LEAD AND CHROMIUM HAIR LEVELS IN A SAMPLE OF EGYPTIAN CHILDREN WITH IDIOPATHIC SHORT STATURE

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Abstract:

Short stature (SS) is a common problem with worldwide concern nowadays. Idiopathic short stature (ISS) is a challenging type of SS as there is no known cause for it. Exposure to toxic metals like Lead and Chromium in humans is rising in developing countries and poses substantial risks to environmental ecosystem systems, and human health and growth. **The aim of this study** is to investigate the association between lead (Pb) and chromium (Cr) hair levels in children with idiopathic short stature. **Subjects and methods:** The study included 100 children from Mansoura University Hospital and Mansoura Insurance Hospital; 50 children were previously diagnosed by ISS while the other 50 were children with normal stature as controls. All children were subjected to history taking, sociodemographic data collection including name, age, sex and residency, Height for age z-score (HAZ) calculation, and hair sample collection. Hair samples were analyzed by Inductively Coupled Plasma Optical Emission Spectroscopy for lead (Pb) and chromium (Cr) hair levels. **Results:** showed that the mean age of the control group was 9.30 ± 2.57 and 10.48 ± 2.4 for the test group. In the control group, Pb level median was 5.8 (IQR, 0.8 - 10.6), while Cr was 34.5 (21.8 - 63.9). In test group, Pb level was 10.2 (IQR, 0.0 - 30.9), while Cr was 127.6 (IQR, 22.7 - 265.0). Higher Pb and Cr levels were significantly associated with ISS (p=0.046, p=0.001 respectively). Correlation between each of Pb and Cr levels with the severity of SS was numerically detected but not statistically significant. **Conclusion:** Public health interventions are required to minimize the exposure of children to Pb and Cr sources, as higher Pb and Cr levels were significantly associated with ISS in a group of Egyptian children.

Keywords: short stature, lead, chromium and heavy metals

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Introduction:

Short stature (SS) is the most common cause of referral to pediatric endocrinologists ***(Polidori et al., 2020)***. Short stature is a significant public health issue that affects 2.86–11.3 percent of children worldwide ***(Yuan et al., 2021)***. Z-score charts are used in pediatric growth follow-up and to compare anthropometrical variables ***(Wang et al., 2015)***.

Idiopathic short stature (ISS) is considered in children with SS, a subnormal growth rate, delayed bone age, no apparent medical cause for growth failure, and normal growth hormone response to provocative testing ***(Gutch et al., 2016)***. Idiopathic short stature is an exclusion diagnosis that is achieved after ruling out other recognizable causes of short stature ***(Inzaghi et al., 2019)***.

Heavy metals absorption rate in children is much higher than in adults. Long-term, low-level exposure to metals results in various metabolic and cognitive disorders, behavioral and developmental impairment, and growth disturbance ***(Mainka & Fantke, 2022)***.

Lead (Pb) has received the most attention, with consistent evidence that higher Pb levels in the blood are associated with shorter stature ***(Signes-Pastor et al., 2021)***. Children that live in areas near Cr processing, and gas processing zones may experience alterations that impede their physical and sexual development ***(Zhumalina et al., 2019)***.

The aim of this case control study is to find out the association between long-term exposure to lead (Pb) and Cr measured from hair samples and ISS in Egyptian children from two hospitals in Mansoura (Mansoura university children hospital and Mansoura insurance hospital).

Subjects and Methods:

Subjects:

A case-control study was conducted at Mansoura University Children Hospital (MUCH). The study was approved by Institutional Review Board (IRB), Faculty of Medicine, Mansoura

University (code MD.20.01.273). Informed written consent was obtained from all children's parents and guardians before enrollment in the study.

Study design:

The study was carried out on 100 children aged from four to fourteen years who were randomly chosen from those attending the outpatient pediatric clinic of endocrinology at MUCH and Mansoura Health Insurance Hospital. Fifty cases were previously diagnosed by ISS (Test group) and another fifty children with normal height for their age and sex (Control group). Children from both sexes were included; other causes of short stature were excluded as disproportionate short stature, familial short stature, constitutional delay of growth, endocrine disorders as growth hormone deficiency (GHD), structural brain abnormalities or pituitary lesions, genetic disorders as Down syndrome or Turner syndrome and Bone diseases as Dwarfism and skeletal dysplasias and chronic disorders as cystic fibrosis and Crohn disease.

