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BIOMONITORING THE PRESENCE OF TRACE ELEMENTS IN THE TOENAILS AMONG ADOLESCENTS OF TOWN BADDI, HIMACHAL PRADESH: A PRELIMINARY STUDY

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ABSTRACT

Present communication assesses levels of trace elements in the toenails of adolescents belonging to Baddi Town of Himachal Pradesh State. Approximately 30 mg of the toe nail samples were collected from adolescents (n=22) aged 16 to 18 years and residing in the industrial town of Baddi, District Solan, Himachal Pradesh. For controls, nails from the normal subjects (n=5) were collected. Detection of trace elements, viz., lead, cadmium and aluminium, in the samples was done using Inductively Coupled Plasma-Mass Spectrophotometer (ICP-MS). Appropriate statistical analysis was performed to evaluate the presence of the trace elements. Data was also analysed on the basis of gender and socioeconomic status. In pooled data, mean levels of lead, cadmium and aluminium (µg/g) were found to be 4.58±1.66, 0.81±0.08 and 37.97±6.27, respectively. Among males, the mean concentration of lead, cadmium and aluminium was found (in µg/g) to be 4.57±2.04, 0.86±0.37 and 37.94±7.71, respectively. In females, mean lead concentration (µg/g) was 4.58±0.966, cadmium was 0.73±0.38 and aluminium was 37.99±3.70. The t-test revealed no significant difference between the mean concentration of these elements in males and females. When the data was divided on the basis of socio-economic status for all the three elements, mean concentration showed increasing trend from higher socioeconomic group to lower socioeconomic group. However, in all the three categories there was no significant difference between the mean concentrations of elements, as depicted by One-way ANOVA.

***Keywords**: Toenails, trace elements, Inductively Coupled Plasma-Mass Spectrometer.*

INTRODUCTION

Rapid industrialization and urbanization over the past years have resulted in increased accumulation of toxic heavy metals in the environment (Ali *et al.*,

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2019). Not only the workers who are in direct contact with these contaminants are at risk but the community at large is highly affected. Although certain trace elements are required for physiological processes, but when present in excess, these elements might be detrimental to human health (Jaishankar *et al.*, 2014). Exposure to heavy metals could lead to diseases like carcinomas Cihan *et al.*, 2011), renal failure (Lentini *et al.*, 2017), behavioural problems , (Ayuso-Álvarez *et al.*, 2019) and many other complications. Recently, in the region of Ropar wetland of Punjab, India, the content of cancer causing heavy metals was found to be exceeding the acceptable limits that pose threat to human life (Sharma *et al.*, 2019). Therefore, it is necessary to monitor the exposure of trace elements in human tissues and their hazards. Such assessments have been found to be useful in identifying the risks of various diseases such as cancer, cardiovascular disease, and osteoporosis (Kobylewski and Jacobson, 2007) Such assessments have also been recognized by agencies like Environmental Protection agency (EPA) to limit the presence of trace elements like arsenic, cadmium, barium, selenium, etc., in drinking water (Calabrese *et al.*, 1985).

Accurate and adequate health risk assessment in most epidemiological studies involves quantification of current and past human exposure to trace elements. It can be calculated qualitatively by analysing behavioural changes in humans and quantitatively through examination of different tissues for chemical accumulation, which is termed as biomonitoring (Clewell *et al.*, 2008; Waseem and Arshad, 2016). Several methods have been used to ascertain the immediate severity of the intake of trace elements on individuals of various age groups, especially in children. Most methods have not appropriately captured the intake because of the minimal amounts and wide range of variations that have been observed (Waseem and Arshad, 2016). Heavy metal exposure among humans can be assessed from different biomarkers including blood, saliva, bones, nails, urine and hair. The appropriate selection of biomarker can reduce the error in epidemiological studies (Slotnick *et al.*, 2005; Slotnick and Nriagu, 2006). Since concentration of metals in nails reflects their mean level in human body during a period of 12–18 months, its use is far from being the universal tool for monitoring long exposures to environmental pollutants. Adair *et al.* (2006) stated that toenails can be a useful biomarker in exposure assessment studies because they are less exposed to water than fingernails and hair. Toenails have been used as a biomarkers in clinical, forensic, epidemiologic, and environmental studies (ab Razak, *et al.*, 2015). Toenails have several advantages over blood and urine like non-invasive sampling as well as easy storage and transportation at room temperature. However, toenails do not have any developed standard methods of collection, preparation, and analysis. Although both finger- and toenails can serve as biomarkers for trace element status; toenails generally provide a larger sample and represent exposures in the more distant past because they take longer to grow. Overall, nails provide valid measurements for ranking subjects according to long-term trace element intake or exposure.

