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Variation in the Distribution of Trace Elements in Renal Cell Carcinoma

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Abstract The development of cancer is a complex, multistage process during which a normal cell undergoes genetic changes that result in phenotypic alterations and in the acquisition of the ability to invade other sites. Inductively coupled plasma optical emission spectroscopy was used to estimate the contents of Al, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, P, Pb, and Zn in healthy kidney and renal cell carcinoma (RCC), and significant differences were found for all elements. Along with the progression of the malignant disease, a progressive decrease of Cd and K was observed. In fact, for Cd, the concentration in stage T4 was 263.9 times lower than in stage T1, and for K, the concentration in stage T4 was 1.73 times lower than in stage T1. Progressive accumulation was detected for P, Pb, and Zn in stage T4. For P, the concentration in stage T4 was 11.1 times higher than in stage T1; for Pb, the concentration in stage T4 was 232.7 times higher than in T1; and for Zn, the concentration in T4 was 8.452 times higher than in T1. This study highlights the marked differences in the concentrations of selected trace metals in different malignant tumor stages. These findings indicate that some trace metals may play important roles in the pathogenesis of RCC.

Keywords Trace elements · Kidney · Carcinoma · RCC · ICP-OES

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Introduction

The incidence of renal cell carcinoma (RCC) has nearly doubled over the past two decades, currently comprising about 2% of all human malignancies [1]. Across the world, more than 100,000 people die annually from RCC [2]. The 5-year survival for patients with RCC has increased twofold over the past 50 years to almost 62%; this is likely due to earlier detection of the tumor, which has led to a prompter start of oncologic treatment [3]. However, the 5-year survival rate for patients with metastatic disease continues to remains dismal at <2% [4].

Cigarette smoking and obesity are the most consistently established causal risk factors, accounting for more than 20% and 30% of renal cell cancers, respectively [5]. Other studies have identified additional risk factors, including hypertension, exposure to asbestos, petroleum products, and heavy metals [6].

The mineral constituents that occur in human or animal organisms in low amounts are called trace elements. These elements enter the human organism by eating, drinking, and breathing. Some trace elements are of physiological importance, either essential or toxic. Essential trace elements have four major functions as stabilizers, elements of structure, essential elements for hormonal function, and enzyme cofactors. Imbalance in the composition of trace metals, recognized to be essential to normal homeostasis, besides accumulation of potentially toxic and nonessential trace metals, may cause disease. Furthermore, it may be expected that a deficiency of trace metals as cofactors of enzymes could impair the host's resistance against carcinogenic stress [7]. Information about trace elements as risk factors for RCC is limited but suggesting that Cd and Ni play a major role in the development of RCC [8].

The aim of this study was to examine the concentrations of trace elements in normal kidney as well as in benign and malignant renal tumor tissues.

Materials and Methods

Kidney Samples

In this study, we examined 50 RCC and 20 normal kidney samples, all of them fixed in formaldehyde. The tumor samples were obtained from the Pathology Department of the Federal University of São Paulo, Brazil, and the normal kidney tissues were obtained from Surgery Department of University of São Paulo. The normal kidney samples were taken cortically because RCC originates from proximal tubuli cells. Both the patients and the controls lived in São Paulo, Brazil.

Clinical Data

A total of 50 samples were taken from patients of different gender, age, and lifestyle. The mean age of the study group was 60.5±13.7 years and that of the control group was 72±10.4 years. Most of the cancer samples were classified as clear cell carcinomas. All RCC patients were histologically diagnosed, and the tumors were staged according to the 1997 TNM classification system [9]. Thirteen samples of T1, 13 of T2, 12 of T3, and 12 of T4 were studied.

Preparation of Samples

High-purity deionized water (resistivity 18.2 MΩ cm) obtained by a Milli-Q® water purification system (Millipore, Bedford, MA, USA) was used throughout the study.

