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Trace Elements in Nails as Biomarkers in Clinical Research

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Abstract

Background—The importance of trace elements in relation to human health has been increasingly recognized. Accurate and adequate quantification of trace elements are crucial in clinical research.

Design—This review was to discuss the rationale of using nail trace elements as biomarkers in clinical studies.

Results—For most trace elements, dietary instruments can not appropriately capture the intakes because of the minimal amounts and wide variations in the same foods grown in different area as well as the non-dietary exposures. Therefore, biomarkers may be essential in studying trace elements. Although there are notable differences among trace elements in the availability of biomarkers, increasing evidence supports that nail particularly toenail concentrations of most trace elements are useful biomarkers of exposure in which a single sample is assumed to represent long-term exposure.

Conclusions—As compared to other potential biomarkers of trace elements, nail measurement has certain advantages in clinical research.

INTRODUCTION

The importance of trace elements in relation to human health has been increasingly recognized. Trace elements have nutritional benefits as essential cofactors for physiologic processes, but some can be toxic to human health. Accumulated evidence suggests that deficiency or excess of certain trace elements may be associated with risk of chronic diseases including cardiovascular diseases, diabetes and cancer.

Accurate and adequate quantification of trace elements are crucial in clinical research. For most trace elements, dietary instruments such as food frequency questionnaire and 24-hour recall can not appropriately capture the intakes because of the minimal amounts and wide variations in the same foods grown in different area. Also, trace element exposures in humans represent both dietary and non-dietary exposure. Thus, biomarkers of trace elements are preferred over other types of measurements in clinical study both as a measure of intake and as a means to validate other forms of exposure assessment.[1]

The availability of biomarkers among trace elements is largely different. Most previous studies assessed trace elements in blood, hair, nail or urine specimens, and for some heavy metals, in bone as well. To date, no single source of measurement is the best across all trace

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elements. Therefore, it is important to understand the strength and weakness of a measurement for trace elements and utilize it appropriately in clinical research.

RATIONALES OF USING NAIL SPECIMENS TO MEASURE TRACE ELEMENT STATUS

Human nails are largely constituted of keratin-rich proteins, which incorporate trace elements in proportion to their dietary intakes and other exposures by various mechanisms including protein synthesis and chemical binding with sulfhydryl groups. Consequently, finger- and toe-nails are useful markers for trace elements and are employed in clinical studies with increasing frequency. For many hypotheses involving trace elements, either as a required dietary nutrient (e.g. Selenium [Se]) or a toxic substance (e.g. Arsenic [As]), the nail specimen is often prospectively collected and stored for months or years prior to being retrieved and analyzed in epidemiological studies relating the trace element concentrations to one or more clinical endpoints.[2, 3]

Although there are notable differences among trace elements in the availability of biomarkers, trace elements in nails provide a time-integrated measure of body intake, especially, toenail clippings may reflect a long exposure time frame given the relatively slow growth rate.[4–6] A recent study found that the average growth rate among American young adults was 3.47 mm/month for fingernails and 1.62 mm/month for toenails, respectively.[7] Of note, nail growth rate may vary depending on age, gender, health conditions, metabolic rate and some other factors such as onychophagia. In addition, a study using data from a human trial indicates that toenail Se level provides a measure of Se intake integrated up to 52 weeks.[8] While most body tissues are in a state of flux, nails are a notable exception. After the nail is formed it is expelled from the nail bed to become isolated from the body's continuing metabolic activities. Thus, nail clippings represent body intake or exposure during the past a few months or a year; one mm of nail sample corresponds to roughly one month of body nutritional status.

Nails particularly toenails are relatively sheltered from environmental contaminants. They are free from or less likely to have contaminants introduced through shampooing, hair treatments, and medication. As for nail polish, the chemicals introduced by polishing can be largely washed out in the laboratory, and the element contents in nails are less likely to be affected. For instance, any external contamination in most nail specimens can be removed using ultrasonic cleaning protocols with both polar and non-polar solvents.

