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# Analysis of Trace Elements in the Hair of Volunteers Suffering from Naso-Pharyngeal Cancer

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## ABSTRACT

This article describes a study where the trace elements (TEs) of four groups of volunteers were analyzed. The volunteers were divided into four groups A, B, C, and D. Group A was made up of healthy subjects, group B was made up of volunteers who had just been diagnosed as having naso-pharyngeal cancer (NPC), group C was made up of volunteers who had been diagnosed as having NPC after 3 mo of treatment, and group D was made up of volunteers who had been diagnosed as having NPC after 6 mo of treatment. In all groups, 11 trace elements in hair were analyzed. Our study shows that the concentrations of zinc (Zn), copper (Cu), manganese (Mn), and cobalt (Co) in group B are less than that of group A, whereas the concentration of titanium (Ti) in group B is higher. Our results also show that the difference in the level of TEs between group A and the other groups is dramatically decreased as the time of the treatment is increased. This may be a reflection of successful treatment.

**Index Entries:** Trace element; naso-pharyngeal cancer; following patient's track; statistic analysis; robust statistics.

## INTRODUCTION

Trace elements (TEs) in human hair have been the subject of many investigations. It has been revealed that there is a correlation between

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TEs and diseases where human hair may record the information of the body status for some intervals of past time *(1–4)*.

From 1983–1987, the American MEDLINE database cited 3327 papers about the relationship between TEs and cancer. Among the most relevant 20 TEs, Se, Zn, and Cu have been most frequently looked at by previous investigators. Concentrations of Se, Zn, Cu, and Mn in the hair of cancerous patients are lower than those of healthy persons *(5)*. A similar result is found in naso-pharyngeal (NPC) patients *(6)*. In the last few years, both Zn and Cu have been studied and used to diagnose cancer *(7–12)*. Also, a pattern recognition analysis of TEs for NPC patients showed that the major contributing TEs in principal component analysis (PCA) are Zn, Cu, Fe, Mn, and Mg *(13)*.

Guangdong province of China has the highest incidence of NPC in the world. Since the 1970s, the scientists from Guangdong have done many studies to reveal the relationship between NPC and TEs, and some valuable information has been obtained *(1)*. Most recently, there have been some interesting findings on the relationship between NPC and TEs *(1,14–16)*.

In our study, 11 TEs, namely Zn, Ti, Cr, Mn, Fe, Co, Ni, Cu, As, Pb, and Sr, were analyzed in four groups of volunteers.

## SAMPLING

Hair samples were obtained from volunteers in a cancer hospital in the Guangdong province. In total there were 130 volunteers, and one sample was taken from each volunteer at the beginning. Thirty samples (23 males and 7 females) were taken for group A as the control samples. There were 100 samples in group B, which consisted of 81 male and 19 female volunteers. After 3 mo treatment to the NPC patients in group B, 53 samples (44 males and 9 females) were taken to make up group C. After 6 mo of treatment, another 23 samples (17 males and 6 females) were taken to make up group D.

About 1 g of hair was taken from the occipital region of the head, 0.5 cm from the scalp. Following immersion in 1% detergent solution, the hair sample was washed and rinsed three times with distilled water in an ultrasonic cleaner and then dried at 80°C for 4 h. It was then ashed at 400°C and digested in mixed acid ($HNO_3$:$HClO_4$ = 10:1). Yttrium was added to meet the internal standard. A drop of PVP solution was placed on polyester fiber film followed by 25 µL of target solution. After drying naturally, the target sample was obtained.

The target samples were fitted to a rack in the target chamber and bombarded with protons. The proton energy was 2.0 MeV, and the current of proton beam 15 nA. The energy resolving power of the Si(Li) detector was about 170 eV for $^{55}Fe$. The system was calibrated by using the first level standard materials of hair, peach leaf, and dong seng. The

detection limit was 0.1 ppm and 5% precision. The concentrations of Zn, Ti, Cr, Mn, Fe, Co, Ni, Cu, As, Pb, and Sr were determined.

## STATISTICS

### *Robust Statistics*

Despite the accuracy of the proton-induced X-ray emission (PIXE) technique, it is possible that some contamination may have occurred. As a result, the measurements are likely to result in excessively large readings or zero readings. Such readings were then discarded in our study. The discarded large value of the element concentration in group A was found to be 1.5 times larger than the "normal value" *(2)*.

