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Fingernails are the Best Indicator Sample for Biomonitoring

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ABSTRACT: To find out the best indicator sample for biomonitoring, whole-blood, urine, scalp hair, fingernail and tooth samples were collected randomly from volunteers of Mysore city and surrounding villages. These subjects (rural or urban) were grouped into two subgroups depending upon their personal/lifestyle factors namely, sex, occupation, food habit, drinking water source, alcohol consumption, betel and nut chewing, sugar level, blood, insulin treatment, economic status and cooking utensils used. The concentrations of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in whole blood, urine, scalp hair and fingernail were determined with the use of ICP-AES and levels of Cr, Cu, Fe, Ni, Pb and Zn were measured in tooth samples with AAS. Mean element concentration for each subgroup in each sample was calculated and the difference in mean value between subgroups was tested for its significance. Significantly differing element levels in each sample shows the influence of personal factors; in other words each sample indicate the effect of various lifestyle factor. Overall comparison was made for suitability of samples for biomonitoring and it is concluded that the sum total number of varied elements in all the influencing factors were higher in fingernails and it reveals that the fingernails are ideal, suitable and recommended indicator sample for biological monitoring of element status among general, occupationally exposed and ill-health subjects.

KEYWORDS: Element concentration, lifestyle factors, subgroups, influence, comparison.

1 BACKGROUND

Biological monitoring of human occupational and environmental exposure to trace elements has been extended to include a number of human samples that are generally grouped under autopsy and biopsy samples. Many human autopsy samples such as endocrine glands, brain, bone, kidney, liver, lung, heart, stomach, spleen, muscle, thyroid, intestine, pancreas and ovary are extensively analysed for the concentrations of trace elements. Biopsy samples are of mainly two types such as non-invasive (tear, hair, urine, milk, saliva, sweat etc.) and invasive (blood, liver, teeth etc.) samples. Among them, non-invasive samples are predominantly used for element analysis. Among them mainly five bio-samples namely whole-blood, urine, scalp hair, fingernail and tooth were selected for the present study and it is very interesting to assess which is the best indicator sample for biomonitoring.

2 MATERIALS AND METHODS

2.1 SUBJECTS

The residents from Mysore city and surrounding villages who visited various hospitals in Mysore for consultation during the sampling period are the subjects. Based upon their places of residences from city and rural areas the subjects were

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mainly sorted out into rural and urban categories. Further, each category (rural or urban) was grouped into two subgroups depending upon their personal/lifestyle factors namely, sex, occupation, food habit, drinking water source, alcohol consumption, betel and nut chewing, sugar level, blood , insulin treatment, economic status and cooking utensils used (personal information were collected through questionnaire).

2.2 SAMPLING

Among the five biological samples (whole-blood, urine, scalp hair, fingernail and tooth) the permanent tooth samples (M=56 & F=60) were alone collected at JSS Dental College and Hospital, Mysore from one group of volunteers since tooth sampling is invasive in nature, painful with bleeding, time consuming and many subjects objected collection of other samples. Similarly, the other four samples, blood (M=100 & F=76), urine (M=68 & F=52), scalp hair (M=68 & F=52) and fingernail (M=68 & F=52) samples were collected from another group of subjects when visited to the Kamakshi Hospital, Bassappa Memorial Hospital and Vickram Hospital, Mysore (all the samples were collected with the approval of ethical committee). Further, the collected blood, urine, scalp hair and fingernail samples were not equal in number because some people refused to donate nail and hair samples in view of their orthodox nature and tradition.

2.3 PREPARATION OF SAMPLE FOR ELEMENT ANALYSIS

2.3.1 WASHING

Following standard procedures reported elsewhere [1] the tooth, scalp hair and fingernails were washed with double distilled water to completely remove loosely adhering external metals associated with fat, sweat and dirt without altering endogenous content of elements of the sample [2].

2.3.2 DRYING

After washing, these samples were dried in hot air oven at 50^{o} C and 3 hr time was required to achieve a constant weight indicating complete desiccation of the samples [3].

