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INAA OF ELEMENTAL CONTENTS IN FINGERNAILS OF ESOPHAGEAL CANCER PATIENTS

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Fingernails of pathologically diagnosed normal people, light esophageal epitheliosis patients, severe esophageal epitheliosis patients and esophageal cancer patients were irradiated and their elemental contents were determined by INAA. Multivariate statistical treatment of Ca, Cl, K, Mg, Se and Zn data shows that esophageal cancer patients are distinguishable from non-cancer patients. The accuracy computed by neural networks is greater than 80%.

The elemental contents in the human body change along with the health condition. The determination of certain compounds or elements in human organs or tissues is utilized clinically in the diagnosis of certain diseases. The relationship between disease and trace element concentration has been extensively investigated. For example, Keshan disease is linked to the deficiency of Se. The correlation of Se content in hair to internal organs has been studied[1] by instrumental neutron activation analysis (INAA). It is easy to collect hair samples, and INAA is sensitive and accurate, and can determine more than 20 elements in one sample. But hair samples are more easily contaminated, especially by hair tonics and conditioners. Fingernails are also easily collected, but less susceptible to contamination. Since fingernails have a larger exposed area, stronger mechanical decontamination methods or reagents can be applied. Although studies utilizing hair are more common than those with fingernails or toenails, the latter have increased[2,3,4,5] in recent years. The advantages of fingernail samples are gradually being recognized. Xiao directed the first study in China of fingernails of rheumatoid patients using INAA.[6]

Experimental

Sampling: Fingernails from 4 groups of people in the esophageal cancer endemic area were collected. The 4 groups were pathologically diagnosed as normal people, light esophageal epitheliosis patients, severe esophageal epitheliosis patients and esophageal cancer patients. Fifty to sixty samples were taken from each group.

Sample Treatment: We tested various methods of sample cleaning,[7] and finally selected 1:2 volume percent of absolute alcohol and 0.2N HNO_3 as the cleaning reagent. Samples were soaked in distilled water for 2 hours, stirred in the cleaning reagent with a magnetic stirrer, washed three times with de-ionized water, dried, weighed, packed in polyethylene film and put into clear irradiation vials.

Irradiation: Samples were irradiated in the mini-reactor and heavy water reactor of the China Institute of Atomic Energy for short, medium and long irradiations as shown in Table 1.

Determination: The radioactivities in the irradiated samples were measured with a computer based HPGe gamma-ray spectrometer. The software was designed in our Institute. The elemental contents were determined and averaged using the computer. The elemental contents were checked with standard reference materials, such as SRM 1577 bovine liver and BCR, and the Chinese human hair and shrimp powder. Twenty-eight elements were determined.

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Table 1.
Irradiation conditions

Neutron flux	Irradiation time	Cooling time	Elements determined
$7x10^{11}$ n/cm^2 sec	5 min	1 min	Al, Ca, Mg, Cu, etc.
$7x10^{11}$ n/cm^2 sec	14 hrs	20 hrs	K, As, Na, Br, etc.
$4x10^{11}$ n/cm^2 sec	50 hrs	7 days	Fe, Zn, Se, Cr, etc.

Results and Discussion

Table 2.
Determined Result of 28 Elements (μg/g)

No.	Elements	Groups	Samples	Arithmetic average	Median	Geometric average
1	Al	0	52	52.08	48.71	45.23
1	Al	1	62	48.60	45.86	44.22
1	Al	2	56	51.13	40.29	47.80
1	Al	3	44	48.66	44.62	43.75
2	As	0	52	0.43	0.32	0.35
2	As	1	82	0.46	0.38	0.40
2	As	2	56	0.52	0.31	0.38
2	As	3	44	0.43	0.37	0.38
3	Br	0	52	1.36	1.24	1.28
3	Br	1	62	1.26	1.21	1.20
3	Br	2	56	1.76	1.59	1.59
3	Br	3	44	1.49	1.56	1.42
4	Ca	0	52	257.9	197.8	194.0
4	Ca	1	62	333.3	280.8	267.8
4	Ca	2	56	451.4	456.7	434.8
4	Ca	3	44	574.2	537.8	496.5
5	Cl	0	52	47.45	38.09	41.39
5	Cl	1	62	43.26	37.87	37.93
5	Cl	2	56	86.48	67.87	73.12
5	Cl	3	44	374.0	354.6	275.0
6	Co	0	52	0.05	0.05	0.05
6	Co	1	62	0.05	0.05	0.05
6	Co	2	56	0.05	0.05	0.05
6	Co	3	44	0.05	0.05	0.05
7	Cr	0	52	1.90	1.46	1.62
7	Cr	1	62	2.04	1.68	1.70
7	Cr	2	56	2.39	2.15	2.07
7	Cr	3	44	2.60	1.78	2.01
8	Cu	0	46	4.65	4.34	4.38
8	Cu	1	61	4.04	4.88	4.63
8	Cu	2	55	5.07	4.84	4.79
8	Cu	3	43	4.72	4.50	4.447

