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Impact of lead exposure on pituitary-thyroid axis in humans

Baljinder Singh[1], Vinod Chandran[2], Harish K. Bandhu[1], Bhagwant R. Mittal[1], Anish Bhattacharya[1], Surinder K. Jindal[1] & Subhash Varma[2]
[1]*Department of Nuclear Medicine,* [2]*Department of Internal Medicine, PGIMER-160 012, Chandigarh, India (Fax: 91-0172-744 401; E-mail: medinst@pgi.chd.nic.in)*

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Abstract

Thyroid function tests (serum levels of thyroxine-T4, triiodothyronine-T3 and thyroid stimulating hormone-TSH) were performed in fifty-eight men (mean age: 31.7 ± 10.6 years; mean duration of lead exposure: 156.9 ± 122.7 months). These subjects were exposed to lead either as petrol pump workers or automobile mechanics. The mean whole blood lead (Pb-B) levels were 2.49 ± 0.45 μmole/l (51.90 ± 9.40 μg/dl) in the lead exposed workers and were approximately 5 times higher than in the control ($n = 35$) subjects. No significant alteration was seen in their mean T3 and T4 levels as compared with the controls. Interestingly, T3 was significantly lower with the longer (210 months) exposure time in comparison with the group having shorter (29 months) exposure duration. The mean TSH levels were significantly ($p < 0.01$) higher in workers exposed in comparison with the control group. This rise in TSH was independent of exposure time, but it was definitely associated with the Pb-B levels. The increase being more pronounced with mean Pb-B levels of 2.66 ± 0.2 μ mole/l (55.4 ± 4.25 μg/dl) when compared with the group having mean levels of 1.51 ± 0.30 μmole/l (31.5 ± 6.20 μg/dl). The rise is TSH associated with Pb-B levels was only statistical valid, however, the levels fall within the normal laboratory range. We thus conclude that the Pb-B levels of ≥ 2.4 μmole/l (50 μg/dl) could enhance the pituitary release of TSH without having any significant alterations in the circulating levels of T3 and T4.

Introduction

Among all the heavy elements that contaminate the environment and pose a potential hazard to public health, lead can result in a wide range of biological effects depending upon the level and duration of exposure (WHO, 1995). Occupational exposure, which results in poisoning, both moderate and clinically symptomatic, still occurs in many countries of the world (Verrula & Moah 1992). In many countries, occupational lead exposure is entirely unregulated and no monitoring of exposure exists. Automobile battery manufacture and repair, radiator repair, secondary smelters and vehicular emission are found in most countries (Kaye *et al.* 1987; Singh *et al.* 1994). Lead used in petrol as organic tetra alkyl lead additive to improve the efficiency of engine, is a major source of wide spread lead contamination at the petrol filling stations, automobile repair market (Singh *et al.* 1994; Goldman *et al.* 1987; Rudolph *et al.* 1990). The major route of lead intake at such places is through respiratory tract. The organic is dealkylated in vivo and the known metabolite are triethl lead and inorganic lead and this conversion probably occurs in the liver (Bolanowska *et al.* 1971). The combustion of alkyl lead is the predominant source of increased lead in all compartments of the environment. This has been hypothesized based upon mass balance studies (Nriagu 1979).

The lead circulating in the bloodstream is mobile, in contrast to that stored in bones and it is this lead that exerts adverse effects on the body. Hence, the concentration of lead in the blood stream (Pb-B) is an important parameter in the characterization of an individual's exposure to lead. But, Pb-B levels represent only recent exposure to lead and do not reflect pre-

vious exposures, which may have been considerably higher. However, it has now been generally accepted that under conditions of more or less constant and prolonged exposure, the Pb-B level reflects the quantity of 'biologically active' form of lead in the body and has positive correlation with the symptoms of lead toxicity (Robinowitz *et al.* 1976). Moreover, evaluation of health risks due to lead exposure is generally based on Pb-B levels. Analysis of Pb-B levels is, therefore, the first choice for the assessment of internal exposure to lead in the lead exposed population (Cullen *et al.* 1990).