2.3.3 DIGESTION

Dried samples were wet acid digested with addition of HNO_3 and $HClO_4$ in 4:1 ratio in Kjeldhal digestive unit following the procedures reported by others [3]. Blood sample was digested as it is and urine was not digested since it is in liquid form. Simultaneously, few blank and quality control samples were digested for every 20 samples. Digested samples, blank and quality control samples were made up to 10 ml by adding DDW, stored in labelled, acid proof high density polyethylene bottles at 4^{o} C and were analysed for element levels within two weeks.

2.4 ELEMENT ANALYSIS

Concentrations of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in whole blood, urine, scalp hair and fingernail samples were determined with the use of Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES), model JY-IYON-2002–2, at the Department of Environmental Engineering, Sri Jayachamarajendra College of Engineering, Mysore. Similarly, the levels of Cr, Cu, Fe, Ni, Pb and Zn were measured in tooth samples with Flame-Atomic Absorption Spectrometer (F-ASS) model - AAS 6 VARIO (make - Analytic jena), at Centre for Environmental Studies, Anna University, Chennai and School of Environmental Science, Jawaharlal Nehru University, New Delhi. Further, due to the non-availability of specific lamps, the amount of Cd, Co and Mn were not measured in tooth samples.

2.5 QUALITY CONTROL STUDIES

Considering the importance of precision and accuracy in element analysis the quality assurance in element analysis is taken care of by adopting 1) use of Certified Reference Material (CRM) – human hair powder as external quality control sample, 2) an in-house reference material as internal quality control sample and 3) participating in the inter laboratory quality control programme [4].

3 RESULTS AND DISCUSSION

Mean element concentration for each subgroup in each sample was calculated and the difference in mean value between subgroups was tested for its significance. Based on the fact that significantly differing element levels (between subgroups) in each sample shows the influence of personal factors or/in other words each sample may indicate the effect of various lifestyle factor, the influence of various personal characters on the levels of elements in different bio-samples (table 1) was discussed individually here under.

3.1 INFLUENCE OF PERSONAL CHARACTERS

3.1.1 GENDER

It is observed from the table 1 that the influence of gender on element levels were indicated in blood (Cr), urine (Ni and Pb), hair (Co, Cu and Zn) and teeth (Pb), but not in nail element levels. However, [5] observed that among the blood, urine, hair and nails samples gender affected only the blood element levels (Pb-B). On the other hand [6] reported that gender did not influence Se status in blood, urine and hair of Canadian subjects.

3.1.2 OCCUPATION

Occupation is related with the element levels in other words, influence of occupation on elements level were indicated in blood (Pb), hair (Cr, Mn and Ni) and nails (Co, Cu, Fe, Ni and Pb). It is observed that many elements' levels varied in nails due to occupation and that nails are good indicator of occupational exposure to elements. Several studies also reported that occupation alters element levels in blood [7], urine [8] and hair [9].

3.1.3 FOOD HABIT

Food habit of Mysore subjects altered their element contents in blood (Cd and Pb), nails (Cd, Cr, Mn and Zn) and teeth (Zn) whereas, urinary and hair element levels were not affected. In support, influence of diet was observed in blood and urinary element (Cd) levels in Japanese general population in Japan [10] and non-vegetarian hair, nails and urine of Japanese women subjects [11]. From the present study it is observed that nails may be a good indicator of diet causing change in the element levels.

3.1.4 SOURCES OF DRINKING WATER

Similarly, the drinking water influenced the element levels of blood (Ni, Co, Fe and Zn), urine (Cd, Cr and Pb) and hair (Co and Fe) and thereby indicating that blood, urine and hair may be used to monitor the influence of drinking water on body element levels (Ni, Co, Fe, Cd and Pb). There are studies stating the influence of drinking water on element levels in blood [12] urine [13].