Table 2. Continued

No.	Elements	Groups	Samples	Arithmetic average	Median	Geometric average
9	Fe	0	52	122.7	120.3	110.2
9	Fe	1	62	118.7	116.8	110.0
9	Fe	2	56	123.2	112.6	116.5
9	Fe	3	44	115.7	114.9	107.4
10	Hg	0	52	0.19	0.19	0.18
10	Hg	1	62	0.21	0.20	0.19
10	Hg	2	56	1.05	0.21	0.21
10	Hg	3	44	0.29	0.25	0.23
11	K	0	50	45.80	42.47	38.89
11	K	1	61	42.91	40.50	39.42
11	K	2	56	49.81	50.70	46.53
11	K	3	43	87.77	70.59	73.90
12	La	0	44	0.03	0.02	0.01
12	La	1	54	0.03	0.02	0.01
12	La	2	51	0.03	0.03	0.01
12	La	3	29	0.03	0.00	0.01
13	Mg	0	52	64.03	65.68	59.43
13	Mg	1	62	67.56	63.16	63.58
13	Mg	2	56	94.10	92.90	91.75
13	Mg	3	44	111.0	111.9	105.5
14	Mn	0	52	1.18	1.07	1.05
14	Mn	1	62	1.26	1.12	1.10
14	Mn	2	56	1.23	1.22	1.18
14	Mn	3	44	1.38	1.38	1.32
15	Na	0	52	31.67	27.41	28.11
15	Na	1	62	32.12	28.83	29.49
15	Na	2	56	73.38	89.25	64.39
15	Na	3	44	174.9	87.48	108.9
16	S	0	52	28800	28730	28640
16	S	1	62	29160	29110	29020
16	S	2	56	28980	29630	28830
16	S	3	44	28440	28140	28240
17	Sb	0	52	0.23	0.16	0.17
17	Sb	1	62	0.26	0.17	0.19
17	Sb	2	56	0.21	0.19	0.17
17	Sb	3	44	0.26	0.17	0.18
18	Sc	0	52	0.02	0.02	0.02
18	Sc	1	62	0.02	0.02	0.02
18	Sc	2	56	0.02	0.02	0.02
18	Sc	3	44	0.02	0.02	0.02
19	Se	0	52	0.68	0.67	0.68
19	Se	1	62	0.68	0.66	0.67
19	Se	2	56	0.71	0.69	0.69
19	Se	3	44	09.85	0.83	0.82

Table 2. Continued

No.	Elements	Groups	Samples	Arithmetic average	Median	Geometric average
20	Ti	0	52	13.87	12.69	12.13
20	Ti	1	61	13.79	13.25	12.56
20	Ti	2	53	14.73	14.74	13.64
20	Ti	3	41	13.65	12.44	13.13
21	V	0	52	0.16	0.14	0.14
21	V	1	62	0.15	0.13	0.14
21	V	2	56	0.16	0.15	0.15
21	V	3	44	0.14	0.14	0.14
22	Zn	0	52	36.42	31.44	31.24
22	Zn	1	62	44.02	34.70	38.56
22	Zn	2	56	63.69	64.21	62.62
22	Zn	3	44	74.45	81.22	69.52
23	I	0	28	0.27	0.25	0.24
23	I	1	27	0.36	0.28	0.28
23	I	2	23	0.28	0.23	0.23
23	I	3	26	0.62	0.45	0.48
24	Sr	0	4	4.55	5.61	4.33
24	Sr	1	8	5.90	6.35	5.55
24	Sr	2	3	7.93	8.88	7.89
24	Sr	3	6	6.77	5.97	6.04
25	Ba	0	8	7.17	4.63	5.89
25	Ba	1	9	5.96	6.70	5.32
25	Ba	2	7	6.11	6.98	5.15
25	Ba	3	35	30.09	11.18	13.78
26	Ce	0	13	0.04	0.04	0.03
26	Ce	1	17	0.04	0.05	0.04
26	Ce	2	14	0.05	0.05	0.04
26	Ce	3	10	0.05	0.04	0.04
27	Rb	0	12	0.39	0.37	0.36
27	Rb	1	11	0.42	0.44	0.40
27	Rb	2	12	0.39	0.39	0.36
27	Rb	3	16	0.42	0.43	0.40
28	Ag	0	11	0.09	0.11	0.08
28	Ag	1	12	0.09	0.09	0.08
28	Ag	2	7	0.11	0.14	0.09
28	Ag	3	10	0.12	0.14	0.10

