

## Original Research Article

# **Obesity Influence on Lead levels in Blood and Scalp Hair among Teenage Smokers in Jordan**

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**Abstract:** Increased incidence of obesity, cardiovascular disorder and smoking habit between teenagers in the undergraduate's society has highlighted on the putative role of metals in the development of chronic disorders in urban area. This study was carried out to assess the influence of obesity on lead deposition in acute (blood) and chronic (hair) storage sites among university students. 99 healthy university students, 41 nonsmokers and 58 smokers were enrolled in this study, 198 biological samples (hair and blood) were analyzed for lead levels using inductively coupled plasma-atomic emission spectroscopy. Mean lead concentration for the study population of H-Pb and B-Pb was 1.613 µg/g and 0.030 µg/L, resp., a significant correlation was demonstrated in levels of lead in hair and blood ( $r=0.360$   $p<0.001$ ). H-Pb levels were significantly influenced by duration of smoking and body mass index ( $p<0.05$ ), whereas, B-Pb levels were only significant with body mass index ( $p<0.05$ ). In addition, there was an exponential increase in H-Pb and B-Pb levels in obese category in proportion of 65% in comparison with normal weight category. Furthermore, there was an increase in levels of H-Pb and B-Pb in a proportion of 32%, 23%, respectively, for smokers more than 5 years in comparison with nonsmokers group. Our data shows discern healthy environment in Jordan; B-Pb levels did not exceed 0.1 µg/L. Finally, Obesity and duration of smoking are playing a plausible role in increasing lead levels in blood and hair of the university students, which in turn may develop clinical manifestations after 10-20 years.

**Keywords:** Lead, Smoking, obesity, Hair, Blood, ICP-AES.

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## **INTRODUCTION**

The incidence of Cardiovascular disorder (CVD) is rising in an alarming rate worldwide. World health organization (WHO) reported CVD as the first cause for mortality in its reports for the last five consecutive years of 2010-2014. In 2012, estimated 17.5 million people were passed away with cardiovascular complications [1].

Globally, smoking is attributed to 12% of all deaths among adults aged over 30 years [2]. Lead plays a significant role in tobacco toxicity through releasing non-radioactive Pb-206 and radioactive Pb-210 which contributes significantly in increasing mortality and morbidity rates of lung cancer in humans [3]. The passive smoking or second smoking of side stream or exhaled smoke contains also Pb-210 increasing mortality rate up to 30% for the second smoker [4]. In the past few decades, there was an ascending concern on monitoring the contamination levels of metals in industry and environment due to its toxic effect on different biological systems [5]. The ubiquitous forms of lead and its abundance in the nature have high contribution on lead contamination in the environment

[6, 7]. Lead finds its way into our bodies through air, water, and surface soil through mining, smelting and refining of lead, recycling, as well as cigarettes smoking [8, 9]. High blood lead levels (BLL) are highly concomitant with behavioral and neurological disorders from learning difficulties to mental retardation. Moreover, high BLL are strongly linked with hypertension and ischemic heart disease [10]. Not only high blood lead levels can influence the prevalence of chronic disorders as CVD; for instance individuals with BLL of 3.62 µg/dl - one third of the standard Centers for Disease Control (CDC), USA definition of a high lead level for children - having more than double the risk of cardiovascular mortality than individuals with a blood lead of less than 1.94 µg/dl [11]. During smoking, approximately 6% of the lead from a cigarette is believed to enter the lungs of a smoker; the effusion rate widely depending on variable factors such as smoking intensity, age, sex and body mass index (BMI) of the smoker [12]. Overweight and obesity are defined as "abnormal or excessive fat accumulation that may impair health". Almost 20% of all deaths among adults in USA is caused by obesity related diseases [13].

Paucity of information about the correlation of lead levels with other demographic parameters as smoking, sex and BMI in Amman province and the dearth of knowledge about lead levels among teenagers; has highlighted on conducting a pilot study to elucidate levels of lead in two biological systems (hair and blood). In addition, we will try to find evidence about the influence of smoking duration on lead levels in smokers and nonsmoker's population.