Table 1. Showing the elements observed at significantly higher levels in the various samples (between subgroups)

SN	Personal Characters	Sub groups	Place	Elements in various samples				
				Blood	Urine	Scalp hair	Fingernails	Tooth
1	Gender	Male	Rural					
			Urban	Cr				Pb
		Female	Rural					
			Urban		Ni, Pb	Co, Cu, Zn		
2	Occupation	Non-industrial	Rural					
			Urban					
		Industrial	Rural					
			Urban	Pb		Cr, Mn, Ni	Co, Cu, Fe, Ni, Pb	

3	Food habit	Vegetarian	Rural					
			Urban				Cd, Cr, Mn, Zn	Zn
		Non vegetarian	Rural	Cd, Pb				
			Urban					
4	Drinking water source	Corporation water	Rural					
			Urban					
		Bore well water	Rural	Ni		Co, Fe		
			Urban	Co, Fe, Zn	Cd, Cr, Pb			
5	Alcohol consumption	Teetotallers	Rural					
			Urban	Cu				
		Alcohol taker	Rural		Co, Fe, Mn, Ni, Pb, Zn			
			Urban	Cr				
6	Smoking habit	Non smoker	Rural					
			Urban					
		Smoker	Rural	Cd, Co, Fe, Pb			Cd, Pb	
			Urban	Pb			Cd, Pb	
7	Betel & nut chewing	Non chewers	Rural			Mn		
			Urban					
		Chewers	Rural				Co	
			Urban					
8	Blood sugar level	Non diabetes	Rural					
			Urban				Zn	
		Diabetes	Rural	Cu		Co, Fe		
			Urban		Zn		Cd, Cr, Cu, Mn, Ni, Pb	Pb
9	Insulin treatment	NIDDs	Rural			Cu		
			Urban				Cr, Cu, Zn	
		IDDs	Rural					
			Urban	Pb			Co	
10	Blood pressure level	Normotensives	Rural					
			Urban			Co, Fe, Pb		Cu
		Hypertensives	Rural					
			Urban				Cu	Pb
11	Economic status	Low income	Rural				Co, Fe	
			Urban					
		Medium income	Rural					
			Urban					Pb
		High income	Rural					
			Urban		Mn	Fe		
12	Cooking utensils used	Stainless steel	Rural					
			Urban					
		Mixed	Rural					Cu
			Urban					

13	Environment	Rural				Co, Fe	
		Urban	Cd, Co, Fe		Ni, Pb	Pb	Cr, Fe, Ni, Pb, Zn

Note: The elements, which were significantly higher, were mentioned. Subgroups with reference to use of cooking utensils were observed only in tooth samples.

3.1.5 ALCOHOL CONSUMPTION

Consumption of alcohol is another personal habit influencing the element levels only in blood (Cu and Cr) and urine (Co, Fe, Mn, Ni, Pb and Zn) but not in other biological matrices. Many studies also reported influence of alcoholism on As-B and Pb-B [14] and urinary element levels [15]. Out of nine elements measured, the variations in six elements levels (Co, Fe, Mn, Ni, Pb and Zn) were observed in urine, which reflected that urine, may be the best suitable indicator for studying the influence of alcoholism on body element levels.

3.1.6 SMOKING

Smoking habit is associated with change in element levels of blood (Cd, Co, Fe and Pb) and nails (Cd and Pb) while, teeth, urine and hair element levels did not differ between non-smokers and smokers. It is noted that the differences in Pb levels were observed in all two samples (blood and nails), which may be used for biomonitoring of elements among smokers. In support, [5] reported that among the blood, urine, hair and nails samples analysed, smoking habit affected Pb levels only in blood and hair of Mansoura residents.

3.1.7 CHEWING OF BETEL AND NUT

The habit of chewing betel and nut showed limited variations in element levels of two samples particularly, Mn level in hair and Co in nails. In the case of other samples (blood, urine and teeth) the element levels did not differ. So these samples may have restricted use for biomonitoring of elements with reference to betel and nut chewing habit.

3.1.8 DIABETES

Diabetes changed the element levels in all the samples. However, single element only altered in blood (Cu), teeth (Pb) and urine (Zn) and two elements in hair (Co and Fe). But, in the case of nails the highest number of seven elements (Cd, Cr, Cu, Mn, Ni Pb and Zn) were altered, which may reflect that nails are an ideal indicator for biomonitoring of multi elements among diabetic patients. In support, other studies reported that the levels of Zn, Mn and Cr were affected in blood, urine and scalp hair of diabetics from Pakistan [16].

