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Environmental exposures of trace elements assessed using keratinized matrices from patients with chronic kidney diseases of uncertain etiology (CKDu) in Sri Lanka

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ABSTRACT

An alarming increase in chronic kidney disease with unknown etiology (CKDu) has recently been reported in several provinces in Sri Lanka and chronic exposures to toxic trace elements were blamed for the etiology of this disease. Keratinized matrices such as hair and nails were investigated to determine the possible link between CKDu and toxic element exposures. Elements Li, B, Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Ba, Hg and Pb of hair and nails of patients and age that matched healthy controls were determined with Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The results showed that trace element contents in the hair of patients varies in the order of Zn > Fe > Al > Mn > Cu > Ba > Sr > Ni > Pb > Cr > B > Hg > Se > Mo > Co > As > Li > Cd while Fe > Al > Zn > Ni > Cu > Mn > Cr > Ba > Sr > B > Pb > Se > Mo > Co > Hg > Li > As > Cd in nail samples. The hair As levels of 0.007–0.165 $\mu g\,g^{-1}$ were found in CKDu subjects. However, no significant difference was observed between cases and controls. The total Se content in hair of CKDu subjects ranged from 0.043 to 0.513 $\mu g\,g^{-1}$ while it was varied from 0.031 to 1.15 $\mu g\,g^{-1}$ in controls. Selenium in nail samples varied from 0.037 $\mu g\,g^{-1}$ to 4.10 $\mu g\,g^{-1}$ in CKDu subjects and from 0.042 $\mu g\,g^{-1}$ to 2.19 $\mu g\,g^{-1}$ in controls. This study implies that substantial proportions of Sri Lankan population are Se deficient irrespective of gender, age and occupational exposure. Although some cutaneous manifestations were observed in patient subjects, chemical analyses of hair and nails indicated that patients were not exposed to toxic levels of arsenic or the other studied toxic elements. Therefore the early suggested causative factors such as exposure to environmental As and Cd, can be ruled out.

1. Introduction

Trace elements play a significant role in the human physiological system according to their essentiality to the body. Being parts of metalloenzymes, some trace elements actively participate in the structural organization of macromolecules, oxygen metabolism, scavenging of free radicals and hormonal activities [1]. Even elements that are considered as being truly beneficial to human health may also cause detrimental health effects, if ingested in excess doses. On the other hand, the human body can tolerate toxic elements up to a certain dosage although these elements are not involved in any biochemical processes [2]. In the meantime, the available concentrations of such elements in biological samples reflect the nutritional status, environmental and occupational exposure and possible causes for existing adverse health effects. Therefore monitoring of trace element intake and its accumulation in the human body is extremely important for exposure assessments and bio-monitoring [3–5]. In recent years, monitoring of trace elements in environmental samples and biological specimens has gained wider attention with the emergence of certain chronic diseases associated with trace element exposures such as kidney diseases [6,7], neurological disorders [8,9], and cancer [10,11]. Different biomarkers with some target molecules or metabolically inactive tissues are used widely for bio-monitoring. From among human tissues that are used commonly as biomarkers such as blood, urine etc., the determination of trace elements

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and environmental toxins in hair and nails provide several advantages [4,12]. Blood and urine are preferable sources for recent heavy metal exposures whereas hair and nails signifies long term exposure [13,14]. Although there are some drawbacks such as potential external contamination, subject intrinsic factors (age, habits, life style etc.) and problems with elaborating reference values, hair and nails are used widely as biomarkers to detect trace element exposure since they express a permanent record of trace elements that correlate to both normal and abnormal metabolism and environmental assimilations [15]. Particularly, the use of hair as a biomarker in environmental screening has been recommended by the World Health Organization (WHO), Environmental Protection Agency (EPA) and the International Atomic Energy Agency (IAEA) [10,16] Furthermore, they are metabolically inactive non-invasive matrices which can be collected easily without special materials or health personnel. In addition, they are stable, easy to handle, transport and store [17–20]. Human hair is considered as one of the exposure indicators of essential and toxic trace elements as its follicles absorb trace elements circulating in the blood during its growth [3,21]. Such samples also provide a historical record of the exposure episodes and hence can be used for forensic investigations and xenobiotic drug exposure assessments [22]. Keratin is the main constitution of hair and cysteine thiol groups of keratin has the affinity to bind various elements [23]. Hence such materials can record changes of trace element concentrations in the body over a long period of time [14]. Therefore information provided by hair is more important in identifying nutritional status, clinical diagnosis, forensic studies and bio-environmental investigations [24,25]. Similarly, nails are also considered as biomarkers since the nail keratin traps and immobilize trace elements. The amount trapped in nails signifies the exposure to such elements over the time of its formation. Diseases and changes in the chemical balance of the body determine the structure, irregularities, strength, shape and color of the nail [26]. Availability of trace elements in hair and nails also signifies occupational exposure over a period of 2–5 months and 12–18 months [27] thus making them better sources to identify concealed causes for prevailing health issues.