Effects at the subcellular level, as well as effects on the overall functioning of the body, have been noted and range from inhibition of enzymes to the production of marked morphological changes. Lead can cause profound hematological, neurological, gastrointestinal, renal, rheumatological and endocrine manifestations in man even at levels previously considered safe (Cullen *et al.* 1983; Pagliuca *et al.* 1990). Lead exposure also causes functional impairment of pituitary-adrenal axis as well as the pituitary-thyroid axis (Lasisz *et al.* 1992; Gustafson *et al.* 1989; Robins *et al.* 1989; Singh *et al.* 1999).

There are conflicting reports in the literature regarding the effect of occupational lead exposure on the thyroid functions. Therefore, the aim of the present study was to evaluate the potential effect of blood lead levels on thyroid indices (triiodothyronine-T3, thyroxine-T4, and thyroid stimulating hormone-TSH) in workers from petrol filling stations and automobile repair market.

Materials and methods

Study subjects

Fifty-eight men (mean age: 31.7 ± 10.6 years) exposed to lead who were engaged in petrol filling and automobile repair jobs, at four different petrol pumps and automobile service stations in the city of Chandigarh, India were enrolled for the present study. Their mean exposure time was 156.9 ± 122.7 months. Thirty-five healthy male volunteers (mean age: 28.8 ± 4.20 years) with no history of organic/inorganic lead exposure were also enrolled as a control group. A written consent was obtained from each individual participating in the study and the ethical recommendations of the Indian Council of Medical Research (ICMR) were strictly followed. Detailed clinical assessment for evidence for thyroid dysfunction was performed in test and control subjects.

Lead estimation

Two milliliters of venous blood was withdrawn from each study subject by venipuncture and collected in non-heparinised lead free vials. For estimation of whole blood lead (Pb-B) levels, a known volume (2.0 ml) of blood was digested in 3:1 mixture of Nitric acid: Perchloric acid (Chemical purity $> 99\%$; Merck, Germany) in a sand bath. The dried powder was reconstituted in 1/10 M nitric acid to obtain a transparent non-turbid solution. The reconstituted solution was analyzed for Pb concentrations by using Atomic Absorption Spectrophotometer (Element AS-AAS-4139, ECIL, Hyderabad, India). The absorbance of standard solution containing known concentrations of lead was measured and a calibration graph prepared. The concentrations of lead in the test blood samples were interpolated from the absorbance-concentration standard curve as a direct read out. All the values obtained were corrected with respect to the values in the reference blank solution. The necessary quality control procedures were performed to check the performance and the accuracy of the instrument.

Estimation of serum thyroid hormonal profile

Five milliliters of venous blood was withdrawn from each subject separately. Serum was separated and samples frozen at -20 °C until analyzed for thyroid hormones. Radioimmunoassay-kits (BRIT, BARC, Mumbai, India) were used to analyze serum thyroxine (T4) and triiodothyronine (T3) levels. Thyroid stimulating hormone (TSH) was estimated by using ultra-sensitive-immuno-radiometric assay (IRMA)-kits (BRIT, BARC, Mumbai, India). Inter and intra assay variation were seen and in all the measurements, the difference was less than 5% and statistically non-significant.

Statistical analysis

The significance of differences between the 2 means was calculated by student's t-test and amongst the different groups by running multivariate analysis (Hotelling's t^2 test). In all the comparisons, differences were considered significant when p was < 0.05.

Results

Serum thyroid hormones (T3, T4, TSH) and Pb-B levels in lead exposed workers as a collective group in comparison with the control group are presented in Table 1. Pb-B levels in lead exposed subjects were significantly ($p < 0.0001$) higher than in the control group by approximately a factor of 5. On the other hand, serum T3, T4 levels were not significantly different from each other. However, TSH concentrations in lead exposed were significantly higher ($p < 0.01$) than in the control group.

