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SHORT COMMUNICATION

COMPARISON OF SOME ESSENTIAL AND HEAVY METALS IN THE TOENAILS AND FINGERNAILS OF SCHOOL-AGE CHILDREN IN KENYA

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ABSTRACT. This paper describes the determination of the levels of lead (Pb), cadmium (Cd), zinc (Zn), calcium (Ca) and iron (Fe) in the toenails and fingernails of children under the age of six years in urban and rural areas in Kenya by atomic absorption spectrophotometer. Lead levels in urban areas ranged from 8.0-49.0 μg/g in fingernails and 7.0-62.0 μg/g in toenails as compared to those in rural areas (5.0-36.5 μg/g and 5.5-31.5 μg/g, respectively). A similar trend was observed for Cd where significantly high levels were found in children in urban areas than those in rural areas. The Fe and Zn levels were significantly higher in children in rural areas than those in the urban areas. The levels of all the metals studied were higher in the toenails except for Fe and Ca where the levels were higher in fingernails; however, the difference in the levels was not significant. These results indicate that either the toenails or fingernails can be used as a reference for levels of metals environmental exposure.

KEY WORDS: Children, Essential elements, Heavy metals, Fingernails, Toenails, Kenya

INTRODUCTION

Nail tissue as a biological indicator of metals environmental exposure has received a great deal of attention in the literature and many researchers have established the correlations that exist between toxic and essential metals concentration in the body and human nails [1-3]. Human nails have been used in some clinical laboratories to determine metabolic disorders, metal environmental exposure and nutritional status [1, 2, 4, 5].

Nail growth in human is a continuous process throughout life, about 0.05-1.2 mm per week, with the toenails growing at a slower rate of 30-50 % and thus provide a longer integration period for the metals [6, 7]. Nail tissue is rich in fibrous proteins that contain α-keratins as cysteine residues [7]. Their roots are highly influenced by health status of the cells [6, 7], whereas blood and other body fluids give transient concentrations, human nails provide a continuous record of element concentration [3, 7]. Further, blood and other body fluids are not suitable to analyse levels of Cd because the metal exists briefly in the medium [3]. Moreover, nails are easier to sample, transport and store since they do not require any external conditions unlike body fluids that are prone to contaminations [6]. Analysis is economical which makes it a more attractive screening and diagnostic tool in developing countries.

According to various studies, anyone could be poisoned by toxic metals but the most risk group are children under the age of six due to their not fully developed central nervous system and other organs, having more hand to mouth activities, untimely outdoor activities, not fully developed hygienic habits and active metabolism [7-9]. Studies have found that nutritional deficiencies of Fe, Ca and Zn, which are very prevalent in children in developing countries, may facilitate Pb and Cd absorption and exacerbate their toxic effects [8, 9]. Early brain growth has been described as a critical period in the development of the central nervous system of the child and the environmental factors during this stage have a long-term effect on health of the child [8].

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Area description. Nairobi, cover an area of 700 km^2 with a population of over 2.1 million [10]. Nationally it is established to have the greatest concentrations of industrial and vehicular air pollution sources [11]. It is reputed to be the fastest growing city of the world and lacks air quality management system [12]. Indeed among the developing countries cities that were sampled for the study on air quality management capabilities, Nairobi was rated the worst [13]. The city is now regarded as a "hot zone" with highest concentration of pollutants which has been influenced by increasing industries, population, construction, heavy traffic density and deforestation of city fringes [12]. In rural setting, Bungoma is situated in western part of Kenya with a population of 0.8 million and covering an area of 2069 km^2 [10]. The schools in this region are located in agricultural region, in which sugar cane (*Saccarum spp*) has been a predominant crop. According to some reports sugar cane production requires considerable amount of fertilizers and pesticides [14]. Therefore it was the aim of this study to compare levels of heavy metals and essential metals in the toenail and fingernail samples of children under the age of six years in different environmental settings to find out whether they accumulate metals differently.

EXPERIMENTAL

For the ethical and legal reasons, the participation of the children in the study was voluntary and appropriate permits and approvals were obtained prior to the survey. Having been granted, approvals and permits, field visits and sampling began in September 2006 and ended in April 2007. Samples were obtained from Bungoma and Nairobi.

