ISSN 0975-6299

Vol 2/Issue 2/Apr-Jun 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

CLINICAL TOXICOLOGY

MODULATION OF IgE LEVELS IN LEAD EXPOSED CHILDREN BY PARENTAL CIGARETTE SMOKING, QUALYOBIA GOVERNATE, EGYPT

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ABSTRACT

Many children possessing blood lead levels (BLL) in excess of centre of disease control and prevention (CDC) guidelines (>10 µg/dL) exist especially in urban areas with a predominance of housing built early to mid-20th century. In addition, many children are exposed in the home to Parental tobacco smoke (PTS). The current study examined the effect of lead-exposure (BLL levels of 1-55 µg/dL) with/without concurrent PTS exposure on immune system function in 318 children aged 6-84 months from some urban areas, Qualyobia Governate, EGYPT. In this population, 62.9% of children came from smoking homes, 36.5% of children possessed BLL levels >10 µg/dL, 51.9% of children were under 2 years of age, and the population was economically homogeneous. Multiple immune function markers including cell counts, and IgE levels were analyzed for correlation with Pb and / or PTS exposure. For children exposed to both Pb and PTS, a marginally significant relationship between IgE and BLL levels was observed (p=0.12). IgE levels increased in children with combined Pb and PTS exposure. For children exposed to Pb without PTS, decreased lymphocyte cell counts, and serum IgE levels, while granulocyte counts (%granulocytes) was increased. A statistically significant correlation between PTS and BLL levels was found.

ISSN 0975-6299 Vol 2/Issue 2/Apr-Jun 2011

KEYWORDS

Pb-exposure, immune function, Ig E, parental tobacco smoke (PTS)

INTRODUCTION

Elevated blood lead level (BLL) levels in children are linked to immunological, behavioral, and cognitive deficits [1]. Children between 12-24 months exhibit greater hand-to-mouth activity and thus are at increased risk of environmental lead (Pb) exposure from dust, soil, and water [2,3,4]. BLL levels have decreased among young children in the many countries of the world due to an effort to eliminate Pb sources in the environment [5,6]. **Jones *et al, 2009*** [2] noted an 84% decrease in the U.S. children with BLL ≥ 10 between 1988-1991 and 1999-2004. However children living in housing built prior to 1950, common in poorer areas, are three times more likely to have BLL >5µg/dL [7].

Pb-exposure suppresses specific lymphocyte cell populations [8; 9] stimulates humoral immunity including B-cell proliferation [10; 11], and increases IgE levels [10] in children. **Sarasua *et al, 2000*** [12] noted significant increases in IgG, IgA, and IgM in children under age three with elevated PbB, but found no relationship between elevated PbB levels and immunoglobulin levels for adults and children over 3 years of age.

Since cigarettes contain heavy metals such as cadmium and Pb, it has been recently suggested that exposure to passive tobacco smoking (PTS) increases the level of Pb in the blood [13, 14]. **Lutz, et al. (1999)** [10] noted that children in higher BLL risk classes were more likely to come from a smoking home.

The current study aimed to examine the immune system of children from urban cities (banha and its surrounding cities), qualyobia governate, Egypt who were tested for environmental Pb exposure with or without concurrent PTS exposure.

MATERIALS AND METHODS

***Study population*:** Three hundred eighteen children with blood Pb levels ranging from 1-70 or more µg/dL (318 parental responses to survey questions related to PTS exposure, 200 (62.9%) with PTS exposure.) were surveyed for markers of immune function including cell counts, IgE levels.

The study children aged 6 months to 7 years (84 months) selected randomly from children attending the outpatient clinics of banha university hospital, Banha city, Qualyobia Governate. A blood samples were tested for elevated BLL levels (≥10µg/dL). To avoid false positive results from capillary sampling, a venous blood sample was drawn to verify BLL levels and children undergoing venous blood sampling were enrolled in the current study. Informed consent for participation was obtained from a parent according to NIH guidelines at the time of the venous sampling.