Methods:

Sociodemographic data were collected, including name, age, sex, and residency. Height was measured in children using a stadiometer. The stadiometer was mounted on the wall for this purpose, and the child was asked to remove any footwear and/or head ornaments before noting the measurement. With the buttocks, the shoulder blades, heels, and the back of the head against the board, the head was oriented in the Frankfurt horizontal plane (FH plane), and the headpiece gets firmly placed on the head. Height is recorded in centimeters to the first decimal place that is the nearest 0.1cm ***(Louer et al., 2017)***. Then Z score of short children was calculated using the following formula: **Actual height - mean of age and sex / Standard deviation of age and sex.** It was assessed according to Egyptian Growth Chart ***(El-Ziny et al., 2003)***.

Sample collection:

Scalp hair samples were collected from cases and controls, samples were cut from the scalp occipital region using stainless-steel scissors with hair length varied between 2-5 cm ***(El-Morsi et al., 2019)***. A minimum of 5–10 mg of hair was collected for the hair analysis assay. The adhesive paper was placed over the end of the hair strands indicating the end of hair closest to the scalp, then samples were placed in a sealed plastic bag and sent to the lab to assess levels of heavy metals.

Sample digestion:

Hair samples were cut into 5-mm pieces then washed according to the procedure recommended by the International Atomic Energy Agency Advisory Group (IAEA), three times using acetone in deionized water (v:v=1:3) to remove surface dirt, grease, and exogenous contaminants due to contact of hair with smoke, cosmetics, sweat, during collection and storage, as well as contaminating trace elements from tap water or hair care products- without damaging the hair cortex and affecting the endogenous metal content ***(Horvath, 2009)***. All samples were then dried before analysis in an oven at 80°C oven ***(Liang et al., 2017)***.

Each sample was placed in a 50 mL acid-washed polypropylene tube and 10 ml of HNO_3 (69%). After centrifuging for 5 minutes, hair samples were transferred into Ethos-Easy Microwave Digestion apparatus, to undergo closed vessel microwave digestion.

Analytical procedure:

Heavy metals lead (Pb) and Chromium (Cr) concentration was determined using Inductively Coupled Plasma Optical Emission Spectroscopy. The analysis was done in the Laboratory of Soil Fertility and Fertilizers Quality Control in Faculty of Agriculture, Mansoura University. Chromium was detected at wavelength 205.560 and Pb at 220.353 after acid digestion using HNO_3 (69%) and HF (40%) in a microwave digestion apparatus ***(Bettinelli et al., 2000)***.

Statistical Analysis:

Data were analyzed using IBM SPSS software package version 28 and Stata version 16. Age was described using mean ± SD. The Chi-Squared test and Independent T-test were used to compare the age between the test and control groups. After testing normality using Kolmogrov-Smirnov test, Pb, Cr, and z-score variables were not normally distributed and were described using median and interquartile range (IQR) (i.e., 25^{th}, 75^{th} percentiles range). Non-parametric Mann-Whitney U test was used to compare Pb and Cr levels between the test and control group in all children and after stratification by gender. Logistic regression was used to detect the association between short stature and each µg/g increase of Pb or Cr in the form of odds ratios. Spearman Correlation was used to estimate the linear relationship between each of Pb and Cr levels with the z-score among the short children. Quantile regression was used to measure the association between each quantile increase in Pb or Cr levels and short children's height z-score. The significance level for any of the used tests, results were considered statistically significant if the p-value was ≤ 0.05

Results:

1- Demographic data among studied groups:

There is no statistically significant difference between age of boys in control and test groups, also, there is no statistically significant difference between age of girls from both groups. While there is significant difference between age of control group and test group in whole (p=0.025*). The percentage of girls to boys shows no significant difference between both groups. Regarding residence there is no significant difference between both groups as shown in **(Table 1)**.

Table (1): Demographic data among all studied groups (n=100).

Variables	Control group (n=50)		Test group (n=50)		Test of Significance
Age (Years) [Mean ± SD]	Girls n=25	10.0 ± 2.1	Girls n=31	10.9 ± 2.3	t= - 1.463 p = 0.15
	Boys n=25	9.5 ± 2.9	Boys n=19	11.1 ± 2.8	t= -1.769 p = 0.09
	Total	**9.30 ± 2.57**	**Total**	**10.48 ± 2.4**	t= -2.363 **P= 0.025***
Gender n (%)	Girls	25 (50%)	31 (62%)		χ^2= 1.461 P= 0.272
	Boys	25 (50%)	19 (38%)		
Residence n (%)	Urban	20 (40%)	15 (30%)		χ^2= 1.099 P= 0.295
	Rural	30 (60%)	35 (70%)		

n: number. SD: standard deviation. %: percentage. χ^2: Chi-square.
t: t-statistics P: probability. *: Statistically significant (p< 0.05).