The results are shown in Table 2, where 0, 1, 2, and 3 represent normal control, light esophageal epitheliosis patients, severe esophageal epitheliosis patients and esophageal cancer patients, respectively. It is seen that the arithmetic average, geometrical average and median value for the same element in each group approximate each other. From the arithmetic averages of each element for the 4 groups, the t values of 0:1, 1:2, 2:3 and 0:3 (omitting 0:2 and 1:3) were calculated. The t values show that there are no significant differences between 0:1 ($P>0.1$) groups for all the 28 elements, indicating that contents of each of the 28 elements in the fingernails between normal control and light esophageal epitheliosis patients have no marked differences. But for the 7 elements Ca, Cl, K, Na, Mg, Se, Zn, there are marked differences between 1:2, 2:3 and 0:3 groups ($P<0.01$ or 0.001). Furthermore, the contents of these 7 elements show a tendency to

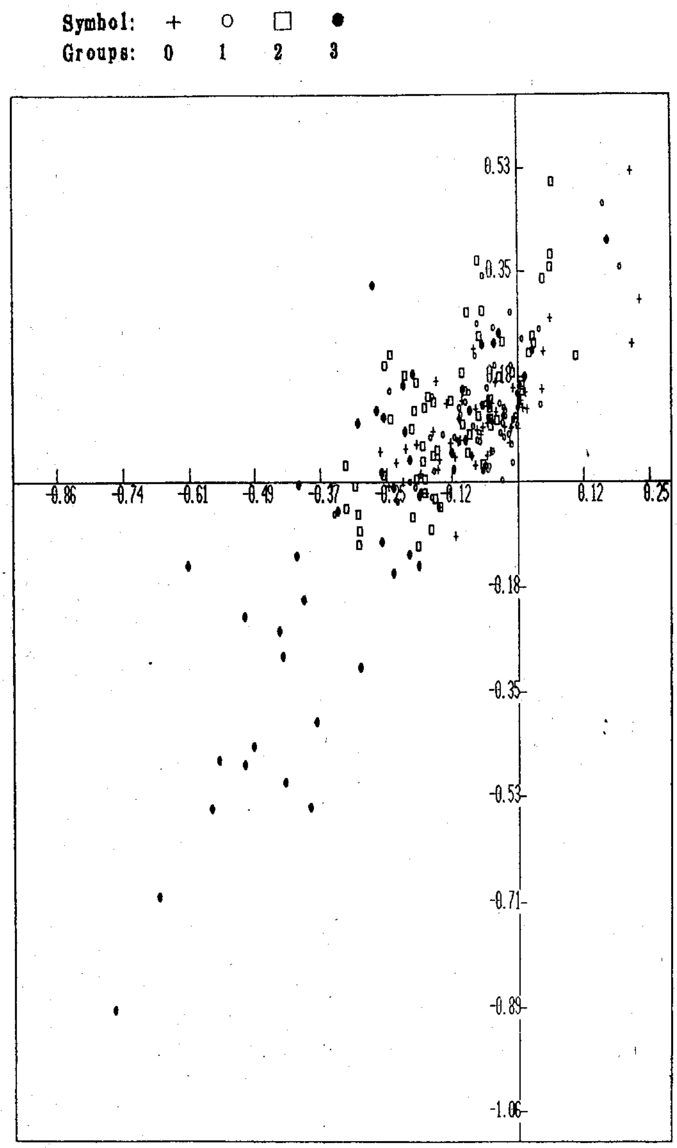

Fig. 1. Principal coordinates of Ca, Cl, K, Mg, Se, Zn

increase from low to high, higher as the groups shift from 1→2→3. Chromium and Mn seem to show the same tendency, but not with marked differences (0.04<P<0.1). For the element I, there are marked differences (P<0.01) between 2:3 and 0:3, but there were only about 20 instead of 50-60 samples in each group. No marked differences were observed between groups for the rest elements.