Analytical reagent grade HNO_3 (Merck, Rio de Janeiro, Brazil) was distilled in quartz sub-boiling stills (Kürner, Rosenheim, Germany). All solutions were stored in polyethylene bottles. Autosampler cups, plastic bottles, and glassware were cleaned by soaking in 1.40 mol/L HNO_3 during 24 h, rinsing five times with high-purity water, and dried and stored in a class 100 laminar flow hood. Stock solutions (1,000 mg/L) were prepared from spectrographically pure reagents (Johnson and Matthey, Royston, UK) in diluted HNO_3. Analytical calibration solutions were prepared by suitable dilution of stock solutions in 0.028 mol/L HNO_3. All operations were performed on a clean bench.

Samples were decomposed in triplicate by using a microwave-assisted method in closed vessels (Milestone, Ethos 1600) and the following procedure: masses between 50 and 500 mg of dried and ground material were accurately weighed in TFM® vessels of the microwave oven and then 6.0 ml of 4.2 mol/L HNO_3 plus 2.0 mL of 30% hydrogen peroxide (*v*/*v*) were added. After decomposition, the TFM® microwave vessels were cooled, the digests were transferred to volumetric flasks, and the volume was completed to 25 mL with water. The microwave oven operational parameters are shown in Table 1. For analyte determinations, an inductively coupled plasma optical emission spectroscopy dual view spectrometer (Optima 3000 XL, Perkin Elmer) was used.

Statistical Analysis

The concentrations of trace elements in normal kidney and in benign and malignant renal tumor tissues were statistically analyzed. For both the study and the control groups, arithmetic mean and standard deviation were recorded. Statistical significance was determined by using the *F* test and Student's *t* test.

Results

Quantitative determination was performed for 13 elements, and the values are expressed in micrograms per gram. The mean profile of elements in RCC tissue compared to healthy kidney cortex is shown in Table 2. Almost all trace elements showed a significant difference between healthy kidney and RCC. The most relevant difference was a decrease detected in Cd, K, and Na in RCC. A significant accumulation of some trace metals, such as Al, P, Pb, and Zn, was also observed in RCC. No statistically significant difference was found for Cu and Mn.

A possible correlation between the malignant disease stages, described as T1, T2, T3, and T4, and trace metal concentrations was analyzed for all trace metals, and the distribution is shown in Fig. 1.

Along with the progression of the malignant disease, a progressive decrease of Cd and K was observed (Fig. 2). In fact, for Cd, the concentration in stage T4 was 263.9 times lower

Table 1 Microwave Heating Program for Sample Digestion[a]

Step	Temperature (°C)	Power (W)	Time (min)
1	160	1,000	3
2	160	0	2
3	230	1,000	5
4	230	1,000	15

[a] Maximum external vessel temperature at 90°C

Table 2 Comparison of Trace Element Concentration (μg/g) in 50 RCC Samples and 20 Normal Kidney Samples (Cortex)

Element	Group	Arithmetic mean ± SD	Healthy kidney cortex vs RCC
Al	Heealthy kidney cortex	<0.046	<0.01
	RCC	8.11±0.07	
Ca	Healthy kidney cortex	329.71±3.03	<0.01
	RCC	511.85±0.93	
Cd	Healthy kidney cortex	14.09±0.08	<0.01
	RCC	2.26±0.017	
Cr	Healthy kidney cortex	<0.005	<0.05
	RCC	1.26±0.02	
Cu	Healthy kidney cortex	8.30±0.09	NS
	RCC	8.0±0.017	
Fe	Healthy kidney cortex	319.60±2.19	<0.01
	RCC	248.16±0.59	
K	Healthy kidney cortex	163.43±3.57	<0.05
	RCC	50.07±0.05	
Mg	Healthy kidney cortex	99.34±1.96	<0.01
	RCC	65.71±0.14	
Mn	Healthy kidney cortex	0.92±0.004	NS
	RCC	0.98±0.006	
Na	Healthy kidney cortex	16,578.05±72.84	<0.001
	RCC	105.01±1.31	
P	Healthy kidney cortex	43.01±0.31	<0.001
	RCC	629.96±1.87	
Pb	Healthy kidney cortex	<0.05	<0.05
	RCC	1.74±0.02	
Zn	Healthy kidney cortex	<0.07	<0.001
	RCC	248.99±3.79	