3.1.9 INSULIN TREATMENT

Insulin treatment changed only one element levels in both blood (Pb) and hair (Cu) and three elements in fingernails (Cu, Cr and Zn) and thus it is evident that nails may be the suitable indicator of insulin influence on element levels.

3.1.10 HYPERTENSION

Influence of hypertension on element levels in teeth (Pb) nails (Cu) and hair (Co, Fe, and Pb) was found, whereas no difference in element levels was observed in other samples. There are studies showing the influence of hypertension on hair element levels for Cu, Mn, Cr and Zn [17].

3.1.11 ECONOMIC STATUS

The economic status of the Mysorians is also a factor affecting the levels of Pb-T, Mn-U, Fe-H, Co-FN and Fe-FN, while it was not a factor for blood elements. However, [6] observed that educational level, as a social status did not alter Se content in blood, urine and hair of subjects from Canada. Since the studies reporting influence of economic status on element levels are meagre, further studies are required to confirm the influence of economic status on element levels.

3.1.12 USE OF COOKING UTENSILS

Tooth sample donors were grouped in to two subgroups namely mixed utensil and stainless steel (SS) utensil users, based on the use of type of metal utensils. But for other sample donors, all the subjects belong to only one category, i.e. SS utensil users. Hence, the difference in element levels was observed only in between the two subgroups of tooth sample donors and significantly higher level of Cu was found in mixed utensil users than stainless steel users. It is reasoned that repeated use of utensils may result in the bioaccumulation of Cu via food and assumed that tooth could be an indicator of bioaccumulation of Cu via food from cooking utensils.

3.1.13 INFLUENCE OF ENVIRONMENT

All the samples except urine indicated rural and urban gradients of the element levels. Variations of three elements in both blood (Cd, Co and Fe) and nails (Co, Fe and Pb) and two elements in hair (Ni and Pb) reflected the environmental influence. Further, out of six elements measured in teeth, five elements (Cr, Fe, Ni, Pb and Zn) differed in their concentrations due to environmental exposure gradients. Therefore, it is opined that the tooth samples may be the suitable indicator of environmental element exposure. In support, [5] reported that environment influenced Pb level in blood, urine, hair and nails of subjects from Egypt.

3.2 OVERALL COMPARISON TO FIND THE BEST SAMPLE FOR BIOMONITORING

The elements, which differed significantly between the subgroups in each sample of the present study, were denoted in '+' mark and the number of '+' marks of various samples were compared in the table 2. It is observed from the table that whole blood sample may be suitable for indicating the exposure of subjects to elements from drinking water, smoking and environment. Similarly, the urine sample may be the good indicator to study the element source from alcoholism, while scalp hair may be useful to study the elemental difference due to gender and hypertension. Out of thirteen influencing factors studied, nails showed the element differences in nine influencing factors. Among them the influences of dietary habit, occupation, diabetes and treatment with insulin were well demonstrated. In the case of teeth, influence of smoking and environment were alone reflected. Further, it appears that teeth are the best indicator for studying elemental exposure of human beings from environment and that the levels of elements measured in the permanent teeth indicate the accumulation of elements incorporated at the different time from tooth formation to development as well as the current status [17].

From overall comparison, it is observed from the table 2 that the total number of '+' mark i.e. the sum total number of varied elements in all the influencing factors were higher in fingernails (29+) and it reveals that the fingernails are ideal, suitable and recommended indicator sample for biological monitoring of element status among general, occupationally exposed and ill-health subjects. In comparison to other biological samples, there are many advantages in utilizing nails as a biomarker of toxic element exposure and nutritional mineral status [18]. These include (1) the ease with which nails can be sampled, stored, transported, and handled [19]; (2) standardized methods are available for collection, washing, preparation, and sophisticated instrumental analysis with use of quality control samples for precision and accuracy [20]; (3) storage of aliquots of sample is simple for reanalysis; (4) once elements are incorporated into keratin of nails, levels remain isolated from other metabolic activities in the body with no fluctuation in element levels due to changing body metabolic activities, unlike the blood.