## **MATERIAL AND METHODS**

### **Study population**

The study was conducted on a group of 99 volunteers were cooperatively involved, 51 males and 48 females aged between 17 and 25 years old (this is the age of university students). The study population was divided into smokers and none smokers; smokers category was divided into two subgroups according to their smoking duration; smokers for more than 1 and less than 5 years and smokers for more than 5 years. Smokers were consuming less than 20 cigarettes per day. Heights and weights were measured using standard medical balance with weight scale (DETECTO, USA). Sampling process took place at University of Petra; Amman, Jordan, two biological samples were obtained from each volunteer (hair and blood).

The study has been approved by the Faculty of Pharmacy and Medical Sciences at University of Petra. This study is conducted on the behalf of community awareness program against smoking.

### **Sample collection**

Samples were collected by healthcare professionals at the medical clinic of the University of Petra following a standard protocol [14]. After collection of hair from scalp position (approximately 0.1-0.3 g) in polyethylene cups with lids (Greiner, Germany), the hair was washed two times with metals free detergent followed by ultrapure water to remove contaminations of metals on the outer hair cuticle. Blood samples were phlebotomy from volunteers in 5.00 ml plain polypropylene tubes and labeled. Samples were stored at -20 °C until analysis.

### **Samples digestion**

Sample preparation was carried out according to Friel and Ngyuen, 1986 guidelines [15].

### **Lead determination**

Hair and blood digested samples were analyzed using inductively coupled plasma-atomic emission spectroscope (PerkinElmer optima 2000) (Department of Health Sciences, University of Hail, Hail, Saudi Arabia). The sample digests were diluted

(100-fold) with 0.2% (v/v) HNO<sub>3</sub> solution before injection into the ICP-AES. Sample solutions were measured in triplicate mean level was adopted for results. For results with RSD $\geq$ 10%, an additional two injections were performed. Calibration standards were also prepared by diluting a commercial multi-element standard solution (PerkinElmer Pure; Part No. N9300244) with 0.2% (v/v) HNO<sub>3</sub> solution

### **Analytical quality control**

The validity and accuracy of the methodology was checked using certified reference materials and using conventional wet acid digestion method on the same certified reference materials (CRMs). The recovery of all studied elements was found to be in the range of 97.5-99.7% of certified reference values of CRMs

### **Statistical evaluation**

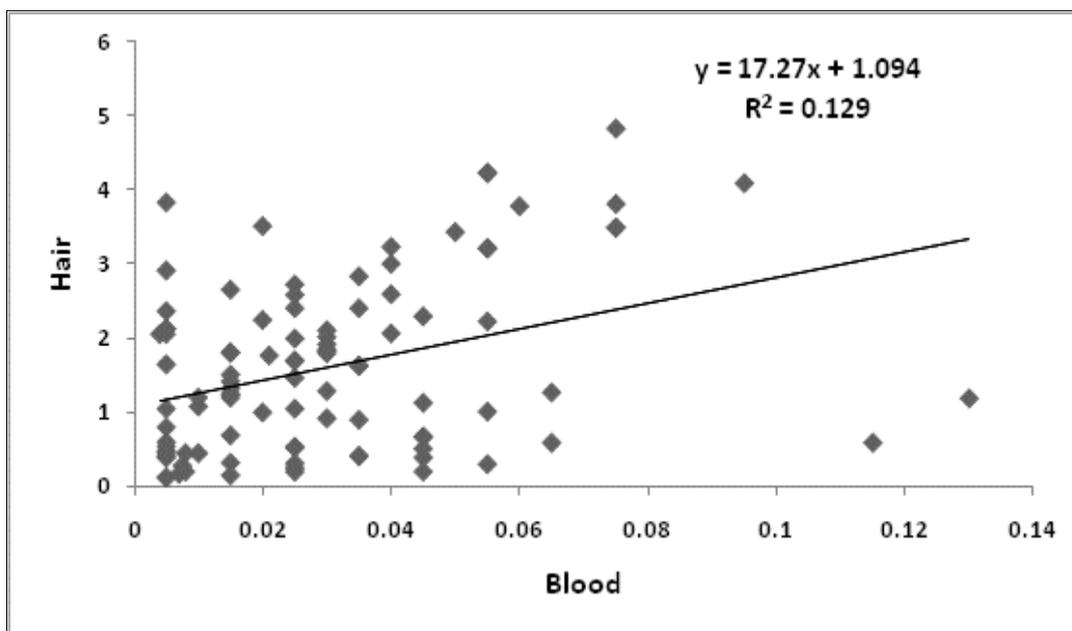
The data was expressed as M $\pm$  SD and analyzed using the SPSS computer software (Statistical Package for the Social Sciences, version 19.0, SPSS Inc., Chicago, IL, USA). Statistical analysis was done using of variance (ANOVA), independent t-test analysis and Duncan's method. Correlation between lead levels in hair, blood sex and duration of smoking was determined by Spearman's correlation. P<0.05 was considered significant. Logistic regression analysis was used to obtain age and gender-adjusted odds ratios (ORs) of obesity for a number of variables. Each of the factors shown in Table 1 was assessed in a separate logistic regression model along with the presence of age (as a continuous variable) and gender. Each factor was also assessed for interaction with gender by inclusion of interaction terms in these models. None of these interactions was significant and were therefore excluded from the models.

