed to asses serum level of Naphthalene and 1,2 Benz-anthracene in children with bronchial asthma and detect the relation to asthma severity and their effect on some of asthma immunologic markers.

2. Subjects and Methods

This is a case control study; it was carried out on 120 children from inpatient and the outpatient clinic of Al-Zhraa hospital, Al-Azhar University during the period from Juan 2013 to December 2013. The study included 60children with bronchial asthma, their ages ranged from 6 to 17 year. They were 39 males (65%) and 21 females (35%). Also it included 60 ages and sexes matched children served as a control group. Children diagnosed as bronchial children according to GINA 2012 [19] and they were sub classified into (mild persistent, moderate persistent and severe persistent) asthma according to GINA, 2008 [20]. Children less than 6 years and children with other causes of wheezy chest were excluded from the study. Informed consent was obtained from the participating parents in adherence with the guidelines of the ethical committee of AL-Zahraa hospital, AL-Azhar University, Cairo, Egypt.

All studied children were subjected to: full history taking included (complaint, residence, present history, past history, family history of asthma or atopy, second hand smoking, bronchial asthma symptoms, triggering factors), full general and local chest examination.

Laboratory investigations:

Blood sampling

-2 ml blood was taken on EDTA vacutainer for complete blood picture with total and differential leukocytic count using Cell-Dyn 1800.

-5 ml of blood was taken on venoject tube. Samples were centrifuged for 20 minutes at 3000 rpm and the serum was separated and divided into two specimens, one for (IL5, IgE and IL13) assay by ELISA and the other for measurement of (Naphthalene and 1,2 Benz-anthracene) using high performance liquid chromatography (HPLC) with UV detector, both samples were stored at -20 till the time of the assay.

Naphthalene and 1,2 Benz-anthracene assay

Serum level of two polycyclic aromatic hydrocarbons (PAHs), Naphthalene and 1,2 benz-anthracene, using high performance liquid chromatography (HPLC) with UV detector.

Sample preparation, for each sample, 0.5 ml of serum was added to 0.5 ml of methanol and vortexed for 10–20 seconds. Then, 5 ml from the mixture of 1:1 hexane: diethyl ether was added and vortexed for an additional 1 minute. It was then centrifuged at 3000 rpm for 5 minutes. Eluent 70%: 45% Acetonitrile: 55% Water [21]. Concentrations of the unknown samples were calculated as:

$$\text{Concentration of unknown sample (ppm)} = \frac{\text{Concentration of Std (ppm) x Area (sample)}}{\text{Area (standard)}}$$

IgE, IL-5 and IL-13: were determined by using commercially available immunoassay. Quantitative Sandwich ELISA technique Bender Med systems GmbH compus Veinna. Biocenter 2A-1030 Vienna, Australia, Europe.

Pulmonary function tests:

It's performed by spirometer (spirdsifts pirometry 5000 FUTUD a NANSHI).

Standardization of the testing environment during spirometry was followed according to Miller et al. [22]. The child torso and head should be erect throughout testing, A nose clip was used and the mouth piece of the spirometer was put between the teeth, making a seal with the lips. After standardization of environmental conditions and removing heavy clothes of the child, he or she takes the deepest inspiration and then closes his or her mouth firmly around the mouth piece; he must blow forcibly down the tube without hesitation, without catching or holding breathing. At least three attempts are made then choosing the best result.

FVC, FEV_1, FEF and PEFR expressed as a percentage of predicted values [21].

Statistical Analysis:

Data were collected, revised, coded and entered to the Statistical Package for Social Science (SPSS) version 17 and the following were done: The qualitative data were presented as number and percentages while the quantitative data were presented as mean, standard deviations and ranges. The comparison between two groups with qualitative data were done by using Chi-square test .The comparison between two groups with quantitative data were done by using independent t-test when the data were parametric and Mann-Whitney test was used in the comparison between two independent group when the data were non parametric. Comparison between more than two groups with nominal data was done by using One Way ANOVA test. Pearson and Spearman correlation coefficients were used to assess the relations between two quantitative parameters in the same group. Receiver operating characteristic curve (ROC) was used to assess the best cut off point with sensitivity, specificity, positive predictive value (+PV) and negative predictive value (-PV).

