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# Sequential Multielement Analysis of Cd, Cr, Ni, and Pb in Human Tissues by Inductively Coupled Plasma Spectrometry

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The role of trace metals in cancer has been the subject of conjecture, and reports of different authors are often conflicting and contradictory. Attention is focused primarily on a) four metals (As, Cd, Cr, and Ni) that have been implicated as human carcinogens on the basis of epidemiological investigations, and b) compounds of nine metals (Be, Cd, Co, Cr, Fe, Ni, Pb, Ti, and Zn) that have been reported to induce cancers in experimental animals (Sunderman 1978; Frust, 1977; Aitio and Tomatis 1991; Magos 1991).

Most toxic elements affect multiple organ systems, with specific biochemical processes and (or) organelles as targets. Their toxic effect usually involves an interaction between the free metal ion and the specific target protein. Cells or organs involved in the transport of trace metals, i.e., muscle, liver, renal tubular, or gastrointestinal cells, are particularly susceptible to toxicity. Most of these elements are concentrated intracellulary; heavy metals deposit in tissue after exposure. There are three principal binding sites for metals on the nucleic acid molecules: the phosphate groups, the heterocyclic bases and the 2-OH groups in the case of RNA (Eichhorn et al 1979).

It is generally accepted that a combination of environmental factors and life-style contribute to about 70-90% of cancer cases. The most important factors are smoking (about 30-40%) and food (30-50%) whereas environmental pollution contributes only a small percentage (1-3%). Augustin and Zejda (1991) found that geochemical factors in the environment such as radioactive elements and heavy metals were possible causes of the high incidence of cancers in some districts in Czechoslovakia.

In view of conflicting reports in the literature, the concentrations of Cd, Cr, Ni and Pb were determined in the kidney and brain of Saudi patients with malignant or benign tumors.

## MATERIALS AND METHODS:

The study included 76 Saudi patients with histological diagnosed malignant or benign tumors who were examined at the Department of Pathology, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia. Biopsy tissue samples of kidney and brain tumors were frozen in the liquid nitrogen at the Histopathology laboratory, and then transferred to Tissue Bank Laboratory at the Research Centre where frozen sections were prepared using cryostat microtome. and stored at -70°C. Profiles of the 120 cases examined are shown in Table 1.

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Table 1. Disease, Sex, and Age of the studied patients.

| Disease | No. of cases | Sex (M/F) | Mean age and range (yr) |
|---|---|---|---|
| Malignant Kidney Tumor Group | 59 | 29/30 | 41.7 (7 months-88) |
| Benign Kidney Tumor Group | 4 | 3/1 | 19.1 (10 months-51) |
| Malignant Brain Tumor Group | 6 | 4/2 | 27.7 (6-55) |
| Benign Brain Tumor Group | 7 | 3/4 | 34.5 (3-72) |

Tissue samples were thawed at room temperature and approximately 0.3-0.59 of wet tumor tissues were digested in Teflon vessels with 3 ml concentrated "selectipur" nitric acid (E. Merck, D-6100 Darmstadt, Frankfurter Strasse 250 Germany) for one hour at 150°C. Furthermore, after adding 2 ml of 30% hydrogen peroxide (Fisher Scientific Co., A.C.S.), the sample solutions were heated at 100°C for half an hour. After digestion, the samples were allowed to cool to room temperature. The clear supernatant was transferred to polypropylene tubes and one volume of digested sample (usually 1 ml) was mixed with four volume of deionised water. Metal contents were expressed as ug/g wet weight.

All metal analyses were performed using an Inductively Coupled Plasma Spectrometry- ICP-701 (ATI Unicam, Cambridge, UK) with 221 XL Gilson autosampler (Gilson, 95400 Villiers le Bel, France). Instrument operating conditions are given in Table 2. Instrument start-up and optimization was carried out as detailed in the operating manual. Spectral lines for the tested elements were scanned to check for spectral interference. Wavelengths to be used for the analysis are given in Table 3. The high temperature of the ICP source causes destruction of sample and excitation of the constituent atoms. The resulting spectrum is separated into the individual wavelengths with a high resolution monochromator and intensities measured using a photoelectric device. Five determinations were made on all samples. If the range exceeded 10% of the mean, further replicates were performed.