Table 2. Overall comparisons for suitability of samples for biomonitoring

SN	Influencing factors	Whole blood	Urine	Scalp hair	Fingernails	Tooth	Inference / Remark
1	Gender	+	+ +	+ + +		+	Scalp hair may be the suitable indicator for gender influence.
2	Food habit	+ +			+ + + +	+	Fingernails may be the indicator to study the influence of dietary habit.

3	Drinking water source	+ + + +	+ + +	+ +			Whole blood indicates the influence of drinking water.
4	Occupation	+		+ + +	+ + + + +		Fingernails may be an ideal indicator of occupational exposure.
5	Alcohol consumption	+ +	+ + + + + +				Urine is a good matrix to indicate influence of alcoholism.
6	Smoking habit	+ + + +			+ +		Whole blood is a suitable indicator of smoking influence.
7	Betel & nut chewing habit			+	+		Further study required to understand clearly.
8	Blood sugar level	+	+	+ +	+ + + + + + +	+	Fingernails may be the most suitable indicator to study the influence of diabetes.
9	Blood pressure level			+ + +	+	+ +	Scalp hair may be considered for biomonitoring among hypertensives.
10	Economic status		+	+	+ +	+	Further study required to ascertain the influence of economic status.
11	Insulin treatment	+		+	+ + ++		Fingernails may be used for studying the influence of insulin impact.
12	Cooking utensils used					+	Further study required to confirm the influence of cooking utensils
13	Environment	+ + +		+ +	+ + +	+ + + + +	Teeth can be the ideal indicator for environmental exposure.
	TOTAL '+'	**19**	**13**	**18**	**29**	**12**	Fingernails may be the best biomarker for biomonitoring among general populations.

Note; '+' mark is to indicate the element levels observed significantly different in the sample for the factors.

4 CONCLUSIONS

As the total number of varied elements in all the influencing factors was higher in fingernails (29+), it may be concluded that the fingernails are ideal, suitable and recommended indicator sample for biological monitoring of element status among general, occupationally exposed and ill-health subjects. Further, it is suggested that the biosample use for assessment of human exposure to elements could be adopted in the following order of preference, fingernail, whole blood, scalp hair, urine and teeth.

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REFERENCES

[1] K. M. Hambidge, M. L. Franklin and M. A. Jacobs, "Hair chromium concentrations: effects of sample washing and external environment," *American Journal of Clinical Nutrition*, vol. 25, pp. 384-389 1972.

