서울特別市 保健硏究所報 第13卷 (1977)
Report of S. I. P. H., 13 : 31~35 (1977)

Trace Element Contents in Korean Human Fingernails by Atomic Absorption Spectroscopy

Won Yong Yoon, Ki Sook Yang and Sung Bae Park

Pharmaceutical Chemistry Division

원자 흡광 분석법에 의한 손톱중의 미량원소 함량

약 품 화 학 과

윤 원 용·양 기 숙·박 성 배

=국문초록=

인체의 무기물인 미량금속원소가 생체조직의 일부인 모발이나 손톱등을 통해 이루어진다는 보고가 있으며 이들의 함량을 분석해서 질병의 진단에 응용하려는 많은 연구가 진행되고 있다.

그러나 우리나라에서도 두발에 관해서는 약간의 보고가 있으나 fingernail에 관한 보고는 거의 없으므로 저자등은 fingernail 중 생체의 무기물 대사를 제시하는 많은 미량금속중 Zn과 Ca의 함량을 원자흡광분석법으로 분석 검토하였다. 즉 정상인 30명의 fingernail 중 Zn, Ca의 함량을 측정한바,

Zn 함량은 남자가 평균 175.77±21.65ppm, 여자는 207.72±22.44ppm로서 여자가 다소 높았으나 유의차는 없었으며, Ca 함량에서는 남자가 평균 3847.94±262.84ppm, 여자는 2917.88±352.54ppm으로서 남자가 다소 높아 성별에 따른 유의차를 인정할 수 있었다($p<0.05$).

A considerable number of reports are to be found in the literature concerning the trace eremental analysis of various biological meterials by A. A. S.

However, little work has been reported on human fingernails, a sample which offers some promise with regard to monitoring trace metal concentration in the body particulary deviations from normal levels. Since metal ions have on affinity for N, S and O-containing groups in proteins[1], metals may accumulate in the nails in cases of excessive intake such as poisoning. Cumulative arsenic poisoning resulting in death has been established by neutron activation studies of nails sections progressively close to the nails matrix. The metal ion concentration of elements associated with certain diseased has also been studied in nails. Harrison and Turee[2] have proposed the determination of Cu in nails for diagnosis of cystic fibrosis. Roboson and Brooks[3] have demonstrated an increase in the sodium and calcium concentration of nails from children suffering from Kwashiorkor[4]. Sodium and potassium levels in the nails of children with cystic fibrosis have been studied using flame photometry and compared to concentrations for siblings and parents as well as normal children and adults. Sodium, Potassium, Calcium and Magnesium were studied by flame photometry and fluorimetry methods in nails of normals and children with Kwashiorkor[4]. A children disease resulting in a lose of potassium and a retention of sodium by the tissue.

The iron contents as influenced by age and anemia was studied in nails using colorimetric techniques[5].

Copper was determined spectrophotometically in normals and those with Wilson's disease[16]. The sulfur content in nomal nails and nails of patients with a variety of diseases affecting the nails was

studied[7].

Calcium was shown to have no effect on the brittleness of nail[3]. Studies on the normal levels of phosphorous, calcium, zinc, magnesium, copper, iron and manganese have been conducted by emission spectroscopy[9]. Silicon has been determined colorimetrically[10]. Neutron activation has been used to study manganese[11, 12], gold, copper, and sodium[12]. Another activation analysis study[13] has reported sodium, chromium, zinc, and antimony in nails.

In the present study, we determined the contents of zinc and calcium in normal human fingernails, and compared to the reported results.

EXPERIMENTAL METHOD

Apparatus; A Hitach Model 207 Atomic absorption spectrophotometer was used to determine the calcium zinc concentration of the digested samples. The conditions for determinations in the flame were as outlined in the Hitach model methods manual[14].

Reagents; Standards were diluted to appropriate concentration from 1000μg/ml commercial standards, (A. A. manual) perchloric and nitric acid(pure grade) were used in the digestion and preparation of standards. All dilutions were made with distilled water. Glassware for race metal analysis was specially cleaned by soaking in 50% nitric acid for 30 min., subsequently rinsed with D. W. and dried in an oven.

Method: The general sample preparation used in this study is a modification of the procedure previously used for hair samples[20]. The samples of clippings from all thirty-fingernails, if available, was scraped with forceps to remove any obious surface contamination such as nail polish. Clipping were washed twice with acetone and twice with water on the mechanical shaker for 30 min., and dried at 100°C for 30 min., and allowed to cool in desiccator. About 30 mg of dried fingernail was weighed on a Mettler balance(Model H34) placed in a 10ml volumetric flask and 1 ml of concentrated nitric acid followed by 0.5ml of 60% perchloric acid were added. The samples were heated gently until clear yellow liquid was obtained, cooled, and diluted to volume with distilled water. Each sample solution was analyzed three times.

RESULTS AND DISCUSSION

As in the case of human hair, there is no simple, standard, and completly satisfactory method for cleaning the nail samples. Prior to analysis, ideally, surface contamination should be completly removed without significantly depleting the concentration.

