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Estimation of blood lead level and its effect on hormonal responses of exposed men

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Abstract

The use of leaded gasoline play a major role in the air pollution in Iraq and most Asian countries that did not band the addition of lead to gasoline, also the increased use of private electricity generators contribute to the lead air pollution. In this study the blood lead level (BLL) of traffic policemen, national guards, electricity generator workers, and a control group for compression was measured**.** The results showed that the highest BLL mean was in the traffic policemen (26.077µg/dl) and the lowest mean was in the control (15.846µg/dl). Also, the reproductive hormones: testosterone, luteinizing hormone and follicle stimulating hormone of the exposed and control men were tested. The testosterone (T) concentration level was significantly decrease with the increase of blood lead level for the exposed and control groups and the results was 1.849, 3.367, 2.640 and 7.070µg/ml, respectively for the traffic police men, national guards, electricity generator workers, and control groups. While, the concentration level of both luteinizing hormone (LH) and follicle stimulating hormone (FSH) were increased with the increase of blood lead level. The concentration for LH were 6.906, 5.32, 5.513and 4.237mlU/ml, respectively for the mentioned groups above. And the concentration of FSH were 9.329, 9.932, 7.486 and 5.280mlU/ml, respectively for the mentioned groups.

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Introduction

The largest source of lead (pb) in the atmosphere has been found to be from leaded gasoline combustion, but with the gradual elimination worldwide of lead in gasoline, a noticeable decrease in air lead levels was accomplished. Other airborne sources include combustion of solid waste, coal, oils, and emissions from iron, steel production, lead smelters, and tobacco smoke (Chen *et al.,* 2007).

Lead is a common environmental contaminant which is used in the preparation of batteries, paints, varnishes and as the antiknock compound in gasoline (Singh *et al.,* 2000). Occupational and environmental exposures to heavy metals have an adverse effect on human health. Evaluation of health risks due to lead exposure is generally based on blood lead levels measurement. An analysis of blood lead levels is, therefore, the first choice for the assessment of internal exposure to individuals exposed to lead (Cullen *et al.,* 1983).

According to the Center for Disease Control, Atlanta, the permissible limit of blood lead level is 10mg/dl (Rosen, 1992).

Heo *et al.,* (1998) suggested that lead is known to have detrimental effects on the central nervous, immune, hematopoietic, and renal systems. Exposure to lead can affect the blood, cardiovascular, kidneys, and nervous, immune, and reproductive systems. Also, Al-Amier et al., (2018) studied the effect of lead on liver and kidney functions among exposed workers.

The environmental impact of chemicals on male reproductive system could happen at different levels: either prenatally (Agarwal *et al.,* 2015) or during postnatal development period continuing throughout the male reproductive life time in a gradual and cumulative manner (Navarro-Costa *et al.,* 2010).

The continues addition of lead to gasoline in order to reduce engine knocking and boost octane ratings, caused severe harm to human body when inhalation the polluted air. The study was conducted to investigate the effect of lead emission from car exhaust on male sex hormones (testosterone, follicle stimulating hormone, and luteinizing hormone).

Materials and methods

Collection of blood samples

All blood samples were collected in the morning period around 6-9 am from 52 males. Their age ranged from 18 to 48 years. 26 person were traffic policemen and national guards exposed to car exhaust emission, 13 person were electricity generator workers directly exposed to diesel exhaust for a period around (4 to 13 years) of exposure, the last 13 person were control negative. Five milliliters of venous blood sample were collected from each individual of the groups in Iraq/Baghdad province in both Al-Karkh (Al-Kadhimiya, Al-Shu'ala and Al-Ghazaliya Area) and Al Rusafa (Al-Jadriya and Al-Adhamiyah). The 5ml were distributed into: 2ml of the blood in an EDTA tube for blood lead level measurement, 2ml in a gel tube for hormonal test. All samples were kept in a cool box and transported to the laboratory.

Blood Lead Level (BLL) measurement

This test was performed in the Medical City/Gazi Alhariri Hospital/Poisoning Consolation Center. Two milliliters of blood in an EDTA tube was collected and kept frozen at -20°C until the analyses.