2-Height for age z-score among all studied groups:

The median value for HAZ score in control group is 0.4 (-0.3-1.5) Regarding test group, the median value for HAZ score is -2.9 (-3.0 - -2.6) as shown in **(Table 2)**.

Table (2): Height for age z-score among all studied groups (n=100).

Variables	Control group (n=50) Median (Range)		Test group (n=50) Median (Range)		Test of Significance
HAZ score	Girls (n=25)	**0.7 (-0.3 - 1.2)**	Girls (n=31)	**-2.8 (-3.3 - -2.5)**	**z = 6.399 P <0.001***
	Boys (n=25)	**0.4 (0.1 - 1.6)**	Boys (n=19)	**-2.9 (-3.0 - -2.6)**	**z = 5.636 P <0.001***
	Total (n=50)	**0.4 (-0.2 - 1.5)**	Total (n=50)	**-2.9 (-3.0 - -2.6)**	**z = 8.628 P <0.001***

HAZ: Height for age z-score n: Number z: Mann-Whitney U-test. P: Probability. *: Statistically significant (p< 0.05).

3-Comparison of Pb and Cr levels (µg/g) among all studied groups:

Regarding Pb there is a statistically significant difference between both groups **(P=0.046)**. While, among girls there is no statistically significant difference found between both groups. Also, among boys, there is no statistically significant difference between both groups.

Regarding Cr, there is a statistically significant difference between both groups **(P=0.001)**. Among girls, there is a statistically significant difference between both groups **(P=0.0006)**. While among boys, there is no statistically significant difference between both groups **(Table 3).**

Table (3): Comparison of Pb and Cr levels (µg/g) among all studied groups (n=100).

Heavy metals	**Control group (n=50) Median (Range)**		**Test group (n=50) Median (Range)**		**Test of Significance**
Pb (µg/g)	Girls n=25	10.6 (4.5 - 11.50)	Girls n=31	9.9 (0.0 - 17.4)	z = -0.124 p= 0.901
	Boys n=25	3.9 (0.8 - 8.1)	Boys n=19	29.8 (0.0 - 54.3)	z = -2.360 p= 0.0183*
	Total n=50	5.8 (0.8 - 10.6)	**Total n=50**	10.2 (0.0 - 30.9)	z= -2.018 P= 0.046*
Cr (µg/g)	Girls n=25	28.0 (20.0 - 35.9)	Girls n=31	101.1 (22.7 - 169.1)	z = -3.420 p= 0.0006*
	Boys n=25	63.9 (33.0 - 96.7)	Boys n=19	261.5 (19.5 - 557.5)	z = 0.0506 p= 0.0506
	Total n=50	34.5 (21.8 - 63.9)	**Total n=50**	127.6 (22.7 - 265.0)	z= -3.426 P = 0.001*

Pb: Lead. Cr: Chromium. µg/g: microgram per gram. n: Number.
z: Mann-Whitney U-test. P: Probability. *: Statistically significant (p< 0.05).

4-Multiple logistic regression of Pb and Cr levels (µg/g) with short stature children:
Lead adjusted analysis:

Each µg/g increase of Pb has 1.05 times the odds of developing short stature in unadjusted and adjusted models for age, gender, and both. All the odds ratios are statistically significant as shown in **(Table 4)**.

Table (4): Association between Pb level (µg/g) and short stature using simple and multiple logistic regression.

	Odds Ratio	**95% Confidence interval**	**P- value**
Unadjusted Model	1.05	1.02 - 1.09	**0.005***
Multiple logistic regression models			
Adjusted for gender	1.05	1.02 - 1.09	**0.003***
Adjusted for age	1.05	1.01 - 1.09	**0.009***
Adjusted for both Age & gender	1.05	1.01 - 1.08	**0.006***

Pb: lead. µg/g: microgram per gram. P: Probability. *: Statistically significant (p< 0.05)

Chromium adjusted analysis:

Each mg/ L increase of Cr has 1.012 times the odds of developing short stature in unadjusted and adjusted models for age. While the odds ratios are higher after adjusting for gender 1.015 (95% CI, 1.007 - 1.023). All the odds ratios are statistically significant as shown in **(Table5)**.