Studies also examined the reliability of toenail measurement.[9, 10] Although attenuation in measures of association may occur, the toenail concentrations of most trace elements are suggested to be useful biomarkers of exposure in which a single sample is assumed to represent long-term exposure.[9]

ABILITY OF NAIL TRACE ELEMENT TO PREDICT CHRONIC DISEASES

The measurements of trace elements in nails have been used in clinical research for decades. For some hypotheses, nail measurements have been demonstrated to be useful in the study of trace element status as it relates to chronic diseases including cancer and CVD. For example, toenail Se level provides a time-integrated measure that is superior to other biomarkers in assessing Se status.[8, 11, 12] A number of studies investigated nail Se in relation to cancer risk. A prospective cohort study examined toenail Se status and the risk of lung cancer,[13] and the results indicated an inverse association between Se status and lung cancer and suggested a modification of the effect of Se by the antioxidants β-carotene and vitamin C. The findings were supported by a nested case-control study in men,[14] but not

another nested case-control study in women.[15] Nevertheless, a meta-analysis quantitatively analyzed Se and lung cancer and reported an overall inverse association between toenail Se and lung cancer.[16] The authors found that the evidence for the potential protective effect was greater in studies of toenail Se than in studies involving other measures of Se status. Studies also examined toenail Se in relation to risk of prostate cancer. The results were inconsistent and inconclusive based on the current evidence.[17–23] Some studies have related toenail Se to other cancers.[24–26] In addition, nail specimens were used as biomarkers for other trace elements in cancer studies. For example, a case-control study examined iron (Fe), zinc (Zn), calcium (Ca), chromium (Cr) and cobalt (Co) in toenails in relation to cancer of upper aerodigestive tract.[27] Although the evidence was weak, the study suggested that there might be differences in those mineral intake or metabolism between individuals who developed some carcinomas of the upper aerodigestive tract and those who did not. Another case-control study investigated toenail cadmium (Cd) and risk of prostate cancer and suggested that Cd exposure increased prostate cancer risk.[28] A recent case-control study also found an inverse associated between toenail Zn level and gastric cancer risk.[29]

Associations between nail trace elements and CVD risk have been reported. Studies indicate that toenail Se concentrations predict risk of myocardial infarction (MI).[30, 31] Also, an inverse association between toenail Cr levels and coronary heart disease (CHD) is observed among diabetics[32] and non-diabetics.[33] In spite of sparse data, toenail Sc levels are suggested to predict acute MI.[34] Although the findings are inconsistent, studies indicate toenail Mercury (Hg) levels predict risk of MI.[31] In addition, studies find that toenail As concentrations are a reliable, long-term biomarker of total Arsenic exposure even for quantifying low level of As exposure.[35–38] In fact, studies indicate nail As levels predict blackfoot disease,[39] which is a unique peripheral vascular disease that is largely due to arteriosclerosis.

COMPARISONS OF NAIL MEASUREMENT TO MEASUREMENTS IN OTHER TISSUES

In addition to nail, trace element assessments in blood, hair and urine specimens have been used in clinical studies. Although comparisons among these markers may not help to determine the accuracy of the measurement of an individual marker since none of them can serve as a gold-standard for others, the measurement in concordance with others may support the capacity of the marker in clinical research. A few studies have been conducted comparing measurements in nail, blood, hair and urine. In general, each of the biomarkers is considered effective for particular clinical studies.[39–41] However, compared with blood or urine sample, nails particularly toenails provide a relatively long-term measure of exposure. For example, Arsenic can appear initially throughout the body, but it clears rapidly from most tissues, including the blood stream. After methylation in the liver, it is excreted in the urine;[42] as a result, blood and urine concentrations reflect only relatively recent exposure.[43] Also, nail clippings are non-invasive and relatively easy to collect especially can be completely self-administered, and easy stored at room temperature. Human hair is formed of the same keratinous tissue of nail and is therefore expected to share some advantages of nail marker. However, hair analysis is prone to be affected by cosmetic procedures such as dyeing, bleaching, and permanent waving which alter trace element content in hair.[44] In fact, hair as a biomarker for assessing trace element status has been questioned.[45–48] Although both finger- and toe-nail 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 out. Overall, nails provide valid measurements for ranking subjects according to long-term trace element intake or exposure.

STRENGTHS AND LIMITATIONS OF USING NAIL MEASUREMENTS

Strengths

The advantages of using nails as biomarkers of trace element status in hypothesis testing clinical studies are well established because nails: 1) are a keratin-rich protein matrix, and incorporating minerals and trace elements proportional to their intake or exposure; 2) integrate relatively long-term intake and exposure in a single specimen; 3) as a biomarker, in addition to estimating intake, also serve as a surrogate to measure status in critical organs; 4) non-invasive specimen collection and may increase participating rate; and 5) easy to be collected, shipped and stored, which will certainly make a study cost-effective.

