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TRACE ELEMENT CORRELATIONS WITH AGE AND SEX IN HUMAN FINGERNAILS

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Concentrations of 17 elements in fingernails of 92 control individuals with ages ranging from 4 months to 93 years living in a relatively non-industrial environment were determined by instrumental neutron activation analysis (INAA). Statistical analysis demonstrated several different patterns of trace element correlation with age and sex. Bromine, Co, Cr, Fe, Na and Sb were found to be negatively correlated ($p < 0.05$) with age, while Zn was positively correlated ($p < 0.05$). Silver, Au, Se, and Zn concentrations were found to be higher in females than in males. Males had higher concentrations of Na and K than females. Significant interelement correlations were also observed. The age and sex variations observed should prove to be useful in the proper interpretation of elemental imbalances associated with degenerative neurological diseases, especially in view of recent reports that markers for AD have been detected in external tissue.

Many studies have recognized that the concentration of essential, non-essential and toxic metals in the body are affected by a wide range of factors such as sex, age, nutrition, dose, retention, chemical form of the metal and their binding sites.[1-3] The elemental composition of tissues is indicative of the nutritional and pathological status of humans.

The relationship between the aging process and trace element concentrations in normal human brain has also received attention recently as related to studies of elemental imbalances in Alzheimer's disease and similar neurological disorders. Other studies have investigated disease-control imbalances in hair and fingernail samples of elderly age-matched patient groups.[4-6] However, little information has been published on possible correlations of nail trace element levels with age and sex in controls, primarily in the middle age group. It is of interest in this work to see if trace elements in nails show any correlation with age or sex. Demonstration of such correlations would be an important step toward elucidating the possible role of trace elements in these neurological diseases.

The analytical technique used for elemental determinations in this study was instrumental neutron activation analysis (INAA). This method, with its sensitive and selective detection of many elements in diverse matrices, is well suited for trace element determinations in biological tissues. Little sample preparation is needed, so the possibility of contamination is minimized. The fact that INAA is a simultaneous multielement technique avoids sampling bias problems that may be associated with interelemental correlation studies that require separate sequential analyses.

Experimental

Sample Collection and Preparation: Fingernail samples were collected from healthy volunteers, with no neurological disease. The group was made up of faculty, staff and students at the University of Kentucky, their spouses and children, who have been living in the Lexington area for

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over a year. A total of 92 samples with an age range from 3 months to 93 years were analyzed. The average age was 30.3 years, with 70% under the age of 45. There were 36 females and 56 male subjects. The samples were collected by the subjects themselves. The subjects were provided with clean *Ziploc* bags for storing the samples. Instructions were given to them in writing for the sample collection procedures (e.g., to use stainless steel nail clippers), and a questionnaire that requested information on age, sex, race, geographical location, occupation, medication (if any), and unusual exposure to trace elements.[8] All the above factors are known to affect trace element levels in nails. All nail clippings were individually scraped with synthetic quartz knives, cut with *Teflon*-coated scisssors and stored in virgin polyethylene scintillation vials. A standard International Atomic Energy Agency (IAEA) method[7] was used for nail washing. The method consists of five successive 10 minute washes, with mechanical shaking, in the sequence acetone-water-water-water-acetone. *Burdick and Jackson Distilled-in-Glass* acetone and distilled and deionized water (ddw) were used. The samples were air dried in a dust free enclosure for approximately 2 to 3 days after washing. All implements that contacted the samples were prewashed using 5% *Instra-Analyzed* nitric acid (J.T. Baker), ddw water, and *Burdick and Jackson Distilled-in-Glass* methanol. Talc-free gloves were worn during all sample handling procedures, and a laminar flow hood was used for sample processing.

Irradiation and INAA Counting Parameters: Samples were packed in pre-washed *Suprasil* quartz vials. Sample masses were determined with a *Mettler* Model M5 micro balance (precision ±0.000005 g), and ranged from 10-25 mg. *Suprasil* synthetic quartz vials used for packaging were prewashed in *5% Ultrex* nitric acid (J.T. Baker) for 30 minutes in an ultrasonic cleaner and rinsed with ddw water and *Burdick and Jackson Distilled-in-Glass* methanol. Samples and standards along with blanks were irradiated at the University of Missouri Research Reactor (MURR) at a thermal neutron flux density of ~5.5 x 10^{13} neutrons cm^{-2} s^{-1} for a period of 40 hours.