Equipment and reagents. Atomic Absorption Spectrophotometer (AAS-Spectr AA-10, Varian-Techron, Austria) was used. Water used throughout the analytical procedures was deionised and distilled. Weighing balance used was Mettler Toledo AG-240. Digester block was 2080/DA, No 935, Volt 220-w Germany, -LIEBISCH BIELEFELD 14. Reagents used in the analysis were of high quality analytical grade. Liquid soap-an Izal product, nitric acid AR, acetone, 4-methyl pentan-2-one and perchloric acid were supplied by Hopkin and Williams, England. The plastic bottles were cleaned with non-ionic liquid soap rinsed with distilled water. They were then soaked overnight in 1:1 nitric acid and rinsed thoroughly with deionised and distilled water. All the glasswares used in this study were decontaminated by soaking them overnight in 5 % HNO_3 and rinsed thoroughly in deionised and distilled water. They were oven dried at 105 ^oC and stored safely. The metal standards from the stock solution (1000 µg/mL) were freshly prepared daily by serial dilution and checked for constancy of the results before taking the readings.

Sampling. Fifty (n = 50) children under the age of six years were randomly recruited with informed consent from their parents/guardians. Purposive sampling strategy was used to select schools in both urban and rural settings. The major criterion for selection of schools in urban areas was influenced by the intensity of pollution. The schools in urban area, Nairobi, were randomly selected and included a school near a highway, city centre and industrial area. In rural setting, two schools that were selected were far from the urban influence. Ten subjects were randomly recruited from each school.

In view of high prevalence of bacterial and fungi infections, each subject was given a labelled stainless steel nail clippers and a towel. All the nails of the subjects were cleaned using surgical spirit followed by non-ionic detergent. They were rinsed with water then dried with a clean towel. In order to minimize secondary contamination with metallic elements the stainless steel nail clippers were washed with analytical reagent grade HCl, diluted at 1:10 then rinsed with distilled water. Seventeen (n = 17) subjects were excluded from this study as either they had nail infections or were unable to get sufficient samples for analysis. Toenails and fingernails

were clipped from the same subject every two weeks for a period of seven months. The samples (n = 66) were kept in a labelled plastic bottle securely closed, until they were analysed.

Laboratory procedure and quality assurance. Analysis of nail samples was carried out using standard methods as reported elsewhere [4, 15].

Sample pre-treatment. Meticulous care was taken to avoid external contamination of samples during analytical procedure. The nail samples were soaked in non-ionic liquid soap in a glass beaker for two hours and washed free from metallic debris following a standardized washing procedure [4, 15]. They were subsequently soaked in acetone for one hour and rinsed five times in deionised and distilled water. The samples were kept in vial tubes, and oven dried at 60 ^{o}C to a constant weight. The polished nail samples were placed into beaker to which 10 mL of 4-methyl pentan-2-one was added and left for 45 min. They were rinsed three times using 4-methyl pentan-2-one before oven drying. The samples were weighed in triplicates and kept in the desiccators.

Acid digestion and AAS analysis. The weighed dry nail samples were transferred into the digesting tubes. Ten mL of 6:1 mixture of concentrated HNO_3 and perchloric acid was added to the samples and kept overnight at room temperature to prevent excessive foaming. The digesting tubes were closed with aluminium foil and placed on the digester block in a fume chamber and subsequently heated at 180 ^{o}C [15]. The samples were digested slowly for about one hour until all nails samples dissolved, leaving a clear solution (complete decomposition of organic matter). The digested sample solution was diluted with 0.1 M HNO_3 in a 25 mL volumetric flask and made up to the mark using deionised and distilled water. The concentration of trace metals was assayed by use of AAS in triplicates. Detection limits of the instrument used for the metals analysis of Pb, Cd, Zn, Fe and Ca were 0.0020, 0.0006, 0.0020, 0.0050 and 0.0210 µg/mL, respectively. A series of standards from stock solution were prepared using deionised and distilled water for instrumental calibration. Before each analysis the instrument was zeroed using the blank prepared by following the acid digestion procedure but without the sample. For quality control, standards were analysed for every five samples analysed. The main instrumental parameter such as bandwidth, lamp current, height of the flame, and wavelength for AAS were optimized separately for each metal. Adequate quality control was ensured by inter-laboratory comparisons of representative samples carried out at Kenyatta University Research Laboratory and Mines and Geology Analytical Research Department, Nairobi. The validity of method was further ascertained by cross-method checks, spiked recovery and replication analysis.