The mean values and their standard deviation of the concentrations of 11 TEs for the four groups are shown in Table 1. In this table, the value $n$ in brackets indicates the valid counts of samples. The value of $P$ is the level of significance between the control group (A) and other groups.

### *Consistent Statistics*

In our studies, it is true that the patients in the second and third rediagnosis group (group C and group D) all have attended the initial diagnosis (group B), but some of the patients in the third diagnosis group have not attended the second diagnosis. In order to obtain reliable results, the patients included in the analysis should satisfy the demand of consistent statistics. This means that only those patients who have attended all three diagnoses should be included in the analysis. In our studies, there are 14 patients who satisfy the above criteria. Although the number of samples may not be enough to represent the result, the statistics were carried out as a comparison with the first method. The result is listed in Table 2.

Previous workers have shown that the concentrations of TEs in the hair vary according to sex and age *(1,2,15)*. Therefore, hair samples from male subjects in the age group of 30–50 were selected from the original samples and analyzed using the previous two statistical methods. The results are listed in Tables 3 and 4, respectively.

## ANALYSIS AND DISCUSSION

The analysis is based on the level of the significance of $a = 0.10$, which is used to indicate the difference between the NPC groups and group A.

Tables 1–4 show that during and after the medical treatment, the metabolism of patients' bodies seems to have a series of complicated changes, as well as the absorption and storage of TEs.

Table 1
Concentrations of TEs in Hair Samples of Healthy Persons and NPC Patients and Their α-Level of Significance

| TEs | Healthy Persons | NPC Patients | | | | | |
|---|---|---|---|---|---|---|---|
| | | Initial Treatment | | Second Treatment | | Third Treatment | |
| | $M^{[1]} \pm D^{[2]}(n)$ | $M \pm D(n^{[3]})$ | $P^{[4]}$ | $M \pm D(n)$ | P | $M \pm D(n)$ | P |
| Zn | 196.72±61.18(26) | 172.58±4.29(100) | <0.01 | 164.37±31.17(53) | <0.01 | 179.18±43.74(23) | >0.20 |
| Ti | 0.71 ± 0.65(25) | 1.84 ± 2.18(91) | <0.02 | 1.14±0.85(51) | <0.05 | 1.54 ± 2.47(22) | >0.10 |
| Cr | 0.09 ± 0.11(11) | 0.39 ± 0.42(22) | <0.01 | 0.20 ± 0.19(26) | <0.05 | 0.39 ± 0.29(8) | <0.01 |
| Mn | 3.53 ± 3.30(26) | 2.63 ± 5.86(97) | >0.40 | 1.91 ± 2.86(53) | <0.05 | 2.72 ± 3.71(23) | >0.40 |
| Fe | 10.98 ± 3.85(26) | 14.58± 8.34(100) | >0.10 | 12.73 ± 9.25(53) | >0.20 | 12.52± 8.20(23) | >0.20 |
| Co | 0.50 ± 0.20(26) | 0.42 ± 0.50(81) | >0.40 | 0.25 ± 0.20(40) | <0.01 | 0.33 ± 0.29(19) | <0.05 |
| Ni | 0.77 ± 0.44(26) | 0.80 ± 1.06(86) | >0.80 | 0.58 ± 0.57(44) | >0.10 | 0.28 ± 0.28(20) | <0.01 |
| Cu | 13.36 ± 3.53(26) | 9.96± 2.16(100) | <0.01 | 9.41 ± 2.54(53) | <0.01 | 9.58 ± 2.13(23) | <0.01 |
| As | 1.09 ± 0.61(26) | 1.73 ± 1.75(79) | <0.10 | 1.50 ± 0.82(43) | <0.05 | 0.58 ± 0.25(17) | <0.01 |
| Pb | 2.89 ± 2.06(25) | 3.54 ± 5.35(81) | >0.40 | 5.84 ± 10.09(47) | >0.10 | 0.69 ± 0.43(13) | <0.01 |
| Sr | 4.25 ± 3.92(22) | 6.17 ± 6.12(53) | >0.10 | 5.20 ± 4.03(25) | >0.40 | 2.73 ± 3.88(12) | >0.20 |

[1]M—arithmetic mean.
[2]SD—standard deviation.
[3]*n*—valid samples number.
[4]*P*—the α-level of significance of Student's *t*-test about patients and healthy persons.