Table 2. ICP-701 parameters for analysis of elements in tissue sample.

| | |
|---|---|
| Power: | 1 Kw |
| Coolant gas flow: | 12 L/min |
| Auxiliary gas flow: | 0 L/min |
| Nebuliser pressure: | 35 L/min |
| Sample introduction rate: | 1 mil/min |

Table 3. The selected wavelength for the tested elements.

| Elements | Wavelength (nm) |
|---|---|
| Cd | 214.4 |
| Cr | 205.5 |
| Ni | 221.6 |
| Pb | 220.3 |

For this study standard stock solutions (1000 ug/ml) were used to prepare multielement calibration standard solution as listed in Table 4. Dilution was carried out with deionised water to a final solution in 7% nitric acid.

Table 4. The concentration range of the multielement calibration solution.

| Calibration Standard Solution | Concentration Range (ug/ml) |
|---|---|
| Cd | 0.0025-0.04 |
| Pb | 0.02-0.16 |
| Cr&Ni | 0.005-0.08 |

A six point calibration curve was established for each element by linear regression analysis of emission intensity versus standard concentration. There was a good linear relation between emission intensity and standard concentration of the screened element. Linearity was evaluated by calculating the correlation coefficient as shown in Table 5.

Table 5. Intercept, slope and the correlation coefficient for the calibration curve of each element.

| Element | Intercept | Slope | Correlation coefficient |
|---|---|---|---|
| Cd | 1.34021e-6 | 1.39233e-3 | 0.999651 |
| Cr | 9.09926e-6 | 5.13016-3 | 0.999142 |
| Ni | 9.000994e-6 | -2.30059-3 | 0.999539 |
| Pb | 3.12012e-5 | -2.55319e-2 | 0.999547 |

The accuracy of the method was determined by measuring the recovery of Cd, Cr, Ni, and Pb added to tissue samples before digestion (Table 6). These spiked tissue samples were run with the test samples using the same analytical procedure. Detection limits for the elements in this study were 0.00097 ug/ml for Cd, 0.0038 ug/ml for Pb, 0.00085 ug/ml for Ni, and 0.00092 ug/ml for Cr.

Table 6. Recovery of metal from the spiked tissue amples.

| Metal | Spiked value (ug) | Determined Value (ug) | Recovery (%) |
|---|---|---|---|
| Cd | 2 | 1.79 | 89.5 |
| | 5 | 4.975 | 99.5 |
| | 10 | 10.613 | 106.1 |
| Cr | 2 | 1.82 | 91.0 |
| | 5 | 4.675 | 93.5 |
| | 10 | 10.075 | 100.8 |
| Ni | 2 | 1.91 | 95.5 |
| | 5 | 5.15 | 103.0 |
| | 10 | 10.619 | 106.2 |
| Pb | 2 | 1.98 | 99.0 |
| | 5 | 5.138 | 102.8 |
| | 10 | 10.013 | 100.1 |

Due to non-Gasussian distribution pattern of the tested metals, nonparametric statistical tests were employed including Wilcoxon matched-pairs test and Kruskal-Wall is one way analysis of variance using Statgraphics software (1992).

## RESULTS AND DISCUSSION

Table 7 shows the results expressed as ug/g wet weight of Cd, Cr, Ni and Pb in kidney and brain organs for subjects with malignant and benign tumors. We subjected the data obtained for Cd, Cr, Ni and Pb to Wilcoxon matched-pairs test, and found that only the average ranks for Cd in the malignant kidney tumor group (n=59) was significantly higher ($p<0.05$) than the malignant brain tumor group (n = 6). The highest ratio for malignant/benign kidney tumor groups was 41.9 for Cd. No significant difference was found between the two benign tumor groups. As previously observed (Sumino et al 1975), Cd was highest in kidney as it is known that Cd is rapidly cleared from the blood and accumulates in the kidney and liver, which contain approximately two-thirds of the total body burden. On the other hand, the distribution of Cr, Ni and Pb in the kidney, and brain are homogenous. For the general population, the two main sources of Cd exposure are diet and tobacco smoking (WHO, 1988). A study by Al-Saleh and Coate (1993) demonstrated that blood Cd was significantly higher in Saudi smokers than nonsmokers.