Sample preparation

The samples were left at room temperature to thaw, and then placed on the shaking device for 30-59 minutes. The blood was mixed with equal amount of trichloric acid (2ml blood with 2ml TCA) and left at room temperature for 10 minutes. After homogenizing the mixture, it was placed in centrifuge for 10 min. at 3000 round per minute. Then the clear suspension was separated from the precipitate after that the clear suspension placed in the atomic absorption spectrophotometer to read the result.

Testosterone level measurement

The procedure was carried out according to CALBIOTECH Company for testosterone ELISA kit.

Assay procedure

25µg of the standers was pipetted into the assigned well then 100µg of working testosterone-enzyme conjugate reagent was Add to all wells. The microplate gently Swirled for 20-30 seconds to mix

the reagents and the plate then covered and incubated for 60 minutes at room temperature. The liquid was removed from all wells, then washed the wells three times with 300µl of 1X wash buffer. Blot absorbent paper towels and 100µl of TMB substrate reagent was added to all wells. The plate then covered and incubated at room temperature for 15 minutes and then 50µl was added of the stop solution to each well and gently mixed for 15-20 seconds. We read the absorbance on ELISA reader for each well at 450nm within 15 minutes after adding the stop solution.

Luteinizing Hormone (LH)

The procedure was carried out according to CALBIOTECH Company for Luteinizing Hormone ELISA kit.

Assay procedure

The desired number of coated strips was placed into the holder. 25µl of LH standards was pipetted. 100µl of enzyme conjugate was added to all wells. The plate was covered and incubated for 60 minutes at room temperature. The liquid was removed from all wells, and washed three times with 300µl of 1X wash buffer. Blot on absorbent paper towels. 100µl of TMB substrate was added to all wells. The plate then was incubated for 15 minutes at room temperature. 50 µl of stop solution was added to all wells. The plate then was gently shaked to mix the solution. The absorbance then was read on ELISA reader for each well at 450nm within 15 minutes after adding the stop solution.

Follicle Stimulating Hormone (FSH)

The procedure was carried out according to Monobind Inc. for Follicle Stimulating Hormone ELISA kit.

Assay procedure

The microplate wells were formatted for each serum reference, control and patient specimen to be assayed in duplicate. 50µl of the appropriate serum reference were pipetted, control or specimen into the assigned well and 100µl of FSH enzyme reagent solution was added to all wells then the microplate was swirled gently for 20-30 seconds to be mixed and covered. The microplate then incubated for 60 minute at room temperature and the content of the microplate was discarded by decantation or aspiration then 350µl of wash buffer was added and this step was repeated two additional times for a total of three washes. 100µl of working substrate solution was added to all wells and the microplate then incubated at room temperature for 15 minutes. 50µl of stop solution was added to each well and gently mixed for 15-20 seconds. The absorbance in each well was read at 450nm in a microplate reader.

Result and discussion

The results in Fig. (1) showed that the highest mean of blood lead level was in traffic police group 26.077±0.400µg/dl. There was a significant difference between national guard's 25.923±0.348µg/dl and electricity generator 23.538±0.369µg/dl groups and between the traffic police and electricity generator groups. While, there was no significant difference between the national guards group and traffic police groups. The lowest mean of blood lead level was 15.846±0.296µg/dl in the control group with high significant difference ($P \leq 0.05$) when compared to all exposed groups. Our data shows that when compared to the reference values the concentration level for the LH, FSH and testosterone are found to be in the expected ranges (1.5-9.3mlU/ ml) (1.0-14.0mlU/ ml) and (3.0-10.0ng/ ml), respectively for all the exposed and control groups. The results indicate that the mean of blood lead level in exposed groups were higher than normal limit value 0-20µg/dl and less than maximum toxic value 80µg/dl (WHO, 2000).

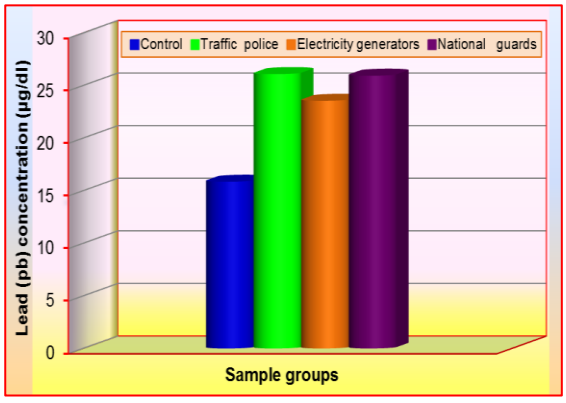

Fig. 1. Measurement of the concentration of blood lead level for the control and lead exposed groups.