NS Statistical difference is not significant

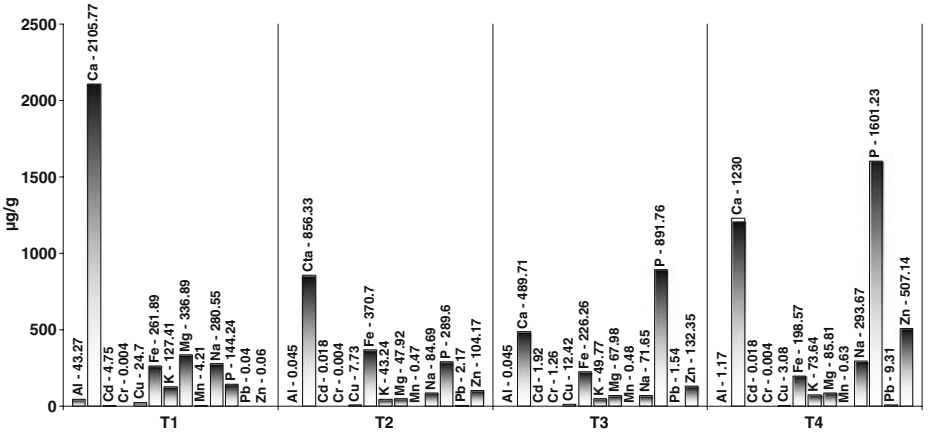

Fig. 1 Concentration of elements depending on the tumor stage

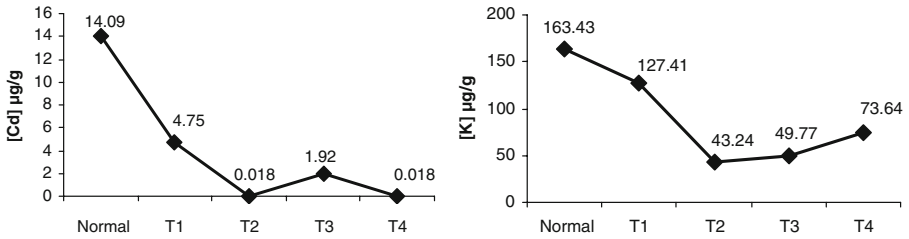

Fig. 2 Concentrations of Cd and K for normal kidney and tumor malignant stages. The mean value of each group sample is shown at the *curves*. The coefficient of variation was lower than 1% for all determinations

than in stage T1, and for K, the concentration in stage T4 was 1.73 times lower than in stage T1.

Progressive accumulation was detected for P, Pb, and Zn in stage T4 (Fig. 3). For P, the concentration in stage T4 was 11.1 times higher than in stage T1; for Pb, the concentration in stage T4 was 232.7 times higher than in T1; and for Zn, the concentration in T4 was 8.452 times higher than in T1.

Discussion

Knowledge about RCC has increased exponentially over the last decades. A clear understanding of RCC is of utmost importance to prevent the disease and improve the outcomes. Large epidemiologic studies have identified cigarette smoking, chemical agents, obesity, hypertension, and end-stage renal disease as risk factors associated with RCC. The identification and confirmation of risk factors may be projected into preventive strategies [10].