Thyroid hormonal profile in lead exposed subjects was also looked at as a function of three other variables viz. work location-4 different locations (Table 2); exposure time (Table 3) and also as a function of Pb-B levels (Table 4).

The Pb-B levels in the workers employed at first three locations (1, 2, 3) were significantly higher as compared to the 4th location (Table 2). However, T3, T4, TSH levels remained unchanged when compared amongst workers at any two locations.

While looking at the data as a function of lead exposure time (Table 3), the Pb-B levels did not differ significantly in lead exposed groups with the mean exposure time of 29.3 ± 15.4 and 210 ± 108 months, respectively. TSH was significantly raised (the values were still within the 'normal' reference limits) with both the lead exposed durations in comparison with the control group. However, no significant change was observed in relation to the length of the exposure. T4 did not differ amongst the two duration related groups, however, T3 was significantly low with the longer mean lead exposure (210 months) in comparison with the group having lower mean exposure of 29 months.

When the data were sorted on the basis of Pb-B levels (Table 4), it was observed that no significant difference was noticed in TSH with the mean Pb-B levels of 1.51 ± 0.30 μmole/l (31.5 ± 6.2 μg/dl) in comparison with the control group. However, with the mean Pb-B levels of 2.66 ± 0.20 μmole/l (55.4 ± 4.2 μg/dl), the TSH was significantly higher in comparison with both the control and the exposed group having moderate Pb-B levels of 1.51 ± 0.30 μmole/l.

Discussion

Lead in organic and inorganic form is a health risk factor. It leads to poisoning after high-grade exposure. It can accumulate in the organism and exert toxic effects, especially on the haemopoietic and nervous systems. Its actions include damage to cell membranes and disorders of the oxido-reductive processes in the cells. Hypothyroidism occurring in subjects with occupational exposure to lead is suggestive of a negative effect of the element on thyroid function (Lasisz *et al.* 1992).

Pb-B levels in lead exposed subjects were significantly ($p < 0.0001$) higher than in the control group by a factor of five. In a recent study (*Bandhu et al.* 2000), we reported that the environmental lead levels in the city of Chandigarh were higher by a factor of 10–12 at the polluted zones as compared to the non-polluted zone.

Despite normal T3 and T4 levels, we did observe a significant rise in serum TSH levels collectively in a cohort of 58 lead-exposed men. The rise in TSH was independent of exposure time. However, the rise in TSH was associated with Pb-B levels, the increase being more pronounced with higher mean Pb-B levels. A dose related depression of thyroid functions had been observed in humans during occupational exposure to inorganic lead (Gustafson *et al.* 1989). Robins *et al.* (1983) found low values for serum thyroxine (T4) and estimated free thyroxine (FT4) in 7 of 12 workers having Pb-B levels above 44 μg/dl. Both the measures were correlated with Pb-B levels in a cross-sectional study of 47 foundry workers. Serum thyrotropin and triiodothyronine levels were within the normal range for the study group (Gustafson *et al.* 1989). No thyroid abnormalities were observed (Tuppurainen *et al.* 1988) and there was no statistically significant relationship between Pb-B and T4. Refowitz (1984) also reported no such relationship in T4, FT4 and Pb-B levels in a study of a one-in-three random sample ($N = 58$) of male employees at a secondary copper smelter. Singh *et al.* (1994) reported that lead administration to rats caused a significant stimulation of I-131 thyroidal uptake and they attributed the rise in iodine-131 uptake to TSH stimulation by lead .