The concentration of blood lead level in the traffic policemen and national guards groups was higher than that of electricity generator group which may attributed to the fact that, since the 1940s, tetraethyl lead (TEL) has been added into gasoline as an antiknock additive (Adriano, 2001). Leaded gasoline became a major source of Pb in the atmosphere with its maximum contribution in the 1970s (Nriagu, 1989). Blood lead level (BLL) is a common biomarker for lead. It reflects the equilibrium between absorption, extraction, and deposition in tissues. There is a linear relationship between blood lead level and exposure to lead (Holstege, 2010). High blood levels of lead even in the controls is probably attributable to the increased amounts of leaded gasoline and to heavy traffic in the roads of the country (Vural and Gu¨vendik ,1988). Abdulhay and Rathi (2017) in a previous local study found that the effect of road vehicle traffic emissions appeared through decreased the lead concentrations in plant leaves and soil as the distance from the road increase.

Testosterone

The results in Fig. (2) shows that the highest mean of testosterone was in the control group 7.070±0.299µg/ ml and there was a significant difference between all exposed group (traffic policemen, national guards and electricity generator workers) 1.849± 0.212, 3.367±0.483 and 2.640±0.554µg/ ml, respectively. The highest mean of blood lead level was 7.070±0.299µg/ ml at the control group with high significant difference ($P \leq 0.05$) when compared to all exposed groups.

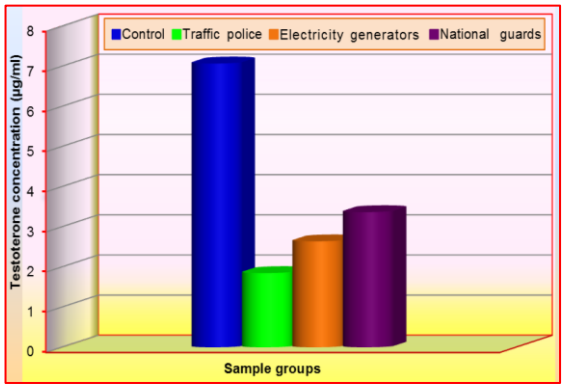

Fig. 2. The concentration of the level of testosterone in the human serum of the control and lead exposed groups.

Luteinizing and follicle stimulating hormones

The results in Fig. (3) shows that the lowest mean of LH was in the control group 4.237 ± 0.356 mlU/ml and the highest mean was in the traffic policemen 6.906±0.453mlU/ml. There was no significant difference between the national guard's 5.321±0.588mlU/ml and the electricity generator workers 5.513±0.366mlU/ml, while there was a high significant difference between the control group and all the exposed groups (traffic policemen, national guards and electricity generator workers and a high significant difference between the traffic policemen and the control group and the national guards and the electricity generator workers.

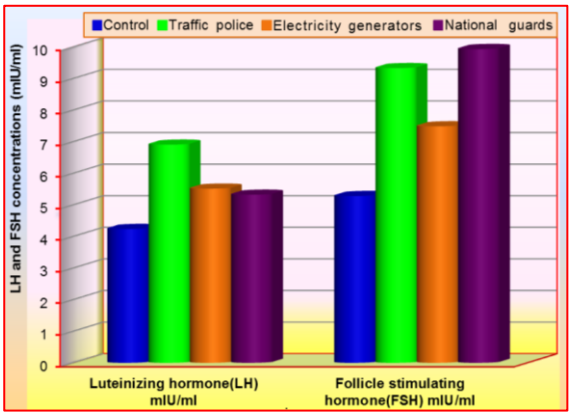

Fig. 3. The concentration level of luteinizing and follicle stimulating hormones in control the and lead exposed groups.