The p-value was considered significant as the following:

P-value > 0.05: Non significant.
P-value < 0.05: Significant.

3. Results

Table 1: shows the anthropometric measurements and demographic data of the studied groups, it revealed no significant difference between both groups regarding weight, height and BMI .However there was a significant difference between both groups as regards residence (urban & rural) as the urban residence is common in cases(85.00%) than in controls (39.00%). Also there is a significant increase in the 2nd hand smoking in patients (60.00%) compared to the controls (15.00%).

Table 2 shows significant increased serum level of naphthalene and 1,2 benz-anthracene in children with bronchial asthma than in controls. Also it shows that all lung functions (FVC, FEV_1, FEV_1/ FVC) were significantly lower in cases than in controls. The same table shows a significant increase in lymphocytes , eosinophilis counts and serum IgE level in children with asthma than in controls, while there was a significant decrease in the serum level of IL 13 and IL 5 in asthma than in controls.

Table 3 shows the relation between residence (urban & rural) areas with serum level of naphthalene and 1,2 benz-anthracene it revealed; significant increase in the serum level of naphthalene and 1,2 benz-anthracene in children with bronchial asthma living in urban than those in rural area.

Table 4 shows the correlation between serum level of (1,2 benz-anthracene and naphthalene) with the studied clinical and laboratory data it revealed; a significant positive correlation between serum level of naphthalene with eosinophilis counts and IgE level ,while a negative correlation with pulmonary functions and IL13 were detected. However there was no correlation regarding the other studied parameters.

Table 5 shows the comparison between bronchial asthma subgroups regarding pulmonary functions and serum levels of (PAHs, IgE, IL 13 and IL5), it revealed; significant decrease in pulmonary functions with significant high level of 1,2 benz-anthracene and naphthalene in children with severe asthma compared to mild and moderate asthma subgroups. Also a significant increase in serum IgE and decrease in IL 13 and IL5 serum level in severe asthma subgroups than in mild and moderate subgroups.

Table 6 & fig 1 show the cut of points for1,2 benz-anthracene and naphthalene between patients and the controls; it revealed that benz-anthracene 81.25% sensitive and 80 % specific followed naphthalene 100% sensitive and 91.67% specific in prediction of bronchial asthma.

Table (1). Demographic data and anthropometric measurements of the studied groups

Variable	Controls N = 60	Cases N = 60	test	
	Mean ± SD	**Mean ± SD**	**t**	**p-value**
Age (year)	11.75±2.63	10.7±3.91	0.996	0.325
BW (kg)	44.1±11.03	37.8±16.74	1.406	0.168
Height (cm)	145.9±11.22	139.1±25.21	1.350	0.182
BMI	20.33±2.71	21.64±3.67	1.281	0.208
Gender	**N %**	**N %**	**X^2**	**P-value**
Females	18 30%	21 35%	0.152	0.696
Males	42 70%	39 65%		
Residence: Urban	21 35%	9 15%	5.378	0.02*
Rural	39 65%	51 85%		
2nd hand smoking: Positive	15 25%	36 60%	13.604	0.001*
Negative	45 75%	24 40%		

* significant: P-value < 0.05

Table (2). Comparison between cases and controls regarding serum level of Naphthalene and 1,2 Benz-anthracene, pulmonary function tests, eosinophilis counts and serum levels of IgE, IL13, and IL5

Variable	Controls N = 60	Cases N = 60	Independent t-test	
	Mean ± SD	Mean ± SD	t / z*	p-value
Naphthalene (ng/ml)	5.13±3.21	24.04±13.03	4.894*	0.001
1,2 Benz-anthracene (ng/ml)	6.99±3.90	16.07±8.97	3.009*	0.006
FVC%	100.7±12.79	85.56±9.33	4.297	0.001
FEV_1%	98.17±13.82	65.02±15.17	7.227	0.001
FEV_1 / FVC	97.53±5.58	74.74±11.29	8.093	0.001
Eosinophilis (cell/mm^3)	2.70±1.13	3.95±2.37	-2.128	0.040
IgE (ng/ml)	14.33±5.25	25.51±6.68	-10.184	0.001
IL13	23.40±3.69	12.73±10.79	7.243	0.001
IL5	10.75±3.87	5.28±2.99	8.652	0.001

t: Independent t-test z*: Mann-Whitney test

Table (3). The relation between residence and serum level of Naphthalene and 1,2 Benzo-anthracene