All samples, standards and blanks were counted twice. The first count of 20 minutes was done immediately upon return of the samples from MURR for arsenic (As), gold (Au), bromine (Br), potassium (K) and sodium (Na). A 3 hour count was done approximately 10-14 days later for silver (Ag), calcium (Ca), cobalt (Co), chromium (Cr), cesium (Cs), iron (Fe), mercury (Hg), rubidium (Rb), antimony (Sb), scandium (Sc), selenium (Se), and zinc (Zn). Gamma rays of the irradiated samples were counted using intrinsic germanium detectors with resolutions of 1.9 keV at ^{60}Co (1333 keV) and relative efficiencies of up to 44%. The detectors were coupled to a *Nuclear Data* ND-683 multichannel pulse-height analyzer through an *ORTEC* 572 amplifier.

The comparator reference materials used in this study were well characterized U.S. National Institute of Standards and Technology (NIST) biological standard reference materials 1571 Orchard Leaves, 1577 Bovine Liver, and 1566 Oyster Tissue. Elemental data for these standards are summarized elsewhere.[8] Bowen's Kale, an international biological standard, was used as a secondary reference standard.[9] The samples of Bowen's Kale had been previously analyzed in our laboratory employing standard solution comparators prepared from high purity materials.[10] Results obtained in this study for Bowen's Kale were in excellent agreement with published compilation values.[8]

All the parameters that could affect the accuracy of the determination of trace elements, such as choice of the best indicator radionuclide and of the counting times were selected very carefully. Spectral interferences and fast neutron interference reactions were also evaluated. A majority of peaks selected for analysis were free of spectral interference. The 279.2 keV photopeak of ^{203}Hg was corrected for the contribution from the 279.5 keV peak of ^{75}Se using the predetermined ratio of the intensities of the interfering peak (279.5 keV) to the clean peak (264.7 keV) of ^{75}Se. A summary of the counting parameters for each element is reported elsewhere.[8]

Results and Discussion

The data were processed using SAS Lifereg procedure (SAS Institute, 1985).[11] This program uses the method of maximum likelihood to estimate sample means and standard errors, and then uses the Chi-square statistic to test for significant differences between groups. The Lifereg procedure is capable of simultaneously studying the effects of several covariates on a response variable. A more detailed description of the procedure has been previously reported.[12] The SAS CORR procedure was used in order to study inter-elemental relationships. The Pearson procedure uses detection limit values as actual values. Therefore, no correlations are reported here where >70% of the values from the data set were limit values. The SAS Univariate procedure was used to test the normality of the data distributions. In our nail samples, only Zn is normally distributed. All other elements showed a log-normal distribution. Therefore, geometric means and standard errors are reported for all elements except Zn, for which arithmetic means are reported.[8]

Nail-Age Correlation: Table 1 summarizes the results of the correlation tests of nail levels with age. The above 45 year age group corresponds to a population most often considered in neurological disease studies. Concentrations of Br, Co, Cr, Fe, Na, and Sb were affected by age, with persons under the age 45 having higher levels than those above the age of 45. Declines of Br, Fe, Na, Sb levels in nail have been reported earlier for the older age group.[5] Others have reported declines of Br, Co, Cr, and Sb with age in control brain and other biological tissues.[3,15] Elevated Br level in hair, nails and brain of AD subjects as compared to control have been reported in many studies.[6,16,17] It is reported that Cr concentrations of tissues and organs, except in lung, also appear to decline with age.[13,15] However, considerable variations of Cr levels in biological tissues are found in the literature.[18]

Table 1
Correlations of elemental concentrations in nail with age

Elements, Conc. Unit	Mean Age <45 y	Mean Age >45 y	p	Corrlation with Age
Br, µg/g	2.8	2.4	0.007	(-)
Co, ng/g	64.7	50.4	0.022	(-)
Cr, ng/g	1120.	978.	0.049	(-)
Fe, µg/g	27.3	22.2	0.033	(-)
Na, µg/g	182.	150.	0.043	(-)
Sb, ng/g	52.6	40.7	0.019	(-)
Zn, µg/g	148.	154.	0.022	(+)

Zinc is the only element that showed a positive correlation with age in this study. However, Zn levels in control nails were only slightly higher in the age group over 45 than for persons under that age (Table 1). A similar correlation was reported by VANCE et al.[5] Age related increases have also been observed in Zn levels in mixed saliva and parotid saliva.[1] However, Zn concentrations in hair have been reported by others to be significantly lower in elderly subjects than in young control subjects.[5,19] No significant correlation has been reported between hair and nail Zn levels.[5] Elevated Zn levels have been reported in the hair and nail of AD subjects, compared to controls.[6] Wenstrup et al.[16] have reported decreased brain temporal lobe Zn levels in AD subjects.[16] Other recent studies have reported abnormalities of Zn metabolism in AD subjects.[20,2]

Nail-Sex Correlations: In nail, the elements Au, K, Na, Se and Zn were found to have a significant correlation with gender. The elements Au (possibly due to more frequent use of Au

jewelry), Se and Zn were found to be higher in females, where as alkali metals K and Na were observed to be higher in males (Table 2). VANCE et al.[5] and KANBROCKI et al.[13], have also observed an elevation of Au, Se and Zn in females compared to males.[5,13] Elevation of alkali metals in males is also reported by KAMAKURA et al.[21] In control hair, Au is reported to be higher in females in comparison to males.[6,22] The elements Ag, As, Ca, Cs, Hg, Rb, and Sc did not show any significant correlation with age or sex.