## **RESULTS**

Our study population was evenly distributed in gender and age, with a median of age 21 and 51% males. More than ninety five percent of B-Pb levels were less than 0.040  $\mu$ g/L with a mean of 0.030  $\mu$ g/L and a median of 0.025  $\mu$ g/L. The H-Pb levels were ranged from (0.121 to 4.84)  $\mu$ g/g with a mean of 1.613  $\mu$ g/g and a median of 1.42  $\mu$ g/g, mean lead concentrations in hair and blood for the study population was illustrated in Table I. There was a significant correlation between levels of H-Pb and B-Pb ( $r = 0.360$   $p < 0.001$ ) (Fig. 1). Mean lead levels in hair and blood of smokers was 1.775  $\mu$ g/g, 0.031  $\mu$ g/L, whereas, lead levels were 1.382  $\mu$ g/g, 0.028  $\mu$ g/L in nonsmokers, respectively. There was no significant difference was observed in males than females.

**Table 1: Mean lead concentration of hair and blood samples in the study population**

		N	Mean Lead $\pm$ SD ( $\mu$ g/g) Hair	Mean Lead $\pm$ SD ( $\mu$ g/L) Blood
Smoking period	Non smoker	41	1.382 $\pm$ 1.249 <sup>a</sup>	0.0283 $\pm$ 0.0199
	1-5 years	39	1.643 $\pm$ 1.169 <sup>ab</sup>	0.0288 $\pm$ .0279
	> 5 years	19	2.047 $\pm$ 0.879 <sup>c</sup>	0.0358 $\pm$ .0259
	p Value		< 0.05	> 0.05
Smoking	Non smoker	41	1.382 $\pm$ 1.249	0.0283 $\pm$ 0.01998
	Smokers	58	1.775 $\pm$ 1.091	0.0311 $\pm$ 0.02721
	p Value		> 0.05	> 0.05
Sex	Male	51	1.751 $\pm$ 1.152	0.029 $\pm$ 0.019
	Female	48	1.464 $\pm$ 1.182	0.031 $\pm$ 0.029
	P- value		> 0.05	> 0.05
BMI	Underweight	17	1.083 $\pm$ 0.827 <sup>a</sup>	0.0196 $\pm$ .01254 <sup>a</sup>
	Normal (healthy weight)	51	1.270 $\pm$ 1.014 <sup>a</sup>	0.0246 $\pm$ .01703 <sup>ab</sup>
	Overweight	23	1.988 $\pm$ 0.837 <sup>b</sup>	0.0376 $\pm$ 0.0335 <sup>b</sup>
	Obesity	8	3.838 $\pm$ 0.630 <sup>c</sup>	0.0644 $\pm$ 0.0189 <sup>c</sup>
	P- value		< 0.05	< 0.05
Age	17-19	32	1.630 $\pm$ 0.821	0.028 $\pm$ 0.027
	20-22	38	1.275 $\pm$ 1.153	0.029 $\pm$ 0.021
	23-25	29	2.033 $\pm$ 1.396	0.032 $\pm$ 0.025
	P- value		> 0.05	> 0.05
Total		99	1.613 $\pm$ 1.169	0.0300 $\pm$ 0.024