Variable	Urban	Rural	Mann-Whitney test	
	Mean ± SD	Mean ± SD	Z	p-value
Naphthalene(ng/ml)	23.56 ± 10.69	12.91 ± 2.84	2.982	0.008
1,2 Benz-anthracene(ng/ml)	16.03 ± 6.05	9.25 ± 0.61	2.957	0.009

Table (4). Correlation between serum level of Naphthalene and 1,2 Benz-anthracene with studied parameters

Variable	Naphthalene		1,2 Benz-anthracene	
	r	p	r	p-value
Age (year)	0.414	0.125	0.400	0.124
BW (Kg)	0.502	0.056	0.375	0.152
Height (cm)	0.382	0.160	0.397	0.128
BMI	0.214	0.444	0.022	0.935
FVC%	-0.581	0.023	-0.457	0.075
FEV1%	-0.772	0.001	-0.647	0.007
FEV1\FVC	-0.835	0.000	-0.671	0.004
Eosinophilis (cell/mm^3)	0.792	0.000	0.464	0.070
IgE(ng/ml)	0.641	0.002	0.550	0.012
IL5	-0.382	0.087	-0.320	0.170
IL13	-0.533	0.013	-0.466	0.038

Table (5). Comparison between bronchial asthma subgroups (mild, moderate, severe) regarding serum level of Naphthalene and 1,2 Benz-antrhacene, pulmonary function tests and asthma immune markers

Variable	Mild N= 15	Moderate N= 21	Severe N= 24	One Way ANOVA	
	Mean ± SD	Mean ± SD	Mean ± SD	F	P-value
FVC%	93.80±3.35	87.86±7.99	78.41±7.92	7.696	0.001
FEV1%	85.28±3.64	66.16±9.68	51.35±5.34	37.079	0.001
FEV1/FVC	90.30±3.70	75.00±5.20	64.80±5.50	39.851	0.001
Naphthalene (ng/ml)	10.83±2.21	11.95±2.63	34.62±7.70	69.676	0.001
1,2 Benz-anthracene (ng/ml)	8.78±1.98	9.13±1.74	23.12±7.48	20.834	0.001
IgE (ng/ml)	19.56±2.41	24.68±7.10	29.95±4.78	17.984	0.001
IL 13	28.80±5.94	6.62±4.86	8.04±5.58	87.786	0.001
IL 5	9.54±1.71	3.43±1.52	4.25±1.77	66.212	0.001

Table (6). Cut off point for the serum level of Benzoanthracene and Naphthalene between patients and the controls

	-PV	+PV	Specificity	Sensitivity	Cut off point
1,2 Benz-anthracene	72.7	86.7	80.00	81.25	>9ng/ml
Naphthalene	100.0	93.7	91.67	100.00	>8 ng/ml

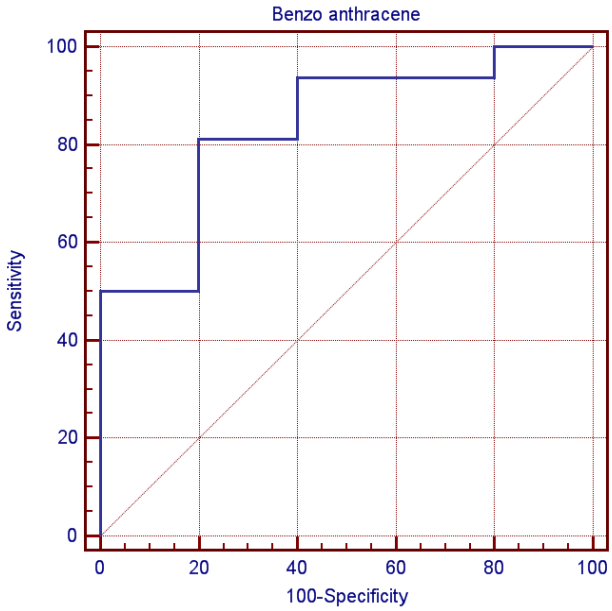

Figure (1). ROC curve for serum level of Naphthalene between patients and the controls