Table 2
Concentrations of TEs in Hair Samples of Healthy Persons and NPC Patients Attending All Three Treatments and Their α-Level of Significance

| TEs | Healthy Persons | NPC Patients | | | | | |
|---|---|---|---|---|---|---|---|
| | | Initial Treatment | | Second Treatment | | Third Treatment | |
| | $M \pm D(n)$ | $M \pm D(n)$ | P | $M \pm D(n)$ | P | $M \pm D(n)$ | P |
| Zn | 196.72 ±61.18(26) | 172.59 ± 9.65(14) | >0.10 | 164.22 ± 7.80(14) | <0.10 | 176.67 ± 0.57(14) | >0.20 |
| Ti | 0.71 ± 0.65(25) | 1.88 ± 1.62(11) | <0.01 | 1.14 ± 0.48(13) | <0.05 | 1.20 ± 1.11(13) | <0.10 |
| Cr | 0.09 ± 0.11(11) | 0.08 ± 0.03(3) | >0.80 | 0.16 ± 0.16(9) | >0.10 | 0.34 ± 0.16(3) | <0.01 |
| Mn | 3.53 ± 3.30(26) | 3.29 ± 6.20(13) | >0.80 | 1.97 ± 2.85(14) | >0.10 | 3.41 ± 4.70(14) | >0.80 |
| Fe | 10.98 ± 3.85(26) | 10.71 ± 4.80(14) | >0.80 | 11.48 ± 3.52(14) | >0.60 | 12.04 ± 4 .21(14) | >0.40 |
| Co | 0.50 ± 0.20(26) | 0.33 ± 0.31(11) | <0.10 | 0.18 ± 0.14(11) | <0.01 | 0.34 ± 0.28(10) | <0.10 |
| Ni | 0.77 ± 0.44(26) | 0.50 ± 0.43(14) | <0.10 | 0.51 ± 0.54(13) | >0.10 | 0.29 ± 0.31(11) | <0.01 |
| Cu | 13.36 ± 3.53(26) | 9.48 ± 1.71(14) | <0.01 | 9.32 ± 1.47(14) | <0.01 | 9.55 ± 2.19(14) | <0.01 |
| As | 1.09 ± 0.61(26) | 1.09 ± 0.84(11) | >0.80 | 1.27 ± 0.62(12) | >0.40 | 0.61 ± 0.24(9) | <0.05 |
| Pb | 2.89 ± 2.06(25) | 1.94 ± 1.55(13) | >0.10 | 4.33 ± 4.12(11) | >0.10 | 0.74 ± 0.34(8) | <0.01 |
| Sr | 4.25 ± 3.92(22) | 6.19 ± 3.94(6) | >0.20 | 6.24 ± 4.28(6) | >0.20 | 1.67 ± 1.51(8) | <0.01 |

Figure 1 shows that in patients who have had 6 mo or more of treatment, the concentrations of Zn, Ti, Co, As, and Mn have the tendency to return to a normal healthy level, and this is more noticeable in the curve of Table 4 (sex and age are considered). However, the concentrations of Ni, Cr, and Pb have notable fluctuations against the healthy level. In addition, there is no dramatic change in the concentration of Cu.

Table 3
Concentrations of TEs in Hair Samples of Healthy Persons and NPC Male Patients Aged from 30–50 yr and Their α-Level of Significance