Sample collection: A blood sample of 8-10mL was taken via venipuncture with a 23 gauge needle and a 10ml uncoated syringe. The samples were immediately transferred to uncoated or heparin-coated Pb-free Vacuatiner tubes (Becton-Dickinson) and stored at ambient air temperature. The samples were transported to Laboratories of Faculty of agriculture, Benha University on the same day for analysis (**Yee et al, 1994**) [15].

Blood Pb analysis: BLL levels were determined by graphite furnace atomic absorption spectrophotometry in Laboratories of Faculty of agriculture, Benha University [14]. Each specimen was diluted tenfold in 5% Triton X solution and run in triplicate with samples of known Pb content (low, medium,

and high; obtained from Laboratory of Hygiene) [15]. Specimens were further diluted if absorbance values exceed the calibrated range.

Cell Counts: A complete blood count was performed on heparinized blood in Benha University Hospitals laboratories to obtain percent lymphocytes, monocytes, and granulocytes [16,17].

Cytofluorimetry: Cytofluorimetry analysis determined percent T and B lymphocyte classes and various cell surface activation antigens [18]. The analysis was performed at Benha University Hospitals laboratories.

Serum IgE: IgE levels were ascertained using micro-particle enzyme immunoassays (MEIA) (IMX system, Abbott Laboratories) at Benha University Hospitals laboratories [19]. Subclass-specific mouse monoclonal anti-human IgE was utilized to avoid cross-reactivity. The IMX system uses six standard calibrator solutions for human IgE, referenced against the WHO Second International Preparation. High (1200 IU/mL), medium (80 IU/mL), and low (1.5 IU/mL) controls were also included with each assay [10].

Statistical analysis: The SPSS was used for data management and analysis and the Microsoft power point for charts. Quantitative data were presented as mean +SD. For comparison of the two groups' means, the Student's t-test was used and Spearmans' correlation coefficient was calculated. All tests were two tailed and considered significant when $p<0.05$. The equality of median values between PbB risk classes were tested using a Kruskal-Wallis test using residuals as data points for the entire population and subpopulations with/without concurrent environmental tobacco smoke exposure. **(Wayne, 1995)[20].**

RESULTS

The data were collected from 2006-2008. Participants were retrospectively assigned to BLL risk categories based on CDC (centre of disease control) guidelines from 1991 modified to include current levels of concern for cognitive impairment (**Table 1**).

Table 1

Risk classifications based on blood lead concentration (BLL)[a]

Class	Blood Pb (µg/dL)	Comment
IA	<5	Not considered to be lead-poisoned
IB	5-9	Not considered to be lead-poisoned; potential cognitive impairment.
IIA	10-14	If many children in this range, community-wide childhood lead poisoning prevention activities triggered. Children in this range need frequent screening.
IIB	15-19	Nutritional and educational interventions and more frequent screening are necessary. If blood lead level persists in this range, an environmental investigation and intervention should be performed.
III	20-44	Environmental evaluation and remediation, and a medical evaluation are needed. Pharmacologic treatment of lead poisoning may be necessary.
IV	45-69	Medical and environmental interventions are needed, including chelation therapy.
V	>70	This is considered to be a medical emergency. Medical and environmental management should begin immediately.

[a] *Modified from the CDC statement on preventing lead poisoning in young children (1991)*

The cohort consisted of 318 children: 56.3% male and 43.7% female (**Table 2A**).

Table 2A

Population demographics age of children from rural areas around Benha city, Egypt enrolled in the current study.

Variable	Percent (%) [a] Total n = 318
Sex	
Male	179 (56.3%)
Female	139 (43.7%)
AGE/ months	
Below 24	165(51.9%)
24-below54	122 (38.4%)
54- below84	26 (8.2%)
Above 84	5 (1.6%)

[a] *Population WIC eligible, indicative of low SES*

More than half of the children were under 24 months of age (51.9%), with 38.4% in the 24-below54 month age group, 8.2% in the 54-below84 month age group, and 1.6% > 84 months. Of the 318 children, parental response to a survey question related to type/quantity of child smoke exposure was collected for 309 participants. Two thirds i.e. 212 (66.7%) of the children were from smoking homes and 97 (30.5%) were from non-smoking homes.