4. Discussion

Attacks of asthma are generated by many factors which include sudden changes in environmental temperature, exercise or exertion, or emotional stress. In children, however, the most common causes are viral infection and exposure to environmental stimulants such as dust particulates and other allergens.

Our study was designed to assess serum level of some polycyclic aromatic hydrocarbons (naphthalene and 1,2 benz-anthracene) as well as some of bronchial asthma immunological markers to evaluate their possible association with bronchial asthma severity.

There is no standard guideline or available data that determine the minimal risk level for a particular PAHs so any amount of PAHs detected in the blood sample was considered as positive in our study.

In the present study there was a significant increase in the serum levels of PAHs (naphthalene and 1,2 benz-anthracene) in children with asthma than in controls. This results concord with Miller et al. who reported that early exposure to airborne PAHs and environmental tobacco smoke can lead to increased respiratory symptoms and probable asthma even as early as at the age of 12 to 24 months. Also our study is in agreement with other studies who found that significant association between exposure to polycyclic aromatic hydrocarbon and asthma in children [23-25].

It is not surprising to find Kids urban residents who suffer from asthma had a high level of (naphthalene and 1,2 benz-anthracene) than kids rural resident this may attributed to more air pollution and exposure to traffic-related polycyclic aromatic hydrocarbons in urban areas . Also we found that children exposed to the second hand (passive smoking) at home, or staying in the same place with a smoker, are at a higher risk of asthma. Smoking and second hand smoking are a direct contributor to PAH exposure among humans. These data conceded with the established data that asthma is more common in children exposed to second hand smoking [26-30]. Passive smoker children therefore may build up an allergic or inflammatory pulmonary response and increased DNA damage due to direct contact with PAH and environmental tobacco smoke [31-32].

In our study lung functions (FVC, FEV_1, FEV_1/ FVC) were assessed and we noticed a significant decrease of lung functions in asthmatic children than the controls, these results were similar to the study done by Barraza-Villarreal, et al; 2014 [33] who concluded that air pollution exposure were inversely associated with lung functions. other studies reported the same effect on lung functions [34-35].

In the present study it is interesting to note that interleukin-5 and 13 levels were reduced in the serum of asthma patients while it is not surprisingly to find a highly significant increase in (eosinophlis, IgE), these results are in consistent with *in vitro* studies that show a potent inhibitory effect of corticosteroids on gene expression and on the release of pro-inflammatory cytokines [36] as corticosteroids have several anti-inflammatory actions [37-39].

Davoodi et al; 2012 [40] suggest the neutral role of IL13 in the inflammatory process and we suggest that these cytokines may be consumed at the site of inflammation and on the same line we found a negative correlation between pulmonary functions (FEV1, FEV_1/ FVC) with eosinophlis and IgE levels as we suggest that the inflammatory response of airways most likely influences the decrease in lung functions, IL-13 is produced by CD4_ T cells, NK T cells, mast cells, basophils, eosinophils, and monocytes. [41-45] ,it is implicated as a central regulator in IgE synthesis, mucus hyper secretion airway hyper-responsiveness (AHR), and fibrosis [46], others such as IL-5 are essential to eosinophil hematopoiesis, activation and survival in tissue [47-51].

In the present study we evaluated and compared the three studied asthma sub groups and according to our results patients with severe asthma had increased PAHs levels, decreased pulmonary functions and increased IgE with significant decrease in IL5 and IL13.

ROC analysis revealed that naphthalene and 1,2 benz-anthracene value at a cut off value of 8ng/mL and 9ng/ml respectively can predict bronchial asthma development in children with high specificity and sensitivity

In conclusions: exposure to PAHs (naphthalene, 1, 2 benz-anthracene) appears to be associated with asthma in children and there is a relation to bronchial asthma severity with a significant interaction with asthma immunological markers. It is essential that efforts are made to reduce pollution to avoid chest allergy.