Interestingly, T3 was significantly low with the mean exposure time of 210 months in comparison with the group having mean exposure time of 29 months. Beidelman (1984) believed that lead induced thyroid dysfunction is simply the 'Sick Euthyroid Syndrome' not the 'True Hypothyroidism'. In a recent report (Singh *et al.* 1999), we have indicated that lead treatment to rats at a dose rate of 50 mg per kg body weight for period ranging from 1–4 months caused a significant depression in T3 and T4 levels. Gustafson *et al.* (1989) reported that most heavily lead exposed

Table 1. Thyroid functions in lead exposed workers and healthy controls.

Group	Number of subjects	Mean age (years)	Mean lead exposure (months)	Whole blood Mean lead levels (μmole/l)	T3 (ng/ml)	T4 (μg/dl)	TSH (μU/ml)
Lead exposed group	$N = 58$	31.7 ± 10.6	156.9 ± 122.7	$2.49 \pm .45$**	1.75 ± 0.47	9.40 ± 2.9	2.2 ± 1.4*
Healthy Control	$N = 35$	28.9 ± 4.20	Nil	0.46 ± 0.42	1.71 ± 0.51	10.7 ± 4.9	1.26 ± 0.86

*$p < 0.01$; **$p < 0.0001$.

Table 2. Thyroid functions in lead exposed workers from 4 different work locations.

Work Location	Number of subjects	Mean age (years)	Mean lead exposure (mo)	Whole blood Mean lead levels (μmole/l)	T3 (ng/ml)	T4 (μg/dl)	TSH (μIU/ml)
1	13	36.7 ± 8.60	184 ± 114	2.62 ± 0.22	1.76 ± 0.44	10.5 ± 2.1	1.96 ± 1.0
2	08	31.9 ± 10.7	99 ± 88	2.63 ± 0.11	1.56 ± 0.47	9.0 ± 1.6	2.33 ± 1.64^{b}
3	20	27.7 ± 11.5^{a}	126.9 ± 143.5	2.7 ± 0.15	1.80 ± 0.50	9.3 ± 2.8	2.50 ± 1.51^{c}
4	17	32.6 ± 9.9	198.3 ± 103	$2.03 \pm 0.6^{b,c,d}$	1.8 ± 0.5	8.9 ± 4.0	2.0 ± 1.55

Work locations:
1 = Petrol pump and automobile service center.
2 = Petrol pump and automobile service center.
3 = Exclusively automobile service center.
4 = Exclusively automobile service center.

[a] $p < 0.02$. Comparison of results amongst locations 1 and 3.
[b] $p < 0.001$. Comparison of results amongst locations 1 and 4.
[c] $p < 0.006$. Comparison of results amongst locations 2 and 4.
[d] $p < 0.0001$. Comparison of results amongst locations 3 and 4.

Table 3. Thyroid functions in lead exposed workers as a function of duration of lead exposure.

Group	Exposure duration (months)	Mean age (years)	Mean lead exposure (months)	Mean lead levels (μmole/l)	T3 (ng/ml)	T4 (μg/dl)	TSH (μIU/mL)
Pb-exposed							
$N = 17$	≤ 60 (12-60)	21.4 ± 4.80	29.3 ± 15.42	2.5 ± 0.4^{a1}	2.0 ± 0.50	8.6 ± 2.3	2.7 ± 1.4^{a}
$N = 41$	> 60 (61-432)	36.0 ± 9.3	209.8 ± 108	$2.46 \pm 0.47^{b1,c}$	1.7 ± 0.44^{c}	9.7 ± 3.2	2.0 ± 1.4^{b}
Control ($N = 35$)	Nil	28.9 ± 4.20	Nil	0.46 ± 0.42	1.7 ± 0.51	10.7 ± 4.9	1.26 ± 0.87

[a] $p < 0.01$.
[b] $p < 0.01$.
[c] $p < 0.01$.
[a1] $p < 0.0001$.
[b1] $p < 0.0001$.
Comparison for lead exposed group $\leq$ 72 mo vs control.
Comparison for lead exposed group $\leq$ 240 mo vs control.
Comparison for lead exposed group $\leq$ 72 mo vs $\leq$ 432 mo.

