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Inhaled Lead Exposure Affects Tracheal Responsiveness and Lung Inflammation in Guinea Pigs During Sensitization

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Abstract Total and differential white blood cells (WBC), and cytokines, levels in serum were examined in guinea pigs exposed to inhaled lead acetate. Different groups of guinea pigs including: control (group C), sensitized group (group S), and exposed animals to aerosol of three lead concentrations during sensitization ($n = 6$ for each group) were studied. Total and differential WBC counts of lung lavage, serum cytokine (IFN γ and IL-4), levels and tracheal responsiveness to methacholine and ovalbumin were measured. All measured values were significantly increased except for IFN γ /IL-4 ratio which was significantly decreased in non-exposed sensitized and those exposed to all lead concentrations compared to control group ($p < 0.05$ to $p < 0.001$). Most measured values in animals exposed to higher lead concentration were also significantly higher than group S except for tracheal responsiveness to methacholine and lymphocyte count. Lead concentration significantly increased in lung tissues of animals exposed to all three lead concentrations ($p < 0.001$ for all cases). These results showed that lead exposure during sensitization can induce greater increase in tracheal responsiveness, total WBC, eosinophil, neutrophil, and basophil counts as well as serum level of IL-4. It can also cause a decrease in lymphocyte count, IFN γ level, and IFN γ /IL-4 ratio especially in its high concentration. Therefore inhaled lead exposure may cause increased severity of asthma during development of the disease.

Keywords Asthma · Tracheal responsiveness · Cytokines · Sensitized animals · Lead acetate

Introduction

The main characteristic feature of asthma is airway inflammation [1], leading to airway hyper-responsiveness (AHR) [1]. Inflammatory cells involved in airway inflammation over-produce inflammatory mediators [3]. T helper 2 (Th2) is activated in asthma disease, and its mediators may contribute in airway inflammation [4].

Lead is the major industrial environmental heavy metal pollutant [5–7] but its exact effects on respiratory system are controversial [8, 9]. The contribution of lead pollution in the pathogenesis of pulmonary cancers, asthma, and chronic obstructive pulmonary diseases has been suggested, but there is not enough evidence in this regard [10, 11]. Increased incidence of asthma in workers exposed to lead and in children living in places with high levels of lead pollution was also reported [12, 13].

Increased IgE and inflammatory cytokines in serum of laboratory models and children exposed to lead, release of inflammatory mediators from cultured cells, and macrophages exposed to lead were reported although decrease in serum immunoglobins of laboratory animals exposed to lead was also indicated [14–19].

It is well established that lead intake, such as through lead vapor inhalation, may cause irreversible effects in several organ systems [20, 21] including immune system [22]. Increased immunoglobulin E (IgE) was documented previously [15, 23, 24]. Significant positive correlation between blood lead concentration and serum IgE level may indicate predisposition to infection and inflammatory disorders [23]. Significant relationship between lead exposure, IgE-mediated allergies, and development of asthma was shown [8, 25]. Although the lung is one of the most important

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portals of entry for lead, there is a limited number of studies regarding the impact of lead on the lungs [10, 17, 26].

Our previous studies showed increased tracheal responsiveness, total and differential WBC counts in lung lavage as well as serum levels of cytokines in nonsensitized guinea pig [27] and after sensitization exposed to inhaled lead [28]. Increased total and differential WBC counts in blood and serum levels of total protein and phospholipase A2 in non-sensitized guinea pig exposed to lead were also shown [29]. In the present study the effects of inhaled lead exposure were examined on tracheal responsiveness, total and differential WBC counts in lung lavage as well as serum levels of cytokines in guinea pigs during sensitization.

Materials and Methods

Animal Sensitization, Their Exposure to Lead, and Animal Groups

Guinea pigs were sensitized to 10 mg ovalbumin (OA, Sigma Chemical Ltd, UK) and 100 mg $\text{Al}(\text{OH})_3$ dissolved in 1 ml saline via i.p. injection on days 1 and 8. From day 14 sensitized animals were exposed to an aerosol of 4 % OA for 18 ± 1 days, 5 min daily [20–32]. The aerosol was administered in a closed chamber, dimensions $30 \times 20 \times 20$ cm (Fig. 1). Nonsensitized animals were treated similarly but saline was used instead of OA solution.

Guinea pigs were exposed to lead acetate by placing animals in a closed chamber (described above) connected to an ultra-nebulizer (Ultra-Neb 99 DeVilbiss) with an air flow of 10 L/min, producing particles of $<1 \mu\text{m}$. Animal were exposed to aerosol of three lead acetate concentrations

of 0.1, 0.2, and 0.4 M (Sigma Chemical Co., St. Louis, MO, USA) for 1 h, three times a week for 2 weeks from the beginning of OA inhalation (Fig. 1), [33].