As shown in **Table 2B**, 15.8% of the participants were categorized as Class IA, 47.5% as Class IB, 24.9 % as Class IIA, 5.5% as Class IIB, and 5.8 % as Class III. When separated by parental report of smoking exposure, a shift towards higher BLL is evident for children from homes where parents smoke cigarettes. For children from smoking homes, 39.6% (85 of 212 child) meet the classification of Pb-poisoned (Class IIa-IV) while 28.9% (28 of 97 child) of children from non-smoking homes are classified as Pb-poisoned. A corresponding significant increase in mean BLL level was observed for children from smoking homes ($p<0.05$). A greater percentage (71.9 %) of children from non-smoking homes (69 from 97) was classified as non-Pb poisoned (CDC Class IA & IB) in comparison with percentage of children from smoking homes (59.9%) {127 child from 212}.

Table 2B

BLL classification distribution, stratified by smoking status, of children included in study.

Pb Class	Total (n)	Total (%)	PTS Exposed			
	309	100	No (n= 97)	Percent (%)*	Yes (n= 212)	Percent (%)*
IA	49	15.8	23	23.7	26	12.3
IB	147	47.5	46	47.4	101	47.6
IIA	77	24.9	19	19.6	58	27.3
IIB	17	5.5	2	2.1	15	7.1
III	18	5.8	7	7.2	11	5.2
IV	1	<0.5	0	-	1	<0.5
Average BLL (µg/dL) (t- test)**		**9.23**	8.62 NS (P > 0.05)		9.57 *** (P< 0.05)	

**Percent of population non-PTS or PTS exposed calculated from total number of children in subgroup. Example: Of 97 non-PTS exposed children, 23 possessed BLL levels in Class IA for a percentage of 23.7% of non-PTS exposed children in Class IA*

***t-test for significance revealed significant difference between BLL values for non-smoking vs. smoking homes (P< 0.05)*

Together, these data support the general observation of higher BLL levels in children from smoking homes. A significant correlation of BLL and PTS exposure (reported as number of cigarettes smoked/day) was evident such that as PTS exposure increased, BLL increased in the study population (**Table 2C**; p=0.05).

Table 2C

Correlation of BLL level and PTS exposure exists for children from studied group

	Correlation coefficient (r)	p-value
PTS and BLL	0.113	< 0.05*

A subset of data from 69 children without either Pb-exposure or PTS exposure (BLL <10 µg/dL; parental report of non-smoking home) was tested for dependence of variables on age since age of the child influences immune system maturity (**Table 3**).

Table 3

Spearman correlation between immune parameters and age for children without PTS exposure and PbB < 10µg/dL, a subset of the study population (n=69).

Parameter	Age	
	Correlation coefficient (ρ)	p-value
% Lymphocytes	-0.54	<0.001**
IgE (IU/ml)	0.61	<0.001**
% Granulocytes	0.40	0.003**
% Total T cells	-0.20	0.14
% Total B cells	0.09	0.51
% Monocytes	0.07	0.60

** *p < 0.05*

* *0.05 < p < 0.10*

Children with either Pb-exposure and/or PTS exposure were excluded since both toxicants also modify immune system activity and maturation. The immune parameters identified as age-related were: % lymphocytes and % granulocytes were found to be significantly age dependent. IgE were found to be non-age dependent. The dataset was split into two subgroups for further analysis: children exposed to PTS or not exposed to PTS. A comparison of correlation of immune parameters and BLL in children from these subgroups is given in **Table 4**.

Table 4
Spearman correlation between immune parameters and blood lead levels (PbB) for children with and without PTS exposure

Parameter	Without PTS exposure		PTS exposure	
	Correlation coefficient (ρ)	p-value	Correlation coefficient (ρ)	p-value
Age Related				
% Lymphocytes	-0.21	0.08[*a]	-0.03	0.67[a]
IgE (IU/ml)	-0.08	0.50[a]	0.12	0.24[a]
% Granulocytes	0.16	0.20[a]	0.04	0.62[a]
% Total T cells	0.09	0.43	0.06	0.42
Non-Age Related				
% Total B cells	-0.14	0.2*	0.01	0.89
% Monocytes	0.004	0.98	-0.07	0.34

