Alteration in the Balance between Type 1 and Type 2 Immune Responses among Lead Exposed Population

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ABSTRACT

Several immunotoxic effects of lead were reported. However, overt cytotoxicity of major immune cell types was not evident. It was suggested that lead might rather affect the balance between T-helper 1 and T-helper 2 immune responses cytokines. Such hypothesis was investigated in the current study.

Aims: Studying the effect of environmental exposure to lead on blood lead level (B-Pb) and investigating the balance between interleukin-4 (IL-4) and Gamma interferon (IFN-γ) and its relation to lead exposure and blood lead levels in the study subjects.

Study design: the study was designed to be cross-sectional case-control study.

Methodology: the study was conducted on 80 subjects, 46 of them were environmentally exposed to lead (exposed group) as a result of living near a secondary lead smelter and the other 34 socioeconomically matched subjects (control group) were recruited from a rural area and were not exposed to lead, neither environmentally nor occupationally. All subjects were interviewed, clinically examined and tested for whole blood lead (B-Pb), serum interleukin-4 (IL-4) and Gamma interferon (IFN-γ)) levels.

Results: A statistically significant higher blood lead (P= .006) and serum IL-4 levels (P < .001) in exposed group (when compared to the control group). On the other hand, serum IFN-γ was of significantly lower levels (P < .001) in exposed group than control group. A statistically significant negative correlation was found between B-Pb level and serum IFN-γ (r= -0.284, P= .011) in all subjects.

Conclusion: Environmental exposure to lead alters the balance between Th1 and Th2 cytokine with a bias towards the later.

Keywords: Lead, environmental exposure, immune response, interleukin-4, Gamma interferon.

1. INTRODUCTION

Lead is an abundant toxic metal that is detectable in practically all phases of the inert environment and in all biological systems [1]. In Egypt, lead smelting facilities are a major source of environmental pollution by lead. In 1999, there were 27 lead smelters in Egypt in seven governorates: Cairo, Qalioubiya, Giza, Alexandria, Dakahliya, Minya and Sharkiya. Most of them are secondary smelting facilities [2].

Lead is known to have several toxic effects on different body systems. However, the neurological and immune systems appear to be most sensitive to lead. Recent literature showed that the immune system may even be impaired by lead levels below those required for neurotoxicity to occur. Thus, many people may suffer from suboptimal immune function due to undiagnosed lead exposure [3].

Many studies have shown that lead exposure can lead to immunodeficiencies [4]. Though, more recently, the spectrum of immunopathologies affected by lead has expanded and reports have suggested that lead exposure contributes also to increased risk of atopy, asthma and some forms of autoimmunity [5].

Lead exposure at low to moderate levels, however, appears not to produce overt cytotoxicity of major immune cell types. Instead, immune-associated health effects result from disrupted regulation and shifts in functional capacity. Both in vivo and in vitro studies suggest that lead exposure at low to moderate levels causes a significant shift in the production of T-helper 1 (Th1) versus T-helper 2 (Th2) cytokines with a bias toward the latter. Production of IL-4, IL-6 and frequently IL-10 is elevated while production of IFN-γ is decreased [4]. However, there were other studies that reported exceptions to lead-induced biasing in favor of Th2. These controversial findings together with the apparent gap of information regarding the effects of environmental exposure to lead on the immune system were the triggers of the current study.

2. METHODOLOGY

The study involved 80 subjects recruited into two groups;

a) An "Exposed Group" consisted of 46 subjects who were environmentally exposed to lead as a result of living a residential area (Al-Molliha) that surrounds one of the oldest and largest secondary lead smelters in Cairo, Egypt. The production from the smelter, however, was stopped 15 years prior to the study conductance. The subjects were selected as a purposive sample from about 10,000 populations living within 500 meters from the smelter. **B) A "Control Group"** composed of 34 subjects recruited from a rural area (Abu Rawash) at the borders of Greater Cairo and are not exposed to lead, neither environmentally nor occupationally.

Twenty eight subjects of the exposed group were females and 18 were males. Age ranged from 11 to 70 years (with a mean of 41.30 and a standard deviation of 16.08 years). On the other hand, control group consisted of 11 females and 23 males with their age ranging from 19 to 62 years (with a mean of 38.94 and a standard deviation of 12.43 years).