Dunkin-Hartley guinea pigs (400–700 g, both sexes) were allowed to get into the habit of new situations for 10 days. The animals were group-housed in individual cages in climate-controlled animal quarters and were given water and food ad libitum, while a 12-h on/12-h off light cycle was maintained. Animals were randomly divided to control group (not sensitized and exposed to the nebulized saline alone instead of lead, group C) and four sensitized groups which exposed to aerosol of following solutions during sensitization; nebulized saline alone (group S), 0.1 M lead concentration (DS+0.1 M Pb), 0.2 M lead concentration (DS+0.2 M Pb), and 0.4 M lead concentration (DS+0.4 M Pb).

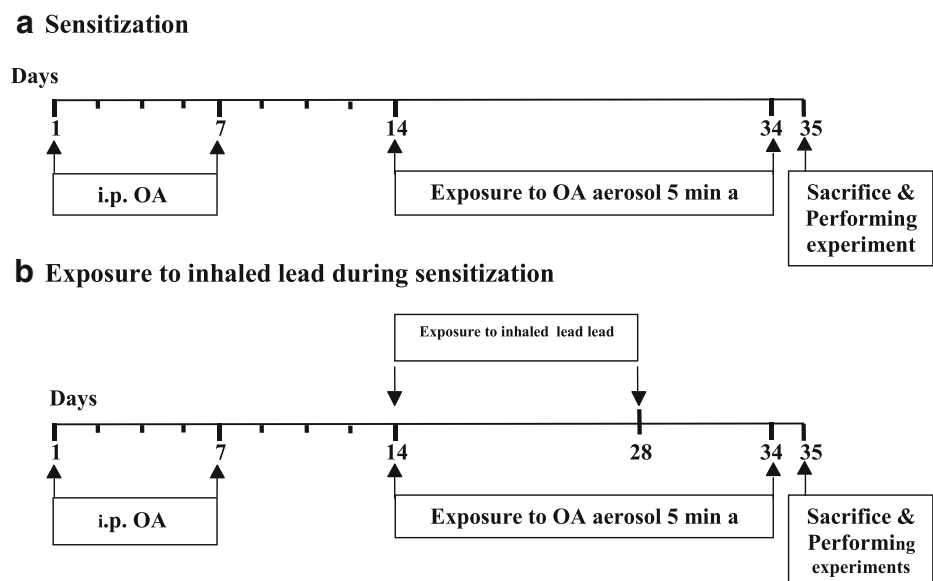
The lead concentrations used in this study [60, 120, and 240 mg/m^3 (0.38, 0.76, and 1.52 g nebulized with 6,000 l air)] were chosen according to several previous animal studies [33, 34]. The solvent of lead was distilled water as used by previous study [34].

Measurements

Tissue Preparation

Guinea pigs were sacrificed by a blow on the neck, trachea was removed and cut into ten rings (each containing two to three cartilaginous rings). The rings were then cut open opposite the trachealis muscle, and sutured together to form a tracheal chain [35, 36]. Tissue was suspended in a 20-ml organ bath (Schuler organ bath type 809, March-Hugstetten, Germany) containing Krebs–Henseliet solution of the following composition (mM): NaCl 120, NaHCO_3 25, MgSO_4

Fig. 1 a, b Method of sensitization and administration of inhaled lead in sensitized groups exposed to lead during sensitization



0.5, KH_2PO_4 1.2, KCl 4.72, CaCl_2 2.5, and dextrose 11, under an isometric tension of 1 g and allowed to equilibrate for at least 1 h. The Krebs solution was maintained at 37 °C and gassed with 95 % O_2 and 5 % CO_2 . Tissue was washed with Krebs solution every 15 min.

All experiments were performed in compliance with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council. The study was approved by the ethical committee of the Mashhad University of Medical Sciences. The allowance number of the relevant ethical committee for the animal experiments is 89174.

Assessment of Tracheal Response to Methacholine

A cumulative log concentration–response curve to consecutive concentrations (including 10^{-7} to 10^{-3} M, added every 2 min) of methacholine hydrochloride (Sigma Chemical Ltd, UK) in each tracheal chain was obtained by recording the contraction at the end of 2 min. The percentage of contraction of the tracheal smooth muscle due to each concentration of methacholine in proportion to the maximum contraction obtained by its final concentration was plotted against log concentration of methacholine.

The effective concentration of methacholine, causing 50 % of maximum response (EC_{50}) was measured from methacholine response curve.

Measurement of Tracheal Response and Contractility Response to OA

Tracheal response to 1 % OA solution was evaluated by measurement of the degree of contraction 15 min after its adding to organ bath and was expressed as a proportion (in percentage) to contraction obtained by 100 μM methacholine. The measurement of tracheal response to methacholine and OA was performed in random order. Tracheal contraction responses were measured using an isometric transducer, connected to a powerlab system (MLT0202 and PowerLab 8/30, ML870, AD Instruments, Australia).