**Fig-1: Correlation of lead concentration in hair and blood in study population. There was significant correlation of lead deposition in blood and hair ( $r=0.360$   $p > 0.001$ ).**

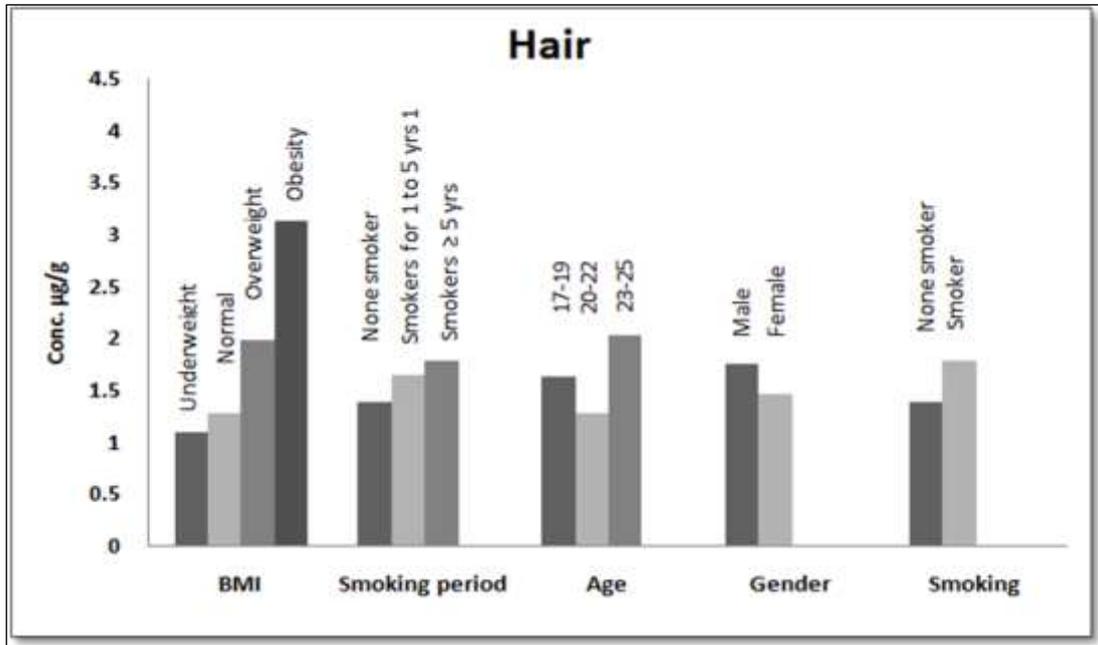


Fig-2: Mean lead concentration in scalp hair according to duration of smoking. There was an increase in cadmium levels in hair with the duration of smoking there was almost two fold increase in the concentration within five years.