[**] *p < 0.05*
[*] *0.05 < p < 0.20*
[a] *age used as a covariate*

Few correlations were evident in either group (age as covariate for parameters identified from **Table 3** as age-dependent). For the children without parental report of PTS exposure, % Granulocytes exhibit a weak trend of positive correlation. In the same subpopulation, the % total B-cells and %Lymphocytes exhibit a trend of negative correlation with BLL levels. This was not seen in children with PTS exposure. The range of values for both the significantly correlated parameters and those whose values trend toward correlation outcomes were similar between the two groups. These outcomes may indicate a modulation of the effect of Pb-exposure on the immune cells by PTS exposure. The correlation of BLL with PTS exposure does not appear to result in a magnification of effect of Pb-exposure on immune cells.

Estimations of the impact of Pb-exposure on median values in Risk Categories were assessed by the Kruskal-Wallis test for age-related immune parameters in the population (**Table 5**; Classes IIB-IV combined for analysis). Three separate population groups are shown. In **Table 5A**, outcomes from the entire population of children enrolled in the current study including both PTS positive and PTS negative reported exposures are tabulated. A positive association between BLL (Risk Class IA-IIB) and IgE levels was found.

In **Table 5B**, outcomes from the age-related immune parameters for the children without reported PTS-exposure are given. In this population subset, significant variation in the %Lymphocytes with BLL levels was observed (p=0.05). Marginally significant variation in IgE levels (p=0.10) and %Granulocytes (p=0.06) between BLL Risk Classes was also observed, with IgE levels tending to decrease over the range of BLL levels. In **Table 5C**, outcomes from age-related immune parameters for the children with reported PTS-exposure are tabulated. IgE levels appear to be positively associated with BLL Risk Class though the Spearman correlation between the two variables was not significant (**Table 4**, p=0.24). This trend is evident as increased IgE levels in Pb-poisoned

In comparison with **Table 5A**, it is apparent that the positive association between

BLL levels and IgE observed in the entire population is driven by the PTS-exposed population subset. This outcome likely reflects the increased number of children in the higher exposure BLL Classifications for the PTS exposure positive population.

Table 5A

Effect of Pb on age related immune para meters; total population (PTS and non-PTS exposure).

Parameter	Risk Class IA		Risk Class IB		Risk Class IIA		Risk Class IIB		Risk Class III		Risk Class IV		Kruskal p-value [a, b, c]
	Median (Min, Max)	N	Median (Min, Max)	N	Median (Min, Max)	N	Median (Min, Max)	N	Median (Min, Max)	N	Median (Min, Max)	N	
% Lymphocytes	51.8 (22.8, 77.5)	37	53.2 (17.2, 78.2)	116	50.4 (10.5, 76.1)	66	58.6 (29.8, 70.1)	12	52.2 (17.2, 78.2)	12	58.3	1	0.35
IgE (IU/ml)	13.0 (0.8, 892)	51	12.0 (0.0, 2008)	152	20.8 (0.40, 611.6)	78	14.9 (4.1, 1756)	18	20.4 (3.6, 235)	18	10.2	1	0.001**
% Granulocytes	41.3 (0.0, 69.8)	37	39.0 (0.0, 73.9)	115	41.4 (10.5, 80.9)	66	33.9 (17.2, 66.8)	12	40.9 (0.0, 73.9)	12	30.0	1	0.61

Table 5B

Effect of Pb on age related immune parameters; subpopulation without PTS exposure.

Parameter	Risk Class IA		Risk Class IB		Risk Class IIA		Risk Class IIB		Risk Class III		Risk Class IV		Kruskal p-value [a, b]
	Median (Min, Max)	N	Median (Min, Max)	N	Median (Min, Max)	N	Median (Min, Max)	N	Median (Min, Max)	N	Median (Min, Max)	N	
% Lymphocytes	54.7 (22.8, 71.7)	16	55.1 (17.2, 72.6)	37	42.9 (17.0, 76.1)	14	58.8	1	50.4 (30.9, 63.2)	4	-	0	0.05**
IgE (IU/ml)	12.0 (2.0, 892)	19	15.1 (0.8, 1545)	40	15.0 (1.6, 388)	17	8.0	1	17.5 (3.8, 69.0)	6	-	0	0.10*
% Granulocytes	7.7 (3.2, 17.1)	16	38.2 (11.1, 72.1)	36	48.3 (17.5, 78.7)	14	33.3	1	41.1 (31.5, 64.3)	4	-	0	0.06*

Table 5C

Effect of Pb on age related immune parameters; subpopulation with PTS exposure.