Lung Lavage and Its White Blood Cell Count

A cannula was located into the remaining trachea and lung was lavaged four times with 5 mL of saline (total, 20 mL). One milliliter of lung lavage fluid (LLF) was stained with Turk solution (consisted of 1 mL of glacial acetic acid, 1 mL of 1 % gentian violet solution in 100 mL distilled water) and counted in duplicate in a hemocytometer (in a Burkner chamber). The remaining LLF was centrifuged at $3,500\times g$ at 4 °C for 10 min. The smear was prepared from the cells of the pellet and stained with Wright-Giemsa. Differential cell analysis was carried out under a light microscope by counting 400

cells in each sample and the percentage of each cell type was calculated.

Measurement of Serum IL-4, $\text{IFN-}\gamma$, IgE, and Histamine Levels and Lead Concentrations in Lung Tissue

Five milliliters of peripheral blood was obtained immediately after the animals sacrificed and centrifuged at $3,500\times g$ at 4 °C for 10 min after placing at room temperature for 1 h. The supernatant was collected and immediately stored at -70 °C until analyzed. IL-4 and $\text{IFN-}\gamma$ were measured using Elisa sandwich (Ab Sandwich) method and the ratio of $\text{IFN-}\gamma/\text{IL4}$ as an index of Th1/Th2 was calculated.

Lung samples were analyzed using a graphite furnace atomic absorption spectrometer (PerkinElmer Mod. 2380). The light source came from a hollow cathode lamp. Accuracy was assured by three random determinations of seven different standard solutions, prepared with the same chemical reactive used during the metal analysis. For Pb, the wavelength was 318.4 nm, the detection limit was 0.37 ppm, and the slit was 0.7 nm. Each sample was analyzed in triplicate [30].

Statistical Analysis

The data were quoted as mean $\pm$ SEM. The comparison of data between groups was performed using unpaired "*t*" test and one-way analysis of variance with Tukey–Kramer post test. Significance was accepted at $p<0.05$.

Results

Leftward shift in concentration–response curves to methacholine were observed in S group and those exposed to all concentrations of lead compared to group C (Fig. 2). Tracheal responsiveness and contractility to both methacholine and OA were significantly higher in S group and those exposed to all lead concentrations compared to group C ($p<0.01$ to $p<0.001$, Figs. 3 and 4). Tracheal responsiveness to OA in S groups exposed to two higher lead concentrations (0.2 and 0.4 M) and maximum response to OA in animals exposed to highest lead concentration (0.4 M) were significantly higher than group S ($p<0.01$ to $p<0.001$, Fig. 4). Tracheal responsiveness to OA in S groups exposed to two higher lead concentrations (0.2 and 0.4 M) were also significantly higher than those exposed to its lowest (0.1 M) concentration ($p<0.05$ to $p<0.001$). Maximum response to OA in animals exposed to highest lead concentration (0.4 M) was also significantly higher than those exposed to its lower (0.1 M) concentration ($p<0.01$, Table 1).

In S group and S animals exposed to all lead concentrations, total and all differential counts of WBC in lung lavage were higher except for lymphocytes percentage which was