The whole studied population was subjected to interview, history taking, medical examination and investigation in the form of whole blood lead level, serum interleukin-4 (IL-4) and serum Gamma interferon (IFN-γ). For performing such investigations, a 7.5 mL peripheral venous blood sample was collected by venipuncture from non-fasting subject. From this sample, 2.5 mL was collected in tubes containing EDTA as an anticoagulant and

was used to determine blood lead level and 5 mL was collected in blood tubes without additives or anticoagulants and was used for analysis of IL-4 and IFN-γ.

Blood samples used for assessment of blood lead level were wet-ashed by heating in the presence of mineral acid, and subjected to analysis by atomic absorption spectrometry (AA Series Spectrometer manufactured by Thermo Electron Corporation, USA) using flame photometer.

Blood collected in tubes without additives or anticoagulants (5 mL) was allowed to clot then centrifuged to obtain serum, which was stored at - 20˚C until use for cytokine levels determination. Serum levels of IL-4 and IFN- γ were measured using an in-vitro enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's instructions (Invitrogen, Belgium).

2.1 Statistical analysis

Study data were summarized using mean, standard deviation, minimal and maximum (range) values. Statistical comparisons between the exposed and control groups were performed using Nonparametric Mann Whitney test while the correlations between the blood lead level and the levels of serum IL-4 and IFN-γ were performed with the Pearson's correlation test. P-values less than or equal to 0.05 were considered statistically significant. All data were coded and processed using the statistical package SPSS version 15.

3. RESULTS AND DISCUSSION

There was a statistically significant difference between exposed and control groups regarding blood lead level (with higher levels in exposed group subjects), serum Interlukin-4 (IL-4) (with higher levels in exposed group subjects), serum Interferon Gamma (IFN-γ) (with lower levels in exposed group subjects) (Table - 1 and Figure - 1).

Table-1: Blood lead level and levels of serum IL-4 and serum IFN-γ in exposed and control groups

Parameter	Exposed Group		Control Group		Statistical difference	
	Range (median)	Mean ± SD	Range (median)	Mean ± SD	Z	*P*
Blood Lead (Pb) (µg/dl)	2.8 - 63.5 (15.5)	20.28±13.99	4.40 – 28 (11)	12.52±5.61	2.75	.006*
Interleukin-4 (IL-4) (pg/ml)	0.07 - 166.6 (26.4)	41.26±38.12	11 – 105 (15.9)	20.16±16.14	3.675	< .001*
Interferon Gamma (IFN-γ) (pg/ml)	2.89 - 85.33 (6.33)	8.96±12.38	11 – 28 (19.79)	19.52±3.5	6.892	< .001*