[2] S. Caroli, A. Alimonti, E. Coni, F. Petrucci, N. Senofonte and N. Violante, "The assessment of reference values of elements in human biological tissues and fluids: a systematic review," *Critical Review of Analytical Chemistry*, vol. 24, pp. 363-398, 1994.
[3] A. Sukumar, "Human nails as a biomarker of element exposure," *Review of Environmental Contamination Toxicology*, vol. 185, pp. 141-177, 2006.
[4] R. M. Parr, R. Schelenz and S. Bellestra, "IAEA Biological reference Materials," *Proceedings of the third International Symposium on Biological reference Materials," Bayreuch FRG*,1988.
[5] W. I. Mortada, M. A. Sobh, M. M. El-Defrawy and S. E. Farahat, "Reference intervals of cadmium, lead, and mercury in blood, urine, hair, and nails among residents in Mansoura City, Nile delta, Egypt," *Environmental Research*, vol. 90, no. 2, pp. 104-110, 2002.
[6] M. Lemire, D. Mergler, G. Huel, C. J. Passos, M. Fillion, A. Philibert, J. R. Guimaraes, I. Rheault, J. Borduas, G. Normand, "Biomarkers of selenium status in the Amazonian context: blood, urine and sequential hair segments," *Journal of Exposure Science and Environmental Epidemiology*, vol. 19, no. 2, pp. 213-222, 2009.
[7] D. Backovic, J. Marinkovic, J. Jorga, M. Pavlica, Z. Maksimovic and M. Nikolic, "Environmental factors, health-related habits, and serum selenium levels in cancer patients and healthy controls," *Biological Trace Element Research*, vol. 67, no. 1, pp. 55-62, 1999.
[8] D. G. Ellingsen, L. Dubeikovskaya, K. Dahl, M. Chashchin, V. Chashchin, E. Zibarev and Y. Thomassen, "Air exposure assessment and biological monitoring of manganese and other major welding fume components in welders," *Journal of Environmental Monitoring*, vol. 8, no. 10, pp. 1078-1086, 2006.
[9] M. Wilhelm, D. Hafner, Q. Lombeck and F. K. Ohnesorge, "Variables influencing cadmium concentrations in hair of pre-school children living in different areas of the Federal Republic of Germany," *International Archive of Occupational and Environmental Health*, vol. 60, pp. 43-50, 1988.
[10] M. Ikeda, C. S. Moon, Z. W. Zhang, H. Iguchi, T. Watanabe, O. Iwami, Y. Imai and S. Shimbo, "Urinary alpha1-microglobulin, beta2-microglobulin, and retinol-binding protein levels in general populations in Japan with references to cadmium in urine, blood, and 24-hour food duplicates," *Environmental Research*, vol. 70, no. 1, pp. 35-46,1995.
[11] T. Ohno, M. Sakamoto, T. Kurosawa, M. Dakeishi, T. Iwata and K. Murata, "Total mercury levels in hair, toenail and urine among women free from occupational exposure and their relations to renal tubular function," *Environmental Research*, vol. 103, no. 2, pp. 191-197, 2007.
[12] A. R. Sharrett, "Water hardness and cardiovascular disease: Elements in water and human tissues," *Science of Total Environment*, vol. 7, no. 3, pp. 217-226, 1977.
[13] M. Ikeda, T. Watanabe, F. Ohashi and S. Shimbo, "Effects of variations in cadmium and lead levels in river sediments on local foods and body burden of local residents in non-polluted areas in Japan," *Biological Trace Element Research*, 2009, (Online).
[14] J. Kristiansen, J. M. Christensen, B. S. Iversen and E. Sabbioni, "Toxic trace element reference levels in blood and urine: influence of gender and lifestyle factors," *Science of Total Environment*, vol. 26, no. 204(2), pp. 147-160, 1997.
[15] E. Rodriquez and C. Diaz, "Iron, copper and zinc levels in urine: relationship to various individual factors," *Journal of Trace Element in Medicine and Biology*, vol. 9, no. 4, pp. 200-209, 1995.
[16] T. G. Kazi, H. L. Afridi, N. Kazi, M. K. Jamali, M. B. Arain, N. Jalbani and G. A. Kandhro, "Copper, chromium, manganese, iron, nickel, and zinc levels in biological samples of diabetes mellitus patients," *Biological Trace Element Research*, vol. 122, no. 1, pp. 1-18, 2008.
[17] A. Sukumar and R. Subramanian, "Characterisation of human teeth for trace elements," *Proceedings of Second National Workshop on development and use of reference materials*, Central Pollution Control Board, New Delhi, pp: 86-88, 1999.
[18] K. L. Chen, C. J. Amarasiriwardena and D. C. Christiani, "Determination of total arsenic concentrations in nails by inductively coupled plasma mass spectrometry," *Biological Trace Element Research*, vol. 67, no. 2, pp. 109-125, 1999.
[19] C. R. Daniel, B. M. Piraccini and A. Tosti, "The nail and hair in forensic science," *Journal of American Academy of Dermatology*, vol. 50, no. 2, pp. 258-261, 2004.
[20] M. Bergomi, M. Vinceti, G. Nacci, K. V. Pietrk, P. Bratter, D. Alber, A. Ferrari, L. Vescovi, D. Guidetti, P. Sola, S. Mlagu, C. Aramini and G. Vivoli, "Environmental exposure to trace elements and risk of amyotrophic lateral sclerosis: a population-based case-control study," *Environmental Research*, vol. 89, no. 2, pp. 116-123, 2002.