Table 3.
Judgement of 0, 1 and 2, 3 by neural networks

Clinical Diagnosis	Judgement with computation results					
	Learning samples			Non-learning samples		
	0, 1	2, 3	Total	0, 1	2, 3	Total
0, 1	44	10	54	43	11	54
0, 3	1	46	47	5	42	47
Total	45	56	101	48	53	101

Table 4.
Judgement of 2 and 3 by neural networks

Clinical Diagnosis	Judgement with computation results					
	Learning samples			Non-learning samples		
	2	3	Total	2	3	Total
2	26	0	26	22	4	26
3	2	19	21	5	16	21
Total	28	19	47	27	20	47

Table 5.
Accuracy and falsehood (%) of judgements

		Accuracy	Inaccuracy	False negative	False positive
Learning samples	01 /	89.11	10.89	18.52	2.13
Non-learning samples	23	84.16	15.84	20.37	10.63
Learning samples	2 /	95.74	4.26	9.52	0
Non-learning samples	3	80.85 (22+16)/47	19.15 (5+4)/47	23.81 5/21	14.38 4/26

In order to further investigate the correlation between esophageal disease development and elemental contents variation, we applied multivariate statistical treatment to the analytical data with statistically significant differences, i.e., Ca, Cl, K, Mg, Se, Zn (excluding Na). This method of principal coordinate analysis is based on point projection of N vectors from M dimensional space to a two dimensional space, preserving the inherent characteristic structure of the points, where N is the number of points determined, and M is the number of elements (here M is equal to 6) analyzed in each sample. This is achieved by fitting N points in the two dimensional space so that their interpoint Euclidean distances approximate the corresponding interpoint Euclidean distances in the M space. We normalized the data before calculation. The principal coordinates of the determined points are calculated from the Euclidean distance matrix by linear transformation. It is interesting to notice in Figure 1 that there are no differences between 0:1, 0:2 and 1:2 or between non-esophageal cancer groups, and that there seem to be differences between 0:3, 1:3 and 2:3 or between esophageal cancer and non-cancer groups. In the upper left region, the 4 groups mix among each other.

Principal coordinate analysis is a kind of non-learning pattern recognition. We then apply the adaptive pattern recognition and neural networks[8] to investigate the experimental data of Ca, Cl, K, Mg, Se and Zn. Half of the 202 samples were taken randomly and utilized their characteristic to establish the learning pattern (the samples thus taken are called learning samples) to recognize the remaining samples which are called the non-learning samples.

To make the effect of recognition more distinct, the recognition proceeds in two steps. First, we took 0, 1 as one group and 2, 3 as another. The judgement of 0, 1 and 2, 3 by neural networks is shown in Table 3. Furthermore, for recognition of 2 and 3, the judgement is shown in Table 4. The accuracy and falsehood (%) of judgement are shown in Table 5. It can be seen that the accuracy is greater than 80%.

The accuracy and falsehood percentages are calculated from Table 3 and Table 4. The fractions in the lower part of the bottom row show, as an example, how the percentages are calculated.

Conclusion

Fingernails from 4 groups of people, the normal control, light esophageal epitheliosis patients, severe esophageal epitheliosis patients, and esophageal cancer patients were analyzed for the elemental contents by INAA. The contents of Ca, Cl, K,Mg, Se and Zn show that there is no marked difference between normal control and light esophageal epitheliosis patients, that the contents show a tendency to increase in the order of light esophageal epitheliosis patients, severe esophageal epitheliosis patients and esophageal cancer patients as the esophageal disease is developing step by step into cancer. Principal coordinates (Figure 1) determined by multivariate statistical treatment show that there is no difference among normal control, light esophageal epitheliosis patients and severe esophageal epitheliosis patients but that differences exist between esophageal cancer and non-esophageal cancer. Neural network analysis shows that the accuracy of judgement by computation and calculation is greater than 80%.

It would be interesting to apply multivariate statistical treatment and the neural networks to the essential trace elements Se, Zn, Cr and Mn and to macroelements Cu, Cl, K and Mg separately.

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