| TEs | Healthy Persons | NPC Patients | | | | | |
|---|---|---|---|---|---|---|---|
| | | Initial Treatment | | Second Treatment | | Third Treatment | |
| | M ± D(n) | M ± D(n) | P | M ± D(n) | P | M ± D(n) | P |
| Zn | 186.70 ± 0.98(17) | 171.62 ± 5.13(47) | <0.10 | 168.87 ± 2.01(27) | >0.10 | 187.11 ± 1.55(15) | >0.80 |
| Ti | 0.62 ± 0.68(17) | 1.15 ± 0.87(43) | <0.05 | 0.93 ± 0.62(26) | >0.10 | 1.09 ± 1.12(14) | >0.10 |
| Cr | 0.08 ± 0.11(12) | 0.33 ± 0.38(9) | <0.05 | 0.15 ± 0.17(14) | >0.20 | 0.24 ± 0.11(4) | <0.05 |
| Mn | 2.34 ± 2.30(17) | 1.15 ± 1.52(45) | <0.02 | 1.10 ± 0.98(27) | <0.02 | 1.81 ± 2.32(15) | >0.40 |
| Fe | 10.66 ± 4.12(17) | 11.17 ± 5.29(47) | >0.60 | 10.99 ± 7.18(27) | >0.80 | 11.45 ± 4.36(15) | >0.40 |
| Co | 0.46 ± 0.16(17) | 0.22 ± 0.24(37) | <0.01 | 0.18 ± 0.13(20) | <0.01 | 0.28 ± 0.26(11) | <0.05 |
| Ni | 0.61 ± 0.26(17) | 0.48 ± 0.53(38) | >0.20 | 0.56 ± 0.56(22) | >0.60 | 0.28 ± 0.28(13) | <0.01 |
| Cu | 13.67 ± 3.74(17) | 10.16 ± 2.54(47) | <0.01 | 9.55 ± 2.98(27) | <0.01 | 10.13 ± 2.36(15) | <0.01 |
| As | 0.91 ± 0.53(17) | 1.96 ± 2.10(41) | <0.05 | 1.34 ± 0.79(23) | <0.10 | 0.66 ± 0.24(11) | >0.10 |
| Pb | 3.07 ± 1.79(17) | 2.85 ± 3.99(38) | <0.05 | 5.99 ± 2.11(24) | >0.20 | 0.63 ± 0.35(7) | <0.01 |
| Sr | 3.09 ± 2.51(14) | 2.39 ± 2.32(20) | >0.40 | 3.80 ± 3.38(11) | >0.40 | 1.62 ± 1.49(6) | >0.20 |

Table 4
Concentrations of TEs in Hair Samples of Healthy Persons and NPC Male Patients Aged from 30–50 yr Attending All Three Treatments and Their α-Level of Significance

| TEs | Healthy Persons | NPC Patients | | | | | |
|---|---|---|---|---|---|---|---|
| | | Initial Treatment | | Second Treatment | | Third Treatment | |
| | M ± D(n) | M ± D(n) | P | M ± D(n) | P | M ± D(n) | P |
| Zn | 186.70 ± 0.98(17) | 178.75 ± 4.01(9) | >0.40 | 173.92 ± 3.66(9) | >0.20 | 186.00 ± 0.29(9) | >0.80 |
| Ti | 0.62 ± 0.68(17) | 1.19 ± 0.95(7) | >0.10 | 0.97 ± 0.35(9) | >0.10 | 1.23 ± 1.32(8) | >0.10 |
| Cr | 0.08 ± 0.11(12) | 0.06 ± 0.00(1) | >0.80 | 0.11 ± 0.06(6) | >0.40 | 0.31 ± 0.00(1) | <0.10 |
| Mn | 2.34 ± 2.30(17) | 1.61 ± 2.88(8) | >0.40 | 1.16 ± 1.36(9) | >0.10 | 2.35 ± 2.90(9) | >0.80 |
| Fe | 10.66 ± 4.12(17) | 9.35 ± 3.85(9) | >0.40 | 10.98 ± 2.26(9) | >0.60 | 12.31 ± 4.85(9) | >0.20 |
| Co | 0.46 ± 0.16(17) | 0.29 ± 0.36(6) | >0.10 | 0.17 ± 0.13(7) | <0.01 | 0.36 ± 0.33(6) | >0.20 |
| Ni | 0.61 ± 0.26(17) | 0.39 ± 0.36(9) | <0.10 | 0.69 ± 0.61(8) | >0.60 | 0.34 ± 0.35(8) | <0.05 |
| Cu | 13.67 ± 3.74(17) | 9.59 ± 1.68(9) | <0.01 | 9.66 ± 1.39(9) | <0.01 | 9.81 ± 2.63(9) | <0.02 |
| As | 0.91 ± 0.53(17) | 0.82 ± 0.68(8) | >0.60 | 1.11 ± 0.59(8) | >0.40 | 0.60 ± 0.25(6) | >0.10 |
| Pb | 3.07 ± 1.79(17) | 1.18 ± 0.38(8) | <0.10 | 3.28 ± 2.00(6) | >0.80 | 0.56 ± 0.40(3) | <0.01 |
| Sr | 3.09 ± 2.51(14) | 3.45 ± 1.83(3) | >0.80 | 5.54 ± 4.33(3) | >0.10 | 1.81 ± 1.88(4) | >0.20 |

Zn, Cu, and Mn are the essential trace elements in the human body. They can enhance the function of immunity and prevent cancer, whereas metals As, Cr, Ni, Be, and Pb are carcinogens (*2*). Taking medication with Zn or Zn/Se enhances the immune system and inhibits the spread of cancerous cells (*17*).