Parameter	Risk Class IA		Risk Class IB		Risk Class IIA		Risk Class IIB		Risk Class III		Risk Class IV		Kruskal p-value [a, b, c]
	Median (Min, Max)	N	Median (Min, Max)	N	Median (Min, Max)	N	Median (Min, Max)	N	Median (Min, Max)	N	Median (Min, Max)	N	
% Lymphocytes	50.6 (23.4, 77.5)	21	51.0 (18.4, 78.2)	76	51.5 (10.5, 72.1)	52	58.3 (29.8, 70.1)	11	41.9 (35.9, 68.3)	8	58.3	1	0.91
IgE (IU/ml)	14.3 (0.8, 104)	26	10.8 (0.0, 2008)	101	22.1 (0.4, 612)	58	17.8 (4.1, 1756)	15	21.0 (3.6, 235)	11	10.2	1	0.002**
% Granulocytes	43.9 (0.0, 69.8)	21	41.8 (0.0, 73.9)	76	39.6 (10.5, 80.9)	52	34.5 (17.2, 66.8)	11	51.4 (25.8, 61.5)	8	30.0	1	0.94

[a] *Risk class IIB, III, and IV combined for analysis*
[b] *Age as a covariate*
[c] *PTS as a covariate*
** *p < 0.05 for difference between risk groups using Kruskal-Wallis tests*
* *0.05 < p < 0.20 for differences between risk groups using Kruskal-Wallis tests*

In **Table 6**, A, B, and C, the effect of Pb on age and non-age related immune parameters; in either total or sub-population show non significant values.

Table 6A

Effect of Pb on non-age related immune parameters; total population (PTS and non-PTS exposure).

Parameter	Risk Class IA		Risk Class IB		Risk Class IIA		Risk Class IIB		Risk Class III		Risk Class IV		Kruskal p-value [a, b]
	Median (Min, Max)	N	Median (Min, Max)	N	Median (Min, Max)	N	Median (Min, Max)	N	Median (Min, Max)	N	Median (Min, Max)	N	
% Total T cells	69.2 (27.9, 84.4)	43	70.2 (34.8, 90.0)	124	73.6 (36.0, 85.2)	68	66.7 (26.2, 89.1)	11	70.4 (34.8, 90.0)	14	70.9	1	0.38
% Total B cells (CD19+)	13.3 (3.5, 33.6)	44	13.0 (2.8, 30.8)	127	12.9 (3.3, 41.0)	72	12.8 (6.4, 39.9)	14	13.0 (2.8, 30.8)	14	13.8	1	0.99
% Monocytes	6.8 (3.2, 17.1)	37	7.4 (3.2, 28.8)	115	7.0 (1.7, 26.6)	66	7.0 (3.4, 19.3)	12	7.4 (2.6, 28.8)	12	11.7	1	0.63

Table 6B

Effect of Pb on non-age related immune parameters; subpopulation without PTS exposure.

Parameter	Risk Class IA		Risk Class IB		Risk Class IIA		Risk Class IIB		Risk Class III		Risk Class IV		Kruskal p-value [a]
	Median (Min, Max)	N	Median (Min, Max)	N	Median (Min, Max)	N	Median (Min, Max)	N	Median (Min, Max)	N	Median (Min, Max)	N	
% Total T cells	69.3 (27.9, 79.8)	19	72.2 (45.3, 90.0)	39	72.2 (53.1, 83.1)	15	76.2	1	62.5 (41.0, 84.3)	6	-	0	0.39
% Monocytes	7.7 (3.2, 17.1)	16	6.6 (3.3, 27.3)	36	7.3 (4.1, 11.3)	14	7.9	1	5.1 (4.5, 12.5)	4	-	0	0.71

Table 6C

Effect of Pb on non-age related immune parameters; subpopulation with PTS exposure.