REFERENCES

[1] Hossny EM, Hasan ZE, Allam MF and Mahmoud ES (2009). Analysis of the filed data of a sample of Egyptian children with bronchial asthma. Egypt J Pediatr Allergy Immunol; 7(2):59-64.

[2] Masoli M, Fabian D, Holt S, Beasley R (2004). Global initiative for asthma (GINA) program. The global burden of asthma: executive summary of the GINA Dissemination Committee report. Allergy; 59:469-78.

[3] Szefler SJ (2008). Asthma progression: Can we and should we measure it? Journal of Allergy and Clinical mmunology; 121:598-600.

[4] Miller RL, Garfinkel R, Lendor C, Hoepner L, Li Z, Romanoff L, Sjodin A, Needham L, Perera FP, Whyatt RM (2010). Polycyclic aromatic hydrocarbon metabolite levels and pediatric allergy and asthma in an inner-city cohort. Pediatr Allergy Immunol; 21: 260–267.

[5] USEPA, (2008). United States Environmental Protection Agency, Office of Environmental Information, Emergency Planning and Community Right-to Know Act – Section 313: Guidance for Reporting Toxic Chemicals: Polycyclic Aromatic Compounds Category, EPA 260-B-01-03, Washington, DC.

[6] Maliszewska-Kordybach, B (1999). Sources, Concentrations, Fate and Effects of Polycyclic Aromatic Hydrocarbons (PAHs) in the Environment. Part A: PAHs in Air. Polish Journal of Environmental Studies; (8), 3, 131-136.

[7] Ravindra K, Sokhi R, Grieken, RV (2008). Atmospheric polycyclic aromatic hydrocarbons: Source attribution, emission factors and regulation. Atmospheric Environment; 42, 2895-2921.

[8] Abrantes R, Assunção JV ,Nóbrega RB (2004). Emission of polycyclic aromatic hydrocarbons from light-duty diesel vehicles exhaust. Atmospheric Environment;38: 1631-1640.

[9] Ravindra K, Wauters E, Taygi, SK, Mor, S , Van Grieken R (2006). Assessment of air quality after the implementation of CNG as fuel in public transport in Delhi, India. Environmental Monitoring and Assessment; 115: 405–417.

[10] Harrison MR, Smith DJT, Luhana L (1996). Source apportionment of atmospheric polycyclic aromatic hydrocarbons collected from an urban location in Birmingham, UK. Environ Sci Technol;30(3):825–32.

[11] Nielsen T (1996). Traffic contribution of polycyclic aromatic hydrocarbons in the center of a large city. Atmos Environ; 30:3481–90.

[12] Rogge WF, Hildermann LM, Mazurek MA, Cass GR (1993). Sources of fine organic aerosol. 2. Non-catalyst and catalyst-equipped automobiles and heavy-duty diesel trucks. Environ Sci Technol; 27:636–51.

[13] Agency for Toxic Substances and Disease Registry (1995). U.S. Department of Health and Human Services. Toxicological profile for polycyclic aromatic hydrocarbons (PAHs). Atlanta.

[14] Downs SH, Schindler C, Liu LJ (2007). Reduced exposure to PM10 and attenuated age-related decline in lung function. N Engl J Med; 357: 2338–47.

[15] McCreanor J, Cullinan P, Nieuwenhuijsen MJ (2007). Respiratory effects of exposure to diesel traffic in persons with asthma. N Engl J Med; 357: 2348–58.

[16] Morgenstern V, Zutavern A, Cyrys J (2008). Atopic diseases, allergic sensitization, and exposure to trafficrelated air pollution in children. Am J RespirCrit Care Med; 177: 1331–7.

[17] Diaz-Sanchez D, Tsien A, Fleming J, Saxon A (1997). Combined diesel exhaust particulate and ragweed allergen challenge markedly enhances human in vivo nasal ragweed-specific IgE and skews cytokine production to a T helper cell 2-type pattern. J Immunol;158(5):2406-13.