Table 2
Correlations of elemental concentrations of nail with sex

Elements, units	Male	Female	p
Au, ng/g	20.7	51.9	0.003
K, µg/g	120.	62.2	0.001
Na, µg/g	169.	142.	0.043
Se, µg/g	0.92	1.12	0.016
Zn, µg/g	140.	157.	0.022

Interelement Correlations: An important advantage of the simultaneous multielement INAA approach is well illustrated by its potential to provide interelement correlation information without sampling bias. Table 3 summarizes the results of the interelement correlation tests. Only significant correlations ($p < 0.05$) are included in this table. To our knowledge this is first study that reports interelement correlations in nail. Therefore, a comparison with other studies is not possible. Positive correlations of the alkali metals, K and Na, with Br are not surprising as these ions occur together in biological tissues. In this study, both Na and Br levels declined with increasing age in nail. Positive K-Br and negative Hg-K correlation have been previously reported in another control brain-nail study.[14] The elements Cr, Co, Fe are positively correlated with each other, but all three are negatively correlated with age.

General Comparisons: A limited amount of data exists for multielement analyses of nail that take into consideration the effects of age and sex. Table 4 shows a general comparison of control nail values observed in this study with those reported by other studies.[5,13,18]

Table 3
Pearson correlations for all nails

Element	Significant Correlations ($p < 0.05$)
Ag	Au (+); Cr (+); Fe (+); Rb (+); Sb (+)
Au	Ag (+); Cr (+); Fe (+); Sc (+); Zn (+)
Br	K (+); Na (+)
Co	Cr (+); Fe (+); Hg (+)
Cr	Co (+); Fe (+)
Fe	Ag (+); Au (+); Co (+); Rb (+); Sb (+)
Hg	Co (+); K (-); Se (+)
K	Br (+); Hg (-); Na (+)
Na	Br (+); K (+)
Rb	Ag (+); Co (+); Cr (+); Fe (+); Sb (+)
Sb	Ag (+); Co (+); Fe (+); Rb (+)
Se	Cr (+); Hg (+); Zn (+)
Zn	Au (+); Cr (+); Fe (+); Sc (+); Se (+)

With few exceptions, concentrations observed in this study agree well with others. As noted previously, this study and also that of VANCE et al.[5] report geometric means for the nail concentrations, except for Zn where the arithmetic mean is reported. In the study by VANCE et

Table 4
Comparison of mean values for nail concentrations with literature values

Element, units	This work* Avg. age (30 y)	Vance[5] * Avg. age (59 y)	Iyengar[18] ** Compilation	Kanabrochi[13] *** Female	Male
Ag, ng/g	60.3	33.1	3 - 1400	740	340
As, ng/g	54.5	31.8	362 - 1970		
Au, ng/g	29.9	23.3	30 - 780	2600	420
Br, µg/g	2.11	2.09	9 - 10		
Ca, µg/g	665	482	368 - 3070		
Co, ng/g	42.1	27.5		70	40
Cr, ng/g	898	1650	6200	6700	4230
Cs, ng/g	5.4	1.7	27 - 347		
Fe, µg/g	19.5	13.5	7270		
Hg, ng/g	124	170	357 - 2800	1900	390
K, µg/g	94.6	49.5	332 - 3010		
Na, µg/g	157	138	3.1		
Rb, µg/g	0.29	0.1	175 - 750		
Sb, ng/g	36.6	24.5	1.14 - 8		
Sc, ng/g	2.7	0.84			
Se, µg/g	1.003	1.002	1.14 - 8	0.88	0.58
Zn, µg/g	147	149	73 - 304	184	154

*Geometric means, except Zn = arithmetic mean, ** Range of values, *** Arithmetic means

al.,[5] the age group for controls was higher (mean = 59 years) and narrower than the age group (mean = 30 years) in this study. KANABROCHI et al.,[13] also used INAA, but report arithmetic means and did not process limit values as reported here. In addition, no wash procedure for the samples was reported.[13] IYENGAR et al.[18] have generally reported ranges, rather than the means for nail trace element concentrations. Our concentration data are obtained from subjects that are from a principally non-industrial area of Kentucky, and not unexpectedly tend to lie towards the lower limits of the ranges compiled by IYENGAR et al.

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