Table 4. Thyroid functions in lead exposed workers as a function of blood lead levels.

Group	Blood lead levels (μg/dl)	Mean Blood lead levels (μ mole/l)	Mean age (years)	Mean lead exposure (months)	T3 (ng/ml)	T4 (μg/dl)	TSH (μUI/ml)
Pb exposed							
$N = 08$	≤ 41.0	1.51 ± 0.29^{a}	34.2 ± 7.8	210 ± 108	1.9 ± 0.51	9.4 ± 4.5	1.21 ± 0.4
$N = 50$	≤ 70.0	$2.63 \pm 0.20^{b1,c1}$	31.3 ± 11.0	148.4 ± 124	1.73 ± 0.47	9.40 ± 2.7	$2.4 \pm 1.5^{b,c}$
Control	range	0.46 ± 0.42^{c}	28.9 ± 4.20	Nil	1.71 ± 0.50	10.7 ± 4.95	1.26 ± 0.87

[a] $p < 0.0001$.
[b] $p < 0.008$.
[c] $p < 0.03$.
[b1] $p < 0.0001$.
[c1] $p < 0.0001$.
Comparison between lead exposed group (lead levels ≤ 41.0 μg/dl) vs control.
Comparison between lead exposed group (lead levels ≤ 70.0 μg/dl) vs control.
Comparison between lead treated groups (lead levels ≤ 41.0 μg/dl and ≤ 70.0 μg/dl).

individuals had higher levels of TSH and their data indicated that there is a complex effect on the endocrine system by moderate lead level exposure, possibly mediated by changes at the hypothalamic-pituitary level (Lasisz *et al.* 1992). Tuppurainen *et al.* (1988) studied 176 African male workers in Kenya and found that the duration of lead exposure correlated negatively with serum total FT4 and total T4. The correlation was strongest for the workers with the highest exposure intensity over time. The mean value for Pb-B was 56.8 μg/dl (21–135 μg/dl), and there was a mean exposure duration of 7.6 years (range 1–20). The authors noted that current Pb-B level, as a point determination, was not associated with total or free T4, T3 or thyrotropin in serum. They proposed that long-term, high-intensity exposure might be associated with depressed thyroid function (Robins *et al.* 1983). Gennart *et al.* (1992) included assessment of thyroid function as a part of a study of lead-exposed workers. Data for serum levels of T3, T4, FT4 and TSH were within normal ranges for a group of 98 workers (mean Pb-B levels 2.44 μmole/l) and 85 controls (mean Pb-B levels 1.0 μmole/l). The lead exposure of this study group was considerably lower than in the workers in Kenya and it was suggested that thyroid function changes might not be indicators of moderate exposure to lead (Refowitz *et al.* 1983).

It is thus concluded that the mean whole blood lead (Pb-B) levels were significantly higher in the lead exposed subjects and were approximately 5 times higher than the control subjects. No significant alteration was observed in their mean T3 and T4 levels as compared to the control. Interestingly, T3 was significantly low with the longer lead exposure time of 210 months in comparison with the group having lower mean exposure time of 29 months. However, TSH was significantly higher ($p < 0.01$) in lead exposed subjects as a collective group with respect to the control group. This rise in TSH was independent of duration of lead exposure. On the other hand, the rise in TSH was associated with Pb-B levels, the increase being more pronounced with mean Pb-B levels of 2.66 ± 0.20 μmole/l when compared with the group having mean levels of 1.5 ± 0.30 μmole/l. In the latter group, the TSH was also not significantly different from the control group. The rise is TSH associated with Pb-B levels (2.66 ± 0.20 μmole/l) was only statistical valid, however, the levels fall within the normal laboratory range. We thus conclude that the Pb-B levels of ≥ 2.0 μmole/l could enhance the pituitary release of TSH without having any significant alterations in the circulating levels of T3 and T4.

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