Table 7. Concentrations (ug/g wet tissue) of Cd, Cr, Ni and Pb in the screened tumor groups.

| Disease Group | Cd | Cr | Ni | Pb |
|---|---|---|---|---|
| Malignant Kidney Tumor Group (n = 59) | | | | |
| Mean ± SD | 0.59 ± 1.7 | 0.14 ± 0.13 | 0.15 ± 0.16 | 0.41 ± 0.55 |
| Median | 0.061 | 0.085 | 0.14 | 0.15 |
| Range | 0-10.47 | 0-0.55 | 0-0.65 | 0-2.4 |
| Benign Kidney Tumor Group (n = 4) | | | | |
| Mean ± SD | 0.014 ± 0.029 | 0.16 ± 0.17 | 0.14 ± 0.083 | 0.32 ± 0.42 |
| Median | 0 | 0.14 | 0.15 | 0.16 |
| Range | 0-0.057 | 0-0.37 | 0-0.65 | 0-0.94 |
| Malignant Brain Tumor Group (n = 6) | | | | |
| Mean ± SD | 0.017 ± 0.033 | 0.2 ± 0.25 | 0.14 ± 0.17 | 0.3 ± 0.37 |
| Median | 0 | 0.11 | 0.064 | 0.146 |
| Range | 0-0.082 | 0-0.67 | 0-0.39 | 0-0.86 |
| Benign Brain Tumor Group (n = 7) | | | | 0.73± 0.69 (0-1.96) |
| Mean ± SD | 0.068 ± 0.1 | 0.16 ± 0.14 | 0.067 ± 0.091 | 0.73± 0.69 |
| Median | 0.00096 | 0.14 | 0.042 | 0.82 |
| Range | 0-0.26 | 0-0.43 | 0-0.25 | 0-1.96 |

No significant differences in Cd, Cr, Ni and Pb concentrations in both kidney and brain malignant or benign tumor groups were observed in relation to sex, age and geographical distribution.

The comparison of the data with other studies is often complicated due to a number of factors involved such as sample collection, expression of values whether wet weight or dry weight, sampling site and analytical procedure. As demonstrated in Table 8, Takacs & Tatar (1987)

reported much higher Cd concentrations from 1072 kidney autopsies than our Cd concentrations in both malignant and benign kidney tumor groups. This may be due to the difference in the screened populations or other analytical factors such as the site of sampling. Bush et al (1995) found intraorgan variability for heavy metals in different areas of kidney and brain. Our values for brain Cd in the both malignant and benign tumor groups, however, are more or less similar to those obtained by Schuhmacher et al (1993).

Table 8. Concentrations of Cd, Cr, Ni and Pb (ug/g) in kidney and brain tissues from other studies

| Element | Kidney | Brain |
|---|---|---|
| Cd | 11.79 ± 10.0 (Takacs & Tatar 1987) | 0.03 ± 0.02*<br>0.01 ± 0.01**<br>(Schumacher et al 1993) |
| Cr | 0.0015***<br>(Lyon et al 1989) | ND |
| Ni | 0.009 ± 0.006<br>(Rezuk et al 1989) | ND |
| Pb | 0.24 ± 0.92<br>(Takacs & Tatar 1987) | 0.09 ± 0.08*<br>0.06 ± 0.02**<br>(Schumacher et al 1993) |

* Mean values for males.

** Mean values for females.

*** Median value.

However, the authors did find significant difference between males and females which our data did not confirm. In contrast, brain Pb concentrations in both malignant and benign tumor groups were much higher than those obtained by Schuhmacher et al (1993) perhaps owing to differences in the analytical techniques or the site of sampling (Bush et al 1995).
On the other hand, the values for the kidney Cr, Ni, and Pb in both malignant and benign tumor group were higher than those reported by others (Takacs & Tatar 1987; Lyon et al 19; Rezuke et al 1987). The cause for this increase is not clear for us at this stage.

Additionally, we were unable to find any reported values for the concentrations of Cr and Ni in brain tissue.

In conclusion, the increased concentrations of Cd in patients with malignant kidney tumors may reflect disturbed metabolic and biochemical activities in the malignant cells. Biologically significant interaction can occur among essential and toxic metals that share similar chemical properties as they can substitute for each other in biochemical reactions involving metalloproteins or metalloenzymes (Hill and Matrone, 1970). The kidney is a major site of antagonistic interaction between essential trace metals and Cd, and a target organ for Cd toxicity (Friberg et al 1985; Bremner 1987). Recent study by Panemangalore (1993) suggest that dietary imbalances of copper and zinc could increase the retention of Cd and slow the induction of metallothionein, which could enhance the risk of Cd toxicity to cellular integrity. Therefore, further research is needed to determine the status of both toxic and essential metals in Saudi patients with malignant and benign tumors.

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