Parameter	Risk Class IA		Risk Class IB		Risk Class IIA		Risk Class IIB		Risk Class III		Risk Class IV		Kruskal p-value [a, b]
	Median (Min, Max)	N	Median (Min, Max)	N	Median (Min, Max)	N	Median (Min, Max)	N	Median (Min, Max)	N	Median (Min, Max)	N	
% Total T cells	69.2 (47.0, 84.4)	23	69.4 (34.8, 86.8)	81	74.0 (36.0, 85.2)	52	66.6 (26.2, 89.1)	9	73.3 (59.9, 80.5)	8	70.9	1	0.32
% Total B cells (CD19+)	13.3 (3.5, 33.6)	24	13.8 (3.1, 30.8)	83	13.3 (5.4, 41.0)	54	16.4 (6.4, 39.9)	12	11.3 (2.8, 21.9)	8	13.8	1	0.98
% Monocytes	6.8 (3.9, 11.2)	21	7.6 (3.2, 28.8)	76	6.8 (1.7, 26.6)	52	6.7 (3.4, 19.3)	11	6.2 (2.6, 10.8)	8	11.7	1	0.34

[a] *Risk class IIB, III, and IV combined for analysis*

[b] *PTS used as a covariate*

[**] *p < 0.05 for difference between risk groups using Kruskal–Wallis tests*

[*] *0.05 < p < 0.20 for differences between risk groups using Kruskal-Wallis tests.*

DISCUSSION

Pb-exposure induces neurocognitive impairment in children at low levels (< 5µg/dL) [(21)] as well as developmental delays [(22)], and has been shown to increase IgE levels at low-to-moderate BLLs [(13; 10; 23).] **Mannino et al, 2003** [6] found that second-hand smoke could be associated with increased blood lead levels in U.S. children aged 4-16 years.

The current study focused on the modifications of immune function induced by Pb exposure with additional emphasis on the effect of concurrent PTS exposure. In this study the average lead level was 9.23 this does not came in accordance with **Elaraby et al 1995** [(24)] who conducted a study in Alexandria city Egypt to determine lead level among children . It was found that the mean BLL was 15 .3 which could be explained by the fact that

Alexandria city is more industrialized urbanized city than Banha

Analysis performed indicate that PTS exposure increases BLL levels in both adults and children with the effect evident for lower BLL classifications [25; 26]. **Willers *et al.*, 1988**[27] also reported a significant association between BLL in children and parental smoking, with a dose-response relationship between number of cigarettes smoked and BLL level. In the current study, a significant correlation of BLL level and parental report of PTS exposure was found though the magnitude of difference between the groups is less than in prior publications. This is likely the result of two factors: a gradual decline in the number of children with elevated BLL levels in this population due to active enforcement of Pb-containing paint abatement in rental housing build before 1950, and public health campaigns against smoking in the home or around children that were initiated during the course of this study. These factors resulted in lower overall BLL levels and less PTS exposure for children enrolled later in the study. The correlation of BLL and PTS exposure remained statistically significant throughout all the study. Epidemiological studies support an association of PTS exposure and low to moderate BLL levels in children.

Friedman et al., 2005 [13] observed an association (p = 0.047, OR = 2.87) with elevated BLL and parents smoking in the home. These results were mirrored in a study by ***Ozden et al., 2007*** [14] who found a strong correlation (p < 0.0001) for hair Pb levels and number of smokers in the home (OR = 0. 229; 0.164–0.321 95% CI). **Lutz, et al. (1999)** [10] noted that children in higher BLL risk classes were more likely to come from a smoking home, and the average BLL level was higher in smoking versus non-smoking homes (12.05 µg/dL and 6.93 µg/dL, respectively).

It is not surprising that PTS exposure leads to elevated BLL levels since cigarettes contain Pb. Graphite furnace atomic absorption spectrometry revealed that side-stream smoke from a reference cigarette (1R4F) contained 43.8 ± 2.0 ng of Pb [28]. Absorption of Pb from particulate matter deposits in the bronchial tree is likely increased with smoke inhalation [29; 30]. Deposition of smoke contaminants on home surfaces that are then ingested by young children with high hand-to-mouth activity represents an additional route of oral Pb-exposure. Reported parental smoking patterns in this study included the estimated total number of cigarettes smoked around the child. Approximately 42% of the children in the study were exposed to greater than one-half pack (15) of cigarettes per day. It appears likely that BLL levels in children from the Qualyobia Governate rural areas are elevated due to a combination of sources of Pb contamination including in many cases PTS exposure.