*Statistically significant correlation

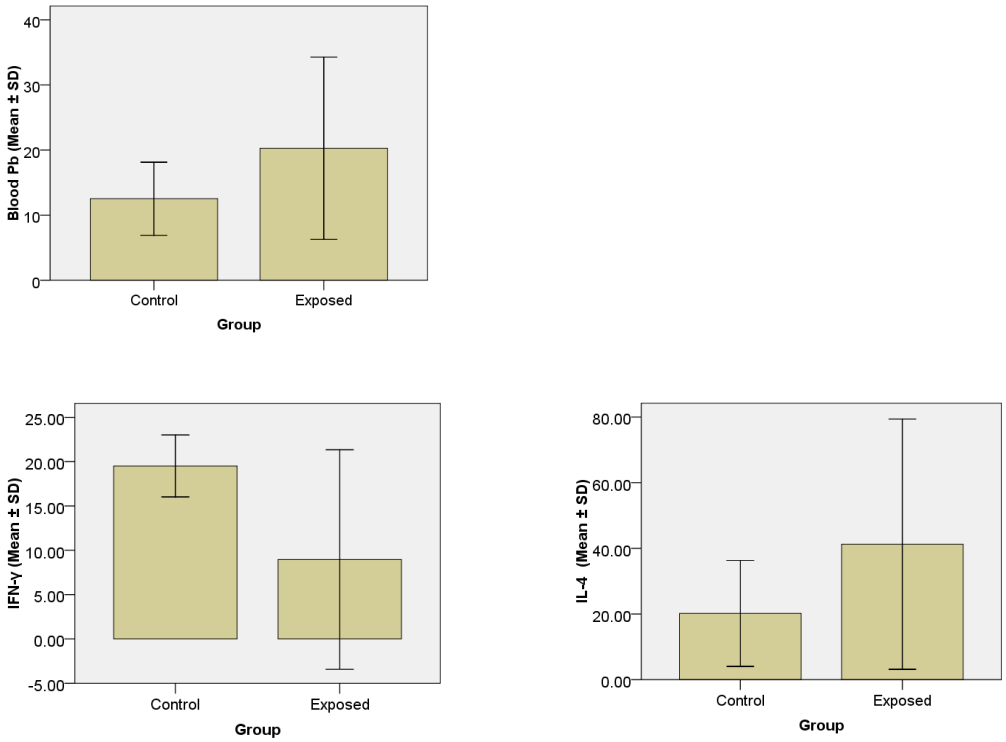

Figure-1: Blood lead level and levels of serum IL-4 and serum IFN-γ in exposed and control groups

When studied in all subjects, there was no statistically significant correlation between blood lead level and serum Interlukin-4 (IL-4). However, a statistically significant negative correlation was found between blood lead level (on one hand) and serum Interferon Gamma (IFN-γ) and the IFN-γ/IL-4 ratio (on the other hand) (Table - 2 and Figure - 2).

Table-2: Correlation between blood lead level and levels of serum IL-4 and serum IFN-γ in all study subjects

Blood Lead (Pb) (µg/dl)		Parameter
P	***r***	
.212	0.141	**Interlukin-4 (IL-4) (pg/ml)**
.011*	-0.284	**Interferon Gamma (IFN-γ) (pg/ml)**
.018*	-0.264	**IFN-γ/IL-4**

*Statistically significant correlation

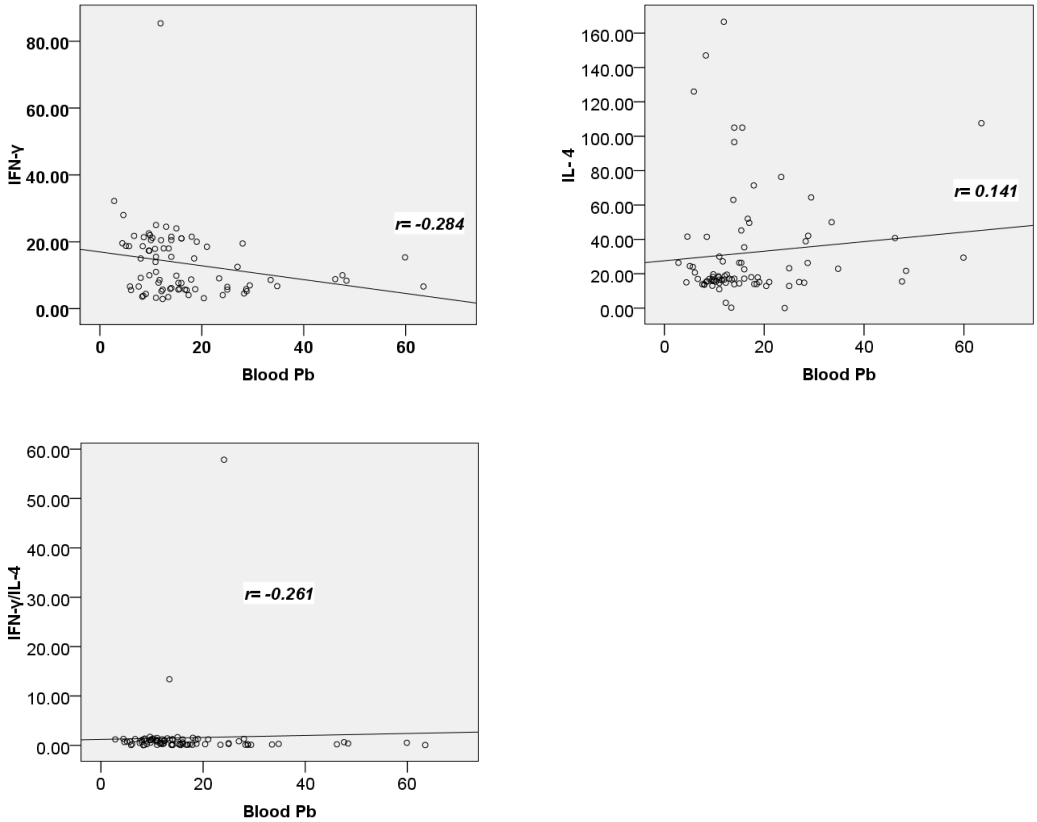

Figure-2: Correlation between blood lead level and levels of serum IL-4 and serum IFN-γ in all study subjects

The results of the present work come in agreement with the findings of several studies that investigated the shift in the balance between Th1 and Th2 immune responses in relation to lead exposure. Kaminska et al (1998) [6] have proven that when the blood leukocyte of cows were induced in vitro to produce IFN-γ and TNF-α in the presence of different concentrations of lead, a significant reduction in IFN-γ titers but enhanced production of TNF-α was observed.