[18] Ngoc LP, Gold DR, Tzianabos AO, Weiss ST, Celedon JC, (2005). Cytokines, allergy and asthma. Curr Opin Allergy Clin Immunol;5:161-6.

[19] GINA (Global Initiative for Asthma),(2012). Available at http://www.ginasthma.org/local/uploads/files/GINA_Pocket_April20_1.pdf.

[20] GINA (Global Initiative for Asthma)) (2008). 'GINA Report, Global Strategy for Asthma Management and Prevention' GINA accessed athttp://www.ginasthma.com/GuidelinesResources.asp?l1=2&l2=0.

[21] Van Schooten FJ, Moonen EJ, van der Wal L, Levels P, Kleinjans JC (1997).Determination of polycyclic aromatic hydrocarbons (PAH) and their metabolites in blood, feces, and urine of rats orally exposed to PAH contaminated soils. Arch Environ Contam Toxicol; 33(3):317-22.

[22] Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten P, Gustafsson R, Jensen, Johnson DC, MacIntyre N, McKay R, Navajas D,. Pedersen OF, Pellegrino R, Viegi G and Wanger J (2005). Standardisation of spirometry. EurRespir J; 26: 319–338.

[23] AL-Daghri NM (2008). Serum polycyclic aromatic hydrocarbons among children with and without asthma: correlation to environmental and dietary factors. International Journal of Occupational Medicine and Environmental Health; 21(3):211 – 17.

[24] Suresh R, Shally A, Mahd AA, Patel DK, Singh VK, and Rita M (2009). Assessment of association of exposure to polycyclic aromatic hydrocarbons with bronchial asthma and oxidative stress in children: A case control study. Indian J Occup Environ Med; 13(1): 33–37.

[25] Al-Daghri, NM, Alokail MS, Abd-Alrahman SH, Draz HM, Yakout SM, and Clerici M (2013). Polycyclic aromatic

hydrocarbon exposure and pediatric asthma in children: a case–control study. Environmental Health; 12:1.

[26] Lu H, Zhu L (2007). Pollution patterns of polycyclic aromatic hydrocarbons in tobacco smoke. J Hazard Mater;139 (2): 193–98.

[27] Ding YS, Yan XJ, Jain RB, Lopp E, Tavakoli A, Polzin GM, (2006). Determination of 14 polycyclic aromatic hydrocarbons in mainstream smoke from US brand and non-US brand cigarettes. Environ Sci .Technol;40(4):1133–8.

[28] Ding YS, Ashley DL, Watson CH (2007). Determination of 10 carcinogenic polycyclic aromatic hydrocarbons in mainstream cigarette smoke. J Agric Food Chem; 55:5966–73.

[29] Lu H, Amagai T, Ohura T (2011). Comparison of polycyclic aromatic hydrocarbon pollution in Chinese and Japanese residential air. Journal of Environmental Sciences; 23(9) 1512–17.

[30] Miller RL, Garfinkel R, Horton M, Camann D, Perera FP, Whyatt RM, et al. (2004) . Polycyclic aromatic hydrocarbons, environmental tobacco smoke, and respiratory symptoms in an inner-city birth cohort. Chest;126:1071–8.

[31] Leem JH, Kim JH, Lee KH, Hong Y, Lee KH, Kang D, et al (2005). Asthma attack associated with oxidative stress by exposure to ETS and PAH. J Asthma; 42 (6): 463–7.

[32] Zalata A, Yahia S, El-Bakary A, Elsheikha HM(2007). Increased DNA damage in children caused by passive smoking as assessed by comet assay and oxidative stress. Mutat Res; 629(2):140–7.

[33] Barraza-Villarreal A, Escamilla-Nuñez MC, Schilmann A, Hernandez-Cadena L, Li Z, Romanoff L, Sjödin A,et al. (2014). Lung function, airway inflammation, and polycyclic aromatic hydrocarbons exposure in mexican schoolchildren: a pilot study. J Occup Environ Med; 56 (4): 415-9.