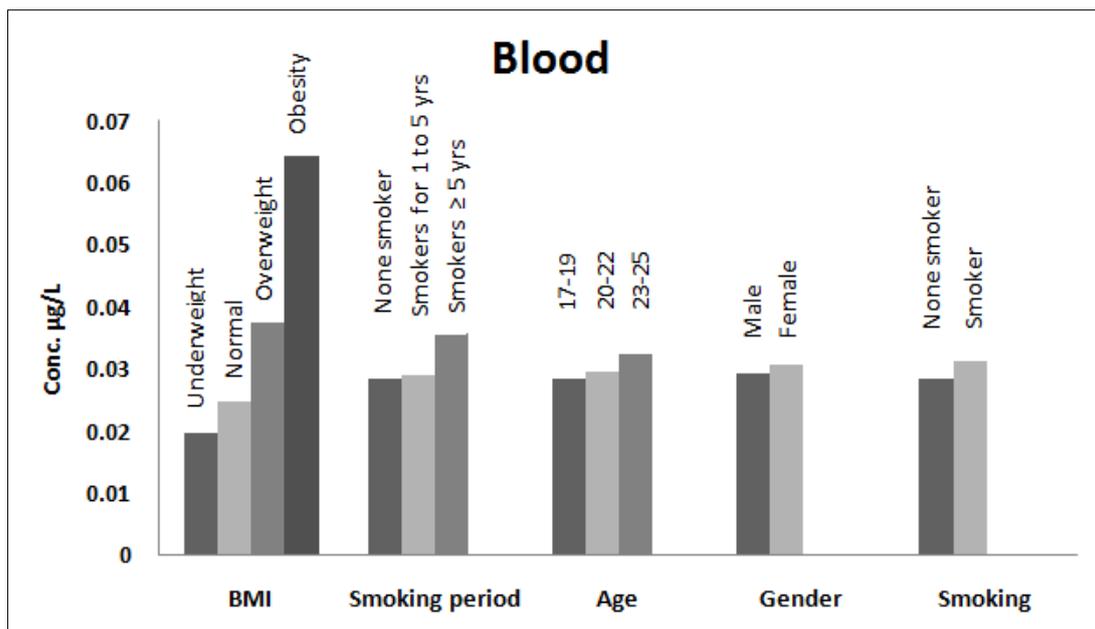


Fig-3: Mean lead concentration in hair and blood samples in different duration smoking groups. There was a significant correlation between both levels of B-Pb and H-Pb ( $P > 0.05$ ) and the levels of cadmium in relation with smoking duration as there was an ascending in cadmium levels with duration of smoking.

**DISCUSSION**

Metals analysis in biological systems is one of the recommended methods for examination of acute and chronic exposure to contaminants in the environment [15]. Blood contains about 1% of the body lead, of which 90 to 99% is associated with the red blood cells [16]. Hair has been widely used as biological marker for the analysis of metals contents in the fields of

toxicology, drug monitoring and environmental exposure [17].

There is a debate about the usefulness and reliability of using hair and fingernails as biological samples to monitor the chronic exposure of metals because of direct contact with contaminants, nevertheless, it is still

approved by the scientific society for their specificity and certainty [18, 19].

Globally, cigarette smoking is basically one of the major causes for the progressive increase in mortality rate among adults and teenagers. Smoking is an exogenous source of metals contamination in human body; smoking increases reactive oxygen species and additionally causes depletion of redox scavengers in peripheral blood [20].

Our study demonstrated low levels of lead contamination among university students on the acute (blood) or chronic (hair) levels as the mean value of B-Pb was less than 0.1 µg/L; which is the recommended limit for B-Pb in children by the Agency for Toxic Substances and Disease Registry (ATSDR) [21]. Additionally, according to statistical analysis of correlation coefficient for our study sample there was a significant correlation between levels of H-Pb and B-Pb ( $r = 0.360$   $p < 0.001$ ) as depicted in Fig 1. Niculescu *et al*; showed there is a significant correlation between lead levels in both hair and blood samples among occupationally exposed workers, furthermore, he showed there is simultaneous increase in H-Pb levels with the spontaneous increase in B-Pb value [22]. In Mexico City, Farias *et al*. demonstrated level of B-Pb in teenagers was 0.075 µg/L [23]. Whereas, Soud *et al*; reported that mean lead level in blood samples for students at Al-Yarmok University of Jordan was 0.057 µg/L [24]. Dabbas *et al*; showed mean lead level in whole blood sample of Jordanian citizens was 0.0196 µg/L [25]. In Lebanon, mean lead concentration in the hair of Lebanese citizens was 4.3 µg/g [26]. Kim *et al*; elucidated the levels of lead in hair samples of Korean population was 1.11 µg/g [27]. According to the previously published data of lead concentrations at different geographic locations worldwide, there is a controversy about lead levels in different countries, and even there is a controversy in the same country as Jordan. These variations in results of blood lead level are still within the recommended limit of ATSDR for lead level in blood. We could not include a recommended limit for lead exposure in hair as ATSDR do not recommend hair as a biological sample for bio-monitoring of metal exposure [21] but lead levels were in an acceptable range for metals exposure in reference to other countries studies [28].