Hemdan et al (2005) [7] also studied the influence of lead on the in vitro cytokine production of human peripheral mononuclear blood cell. The results showed that expression of interferon-γ (IFN-γ), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α) was reduced whereas IL-4, IL-6, and IL-10 secretion was increased in the presence of lower lead doses. Chen et al (1999) [8] have also demonstrated that lead suppressed Th1 type responses (delayed-type hypersensitivity and IFN-γ production) and enhanced Th2-type response (IL-4 and increased TNF-α production) from macrophages in mice.

In humans, Yucesoy et al (1997) [9] reported that chronic lead exposure in lead-exposed workers resulted in significant suppression of the serum IL-1β level and IFN-γ, but did not alter IL-2 and TNF-α level.

Valentino et al (2007) [10] also found that low lead-exposed workers had significantly higher plasma IL-10 level, T cell cross-regulator factor produced by Th2 cells leading to reduced Th1 cytokine production, and higher plasma TNF-α level compared to non-exposed workers.

The results of the present work and the previously mentioned consistent studies were not in agreement with two exceptional studies. One of them conducted by Goebel et al (2000) [11] who reported a local bias towards Th1 response with increased IFN-γ production in the intestinal tract of specialized autoimmune diabetes prone strain of mice treated with $PbCl_2$ via oral administrations in drinking water.

The other study was conducted by Mishra et al (2003) [12] on workers occupationally-exposed to lead. Investigators found a significant increase in IFN-γ production in phytohaemagglutinin (PHA) stimulated culture supernatant of peripheral blood mononuclear cells (PBMCs) obtained from lead exposed individuals as compared with unexposed controls with a positive correlation between blood lead level and IFN-γ production in culture.

The findings of these two studies, however, should not be considered contradictory to the current and previously mentioned studies. This is because, in Mishra and Colleagues' work, the authors themselves attributed the results to the very high lead levels (mean B-Pb level of 88.269±98 µg/dl) considered in the study. Whereas, in Goebel and colleagues' study, the results as a whole suggest that with some genotypes or additional disease conditions, an imbalance might occur in the direction of a gut-associated Th1 environment.

In the present work, and although the correlation between B-Pb level and serum IL-4 was not statistically significant (r=0.141, P= .21), the sample size may have played a role in that as IFN-γ/IL-4 ratio had a statistically significant negative correlation to blood lead level (r=-0.284, P = .01). This later correlation may serve as a better proof for the hypothesis of the present work as IFN-γ/IL-4 ratio can be taken as an individual index of Th1/Th2 balance in each subject recruited in the study.

4. CONCLUSION

Environmental exposure to lead alters the balance between Th1 and Th2 cytokine with a bias towards the later.

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COMPETING INTERESTS

Authors declare that no competing interests exist.

AUTHORS' CONTRIBUTIONS

Dr. Yehia A. Zakaria, Dr. Amal M.K. El Safty and Dr. Essam E. Abdel-Rafaa designed the study and wrote its protocol. Dr. Yehia A. Zakaria and Dr. Amal M.K. El Safty supervised the conductance of the study. Dr. Essam E. Abdel-Rafaa conducted the field work (patient interviewing, clinical examination and sample collection), performed the statistical analysis, managed the literature searches and wrote the drafts of the manuscript. Dr. May M. Sherif managed the analyses of the collected samples and the laboratory work. All authors read and approved the final manuscript.

CONSENT

Authors declare that written informed consent was obtained from subjects recruited in this study.

ETHICAL APPROVAL

All authors hereby declare that study protocol was examined and approved by the Research Ethics Committee (REC) of the Faculty of Medicine, Cairo University (Study number N-55-2013) and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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DEFINITIONS, ACRONYMS, ABBREVIATIONS

Whole blood lead (B-Pb), Interleukin-4 (IL-4), Gamma interferon (IFN-γ), T-helper 1 (Th1), T-helper 2 (Th2).