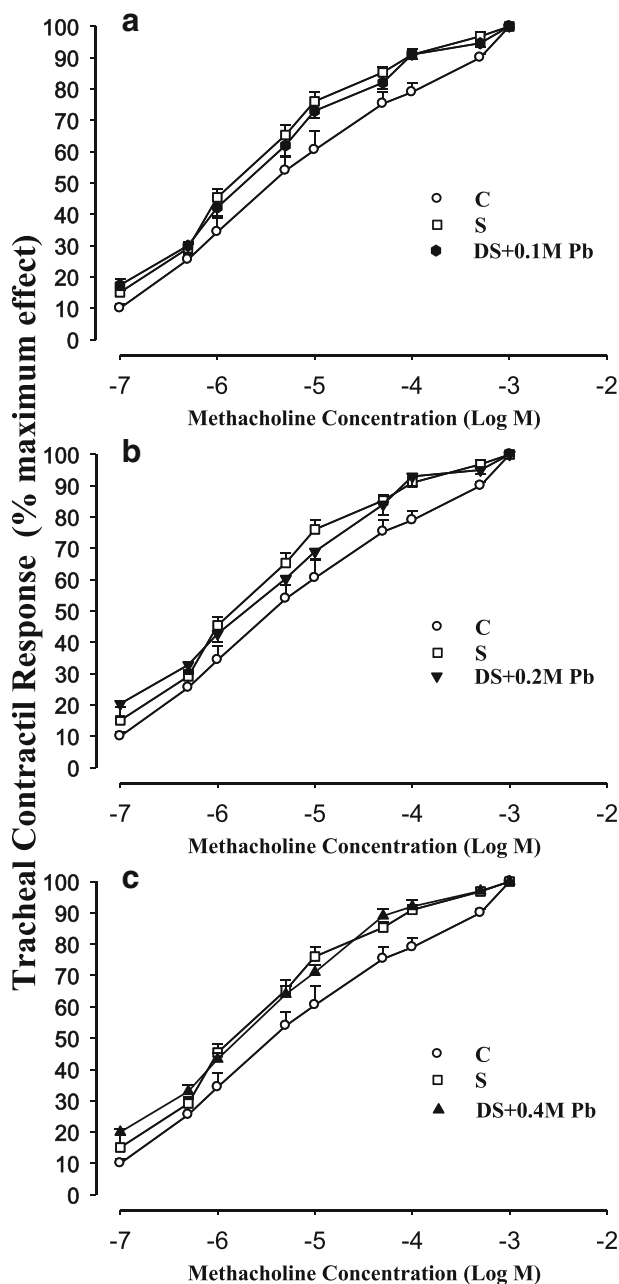


Fig. 2 Cumulative log concentration–response curves of methacholine-induced contraction of isolated trachea in the control (C), sensitized (S), and Pb-exposed during sensitization (DS + Pb) guinea pigs to 0.1 (a), 0.2 (b), and 0.4 M (c) lead concentration (for each group, $n=6$)

lower compared to those of group C ($p<0.05$ to $p<0.001$, Fig. 5a and b). Eosinophil, neutrophil, and basophil counts in lung lavage of animals exposed to two high lead concentrations were significantly higher but lymphocyte was lower than those of group S ($p<0.5$ to $p<0.01$, Fig. 5a and b). Total WBC number in animals exposed to all three lead concentrations, eosinophil in those exposed to two higher lead concentrations as well as monocyte and basophil exposed to only highest concentration were also significantly higher than group S

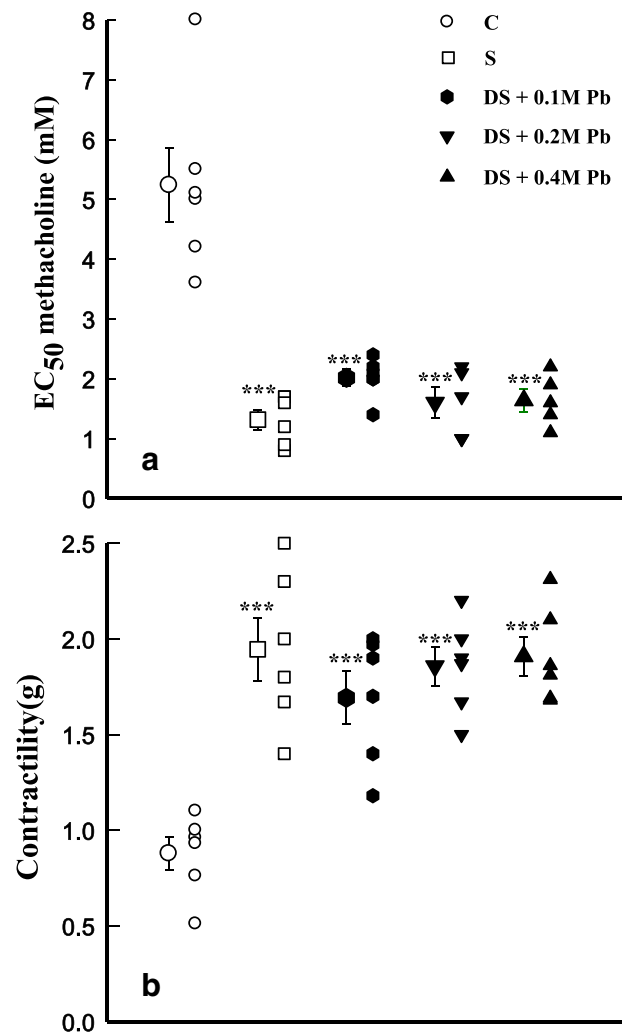


Fig. 3 Individual values and mean $\pm$ SEM (big symbols with bars) of tracheal response to methacholine (EC_{50}) (a) and tracheal contractility response to 10 μ M methacholine (b) induced contraction of isolated trachea in the control (C), sensitized (S), and Pb-exposed during sensitization (DS + Pb) guinea pigs which were exposed to 0.1, 0.2 and 0.4 M lead concentration, (for each group, $n=6$). Statistical significance for the difference between the data of control vs other groups: *** $p<0.001$. There was not any statistical significance difference between the data of sensitized vs lead-exposed groups

($p<0.01$ to $p<0.001$; Fig. 5a and b). Although the percentage of lymphocyte was decreased in sensitized and lead-exposed animals, its absolute counts in sensitized ($1,454.05 \pm 16.59$) and lead-exposed animals ($2,460.06 \pm 33.15$, $1,667.37 \pm 40.30$, and $1,636.55 \pm 22.58$ for low, medium, and high lead concentrations, respectively) were significantly increased compared to control animals ($1,123.76 \pm 66.59$).