A variety of methods have been reported, Kanabrocki et al[12]. scraped the nail with a stainless steel blade and washed the sample three times with 10 ml of ammonium barbituric acid buffer(pH 7.35) Goldblum et al[9] removed surface contamination from samples by washing in a 10% Tween 80 solution on a mechanical shaker for 30 min, followed by three 10min, rinses in distilled water. Fregert[10] reported no specific cleaning procedure prior to digestion. Antonelli et al scraped all visible dirt from the surface before analysis. Kile[8] washed 300mg samples of nail twice by shaking vigorously in a 1% solution of Triton X-100 with 10 subsquent rinses in distilled water. Kopito etal[10] scraped excess dirt from the surface of the nail and washed the sample in 0.1N-HCI(1ml to each 5mg of nail) for extracting 2min. Patushkov et al[13] washed their nail samples first D. W. and then acetone.

Martin[11] scraped the surface of the nails and washed the sample in successive changes of deionized water. Jacobes and Jenkins[5] removed surface contamination by scraping and washed the nail sample in a dilute solution of Teepol followed by three successive ether washes and three portions of distilled water.

Wash comparisons: The two general type of wash procedures previosly used seemed to be 1) detergents, 2) organic solvents.

A comparison to determine the utility of these methods can be evaluated by use of identical samples one analyzed after only scraping clean, the other subjection to the test washing and then analyzed to determined the change in trace element content. Preparation of such samples involves certain precautions since it has been reported that concentrations can vary from one finger to another.

Our comparison samples resulted from splitting each individual nail clipping cross with respect to the axis of growth. Harrison et al[15]. washed for 30min, and analyzed. An 1% solution of 7X-O-Matic was used for the detergent phase and absolute ethylalchol for the organic wash. Of the five elements studied, each was reduced in concentration at least somewhat by both types of wash.

Consistent with the data from human hair washings[14], copper was altered in concentration only slightly, usually 5—10%, while magnesium and iron were significantly reduced, particulary with the detergent, as much as 40—50%. Zinc and calcium usually showed reductions in the 15—25% range. The alcoholic wash rather consistently showed less reduction in elemental concentration than the detergent, but this could be due to a less efficient remove of surface contamination. On the detergent may be extracting more of each element from the structure of the nail.

The results might seem to be suggested that more reliable data could be obtained by use of unwashed samples until it is recalled that nails samples, before taken, are subjected to frequent hand washes. This would seem to agree with the suggestion of Bate and Dyer for human hair that detergent washings are more advantageous in that they correspond more closely to in washing and should tend to reduce spurious effects resulting from variations in time since a prior wash before the sample was taken.

Tabel 1. Calcium and Zinc Contents (ppm) in Fingernails

Component	Calcium	Zin
Male (14)	3847.94±262.84[a] (2331.1~5332.8)	175.77±21.65[c] (80~333.3)
Female (16)	2917.88±352.54[b] (1609.8~3889.6)	207.72±22.44[c] (93.3~499.9)

Mean±SE, Numbers in parentheses indicate the number of samples.

a, b : Means significantly difference at $P<0.05$.

c : No significantly difference between means.

Analytical values : The nails used in this study were taken from individuals, both male and female in the 20 to 50 age range. Nails that were extremely solid or coated with fingernail polish were rejected.

Table. 1 shows the mean values of the analysis elements for each of the 30 individuals of 16 female and 14 male subjects.

The variation in individual means is also indicated by including the range and median in Table. 1.

Male-female contents differences : Zinc contents, in male, was 175.77±21.65ppm in average and its variation range was 80.0~333.3ppm, in female, was 207.72±22.44ppm in average and its variation range was shown 93.3~49.9ppm.

A little more Zn contents was shown in female than male, but difference between them was not significant. Meanwhile, Ca contents in male was 38 47.94±262.84ppm in average and its variation range was 2333.1~5332.8ppm. On the contrary, in female, Ca contents was 291.88±352.54 ppm and its variation range was shown 1609.8~3889.6ppm. Much more Ca contents was shown in male than female and so difference between them was significant($P<0.05$). Differences between the elemental concentrations in males and females could arises from a variety of reasons.

The presumably higher level of outdoor physical activity for males would expose the nails to a wider variety of surface contaminants. The nails from a female would be more likely to have frequent exposure to detergent and various agents related to cleaning activities. In an attempt to limit as many as possible, zinc and calcium show no systimatic differences although more data would be required before further speculation.

Comparison to literature values: Relatively few studies have been made on elemental concentration in nails. As stated previously, difference in sample preparation and analysis method make exact comparison to literature values.

Goldblum et al[9] using nail samples from both the fingers and toes, reported similar calcium values to ours using A.A methods for 14 male subjects. Leonard et al[4] used fluorometric methods to obtain a calcium figure also similar to ours in 25 children.