Data analysis. Statistical calculations were done using statistical SPSS program (Statistical Package for Social Sciences Version 11.5).

RESULTS AND DISCUSSION

The levels of metals were compared in the toenail and fingernail samples of children (n = 33) in both rural and urban areas using t-test at p = 0.05 (Table 1 and Table 2).

A higher Pb level of 26.9 ± 7.6 µg/g was obtained in toenail samples compared to those of fingernail samples in urban areas (24.7 ± 6.6 µg/g), similarly a mean level of 20.0 ± 4.1 µg/g Pb was obtained in toenails as compared to those of fingernails samples (19.1 ± 3.2 µg/g) in rural areas (Table 1). Similar observations were made for most of the metals studied which indicated elevated level in the toenails than in fingernail samples in both urban and rural children. However, few exceptions were made for Ca and Fe, where levels were higher in the fingernail samples (103.5 ± 20.5 µg/g Ca in urban areas and 112.6 ± 19.7 µg/g Fe in rural areas) than those

of the toenail samples (97.9 ± 19.5 µg/g Ca and 99.4 ± 15.0 µg/g Fe, respectively). Comparing the levels of the metals in fingernail and toenail samples in both rural and urban areas using t-test (p = 0.05), there was no significance difference in the levels.

Table 1. Comparison of metal levels (µg/g ± se) in fingernail and toenail samples of children in urban and rural areas.

Sampling area	Metals	Fingernail range	Fingernail mean	Toenail range	Toenail mean
Urban areas (n = 21)	Pb	8.0-49.0	24.7 ± 6.6	7.0-62.0	26.9 ± 7.6
	Cd	0.0-2.45	0.614 ± 0.15	0.1-3.20	0.790 ± 0.17
	Fe	42.5-108.0	67.4 ± 6.3	36.5-132.0	72.0 ± 7.7
	Zn	53.8-134.0	85.4 ± 8.4	47.3-184.0	86.5 ± 19.5
	Ca	42.0-344.5	103.5 ± 20.5	37.5-342.5	97.9 ± 19.5
Rural areas (n = 12)	Pb	5.0-36.5	19.1 ± 3.2	5.5-31.5	20.0 ± 4.1
	Cd	0.10-0.95	0.372 ± 0.13	0.0-1.10	0.385 ± 0.16
	Fe	40.0-162.0	112.6 ± 11.7	35.0-281.0	99.4 ± 15.0
	Zn	70.2-173.0	104.4 ± 8.0	61.0-234.3	119.6 ± 19.7
	Ca	35.0-158.0	70.2 ± 8.8	24.5-135	76.4 ± 9.8

Table 2. Comparison of metal levels (µg/g ± se) in fingernail and toenails samples of urban and rural areas.

	Metals	Urban areas (n = 21)	Rural areas (n = 12)
Mean fingernail samples	Pb	24.7 ± 6.6	19.1 ± 3.2
	Cd	0.614 ± 0.15	0.372 ± 0.13
	Fe	67.4 ± 6.3	112.6 ± 11.7
	Zn	85.4 ± 8.4	104.4 ± 8.0
	Ca	103.5 ± 20.5	70.2 ± 8.8
Mean toenail samples	Pb	26.9 ± 7.6	20.0 ± 4.1
	Cd	0.790 ± 0.17	0.385 ± 0.16
	Fe	72.0 ± 7.7	99.4 ± 15.0
	Zn	86.5 ± 19.5	119.6 ± 19.7
	Ca	97.9 ± 19.5	76.4 ± 9.8