Our investigation shows that initially the concentrations of Zn, Cu, Mn, and Co in groups B, C, and D are lower than those of group A. However, after successive treatments, these elements tend to return to

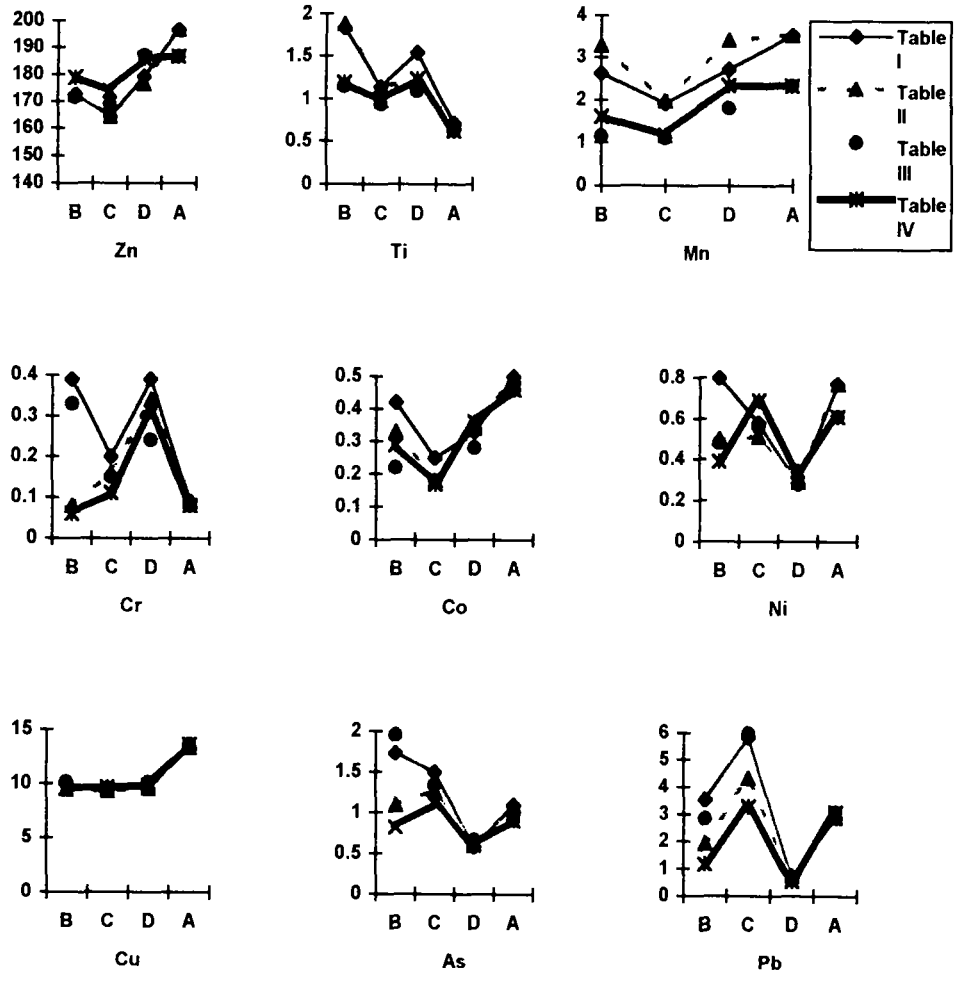


Fig. 1. The tracking of TEs in hair samples of NPC patients.

the normal level, except for the Cu. Also the concentrations of Ti and As, which were higher than the normal level, were back to their normal level after the treatments.

From above, it is clear that the concentrations of TEs in the hair can reflect the general level of health in a human. By tracking the concentrations of TEs, certain changes of body status can therefore be monitored.

However, the human body is a complex system. The variation in the concentrations of TEs in the human body are also very complicated. Although single-element analysis and simple multielement analysis on large numbers of subjects could certainly provide some useful information, the application of this technique to individual patients still needs further investigation. It would be valuable to elucidate the relationship between TEs and human body in general, rather than only to analyze the difference of the levels TEs between healthy persons and patients.

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