In the last two decades, there has been a wider interest in the alarming increase of chronic kidney disease (CKD) in several tropical regions such as Sri Lanka, India, El Salvador and Nicaragua [28–31]. The etiology of these emerging nephropathies is not related to any known risk factors such as diabetes mellitus, chronic or severe hypertension, snake bite, glomerulonephritis and urological diseases. Hence the disease is described as Chronic Kidney Disease of Unknown etiology (CKDu), which is most common among low socioeconomic farming communities. The prevalence of the disease in Sri Lanka is restricted to certain parts of the country where about 13% populations are effected [32]. The histopathological investigations indicated that the disease is tubulointerstitial which may be caused by an involvement of one or more nephrotoxic agents [33]. The prevalence of CKDu is shifting towards the younger generation creating a massive negative impact on the sociological wellbeing of the country. As a whole, it affects the economy of Sri Lanka since end stage patients have to be managed with renal dialysis and kidney transplantation. The pathophysiological etiologies on CKDu remain elusive and controversial, but both genetic and environmental factors have received significant attention [34]. Since the disease is confined to a specific geographical region of Sri Lanka, most early studies have hypothesized environment – related risk factors particularly the involvement of toxic trace elements such as aluminum [35], cadmium [32,36] and arsenic [37]. The environmental exposures to agrogenic cadmium and arsenic have been widely suspected as risk factors for the pathogenesis of CKDu [32,36,37]. Jayasumana et al., [37] observed dermatological manifestations in CKDu patients and argued that the chronic arsenic exposure is the main etiological factor for this disease.

Since elevated levels of trace elements are suspected as an etiological candidate of CKDu, we hypothesized that hair and nail analysis will perhaps provide better information on variable environmental exposures. In this study, we investigated the levels of trace elements in hair and nails in both CKDu patients and healthy controls. Trace element levels of hair and nails in this study were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) which can be effectively and reliably be used in ultra-trace levels of elements in clinical materials. Simultaneously, we investigated the cutaneous manifestations of CKDu patients and observed any changes on the skin due to chronic arsenic exposure. To the best of our knowledge, this is the most detailed study concerning the trace element contents in hair and nails in Sri Lankan population.

2. Materials and methods

Ethical approval for this study was obtained from the Ethical Review Committee of the Faculty of Medicine, University of Peradeniya while written consent was obtained from all participants. Samples of hair (77) and nails (76) were collected from biopsy proven CKDu patients who attended the Renal Care Clinic of the Girandurukotte Base Hospital (7.46684°N; 81.0507°E) from which 41 samples were obtained from male patients. Control samples were collected from age matched volunteer individuals from the Kandy region (7.29067°N; 80.634°E) where CKDu is considered as non-endemic [28]. All patients and controls were carefully examined by a consultant dermatologist (second author) for any skin manifestation. Usually chronic arsenic exposures are associated with cutaneous changes such as hyperpigmentation, hyperketosis, premalignant skin lesions and non-melanoma skin cancers [38]. General information on education, age, sex, occupational history, source of drinking water, agrochemical usage, types of agrochemical, exposure to agrochemicals, habits and medical history were gathered using a structured survey questionnaire. About 10 g of hair were clipped close to the scalp from the occipital area using a pair of sterilized ceramic scissors while the fingernails were collected using a stainless steel nail clipper. Both hair and nails were stored immediately in zip lock polyethylene bags. In the laboratory, all samples were washed following the washing procedure recommended by the International Atomic Energy Agency (IAEA) [15,39,40] in which hair samples were washed few times with deionized water and acetone before cutting into small pieces. Then pieces were thoroughly rinsed several times with deionized water ($<0.055\ \mu S\ cm^{-1}$), followed by washing again with ultra-pure acetone ($\geq$99.5%; Sigma-Aldrich, Germany). Finger nail samples were sonicated for 15 min in acetone then washed several times with deionized water. The cleaned samples were dried at 55 °C overnight and stored in a desiccator.

0.10 g of subsamples were digested with 4.5 mL of HNO_3 ($\geq$69.0% TraceSelect; Fluka, Switzerland) and 0.5 mL of H_2O_2 (35 wt.%; Sigma-Aldrich, Germany) using Mars-6 microwave digester (CEM; Matthews, NC) equipped with EasyPrep-Plus high pressure vessels. Final solutions were diluted to 25 mL using deionized water ($<0.055\ \mu Scm^{-1}$) obtained from Thermo Scientific Smart2Pure system. Samples were stored in high density polyethylene bottles at 4 °C till the analyses were performed. All samples were filtered through 0.45 μm membrane filters before performing the analyses. Concentration of trace elements ^{7}Li, ^{11}B, ^{27}Al, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{75}As, ^{78}Se, ^{88}Sr, ^{95}Mo, ^{111}Cd, ^{138}Ba, ^{202}Hg and ^{208}Pb were determined by Thermo ICapQ (Thermo-Fisher Scientific Inc., Bremen, Germany) Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The instrument was optimized with a solution containing Ba, Bi, Ce, Co, In, Li and U for sensitivity, resolution and mass calibration. Polyatomic isobaric interferences were mitigated with the