In lung lavage of guinea pigs exposed to highest lead concentration, total and all differential WBC counts were significantly higher but lymphocyte was lower than those exposed to its medium and low concentrations ($p<0.05$ to $p<0.001$, Table 2). Lymphocyte and basophil counts in animals exposed to medium lead concentrations were also different from those

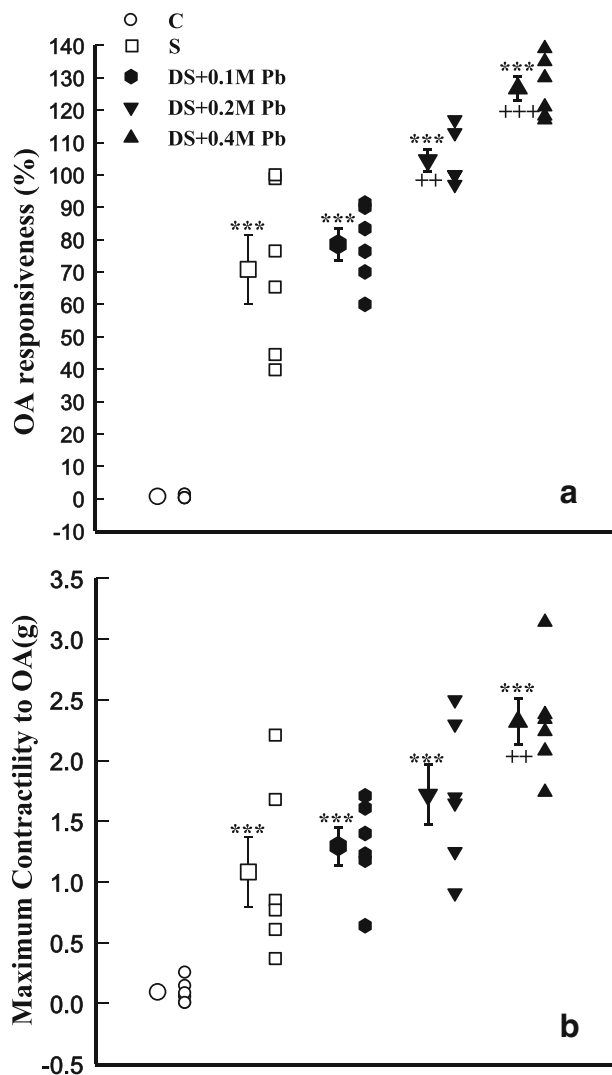


Fig. 4 Individual values and mean $\pm$ SEM (big symbols with bars) of tracheal response to ovalbumin (percent concentration in proportion to contraction obtained by 100 μ M methacholine) (a) and maximum tracheal contractility response to ovalbumin (b) in control (C), sensitized (S), and Pb-exposed during sensitization (DS + Pb) guinea pigs which were exposed to 0.1, 0.2, and 0.4 M lead concentration (for each group, $n=6$). Statistical significance for the difference between the data of control vs other groups: *** $p<0.001$. Statistical significance for the difference between the data of sensitized vs Pb-exposed groups: ++ $p<0.01$; +++ $p<0.001$

Table 1 Values of tracheal response to methacholine (EC_{50} μ mol) and ovalbumin (%), maximum contractility to both agents (g), and Pb lung concentration (μ g/L) in control (C), and sensitized (S) guinea pigs (for each group, $n=6$)

Parameters	C	S	DS+0.1 M Pb	DS+0.2 M Pb	DS+0.4 M Pb
EC_{50} (μ mol)	5.21 ± 0.62	1.31 ± 0.15	1.80 ± 0.09	1.60 ± 0.25	1.61 ± 0.16
Contractility to methacholine (g)	0.86 ± 0.08	1.94 ± 0.16	1.69 ± 0.13	1.85 ± 0.10	1.90 ± 0.11
OA responsiveness	0.35 ± 0.23	78.51 ± 9.98	76.46 ± 4.96	104.51 ± 3.39 *	126.69 ± 3.79 *** +
Maximum contractility to OA (g)	0.16 ± 0.07	1.28 ± 0.37	1.35 ± 0.11	1.87 ± 0.18	2.51 ± 0.16 **
Lung Pb concentration (μ g/L)	0.00 ± 0.00	0.00 ± 0.00	114.65 ± 3.30	173.88 ± 5.22 ***	314.35 ± 50 *** +++

Values are presented as mean $\pm$ SEM. OA responsiveness was expressed as the ratio of percentage contraction due to 0.1 % solution of OA to contraction obtained by 100 μ M methacholine. Statistical significance for the difference between the data of 0.1 M Pb vs 0.2 M and 0.4 M groups: * $P<0.05$; ** $P<0.01$; *** $P<0.001$. Statistical significance for the difference between the data of 0.2 M Pb vs 0.4 M Pb group: + $P<0.05$; +++ $p<0.001$

exposed to its low concentration ($p<0.01$ for lymphocyte and $p<0.05$ for basophil; Table 2).

There were significant increase in serum levels of IFN γ and IL-4 in group S ($p<0.01$ for IFN γ and $p<0.001$ IL-4) but the IFN γ /IL-4 ratio was significantly decreased ($p<0.05$) compared to group C (Fig. 6). Levels of IFN γ and IL-4 in animals exposed to low and high lead concentrations were significantly higher but the IFN γ /IL-4 ratio was lower than group C ($p<0.05$ to $p<0.001$, Fig. 6). Levels of IFN γ and IFN γ /IL-4 ratio in animals exposed to high lead concentration were significantly lower but IL-4 was higher than those in group S ($p<0.05$ to $p<0.001$, Fig. 6).