Table (5): Association between Cr level (µg/g) and short stature using simple and multiple logistic regression.

	Odds Ratio	**95% Confidence interval**	**P- value**
Unadjusted Model	1.01	1.01 - 1.02	**0.001***
Multiple logistic regression models			
Adjusted for gender	1.02	1.01 - 1.02	**<0.001***
Adjusted for age	1.01	1.01 -1.02	**0.001***
Adjusted for both Age & Gender	1.01	1.01- 1.02	**0.001***

Cr: Chromium. µg/g: microgram per gram. P: Probability.
*: Statistically significant ($p< 0.05$)

5- Correlation between Pb and Cr levels (µg/g) and height for age z-score (HAZ) in test group:

There is a weak non-statistically significant negative correlation between Pb levels (µg/g) and Z-score among short children with spearman correlation coefficient of -0.192 and p-value of 0.181. Also, there is a very weak non-statistically significant negative correlation between Cr levels and Z-score among short children with spearman correlation coefficient of -0.012 and p-value of 0.936 **(Figures 1 and 2)**.

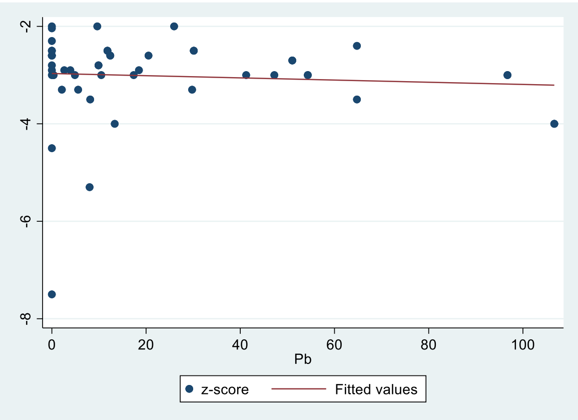

Figure (1): Scatter plot showing the correlation between lead (Pb) level (µg/g) and Z-score values among the short stature children (n=50).

Figure (2): Scatter plot showing the correlation between chromium (Cr) level (µg/g) and Z-score values among the short stature children (n=50)

Among test group (short stature children), the association between each increase in Pb ordinary variable levels and Z-score is not statistically significant. Also, the Cr ordinary variable levels do not show statistically significant association with the Z-score (**Tables 6 and 7**).

Table (6): Quantile regression for the relation between Pb level (µg/g) and Z-score among short stature children (n=50).

	Difference in Z score Median	**P Value**	**95% Confidence interval**
Unadjusted	0.1	P= 0.056	-0.003 - 0.2
Multiple Quantile regression			
Adjusted for gender	0.1	P= 0.170	-0.04 - 0.24
Adjusted for age	0.1	P= 0.118	-0.03 - 0.23

Pb: lead µg/g: milligram per gram. n: Number. P: Probability.

Table (7): Quantile regression for the relation between Cr level (µg/g) and Z-score among short stature children (n=50).

	Difference in Z score Median	**P-value**	**95% Confidence interval**
Unadjusted	0.03	P= 0.647	-0.11 - 0.18
Multiple Quantile regression			
Adjusted for gender	0.03	P= 0.680	-0.13 - 0.19
Adjusted for age	0.03	P= 0.636	-0.10 - 0.16

Cr: chromium µg/g: milligram per gram. n: Number. P: Probability

Discussion

In children, short stature (SS) is a prevalent issue. Social stigma and mental stress may affect children with SS. In Africa and Asia, the prevalence of stunting among school-age children was estimated to be 37% and 23%, respectively ***(de Onis et al., 2012)***. **El-Shafie et al. (2020)** estimated that roughly 17% of Egyptian elementary school students are short-stature.

In upper Egypt, according to **Hussein et al. (2017)**, familial SS constituted 68.2% of the nonpathological causes of short stature. In addition, 2.8% of the kids had idiopathic short stature (ISS), 3.3% were small for gestational age and 25.7% showed constitutional growth delay. Idiopathic short stature (ISS) is one of the most problematic types of SS due to its difficulty in diagnosis and treatment ***(Yuan et al., 2021)***.