The immune system is developing in these children resulting in a broad range of measures for each test. To examine whether increased BLL levels altered the distribution of immune parameter outcomes, the median values from risk categories were compared using the Kruskal-Wallis test with the covariates of age and PTS as appropriate. Though artificial compression of groups may mask statistically significant outcomes, we have chosen to combine the study highest three exposure groups (Class IIB, III, and IV) for statistical analysis due to the small numbers of children in each of these groups. This resulted in a 4 group comparison of means (Class IA, IB, IIA, and Combined IIB-IV) descriptive of variations in immune function parameters associated with low to moderate Pb-exposure. The mean BLL level of the study population was centered near the division between non-Pb-poisoned and Pb-poisoned (Class IB and Class IIA).

The statistically significant correlation of IgE and BLL as well as the differences in IgE

between BLL risk classes reported in the earlier study is confirmed late in the study. This association is predominant in the children with concurrent Pb and PTS-exposure with a lesser trend also evident in the Pb-exposure without PTS subgroup of this population. **Lannerö *et al, 2008*** [29] noted a dose-response relationship for PTS exposure and IgE sensitization (p = 0.019) and **Krämer *et al, 2004*** [30] noted a positive association between IgE and cotinine levels (nicotine metabolite; OR 1.39-1.62). Findings from the current study raise the question of whether the link between Pb-exposure and IgE levels is at least partially attributable to concurrent exposure to PTS.

Lutz *et al,* 1994 [11] noted strong correlations between BLL and IgE (p = 0.0004) as well as significant differences of IgE across BLL risk classes (p = 0.05) among children aged 9 months to 6 years. Elevated PbB also appears to impair bronchial response in an IgE dependent manner [10]. Thus, the immunomodulatory effects of developmental Pb-exposure may predispose children to immune mediated respiratory diseases including asthma [11,12].

In previous studies, Pb has shown a positive correlation with IgE levels [10; 31] by acting upon IL4 synthesis (cytokine controlling immunoglobulin class switch to IgE production) and leading to an up-regulation of IgE in B cells [31]. In the current study, a non-significant trend toward increased IgE levels with increasing BLL levels was observed in both population subs PTS (Pb-exposure with and without PTS exposure). This finding is consistent with prior reports from this cohort [8, 10, 11] that found only significant associations of Pb-exposure and IgE but a lack of significant impact on other immune parameters.

Of additional interest, children with only Pb-exposure showed decreased cell counts for % lymphocytes and a mixed effect on % granulocytes (trend; not significant). These outcomes support prior reports of the immunotoxic action of Pb [32; 33;34;35 8; 11]. For children with PTS- and Pb-exposure, no effect of combined exposure on lymphocyte and granulocyte cell numbers was found. Studies have shown nicotine modulates the immune system through its effect on B cells [36]. Nicotine stimulates both T cells and B cells; this causes B cells to switch production of IgG1 to IgE [37; 38;39]. In conjunction, these effects manifest increased risk of allergies and asthma in PTS-exposed children. The stimulation of IgE production by PTS was also observed in our study as exhibited by a stronger association of IgE level and BLL level for children exposed to PTS than for children without exposure to PTS. Whether the current finding of a lack of impact of combined Pb- and PTS-exposure on cell counts reflects a blunting of the immunosuppressive effect of Pb or an unidentified confounder cannot be determined from the presented data.

The current study reports a magnification of effect by PTS exposure on Pb-exposure associated elevated IgE levels without a similar multiplicative effect on other immune cells. Though an apparent correlation exists, a clear cause and effect relationship has not been proven between BLL, PTS exposure, and IgE levels. The children in the current study were not undergoing chelation treatment and thus the impact of reduced Pb-levels (due to medical intervention) on IgE levels with/without PTS exposure could not be assessed.

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