In guinea pigs exposed to high lead concentration, the levels of IFN γ and IFN γ /IL-4 ratio were significantly lower than those exposed to its low concentration ($p<0.01$ to $p<0.001$, Table 3).

Lead concentration in the lung of all test animals was significantly higher than that in group C ($p<0.001$ all cases, Fig. 7). Significant differences in lead concentration in the lung between animals exposed to three lead concentrations was also observed ($p<0.001$ for all cases, Table 1).

Discussion

The increased total WBC number and eosinophil count but decreased lymphocyte count seen in the present study are well-known changes in asthma [7]. Increased total WBC and eosinophil but decreased lymphocyte count in lung lavage of sensitized guinea pigs [8, 9] have been previously shown in asthma, asthmatic patients, and sensitized animals. Therefore, further increase in total WBC and eosinophil counts, decreased lymphocyte as well as increased serum histamine and IgE levels in sensitized animals exposed to two higher lead concentrations indicate that lead exposure during sensitization (development of asthma) could increase lung inflammation, the important index of asthma severity. The reason of a reduction in lymphocyte is their expression in percentage of total WBC count. However, the absolute counts of lymphocyte in sensitized and

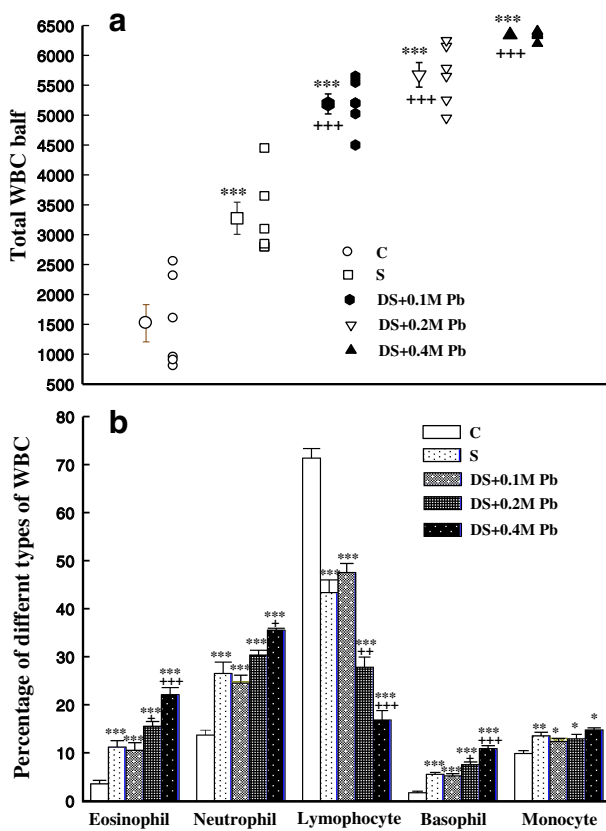


Fig. 5 Individual values and mean $\pm$ SEM (big symbols with bars) of total WBC (a) and percentage different type of WBC (b) in LLF in the control (C), sensitized (S), and Pb-exposed during sensitization (DS+ Pb) guinea pigs which were exposed to 0.1, 0.2, and 0.4 M lead concentration (for each group, $n=6$). Statistical significance for the difference between the data of control vs other groups: * $p<0.05$; ** $p<0.01$; *** $p<0.001$. Statistical significance for the difference between the data of sensitized vs Pb-exposed groups: + $p<0.05$; ++ $p<0.01$; +++ $p<0.001$

lead-exposed animals were significantly increased compared to control animals.

The main pathological feature of asthma is usually airway inflammation, [1] which leads to AHR [2]. Increased specific and nonspecific tracheal responsiveness to methacholine and OA respectively was observed in sensitized group in the

present study which was supported the sensitization of animals by the findings of previous studies using similar methods of sensitization [37, 38]. Specific AHR was also further increased in guinea pigs exposed to inhaled lead which is another evidence regarding the effect of lead exposure on increased AHR, the main characteristic of asthma severity. It was shown that exposure to lead induces contraction of airway smooth muscle [39]. Increased tracheal responsiveness was also observed in animal models exposed to lead [25].

Similar to previous studies [37, 38], this study also showed increased serum IL-4 and IFN γ levels but decreased the IFN γ /IL-4 ratio in sensitized guinea pigs. One of the immunologic features of asthma is shifting from T helper 1 (Th1/Th2 balance) which is proved to inhibit Th2 response [40]. Th1 cells produce interleukin 2 (IL-2) and interferon- γ (IFN γ), but Th2 cells produce IL-4 and IL-10 [41]. Th2 cells promote the activity of macrophages and regulate the pro-inflammatory response, whereas Th1 cells regulate the anti-inflammatory response [42]. Increased IL-4 levels, a decrease in the IFN γ /IL-4 ratio, and especially decreased IFN γ levels in guinea pigs exposed to inhaled lead also indicated enhancing effect of environmental lead pollution on asthma severity. The effect of lead exposure on altering T helper subset development and/or function was observed [16], which confirmed the results of our study. Although, in most studies, measurement of IL-4 is used as marker of Th2 activity, in further study the effect of lead exposure on IL5 and IL-13 should be examined to confirm Th2 skewed inflammatory profiles.