Heavy metals can affect the development and growth of almost all body systems ***(Li et al., 2020)***. Exposure to heavy metals is one of the plausible causes of ISS. However, the previous studies focused mainly on studying blood and urine samples to confirm this hypothesis, and up to our knowledge, no studies had included Chromium (Cr) as a potential risk factor for ISS.

The aim of this case control study is to find out the association between long term exposure to lead (Pb) and Cr measured from hair samples and ISS in Egyptian children.The median Z-score in the test group children in this study was -2.9 (-3.0- -2.6) **(Table 2).**

The current study included children of both sexes with a mean age of 9.0 ± 2.6 years for the control group, while the mean age of the test group was 10.5 ± 2.4 years. Children were collected from urban and rural areas **(Table 1)**. The age in this study is similar to the age in **AbuShady et al. (2017)** study in Egypt, in which the mean age was 9.40 ± 2.33 years, children from both sexes were collected from two regions Dokki and Helwan. Also, in the **Signes-Pastor et al. (2021)** study done on United States participants, the median age was 9.0 (7.0–10.0) in both sexes. In contrast, in a study done by **Dallaire et al. (2014)** on Canadian participants, the children were slightly older with a mean age of 11 years in both sexes.

Regarding heavy metals, in the present study, higher Pb levels were significantly associated with ISS after comparing the median Pb level in the test group with the control group. The Pb level was higher in the hair of the test group with ISS, with a median of 10.2 μg/g **(Table 3**). There was a statistically significant difference in the median Pb level in children with ISS. The association was persistent even after adjusting for the discrepancy in age and gender among groups **(Table 4).**

Similarly, **Zeng et al (2019)** detected that Pb concentrations measured during childhood are linked to ISS. However, blood samples instead of hair samples were used in this study.

AbuShady et al. (2017) found a correlation between short stature and high blood Pb levels (BLLs) (10 g/dL) in their cross-sectional investigation of school children in two districts of Egypt. Also, a cross-sectional Italian study by **Ashley-Martin et al. (2019)** on children aged 11–13 reported that BLLs (mean = 9 μg/dL in boys and 7 μg/dL in girls) were inversely related to height and weight in boys and with height in girls. Similarly, **Anticona and San Sebastian (2014)** in another cross-sectional study of children and adolescents aged 0–17 years from six communities in Argentina found that children and adolescents with BLLs > 5 μg/dL had twice the risk of stunting when compared with those with lower BLLs. In contrast to the above studies, **Dallaire et al. (2014)** had not found cross-sectional study associations between childhood BLLs and height in Canadian children.

However, the effect on Pb was not only restricted to the height but also extended to other anthropometric measures. According to **Zeng et al. (2019)**, preschool children's BLLs were adversely correlated with their height, weight, head circumference, and chest circumference. This finding supports the idea that Pb exposure can hinder children's physical development and growth. A negative correlation was also discovered by **Signes-Pastor et al. (2021)** among United States children included in the National Health and Nutrition Examination Survey (NHANES) in 2013–2016 between BLLs and children's standing height, particularly among boys.

Furthermore, according to two studies, one conducted in Poland and the other in Egypt, children with greater BLLs may experience delayed puberty ***(Tomoum et al., 2010; Gomula et al., 2022)***. These findings may endorse the hormonal theory of the Pb effect on the body. Moreover, **Hauser et al. (2008)** in a Russian study found that elevated BLLs were related to shorter height but not to weight or body mass index (BMI) in Russian boys aged from 8 to 9 years. In line with these findings, **Min et al., (2008)** revealed that BLLs correlate with height and/or head circumference but did not correlate with weight or BMI among Korean children aged 5 to 13 years. Another study done by **Moody et al. (2020)** on 246 child aged 3 to 8 years from Uganda, found that Pb levels in the blood and bones had negative correlations with weight and height.

The potential explanation for the effect of Pb on height is that Pb may interfere with the normal growth and development of children ***(Zeng et al., 2016)***. Pb may alter circulating levels of hormones required for bone development and maintenance (e.g., 1,25-dihydroxyvitamin D3), as well as the ability of bone cells to respond to hormonal regulation, leading to impaired bone formation. Furthermore, exposure to Pb may have endocrine-disrupting capabilities by reducing responses to hormones that are necessary for growth, such as insulin-like growth factor, and inhibiting the hypothalamic-pituitary-growth axis ***(Deierlein et al., 2019)***.

