

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

Yehia A. Zakaria¹, Amal M.K. El Safty¹, Essam E. Abdel-Rafaa^{1*} and May M. Sherif².

¹Department of Occupational and Environmental Medicine, Faculty of Medicine - Cairo University, Cairo – Egypt.

²Department of Clinical and Chemical Pathology, Faculty of Medicine - Cairo University, Cairo – Egypt.

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.

* Telephone: +201005284015, +201118744405. E-mail: connectessam@kasralainy.edu.eg, connectessam@outlook.com.

22 1. INTRODUCTION

23

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

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

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

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

48

49 2. METHODOLOGY

50

51 The study involved **80 subjects recruited** into two groups;

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

60 Twenty eight subjects of the exposed group were females and 18 were males. Age ranged
61 from 11 to 70 years (with a mean of 41.30 and a standard deviation of 16.08 years). On the
62 other hand, control group consisted of 11 females and 23 males with their age ranging from
63 19 to **62** years (with a mean of 38.94 and a standard deviation of 12.43 years).

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

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

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

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

80 2.1 Statistical analysis

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

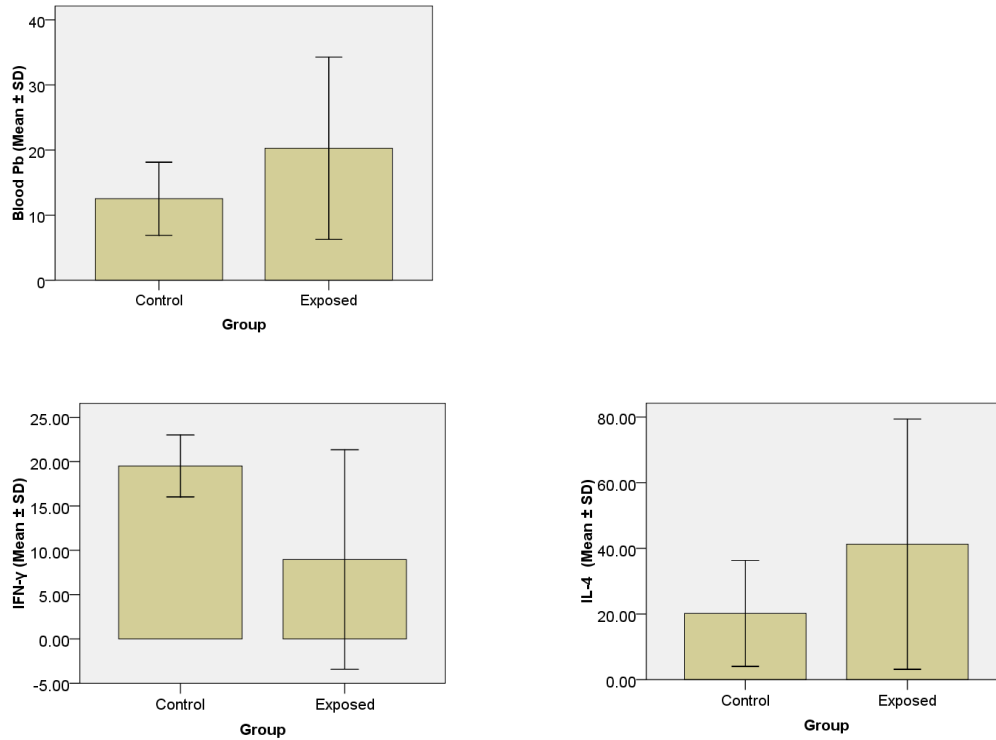
87 3. RESULTS AND DISCUSSION

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

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

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

*Statistically significant correlation



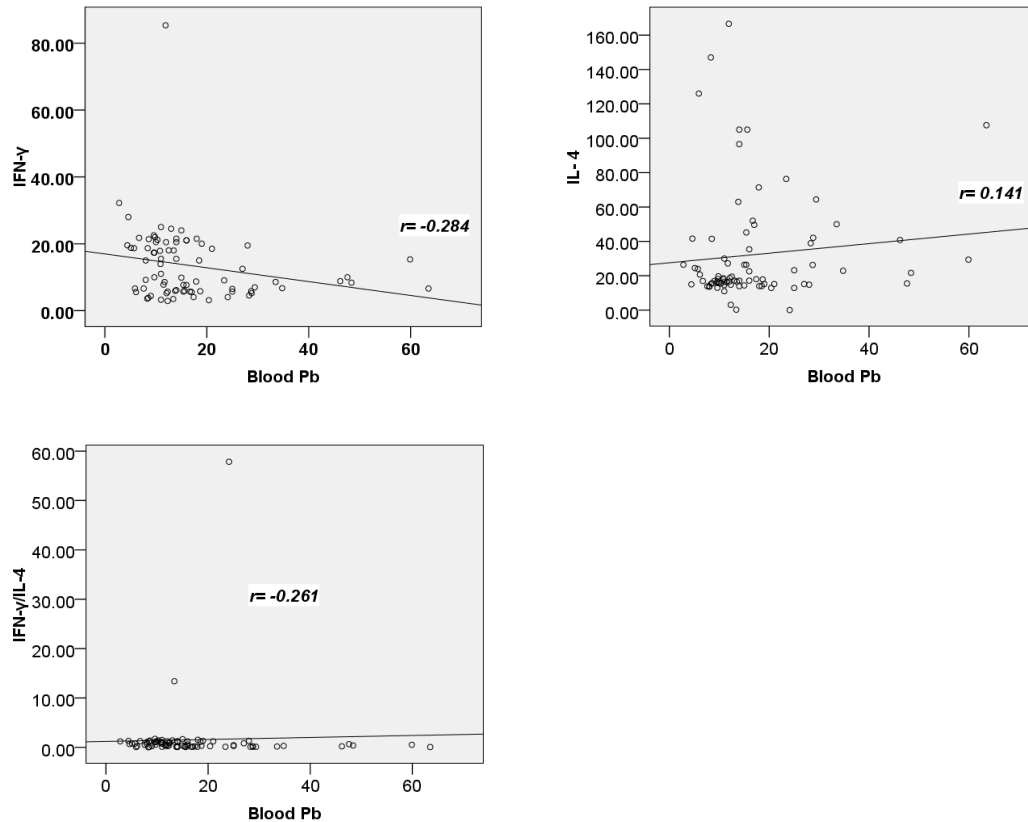
97 **Figure-1: Blood lead level and levels of serum IL-4 and serum IFN-γ in exposed and**
 98 **control groups**