The effect of inhaled lead exposure on increased different indices of sensitized animals seen in the present study was supported by a higher prevalence of respiratory symptoms for phlegm, shortness of breath, and diagnosed asthma in industrial workers exposed to lead [43].

The results of this study also showed a concentration-dependent increase in lead concentration in lung tissues of animals exposed to inhaled lead. Severe smoke inhalation by victims of closed-space fires in an urban tertiary burn center was also associated with an increase in blood lead levels [44],

Table 2 Values of total WBC and differential count of WBC in LLF in control (C), sensitized (S), and Pb-exposed during sensitization (DS+Pb) guinea pigs (for each group, $n=6$)

Cell type	Control	S	DS+0.1 M Pb	DS+0.2 M Pb	DS+0.4 M Pb
Total WBC	1,558 $\pm$ 300	3,300 $\pm$ 229	5,187 $\pm$ 167	5,674 $\pm$ 205	6,341 $\pm$ 30***,+
Eosinophil	2.99 $\pm$ 0.66	11.16 $\pm$ 1.35	10.50 $\pm$ 1.61	15.51 $\pm$ 0.99	22.11 $\pm$ 1.47***,+
Neutrophil	13.60 $\pm$ 1.02	26.51 $\pm$ 2.36	24.50 $\pm$ 1.66	30.33 $\pm$ 1.02	35.50 $\pm$ 0.42***,+
Lymphocyte	71.35 $\pm$ 1.97	43.34 $\pm$ 2.65	47.51 $\pm$ 1.89	27.80 $\pm$ 2.10**	16.82 $\pm$ 1.96***,+
Basophil	1.66 $\pm$ 0.33	5.50 $\pm$ 0.42	5.16 $\pm$ 0.47	7.50 $\pm$ 0.56*	10.85 $\pm$ 0.66***,++
Monocyte	9.83 $\pm$ 0.61	13.51 $\pm$ 0.76	12.33 $\pm$ 0.65	12.91 $\pm$ 0.94	14.73 $\pm$ 0.47**,,+

Values are presented as mean $\pm$ SEM. The data of WBC is their count in 1 mL of LLF and those of each type is the percentage of total WBC. Statistical significance for the difference between the data of 0.1 M Pb vs 0.2 M and 0.4 M groups: * $p<0.05$; ** $p<0.01$; *** $p<0.001$. Statistical significance for the difference between the data of 0.2 M Pb vs 0.4 M Pb group: + $p<0.05$; ++ $p<0.01$

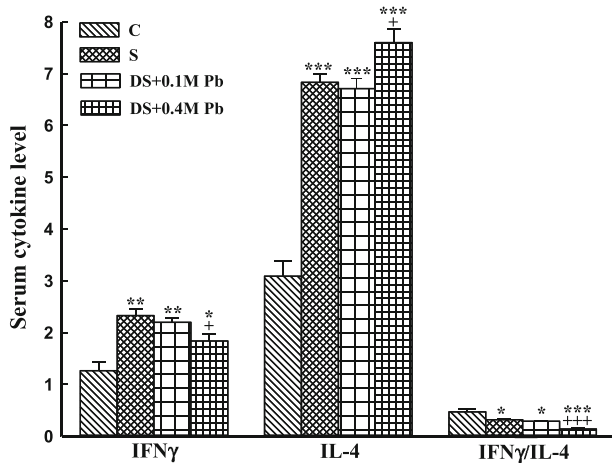


Fig. 6 Serum levels of IL-4, IFN γ (pg/ml), and IFN γ /IL-4 ratio in control (C), sensitized (S), and Pb-exposed during sensitization (DS + Pb) guinea pigs which were exposed to 0.1 and 0.4 M lead concentration (for each group, $n=6$). Values are presented as mean $\pm$ SEM. Statistical significance for the difference between the data of control vs other groups: * $p<0.05$; ** $p<0.01$; *** $p<0.001$. Statistical significance for the difference between the data of sensitized vs Pb-exposed groups: + $p<0.05$; +++ $p<0.001$

which confirmed the result of present study. The results also showed higher tracheal responsiveness, total differential WBC count, change in serum IL-4 and IFN γ levels in animals exposed to the highest inhaled lead concentration which indicate a concentration-dependent effect of inhaled lead exposure on asthma severity. The association of blood lead concentration with increased bronchial hyper-responsiveness [45] was also shown which supports these results. The reason of absence of increased nonspecific tracheal responsiveness (responsiveness to methacholine) in lead-exposed sensitized animal is uncertain to us and should be clarified in further studies.