By studying the association between Pb level and severity of short stature, the results of this study sub-analysis measured the associations between the hair level and the severity of ISS, in contrast to previous cross-sectional and cohort studies that measured the BLL in all children. The association between Pb level and the severity of the SS scale was numerically detected but not statistically significant. This may be due to the restricted population that may be resulting in underpowered statistical analysis. Because of the case-control design, we did a sub-analysis restricted to children with ISS only (n=50) **(Table 6).**

In the cross-sectional study done by **Moody et al. (2020)** in Uganda, on a sample of 97 children, the sample included 22 children who were stunted. Blood samples were analyzed for Pb, Arsenic, and Cadmium. The toxic mixture had a considerably decreased HAZ (p value= 0.03), with Pb accounting for 62% of the effect.

In **(2020), Zhou and his colleagues** conducted a study in China which stated that children's BLLs were significantly adversely linked with HAZ (P = 0.002) across all individuals. Children were divided to three tertiles, children in the highest (>50 g/L) and second (25–50 g/L) tertiles of the BLLs had lower HAZ than those in the lowest tertile (25 g/L). These associations persisted after further accounting for dietary and pica behavior patterns. Furthermore, the effect of Pb on HAZ starts at a very early age. In a two-site study of 618 rural Bangladeshi toddlers aged 20 to 40 months, **Gleason et al. (2016)** discovered that concurrent BLLs were linked to higher probabilities of stunting among Bangladeshi children.

The results of prospective cohort research in Russian by **Burns et al. (2012)** are consistent with these findings, indicating that boys aged 8 to 13 with BLLs concentrations of 50 μg/g had a substantially lower mean height z-score (-0.44) than boys with blood concentrations of less than 50 μg/g. However, according to **Burns et al. (2017)**, no specific blood level for Pb is considered as safe. Boys with very low Pb levels such as 5 g/dL in blood were typically noticeably shorter.

Furthermore, **Ignasiak et al. (2006)** found a dose-related loss in stature of 1 to 3 cm for every 10 mg/dL increase in blood levels of Pb in polish children. Also, **Deierlein et al., (2019)** reported that each 10 ug/dL increase in BLL concentrations was associated with 1.2-1.6 centimeters (cm) shorter heights.

Regarding Cr, in the present study, higher Cr levels were significantly associated with ISS after comparing the median Cr level in the test group with the control group. The Cr level was higher in the hair of the test group with ISS with a median of 127.6 µg/g (**Table 2)**. There was statistically significant difference in median Cr level in children with ISS. The association was persistent even after adjusting for the discrepancy in age and gender among groups **(Table 5).**

Chromium exposure was one explanation for the prevalent short stature in children living in the West Kazakhstan region ***(Zhumalina et al., 2019)***. **Hashemi et al. (2023)** in a study done in Iran, detected that Cr in maternal urine had the most prominent negative association with weight and length in newborns. Their analysis suggested a negative association between increasing levels of Cr and a newborn's weight and length. However, **Zeng et al. (2019)** revealed that there is no significant correlation between blood Cr level and height in preschoolers in his study conducted in China.

This result could be explained by **De Lucca et al. (2009)** who reported that Cr has a definite toxic effect on bone, as shown by the observed reduction in body growth, which is accompanied by a reduction in mandibular growth and a delay in tooth eruption.

Conclusion:

Higher Pb and Cr levels were significantly associated with ISS in a group of Egyptian children. Several risk factors suggested higher levels of Pb and Cr in this study participants. However, the relation between Pb and Cr levels and their negative effect on HAZ score was not evident.

Strengths:

The main advantage of the current study is using hair samples to quantify heavy metals exposure, in contrast to the other previous studies that used blood lead level (BLL) ***(AbuShady et al., 2017; Zamani et al., 2019),*** which may reflect only recent exposure to those hazardous substances. Hair samples were used to evaluate long-term exposure to Pb and Cr**.** ***(Sanna & Vallascas, 2011)***.

Limitations:

- The main limitation of this study is the absence of prospective follow-up of children.
- Also, the unavailability of measurements of the Cr subtypes is considered an important limitation. There is a quite difference in the toxicities between chromium (III) and of chromium (VI). However, up to our knowledge, this was the first study systematically investigating the effect of hair Cr on short stature.

Recommendations:

- Public health interventions are required to minimize the exposure of children to Pb and Cr sources.
- Further studies are needed to assess other heavy metals effect on growth and development.

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