Also, the lowest mean of FSH found in the control group 5.280±0.409 mlU/ml and the highest mean in national guards group 9.932±0.664 mlU/ml. No significant difference appeared between the national guards and the traffic policemen group 9.329±0.598 mlU/ml, while there was a high significant difference between the electricity generator workers 7.486±0.610 mlU/ml when compared to the other groups (control, traffic policemen and national guards). When compared our data to reference values; the concentration level for LH, FSH and testosterone were within the expected ranges (1.5-9.3mlU/ ml) (1.0-14.0mlU/ ml) and (3.0-10.0ng/ ml), respectively for all the exposed and control groups. In addition the increased BLL of the traffic policemen and national guards may be attributed to the increase

amount of hours they spend in the streets and thus the testosterone percent decrease and increase in the LH and FSH percent when compared to the control and electricity generator workers which present normal and close to normal values respectively.

Many researchers studied the correlation between lead exposure and sex hormones. Jia *et al.,* (2009) exposed *Rana nigromaculata* to different lead concentration for successive 30 days, finding that in the lead concentrations ranging from 0.1-1.6mg/L, the testosterone concentration decreased obviously with lead concentration increasing. Yu *et al.,* (2011) studied the effect of lead exposure on the male reproductive function and found that the FSH and LH levels in the lead exposure males were higher than those in the control group, while the testosterone level was lower than that in control group. Excessive lead exposure can affect the reproductive system, both in men and women. The effects of lead in men include reduced motility as well as number of sperms with retarted sperm activity. Reduced libido has also been reported with high lead levels. Male infertility and changes in levels of serum testosterone are also reported. Lead is also known to affect the functions of the prostate gland. Most of these effects have been seen in lead exposed workers, and some are at levels presently considered 'acceptable' (Xuezhi *et al.,* 1992).

Sokol (1987) found that lead showed no significant effect on the LH levels, but significant decrease in testosterone. This suggests that lead exerts its effect more at the testicular level, this study also supports the finding Taiwo *et al.,* (2010) asserted that lead targets the spermatogenesis and sperms within the epididymis by producing reproductive toxicity rather than acting within the hypothalamic-pituitary-testicular axis. They also suggested that the gonad toxic effects of lead are on the intra-testicular sites with minimal effects on hormonal levels and no effect on extra testicular sites. Lead induced imbalances in the HPT hormonal axis, which causes pituitary cells to release inappropriate levels of LH and change the steroid negative feedback loop (Ronis *et al.,* 1996), usually at the hypothalamus level (Grattan *et al.,* 1996).

Yan *et al.,* (2013) found in their study that the mean of FSH and LH concentrations in the males exposed to lead were 19.17 and 12.21mIU/ ml, which were apparently higher than those in the males with no lead exposure (15.19 and 11.03mIU/ ml).

In contrast, the average value of the T concentration in the males with lead exposure was 16.30μmol/L, lower than that in the males with no lead exposure (20.96μmol/L). Chen *et al.,* (2007) adopted meta method to study the effect of lead on the male hormone secretion. The result revealed that the male LH level rose while the male (T) level fell with lead concentration growing. And there was significant correlation between the LH, and T levels and lead concentration ($P < 0.05$).

Gautam *et al.,* (2001) and Acharya (2003) carried out a lead exposure experiment on adult male mice, finding that lead could result in testicle degenerating and clearly reduce the average weight of the testicle and the epididymis which were the male (T) secreting organs. This was strong evidence that lead caused the male T concentration to decrease. Many other reports revealed that lead could directly increase the male hormone binding protein of the epididymis and damage the function of interstitial cell compounding T (Wang and Jia, 2006). In conclusion, the damage of lead to the testicle and the epididymis and the effect on reproductive endocrine function were the major reasons why the male T concentration decreased with blood lead concentration increasing.

Yu *et al.,* (2010) discovered that lead could inhibit inhibin B from secreting. Inhibin B had a negative feedback effects on FSH and LH levels. Also, the testosterone (T) also had negative feedback effects on the FSH and LH. Thus, lead weakened the negative feedback effect of inhibin B on the FSH and LH by restraining testosterone(T) and inhibin B from secreting, causing the FSH and LH concentrations in the male to grow with blood lead concentration rising.

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