The results of this study are inconsistent with other published reports that found significantly high levels of metals in the fingernails than toenails samples, probably due to toenails being affected less by exogenous contaminations [6, 7]. However, in this study both the toenails and fingernails samples of children seem to have been exposed to similar exogenous contaminations. For instance children do not engage in rigorous household chores therefore their fingernails may not be exposed to high concentrations of external contaminations. It was observed that most of the children in rural areas in this study either walked to school without shoes or wore open shoes. Observations were also made that the children in urban areas in this study played without shoes. This implies that external exposure from the environment could affect both their toenails and fingernails in a comparable manner. Therefore the toenails and fingernails of children in this study were probably exposed to similar exogenous contamination from the environment. In addition, the sensitivity of the children to toxic metals has been strongly linked to their distinct behaviour while playing [8].Metal levels were compared in both rural and urban areas in order find out whether fingernails or toenails as biological indicators accumulated metals differently. Cadmium levels were found to be higher in both the toenails (0.790 ± 0.17 µg/g) and fingernails samples (0.614 ± 0.15 µg/g) among children in urban areas as compared to those in the toenail samples (0.385 ± 0.16 µg/g) and fingernail (0.372 ± 0.13 µg/g) in rural areas. A similar trend was observed for Pb, where the levels were higher in both the toenails and fingernails samples in urban areas than those in rural areas, respectively. The t-test results (p = 0.05) showed that the

difference in heavy metals levels was significant. This could be attributed to the population of urban areas living closer to the sources of pollution. It is fairly well documented that nationally, Nairobi is established to have the greatest concentration of pollution [11, 16].

Another report by the UNEP revealed that average soil Pb in the Central Business District of Nairobi are higher than those in the nearby rural areas by a multiple of 95 [16]. Substantial amount of Cd in the industries has been attributed to production of steel, batteries and cement [17]. This finding concurs with an established report that industrial area in Nairobi was among the highly polluted site in the city [18]. The results agrees with the published report of [19] and [20] who revealed that children in the industrial areas and inner cities have high levels of Cd and such sites were considered as "hot pots" as they were known to use antiquated technology, where pollution control devices were inadequate or even non-existent. It was further established that Pb in the inner city soils exhibited a distance decay characteristic of high level in the inner city which decreased towards the outer part of the city [8]. These results agree with those obtained elsewhere [21], where levels of heavy metals were higher in the fingernail samples in urban areas than those obtained in rural areas [8, 20]. This suggests that Pb and Cd concentrations in this study could be influenced by the environmental factors.

The concentrations of essential metals were found to be lower in urban areas in both the fingernail (67.4 ± 6.3 μg/g Fe and 85.43 ± 8.4 μg/g Zn) and toenail samples (72.0 ± 7.7 μg/g Fe and 86.5 ± 9.5 μg/g Zn) than those of rural areas (112.6 ± 11.7 μg/g Fe and 104.4 ± 8.0 μg/g Zn in fingernails) and toenail samples (99.41 ± 15.0 μg/g Fe and 119.6 ± 19.7 g/g Zn). The t-test results ($p = 0.05$) showed that the difference in the essential metals levels was significant. However, the level of Ca was higher for urban population than rural but the difference in the levels was not significant. These results agree with those obtained elsewhere, [21] where levels of Fe, and Zn were lower in the fingernail samples in urban areas than those obtained in rural areas [1, 9]. The significant high levels of toxic metal and low levels of essential metals in fingernail samples of children in urban areas than those in rural areas could probably be due to influence of heavy metals exposures and nutritional factors caused by different environmental settings. These results are in agreement with those of [9] and are supported by those of [1] who found that low dietary levels in Fe, Zn, Ca and phosphorus increased the absorption of toxic metals from the gastrointestinal tract. It is therefore clear from these observations that children who are malnourished with low levels of body Fe stores and marginal Zn could be at a greatest risk of exposure of heavy metals.

Elemental concentrations in biological samples in the literature were found to vary considerably due to geographical differences, nutritional status and the method of analysis [7, 22]. It is hard to establish reference ranges because of above factors as they impose restrictions on the interpretations of the results. This therefore complicates the comparison of the results obtained in this study and those that were conducted elsewhere [21]. However, our study results on heavy metal levels were compared with some results obtained from the other study in the literature with a comparable background. The levels of heavy metals in our study group in toenail samples in urban areas (26.9 ± 7.6 μg/g Pb and 0.790 ± 0.17 μg/g Cd) is significantly higher than those of 8.5 μg/g Pb and 0.457 μg/g Cd obtained by [23] in the toenail samples of children (aged 3-7 years) living in the industrialized areas using AAS for analysis. The results indicate that our environment is highly polluted including the rural areas than elsewhere [7, 21]. Therefore more studies are required to establish the extent of pollution.

CONCLUSIONS

From these results it can be concluded that both the toenails and fingernails give comparable results as biological reference material in determination of metal levels in the environment.

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