It is known that trace elements play an important role in a number of biological processes. These include the activation or inhibition of enzymatic reactions, competition

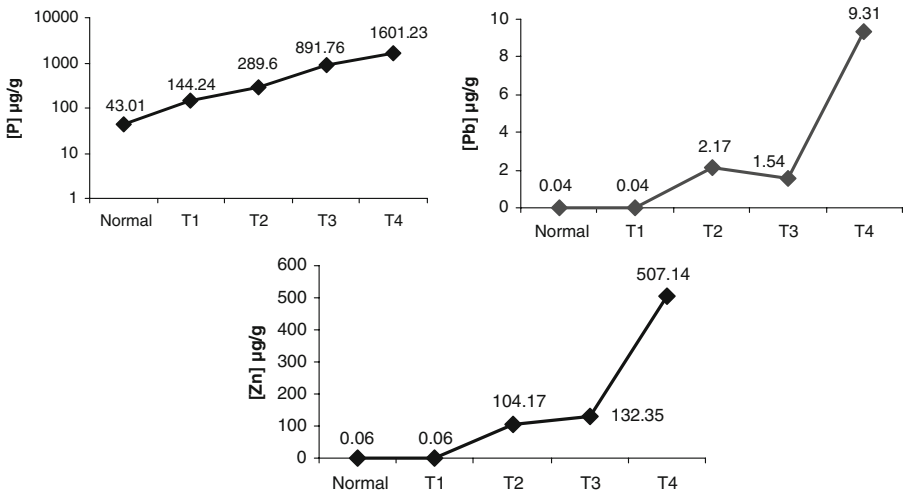

Fig. 3 Concentrations of P, Pb, and Zn for normal kidney and tumor malignant stages. The mean value of each group sample is shown at the *curves*. The coefficient of variation was lower than 1% for all determinations

between elements and metal proteins for binding positions, and modifications in the permeability of cellular membranes. These elements may also influence carcinogenic processes. Determining the trace element concentrations in healthy and neoplastic tissues might help in the establishment of a diagnosis and of its etiology, as well as in the prognosis of the disease [11].

Our findings showed that Cd, Fe, K, Na, and Mg were decreased in the neoplastic mass. Lower concentrations of Cd and Fe in RCC tissue were previously reported by Dobrowolski et al. [12]. In human prostate cancer, Drash et al. [13] demonstrated that the excessive accumulation of Cd in the prostates of smokers along with suboptimal Se intakes could explain why smokers develop more aggressive and lethal forms of prostate cancer than nonsmokers. Our results regarding K in the cancerous kidney samples are in disagreement with the data reported by Dobrowolski et al. [12] and Al-Ebraheem et al. [14]. In another study, Mg deficiency, induced in rats with palpable mammary adenocarcinomas, decreased tumor growth [15]. This effect has recently been confirmed in athymic nude mice, made Mg-deficient before and after inoculation with a human mammary cancer cell line [16].

Our data indicate significantly elevated Al, Ca, Cr, P, Pb, and Zn levels in tumor tissue compared to healthy kidney. Al is a genotoxic element that is bound by DNA and has been shown to be carcinogenic in animal models [17]. Cr exposure has been associated with adverse health effects. At the genomic level, Cr genotoxicity manifests as gene mutations, several types of DNA lesions, and inhibition of macromolecular synthesis. At the cellular level, Cr exposure may lead to cell cycle arrest, apoptosis, premature terminal growth arrest, or neoplastic transformation [18]. Higher Pb contents in dietary intake were linked to certain types of cancer, including RCC [19]. Pb and Cd demonstrated to abolish the anticarcinogenic effects of Se on mammary tumor development and growth in mouse mammary tumor virus-infected female mice [20, 21]. These studies revealed the need to consider the interactions of Se with other trace elements in discussions of its mechanism on anticarcinogenic action. Some studies on experimental animals such as mice suggested a relationship between higher Cr and Zn concentrations and accelerated tumor growth [19, 22–24].

The present study revealed marked differences in the concentrations of selected trace metals in different malignant tumor stages. These findings indicate that some trace metals may play important roles in the pathogenesis of RCC. In conclusion, research in this field is disclosing differences in cellular biology among the different malignant stages that could lead to a better understanding of the etiology of tumors and result in valuable practical information that can be applied to clinical medicine.

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