[34] Tager IB (1999). Air Pollution and Lung Function Growth Is It Ozone? Am J Respir Crit Care Med; 160: 387–89.

[35] Moshammer H, Hutter HP, Hauck H, Neuberger M (2006). Low levels of air pollution induce changes of lung function in a panel of schoolchildren. EurRespir J; 27:1138–1143.

[36] Rolfe FG, Hughes JM, Armour CL, Sewell WA (1992). Inhibition of interleukin-5 gene expression by dexamethasone. lmmunol, 77:494-499.

[37] Morgenstern V, Zutavern A, Cyrys J, Brockow I, Koletzko S, Kramer U, Behrendt H, Herbarth O, von Berg A, Bauer CP, Wichmann HE, Heinrich J (2008). Atopic diseases, allergic sensitization, and exposure to traffic-related air pollution in children. Am J Respir Crit Care Med; 177:1331–1337.

[38] Nel AE, Diaz-Sanchez D, Ng D, Hiura T, Saxon A (1998). Enhancement of allergic inflammation by the interaction between diesel exhaust particles and the immune system. J Allergy Clin Immunol; 102:539–554.

[39] Takenaka H, Zhang K, Diaz-Sanchez D, Tsien A, Saxon A (1995).Enhanced human IgE production results from exposure to the aromatic hydrocarbons from diesel exhaust: direct effects on B-cell IgE production. J Allergy Clin Immunol; 95:103–15.

[40] Davoodi P, Mahesh PA, Holla AD, Vijayakumar GS, Jayaraj BS, Chandrashekara S, Ramachandra NB (2012). Serum levels of interleukin-13 and interferon-gamma from adult patients with asthma in Mysore, Cytokine; 60(2):431-7.

[41] Deng WP, Nickloff JA (1992): Site-directed mutagenesis of virtually any plasmid by eliminating a unique site. Analytical Biochem; 200:81–88.

[42] Madhankumar AB, Mintz A, Debinski W (2002). Alanine - scanning mutagenesis of _-helix D segment of interleukin-13 reveals new functionally important residues of the cytokine. J Biol Chem; 277:43194–43205.

[43] Jain E, Bairoch A, Duvaud S, Phan I, Redaschi N, et al.(2009). Infrastructure for the life sciences: design and implementation of the Uni Prot website. BMC Bioinformatics, 10:136. http://www.uniprot.org/uniparc.

[44] Neill DR, Wong SH, Bellosi A, Flynn RJ, Daly M, et al. (2010). Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. Nature;464:1367–1370.

[45] Fahy JV (2002). Goblet cell and mucin gene abnormalities in asthma. *Chest* ; 122(Suppl.):320S–326S.

[46] Munitz A, Brandt EB, Mingler M, Finkelman FD, Rothenberg (2008). Distinct roles for IL-13 and IL-4 via IL-13 receptor _1 and the type II IL-4receptor in asthma pathogenesis. Poceedings of the National Academy of Sciences; 105:7240 –724.

[47] Krishnaswamy G, Liu MC, Su SN, Kumai M, Xiao HQ, Marsh DG, Huang SK(1993). Analysis of cytokine transcripts in the broncho-alveolar lavage cells of patients with asthma. Am J Respir Cell MolBiol; 9(3):279-286.

[48] Huang SK, Krishnaswamy G, Su SN, Xiao HQ, Liu MC (1994).Qualitative and quantitative analysis of cytokine transcripts in the bronchoalveolar lavage cells of patients with asthma. Ann N Y AcadSc; 725:110-117.

[49] De Diego A, Martinez E, Perpina M, Nieto L, Compte L, Macian V, Senent L (2005). Airway inflammation and cough sensitivity in cough-variant asthma. Allergy; 60(11): 1407-1411.

[50] Lehtimaki L, Kankaanranta H, Saarelainen S, Turjanmaa V, Moilanen E (2005). Peripheral inflammation in patients with asthmatic symptoms but normal lung function. J Asthma; 42(7): 605-609.

[51] Simpson JL, Wood LG, Gibson PG (2005). Inflammatory mediators in exhaled breath, induced sputum and saliva. Clin Exp Allergy;35(9):1180-1185.