There are many applications of assessment of levels of trace elements, the foremost being that toenails provides valuable insight into an individual's exposure to environmental toxins like lead, mercury and cadmium (Khairnar *et al.*, 2016). The information obtained from these assessments can be used to develop public health policies and regulations to minimize the exposure of the population to harmful toxins. The assessment of levels of trace elements in toenails provides a means to evaluate the risks of exposure, and thereby helps the policy makers to frame and implement mitigation strategies to minimize the impact on public health (Mass *et al.*, 2018). Moreover, early interventions can be initiated to minimize the exposure to these trace elements by monitoring sources of intake such as diet or household products (Mass *et al.*, 2018).

The present study aims to assess the levels of trace elements namely, lead, cadmium and aluminium using Inductively Coupled Plasma Mass Spectrometer in the toenails of the adolescents belonging to the industrialized town of Baddi, Solan District, Himachal Pradesh, India.

MATERIALS AND METHODS

The present research was carried on the adolescents of age ranging between 16 and18 years. The subjects belonged to the Baddi, an industrial town in the Solan District of Himachal Pradesh. Before sampling, it was ensured that the subjects were born and brought up in Baddi Town only. Subjects below 16 years of age and above 18 years, who were not born in Baddi, and found to have a family history of any psychological ailment were excluded from this study.

Collection of the samples: A total of 22 subjects (13 males and 9 females) were recruited for the present study. Before data collection, a written consent of the parents and the subjects was obtained. The toenail samples from all subjects were collected and analysed for the elemental concentration of lead, aluminium and cadmium. The details regarding name, age and family income were also noted. Based on the income of the family, socio-economic status of an individual was assessed according to a revised socioeconomic scale of BG Prasad proposed by Debnath and Kakkar (2020). This revised scale proposed classification of the subjects in five social classes, i.e., Upper class, Upper middle class, Middle class, Lower middle and Lower class. In view of the small sample size of the present study, the upper class and upper middle class were clubbed into Upper class, the Middle class and Lower middle class were clubbed to form Middle class. Thus, the sample of the present study was divided into three social classes: Upper, Middle and Lower socioeconomic classes (Table-1).

Table 1: Demographic and socioeconomic details of the adolescents in the present sample from industrial town of Baddi, Himachal Pradesh

		Number (N)	Percentage (%)
Sex	Male	35	55.6
	Female	28	44.4
Socioeconomic status	Low	20	31.7
	Middle	33	52.4
	High	10	15.9

Collection and handling of nail samples: Approximately 30 mg of the toe nail sample was collected from each subject. After collection, each sample was placed in an air tight transparent plastic bag. To ensure the blind study, the bag was given a unique identification number. The samples were stored at room temperature till further analysis.

Analysis of nail samples: The nail samples were chemically analysed for three trace elements, viz., lead, aluminium and cadmium. The elemental analysis was performed on Inductively Coupled Plasma-Mass Spectrophotometer (ICP-MS) available at Punjab Biotechnology Incubator Laboratory, Mohali. Prior to digestion, the nail samples were washed ultrasonically with acetone and then with doubled distilled water for 4-5 times to remove the extraneous matter like dust, nail polish, etc. The washed nails were dried overnight at 60ÚC in hot air oven. The dried samples were transferred to titration flask or beaker. Initially, about 3 ml concentrated nitric acid and 2-3 ml hydrogen peroxide was added and the flask was heated occasionally on a burner for 1-2 minutes and the contents were completely digested for 15 minutes. After digestion, the contents were cooled and transferred to 50ml falcon tube. The total sample content was diluted by adding double distilled water to make the final volume to 25ml. Finally, the sample was analysed by ICP-MS for lead, aluminium and cadmium concentrations.

Statistical analysis: Data have been presented in the form of mean±standard deviation. Unpaired t-test has been used to find the significance of difference between of elemental concentration in different sets of data. One-way ANOVA analysis was used to compare mean values of elemental concentrations of different socioeconomic groups.

RESULTS

Assessment of trace element concentration:

It was seen that in pooled data, mean levels of lead, cadmium and aluminium were 4.58 ± 1.66µg/g, 0.81± 0.08µg/g and 37.97 ± 6.27µg/g, respectively. The minimum value was 1.00µg/g and the maximum 6.98µg/g. The level of cadmium was 0.28µg/g (minimum) and 1.41µg/g (maximum). The aluminium was present from a minimum of 14.70µg/g and maximum of 46.03µg/g (Table-2).