The result showed significant increased in the level of IL-4 but decreased IFN- γ and IFN- γ /IL-4 ratio. Total and differential WBC counts specially eosinophil as well as tracheal response to OA were also significantly higher in sensitized animals exposed to highest concentration of lead compared to nonexposed sensitized group. Therefore, the results of the present study suggest that inhaled lead exposure may alter

Table 3 IL-4 (pg/mL) and IFN γ (pg/mL) levels in serum of control (C), sensitized (S), and Pb-exposed during sensitization (DS + Pb) guinea pigs (for each group, $n=6$)

Group	IFN- γ (pg/ml)	IL-4 (pg/ml)	IFN- γ /IL-4
C	1.19 $\pm$ 0.15	2.99 $\pm$ 0.43	0.39 $\pm$ 0.04
S	2.43 $\pm$ 0.17	7.10 $\pm$ 0.13	0.34 $\pm$ 0.03
DS+0.1 M Pb	2.22 $\pm$ 0.08	6.71 $\pm$ 0.20	0.29 $\pm$ 0.01
DS+0.4 M Pb	1.80 $\pm$ 0.15	7.61 $\pm$ 0.26	0.14 $\pm$ 0.01***

Values are presented as mean $\pm$ SEM. Statistical significance for the difference between the data of two concentrations of Pb: * $p<0.05$; *** $p<0.001$

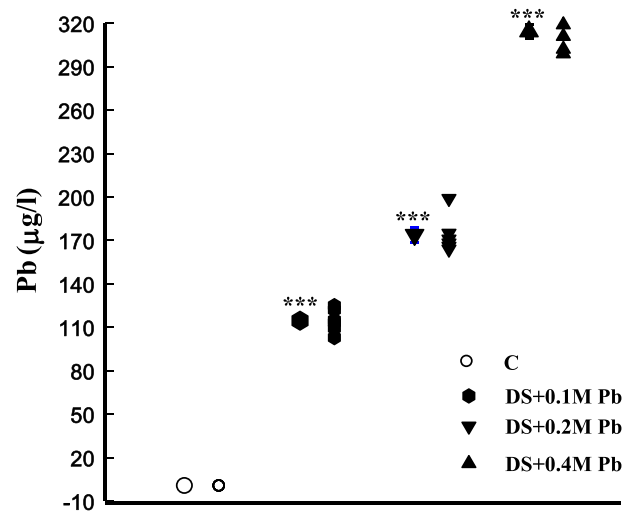


Fig. 7 Individual values and mean $\pm$ SEM (big symbols with bars) of Pb concentration in the lung of the control (C), and Pb-exposed during sensitization (DS + Pb) guinea pigs which were exposed to 0.1, 0.2, and 0.4 M lead concentration (for each group, $n=6$). Statistical significance for the difference between the data of control vs other groups: *** $p<0.001$

Th1/Th2 balance toward the increasing Th2 activity. This can cause lung inflammation (increased total and differential WBC count) and increase specific tracheal responsiveness. However, the exact mechanism of action of inhaled lead should be examined in further studies. In addition, pathological changes of the lung and trachea before and after lead exposure were not evaluated in the present study and it should be investigated in further studies.

Although inhaled lead concentrations used in the present study were much higher than lead concentration in lead industrial environment of 0.089 to 0.092 mg/m³ [46, 47]. The studied animals were exposed to lead for 14 days each day 60 min while workers in industrial environment of lead pollution exposed to inhaled lead for several years each day about 8 h.

Increased tracheal responsiveness, total and differential WBC counts in lung lavage as well as serum levels of cytokines in nonsensitized guinea pig [27] and after sensitization [28] exposed to the same lead concentrations were observed in our previous studies. Increased total and differential WBC counts in blood and serum levels of total protein and phospholipase A2 in nonsensitized guinea pig exposed to inhaled lead were also shown [29]. The changes in nonsensitized guinea pigs exposed to inhaled lead [27] were lower than those observed in the present study (exposed to lead during sensitization). These results suggest a further effect of inhaled lead in lung inflammation and tracheal responsiveness in sensitized animals during sensitization. However, the change in most parameter especially in tracheal responsiveness to OA and total WBC in the present study were lower than those of post sensitized animals exposed to inhaled lead. These findings suggest that environmental lead may affect manifested asthmatic patients greater than those during

development of asthma. However, further studies needed to examine the long-term effect of exposure to lead during sensitization.

In conclusion, the results suggest greater increase in tracheal responsiveness, total WBC, eosinophil, neutrophil, and basophil counts as well as serum levels of IL-4 due to lead exposure during sensitization. Lead exposure during sensitization can also result in IFN γ level and the IFN γ /IL-4 ratio especially in its high concentration. Therefore, environmental lead pollution may increase severity of asthma during development of the disease.

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Authors' Role Boskabaddy MH: Study design, supervision of the experiments, statistical analysis, manuscript preparation

Farkhondeh T: Performing of experiments, help in statistical analysis, help in manuscript preparation

Conflict of Interest Statement There is no conflict of interest in this study.

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