Levels of both H-Pb and B-Pb were higher in smokers than nonsmokers group; there was an increase of 22% and 10% in levels of lead respectively, statistical analysis revealed no significance for these results. Even though our statistical results showed no significance for the relation of smoking and lead levels in hair and blood, we believe the low significance was because of the short smoking period as 80% of our population was nonsmokers or smoking for one and less

than 5 years. To rollout this issue, we used one way ANOVA test to measure the correlation between the nonsmokers and the two smoking subgroups. Significantly, levels of H-Pb and not B-Pb for smokers of 1 and less than 5 years and nonsmokers group have increased in a proportion of 16%. On the other hand, the proportional increase in H-Pb levels between the group of smokers for more than 5 years and smokers for 1 and less than 5 years revealed a ratio of 20%. Whereas, there was almost two fold increase in levels of H-Pb in a ratio of 33% for smokers of more than 5 years in comparison with nonsmokers group. Our results are consistent with data published by *et al*; he reported a difference of 30% in lead levels between nonsmokers and smokers in USA. an epidemiological study for lead levels in hair and blood of school students in Turkey demonstrated a higher levels of H-Pb and B-Pb in smoker in comparison with nonsmokers of the same age group [28]. In Lebanon, there was a 5-6 folds increase in the difference between lead concentration in the hair of smokers and none smokers 5.3 µg/g and 3.6 µg/g, resp. [26]. While reviewing literature, there was a prognostic effect of lead concentrations on the correlation with cause of death for heart-related disease vs. non-heart-related disease [29]. Navas *et al*; found that high concentration of lead was deposited in the veins and arteries of the cardiovascular system leading to tendency for atherosclerosis [30].

In our study, more than fifty percent of the university students were ranked in the normal (health weight) category. The proportion of overweight and obesity in our study were 23% and 8% resp. this implicates a good demographic distribution of results on the BMI scale. World widely, this result is considered as a low percentage of obesity in a study population, for instance, in USA estimated 36% of adults are ranked in the obese category on BMI scale [31]. In KSA, Ahmed *et al*; showed a high prevalence for obesity in the Saudi population 63.6% [32]. In Jordan, the percentage of obesity did not exceed the 35 % in the Jordanian society [33]. Khader *et al*; showed the percent of overweigh and obese student in Jordan aged 12-21 years is around 26% [34]. These results demonstrate congruent percentage of obesity among university students in the age group of 17-25 years. The student life style of enrolling in physical and social activities beside the malnutrition for expatriate students has its impact on the BMI readings. Unfortunately, we noticed an ascending pattern for BMI readings in the Jordanian society as it was 26% in students aged 12-21 [34], in our study 31% and in adulthood the percentage was 35% [33].

In summary, the increase in BMI readings has its impact on lead levels in both hair and blood samples; there was an exponential increase in H-Pb and B-Pb levels spontaneously with BMI increase, a twofold

increase was noticed in lead levels in obese students than both healthy and underweight students as the ratio was almost 65% as depicted in Fig 2, 3. These results demonstrate an conceivable role of obesity and overweight in the pathogenesis of obesity related diseases as cardiovascular events through direct effect of lead on oxidative stress on LDL oxidation and injury of smooth muscles of cardiovascular system [35].

#### CONCLUSION AND RECOMMENDATIONS

From our data, we found that lead deposition in hair and blood tissues in the study population has showed a dramatic increase in relation with both obesity and duration of smoking. In addition, we show discern healthy environment in Jordan; B-Pb levels did not exceed 0.1 µg/L. even though, B-Pb levels were with the recommended limits, the development of clinical manifestations may arise after 10-20 years. It could be concluded that, despite the low lead concentration in hair and blood of university students there should be enforcement for smoking prohibition law in Jordan as the levels of lead were increasing with duration of smoking.

#### Recommendation

- Applying effective law for smoking prohibition in Jordan.
- Increasing awareness program against obesity and smoking side effects.

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