Table-2: Concentration of trace elements in the toenail samples using ICP-MS analysis

Concentration of trace elements (in µg/g)	N	Mean	Standard Deviation of Mean	Standard Error	Median	Minimum	Maximum
Lead	22	4.58	1.66	0.35	4.81	1.00	6.98
Cadmium	22	0.81	0.38	0.08	0.98	0.28	1.41
Aluminium	22	37.97	6.27	1.34	39.23	14.70	46.03

Data was segregated on the basis of gender and it was observed that mean concentrations of lead were approximately similar for males and females (4.5µg/g). Among males, the mean level of cadmium was 0.86 ± 0.37µg/g and females showed a mean concentration of 0.73 ±0.38µg/g. No significant gender difference was found in the mean values of these elements ($p>0.05$) (Table-3). These findings suggest that there is no need to separate data on the basis of sex of an individual.

Table-3: Concentration of trace elements in males and female

Trace elements	Sex	N	Mean	Std. Deviation	Std. Error Mean	t-statistic	p-value
Lead	Male	13	4.57	2.04	0.56	-0.015	0.987
	Female	9	4.58	0.96	0.32		
Aluminium	Male	13	37.94	7.71	2.14	-0.019	0.984
	Female	9	37.99	3.70	1.23		
Cadmium	Male	13	0.86	0.37	0.10	0.764	0.453
	Female	9	0.73	0.38	0.12		

When the data was divided on the basis of socioeconomic status, an increasing trend in the concentration of all three elements was observed from higher to lower socioeconomic group. Results revealed that lead showed a mean value of 3.69±2.24µg/g in higher socioeconomic group that increased to 4.61µg/g in the middle and reached to 4.88µg/g in the lower socioeconomic group. Similarly, we observed an increase in the mean concentration of cadmium in high socioeconomic group (0.74 ±0.55µg/g) to middle (0.76± 0.29µg/g) and 0.86 ±0.29µg/g in the lower socioeconomic group respectively. Likewise, aluminium showed a mean concentration of 32.67 ± 12.62µg/g in the higher income group that was found to be elevated in the middle-income group (39.11± 3.32µg/g) and in lower income group (39.16 ± 3.78µg/g). However, One-way ANOVA showed no significant difference between the mean concentrations in these elements in the three categories (Table-4). This suggests that there is no need to segregate data on the basis of socioeconomic status of an individual.

Table-4: Mean concentrations of lead, cadmium and aluminium in different socioeconomic groups

Lead	N	Mean	Std. Deviation	Std. Error	F-statistic	p-value
Low income group	11	4.88	1.52	0.45	0.73	0.491
Middle income group	7	4.61	1.59	0.60		
High income group	4	3.69	2.24	1.12		
Aluminium						
Low income group	11	39.16	3.78	1.14	1.89	0.177
Middle income group	7	39.11	3.32	1.25		
High income group	4	32.67	12.62	6.31		
Cadmium						
Low income group	11	0.86	0.38	0.11	0.21	0.812
Middle income group	7	0.76	0.29	0.11		
High income group	4	0.74	0.55	0.27		

Moreover, it was seen that all the elements studied were significantly correlated to each other. For lead, a positive significant correlation coefficient of 0.72 ($p<0.05$) and 0.69 ($p<0.05$) was observed with cadmium and aluminium respectively. The concentration of aluminium was positive and significantly correlated to cadmium ($r=0.65$; $p<0.05$).

Table-5: Correlation analysis of the trace elements

	Pearson Correlation			
	Lead	Cadmium	Aluminium	
Lead	Pearson Correlation (r)	1.00000	0.72896**	0.69095**
	p-value	-	**0.00012**	**0.00037**
Cadmium	Pearson Correlation (r)	0.72896**	1.00000	0.65379**
	p-value	**0.00012**	-	**0.00097**
Aluminium	Pearson Correlation (r)	0.69095**	0.65379**	1.00000
	p-value	**0.00037**	**0.00097**	-

DISCUSSION

Industrial unmanageable hazardous waste including heavy metals pose health risks not only to the adults but also to the developing adolescents as well. To ascertain the levels of exposure to the heavy metals, it is imperative to evaluate various biological tissues that can serve as a biomarker for exposure. This may include biological tissues and its fluids such as breast milk, blood, urine, bone, hair, nails and teeth. Not only such biomarkers should be specific but their detection should reproducible. Using these biomarkers, the extent of exposure can be determined such as heavy metals in blood (2-3 hours) and urine (3-4 days) can be used as powerful biomarkers for acute exposure, while hair and nails reflects the long term exposure (Masjedi *et al.*, 2021). Based on these findings, a recent study on toenail bound heavy metals suggested the direct relation between the DNA damage and oxidative stress (Bellinger, 2008). In the present study, exposure of the adolescents to lead, cadmium and aluminium were studied in the industrialized town of Baddi, Himachal Pradesh.