Limitations

The nail biomarker has the following potential limitations in clinical study: 1) the response of the nail as an elemental biomarker is not as well characterized for some elements (e.g., Al, magnesium [Mg], copper [Cu], and Zn) as for others (e.g., Se, As, Cr, Hg, Cd). However, while this is a limitation, previous studies indicate that some useful information can be obtained for these elements; 2) in the study populations of developed countries, nails are generally environmentally sheltered and frequently washed as a result of the prevalent hygiene practices. However, nails can still become contaminated through the use of some medications and nail polishes. Elemental contamination can also be imparted to nail samples by the cutters used to produce the clippings. These limitations (polish and cutters) can largely be overcome by employing ultrasonic cleaning procedures with polar and non-polar solvents. Previous study indicates that the cleaning procedures are effective in removing or substantially reducing contamination from polishes and cutters;[49] 3) nail specimens collected in large cohort studies may include some small nail mass samples, for instance, with nail masses of 20 milligrams or less. In these small samples, some of the elements may be below the detection limits. Nevertheless, the small sample may be able to be analyzed by serial assessments of the same sample to obtain or generate the robust database for hypothesis tests.

COLLECTION AND STORAGE OF NAIL SAMPLES

To obtain more nail masses, participants should be asked in advance not to trim their nails for a couple of weeks or longer. Nails are collected by clipping with a stainless steel clipper from the two great toes (or thumbs) and small toes (or other fingers). Instruction need to be given to the participants to obtain as much nail as possible and clippings should be from both feet and / or hands. The nail clippings from the great toes or thumbs and the rest of the toes or fingers are better to be stored separately since the time frame represented by the great toe or thumb is different from the rest of the toenails or fingers. These nail samples can be placed in a labeled envelope and stored at room temperature in the driest condition possible in a pre-designated area until the samples can be analyzed in the future. Participants can cut their nails at clinic or at home and then mail their sample in. No need to remove nail polish when collect nails samples.

Since trace elements levels in nails reflect both dietary and non-dietary exposure and the concentrations may be affected by a number of factors, it is useful to design a nail questionnaire to obtain relevant information on nail polish, medication on nails, and the frequency of using stainless steel cooking wares as well as wearing socks and shoes. Particularly, participants should be asked how often they have had polish on the nails. If the color of the polish is silver, gold, copper or bronze, the participant should record the polish whether or not she or he knows for certain that it contains a metal. As for using of medication on the nails, the use of foot powder, whether medicated or not, may not be

recorded as it will not affect the nail samples. All this information collected may be useful for adjustment for potential confounders in the data analysis.

EXPERIMENTAL METHODS FOR QUANTIFYING TRACE ELEMENTS IN NAILS

Experimental methods are not this review's focus. Briefly, nail trace elements can be assessed by the most sensitive Instrumental Neutron Activation Analysis (INAA)[50] and / or Inductively-Coupled-Plasma Mass Spectroscopy (ICP-MS)[51] methodologies. INAA, at a high-flux steady-state research reactor, is a very sensitive trace element analysis technique. Neutron irradiations can be done using a pneumatic-tube system (for short irradiations) or in light-water / graphite moderated irradiation positions in close proximity to the reactor core (for long irradiations). Fundamentally, INAA protocols have five sequential sub-protocols: sample preparation, neutron irradiation, planned decay of induced radioactivity, measurement of residual radioactivity, and data reduction. With few exceptions, INAA protocols measure decay gamma rays (γ_d), which can be detected with excellent energy resolution and high sensitivity using state-of-the-art high-resolution gamma-ray spectroscopy (HRGRS). In addition, ICP-MS is a very powerful tool for trace (ppb-ppm) and ultra-trace (ppq-ppb) elemental analysis. In ICP-MS, a plasma or gas consisting of ions, electrons and neutral particles is formed from Argon gas. The plasma is used to atomize and ionize the elements in a sample. The resulting ions are then passed through a series of apertures (cones) into the high vacuum mass analyzer. Of note, in epidemiological studies, the nail samples are likely limited in mass. Consequently, a serial approach, using the same sample passed through each individual analysis protocol, can be used. The INAA and ICP-MS methodologies are ideally suited to this approach.

CONCLUSION

Accumulative literature supports that nail particularly toenail concentrations of most trace elements are useful biomarkers of exposure in which a single sample is assumed to represent long-term exposure. Previous studies indicate the ability of trace elements measured in toenails predicting chronic diseases including cancer and CVD in clinical studies. As compared to other potential biomarkers of trace elements, toenail measurement provides the most time-integrated measure and is less likely to be contaminated. The chemicals introduced by nail polishing can be largely washed out in the laboratory, and the element contents in nails are less likely to be affected. Nail trace element concentrations can be accurately quantified by INAA and ICP-MS.

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