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

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

Blood Lead (Pb) (µg/dl)		Parameter
<i>P</i>	<i>r</i>	
.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



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

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

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

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

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

127 The results of the present work and the previously mentioned consistent studies were not in
128 agreement with two exceptional studies. One of them conducted by Goebel et al (2000) [11]
129 who reported a local bias towards Th1 response with increased IFN- γ production in the
130 intestinal tract of specialized autoimmune diabetes prone strain of mice treated with PbCl₂
131 via oral administrations in drinking water.

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

137 The findings of these two studies, however, should not be considered contradictory to the
138 current and previously mentioned studies. This is because, in Mishra and Colleagues' work,
139 the authors themselves attributed the results to the very high lead levels (mean B-Pb level of
140 88.269 $\pm$ 98 μ g/dl) considered in the study. Whereas, in Goebel and colleagues' study, the
141 results as a whole suggest that with some genotypes or additional disease conditions, an
142 imbalance might occur in the direction of a gut-associated Th1 environment.

143 In the present work, and although the correlation between B-Pb level and serum IL-4 was
144 not statistically significant ($r=0.141$, $P=.21$), the sample size may have played a role in that
145 as IFN- γ /IL-4 ratio had a statistically significant negative correlation to blood lead level ($r=-$
146 0.284, $P=.01$). This later correlation may serve as a better proof for the hypothesis of the
147 present work as IFN- γ /IL-4 ratio can be taken as an individual index of Th1/Th2 balance in
148 each subject recruited in the study.

149

150 4. CONCLUSION

151

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

154

155 ACKNOWLEDGEMENTS

156

157 Authors acknowledge the role of Dr. Dalia Ahmed in revising the statistical analysis of this
158 study. It is to be noted that this study is self-funded by the authors.

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.

REFERENCES

- [1] Goyer RA, Clarkson TW. Toxic effects of metals. In: Klaassen CD, editor. Casarett and Doull's toxicology: the basic science of poisons. 6th ed. New York, NY: McGraw-Hill; 2001.
- [2] Chemonics International. Lead Smelters and Lead Foundries in Egypt. 1999. Accessed 23 August 2014. Available: http://pdf.usaid.gov/pdf_docs/PNACY045.pdf.
- [3] Lawrence DA, McCabe Jr. MJ. Immunomodulation by metals. International Immunopharmacology. 2002;2(2-3):293-302.
- [4] Dietert RR, McCabe Jr. MJ. Lead immunotoxicity. In: Luebke R, House R, Kimber I, editors. Immunotoxicology and immunopharmacology. 3rd ed. Boca Raton: CRC Press; 2007.
- [5] Dietert RR, Piepenbrink MS. Lead and immune function. Critical Review Toxicology. 2006;36(4):359-385.
- [6] Kaminska T, Filar J, Madej E, Szuster-Ciesielska A, Kandefer-Szerszen M. Modification of bovine interferon and tumor necrosis factor production by lead in vivo and in vitro. Archivum Immunologiae et Therapiae Experimentalis [Arch Immunol Ther Exp (Warsz)]. 1998;46:323-328.
- [7] Hemdan NY, Emmrich F, Adham K, Wichmann G, Lehmann I, El-Massry A, et al. Dose-dependent modulation of the in vitro cytokine production of human immune competent cells by lead salts. Toxicological Science. 2005;86(1):75-83.
- [8] Chen S, Golemboski KA, Sanders FS, Dietert RR. Persistent effect of in utero meso 2, 3-dimercaptosuccinic acid (DMSA) on immune function and lead induced immunotoxicity. Toxicology. 1999;132:67-79.
- [9] Yucesoy B, Turhan A, Ure M, Karakaya A. Effects of occupational lead and cadmium exposure on some immunoregulatory cytokine levels in man. Toxicology. 1997;123:143-147.

- 210 [10] Valentino M, Rapisarda V, Santarelli L. Effect of lead on the levels of some
211 immunoregulatory cytokines in occupationally exposed workers. Human & Experimental
212 Toxicology. 2007;26:551-556.
213 [11] Goebel C, Flohé SB, Kirchhoff K, Herder C, Kolb H. Orally administered lead chloride
214 induces bias of mucosal immunity. Cytokine. 2000;12(9):1414-1418.
215 [12] Mishra KP, Singh VK, Rani R. Effect of lead exposure on the immune response of some
216 occupationally exposed individuals. Toxicology. 2003;188:251-259.
217

218 **DEFINITIONS, ACRONYMS, ABBREVIATIONS**

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