Our study showed that, there was an increased level of lead, cadmium and aluminium beyond recommended levels in the toenails of the subjects recruited. The trace element exposure in humans represents both dietary and non-dietary exposure, for example Lead has surreptitiously entered human bodies as a trace element and tends to interfere with the overall process of growth and development of human brain. Environmental toxicity has a severe impact on immature, developing brain largely because the mature brain is equipped with a barrier of cells that prevent entry of neurotoxins from the bloodstream into the brain. A report of WHO 2009, states that lead poisoning alone contributes to 0.6% of overall child neuro diseases. Researchers agree that neuro damage during early years of life was responsible for cognitive and behavioural development (Lanphear, *et al.*, 2005), violence, IQ deficit (Mielke and Zahran, 2012), anti-social behaviour, aggression, and delinquency among children (ACCLPP, 2000). The levels of lead toxicity in the blood has been recommended by the Centers for Disease Control and Prevention (CDC for adults to be 10 ìg/dL and for children 5 ìg/dL (Bellinger, 2008). Recent research on morbidity rates due to lead toxicity has declared the safe level of lead in blood to be 2µg/dl (Gilbert and Weiss, 2006).

In our study, the levels of lead in the toenails was observed to be approaching the toxic levels which showed a decreasing trend towards the adolescents of high-income group. Similar to our study, a recent study conducted in the hair and nail samples of the children from the gold mines of Northwest Nigeria revealed that the contamination for the heavy metal lead was found to be above recommended levels (Radulescu and Lundgren, 2019). In another study conducted on the automobile workers in Pakistan, the exposure to lead was assessed in blood (65µg/dL), hair (24 mg/kg) and nails (30mg/kg) and was found to be significantly raised compared to control (Adewumi, *et al.*, 2020). Radulescu and Lundgren (2019) reiterated that sustained low level exposure to lead resulted in a prominent deficiency in cognitive abilities of children rather than higher doses of lead toxicity (Ahmed *et al.*, 2018).

Previous study on toenails revealed the increased levels of cadmium and risk of pancreatic cancers, we sought to study the levels of cadmium in our subjects (Camargo, *et al.*, 2019). In our study subjects, low cadmium levels below the safe limit was found suggesting that the adolescents residing in this area did not show bioaccumulation of this heavy metal. Further, these values did not change significantly irrespective of the socio-economic status of the subjects that were recruited. Similar to our study, in the previous study conducted using ICP-MS on hair and toenail samples of Pakistan, it was found that low levels of cadmium was present in hair (0.08 ppm) and toenail (0.05 ppm) samples (Anwar, 2005). In our study, ICP-MS analysis also showed elevated levels of aluminium in the toenail samples of the subjects. Additionally, these levels were declined only in the subjects belonging to high income group. Since concentration of aluminium above the tolerance level can modify the enzymatic activities within the cells and results into oxidative stress (Kumar and Gill, 2009), concentration of 1–3 ìg/L of aluminium in serum is considered as normal (Exley, 2016). In another study, the safe exposure levels of aluminium to the children using the inductively coupled plasma mass spectroscopy states the levels to be 0 to 6 ìg/L to <42 ìg/L in serum, 0 to 10 ìg/L to 0 to 15 ìg/L in plasma while 0 to 7 ìg/L to 5 to 30 ìg/L in the urine (Zeager *et al.*, 2012). Aluminium after its absorption, gets accumulated in bones about one half of the total body burden, liver, kidney, lungs and brain (Willhite *et al.*, 2012). This group have also shown in their recent study that there was exposure-response relationship between aluminium and Alzheimer's disease. In a previous study on exposure of welding fumes of aluminium, the CRP levels were found to be increased when the subjects were exposed for short term (6 hours) (Hartman *et al.*, 2014).

Based on these facts, toenails can be an emerging biomarker. A very recent study performed on the school children near the petrochemical and gas area of the Central District of Asaluyeh County located in the Kaki District highlighted the presence of elevated metalloids compared to the urban area (Parhizkar, *et al.*, 2021).

Overall, our study indicates that the toenails of the adolescents can be used as an emerging biomarker of exposure to heavy metals to ensure the safety of the individuals in the industrialized areas.

Future recommendations: Assessing the presence of trace elements in toenails has shown to be a valuable tool in assessing the long-term exposure to environmental pollutants. However, there is a need for further research to establish standard protocols for toenail collection, preparation, and analysis to minimize potential sources of variability. Additionally, more studies are needed to investigate the relationship between toenail levels of trace elements and health outcomes. Future recommendations should also explore the use of other biological matrices, such as hair and blood, to corroborate findings in toenail analysis. Overall, the use of biomonitoring in identifying environmental exposure to trace elements can have significant implications for public health.

Further, more studies are required to assess the applicability of biomonitoring of trace elements in toenails for forensic investigations. In forensic cases, measuring trace elements in toenails can provide valuable information about the individual's environmental history and exposure to toxins, which can help to establish exposure timelines and potential sources of toxins. It can also aid in providing evidence in cases of environmental crimes and toxicological investigations.

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