Harrison and Tyree[2] used A.A.S. to obtain a

Table. 2. Comparison to previously reported values($\mu g/g$ nails)

Element	Method	Sample	Mean	Range
Ca	Emission spec[14]	9(Male)	—	940—5900
	Fluorimetric[9]	25(Children)	3070	1060—5080
	Atomic abs[21]	82(Total)	870	687—1270
	Atomic abs[21]	19(Female)	821	701— 982
	Atomic abs[21]	63(Male)	904	687—1270
	Atomic abs*	16(Female)	2917.88	1610—3889.6
	Atomic abs*	14(Male)	3847.64	2333.1—5332.8
Zn	Emission spec[14]	9(Male)	—	116—3080
	Fluorimetric[8]	25(Children)	2480	130— 391
	Atomic abs[21]	82(Total)	108	130— 360
	Atomic abs[21]	19(Female)	111	135— 391
	Atomic abs[21]	63(Male)	106	1200—2700
	Atomic abs*	16(Female)	207.72	93.3— 203.3
	Atomic abs*	14(Male)	175.77	80— 333.3

* This investigation.

calcium lower than ours in total 82. Goldblum[9] also reports a much wider range for zinc. We didn't detect copper, iron in cur nail studies.

Comparison to concentration in hair in previously reported. Hair and nails are both principally composed of Keratin and both arise from the epidermis by the process of Keratization. Thus it would not be unreasonable to expect that the trace element concentration should be similar. No values were obtainable for calcium by A. A. although the Goldblum study[9] lists a value of 700—4900 $\mu g/g$ for hair which is slightly lower than the range he lists for nails. For zinc in hair a mean value of 176$\mu g/g$ with a range of 70 to 250$\mu g/g$ was reported by Backer. Thus the value reported for hair apparently tend to be lower than those found for nails. This may be partly due to a larger amount of the elements being leached out in washing because of the larger surface area in hair per unit of weight.

The supply of elements to the site of Keratization for hair and nails may also very well be different which would account for differences in concentration. Since the exact mode of incoporation of inorganic elements into the Keratin is not well known. However a great deal more data is required from entersive studies of a large number of cases to clearly establish normal values for both hair and nails.

CONCLUSION

1) Zn contents in average was 175.77±21.65ppm in male and 207.72±22.44ppm in female but difference between them was not significant.
2) Ca contents in average was 3847.64±262.84ppm in male and 2917.88±352.54ppm in female, and difference between them was significant ($P<0.05$).

References

1) Martin, R. B, : The affinity for N, S and O-Containing groups in proteins. Introduction to Biophysical Chemistry, Mc Grow Hill Book Company. pp. 335—348 (1964)
2) Harrison, W. W., Tyrce, A. B. : The determination of trace elements in biological materials

by A. A. S. *Clin. Chim. Acta.* 31 : 63 (1971)

3) Roboson, T. R. K. and Brooks, G. J. : The determination of Na and Ca concentration of nails from children. *Clin. Chim. Acta,* 55 : 255 (1974)

4) Jacobs, A. and Jenkins, D. J. : The determination of Na, K, Ca, Mg by flame photometry and fluorimetry. *Brit. J. Dermatol.* 724 : 145 (1970)

5) Shapiro, H. A. : The iron contents in nails using colorimetry. *J. Forsenic Med.* 14 : 65 (1967)

6) Martin, G. M. : The determination of copper in normals and those with Wilsons disease. *Nature* 202 : 903 (1964)

7) Klauder, J. V. and Browo, H. : The sulfur content in normal nails of patients with a variety of diseases. *Arch. Dematol, Syphilis,* 31 : 26 (1935)

8) Kile, R. L. : The effect of calium on the brittleness of nails. *Arch. Dermatol,* 70 : 75 (1954)

9) Goloblum, R. W. Derbey, S. and Lerner, A. B. : Studied on the normal levels of trace elements by emission spectroscopy. *J. Invest, Dermatol,* 20 : 13 (1953)

10) Fregert, S. : The determination of silicon by colerimetry. *J. Invest, Dermatol* 31 : 95(1958)

11) Cotzias, G. C., Papavasilon. P. S. and Miller, S. T. : The study of manganese by neutron activation *Nature* 201 : 1228 (1969)

12) Kanabrocki, E., Case, L. F., Grabam, L. A. : The study of Au, Cu, Na, by neutron activation. *Fields, J. Oester* 9 : 478 (1968)

13) Petushikov, A. A., Linekin, D. M., Balcius, J. F. and Browntli, G. C. : The study ef Na, Br, Zn, Sb, in nails by activation method. *J. Nuclear Med.* 10 : 730 (1969)

14) Hitachi: Analysical Methods for Atomic absorption spectrophotometry.

15) Harrison, W. W., Yurachek, J. P., and Benson, C. A. : Determination of trace elements in human hair by A. A. S. *Clin. Chim. Acta.* 23 : 83 (1969)

16) Backe, E. T. : Determination of zinc in fingernails by non flame atomic absorption spectroscopy. *Clin. Chim. Acta.* 24 : 233 (1969)