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Increased urinary zinc excretion in cancer patients is linked to immune activation and renal tubular cell dysfunction

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Urinary zinc excretion is known to be increased in cancer patients, but the pathogenesis of this phenomenon remains uncertain. Both skeletal muscle catabolism and renal tubular cell dysfunction have been proposed to explain this observation. We have investigated urinary zinc and *N*-acetyl-β-D-glucosaminidase (NAG), an indicator of renal tubular cell dysfunction, as well as serum neopterin, an index of systemic immune activation, in 22 patients with cancer and seven controls. Both serum neopterin and urinary zinc were significantly elevated in cancer patients (15.8 ± 12.7 versus 7.3 ± 2.3 nmol l^{-1} and 1.77 ± 0.80 versus 1.21 ± 0.41 mmol mol^{-1} creatinine, $P < 0.02$ and $P < 0.05$, respectively), while NAG was similar in cancer patients and the controls (13.58 ± 13.80 versus 13.68 ± 12.19 μkat mol^{-1} creatinine). A significant correlation was observed between serum neopterin and urine zinc ($rs = 0.5119$, $P < 0.02$), serum neopterin and urine NAG ($rs = 0.6761$, $P < 0.002$), and urinary zinc and NAG ($rs = 0.6348$, $P < 0.002$). In conclusion, the present data indicate a link between urinary zinc excretion and immune activation as well as renal tubular cell dysfunction. In addition, renal tubular cell dysfunction appears to be linked to immune activation.

Keywords: cancer, *N*-acetyl-β-D-glucosaminidase, neopterin, urine, zinc

Introduction

Urinary zinc excretion is known to be elevated in many different disorders, including cancer (Horčičko *et al.* 1980, Voyatzoglou *et al.* 1982, Melichar *et al.* 1993). Hyperzincuria was believed to be caused by the increased release of zinc bound to low molecular weight ligands, resulting from skeletal muscle catabolism (Fell *et al.* 1973). Recently, however, increased urine zinc was shown to be associated with renal tubular cell dysfunction (Yuzbasiyan-Gurkan *et al.* 1989, Boosalis *et al.* 1991). Elevated urine zinc excretion was also shown to accompany experimental renal tubular cell necrosis (Chmielnicka *et al.* 1992). Urinary zinc excretion in cancer patients was demonstrated to correlate with urine concentrations of neopterin, an index of systemic immune activation (Melichar *et al.* 1993). The aim of the present study was to investigate the relation between urinary zinc excretion, urinary activity of *N*-acetyl-β-D-glucosaminidase (NAG), an indicator of renal tubular cell dysfunction (Guder & Hofmann 1992, Price 1992), and serum neopterin.

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Material and methods

Patients

Twenty-two previously untreated cancer patients (12 females and 10 males) aged 65 ± 9 (range 46–77) years were included in the study. Eight patients presented with colorectal cancer, five with gastric cancer, four with breast cancer, two with esophageal cancer, two patients with thyroid cancer and one with gastric leiomyosarcoma. Seven patients (six males and one female) aged 58 ± 12 years presenting for elective operation of inguinal hernia served as a reference group.

Sample collection

First morning urine samples were collected in clean plastic tubes and stored at −20 °C until analysis. Simultaneously, a serum sample was taken and stored at −20 °C until neopterin determination was performed.

Zinc measurement

Zinc was measured by atomic absorption spectro-photometry (Sunderman 1973) in air/acetylene flame using a Varian AA 175 spectrophotometer (Springvale, Australia) at 213.9 nm wavelength after dilution of the specimen 1:1. A hollow cathode lamp with a working current of 5 mA was used. Background correction was performed simultaneously by using a hydrogen lamp. A calibration curve was constructed using zinc powder (Merck, Darmstadt, Germany) diluted in hydrochloric acid at concentrations of 200, 300, 500 and 1000 $\mu g\,l^{-1}$. Commercial urine control samples (Lyphocheck, Bio-Rad, Anaheim, CA) were used to test the reliability of the measurements and the concentrations determined were within the given limit.

NAG determination

NAG was measured by a fluorimetric method (Leaback & Walker 1961). Aliquots of 500 μl of the urine sample diluted 1:20 by the citrate buffer were incubated with 500 μl of a 2 $mmol\,l^{-1}$ solution of 4-methyl-umbelliferyl-*N*-acetyl-β-D-glucosaminide (Sigma, St Louis, MO) at 37 °C. After 1 h the reaction was stopped by addition of 1000 μl of the glycine buffer. The fluorescence was read with an excitation wavelength 349 nm and an emission wavelength 460 nm, detecting the concentration of 4-methyl-umbelliferone. A standard curve was constructed using 4-methylumbelliferone (Sigma) in glycin buffer at concentrations of 10, 2, 0.5 and 0.25 $\mu mol\,l^{-1}$. A NAG standard with known activity (Boehringer, Mannheim, Germany) was also used.

Creatinine determination

The urinary concentration of zinc and NAG were expressed as zinc/creatinine and NAG/creatinine ratios (mmol mol^{-1} creatinine and $\mu kat\,mol^{-1}$ creatinine, respectively). Urinary creatinine was determined on a Hitachi 704 analyzer after dilution of the sample 1:50 using a commercial kit (Boehringer).

Neopterin determination

Neopterin was determined by a commercial radio-immunoassay (generously provided by Henning, Berlin, Germany) as described (Werner *et al.* 1987).

Statistical evaluation

The Mann Whitney *U*-test was used for statistical comparison of differences between the patients and controls. The correlation between the variables was studied by Spearman's rank coefficient. In both methods, the decision was based on a $P = 0.05$ significance level.

Results

Both serum neopterin and urinary zinc were significantly higher in cancer patients ($P < 0.02$ and $P < 0.05$, respectively; Table 1). While NAG was similar in both groups, a significant correlation was observed between NAG and neopterin ($rs = 0.6761$, $P < 0.002$, Figure 1) and NAG and zinc ($rs = 0.6348$, $P < 0.002$, Figure 2). In addition, urinary zinc correlated significantly with serum neopterin ($rs = 0.5119$, $P < 0.02$, Figure 3).

Discussion

As expected, serum neopterin and urinary zinc were significantly higher in the patients. While increased serum or urinary neopterin is known to reflect the immune

Table 1.

	Cancer patients ($n = 22$) (range)	Reference group ($n = 7$) (range)
Serum neopterin ($nmol\,l^{-1}$)	15.8 ± 12.7 (4.7 60.6)	7.3 ± 2.3 (5.0–10.3)
NAG ($\mu kat\,mol^{-1}$ creatinine)	13.58 ± 13.80 (2.70–64.46)	13.68 ± 12.19 (2.03–40.20)
Zinc (mmol mol ^{1} creatinine)	1.77 ± 0.80 (0.52–3.97)	1.21 ± 0.41 (0.87–2.05)

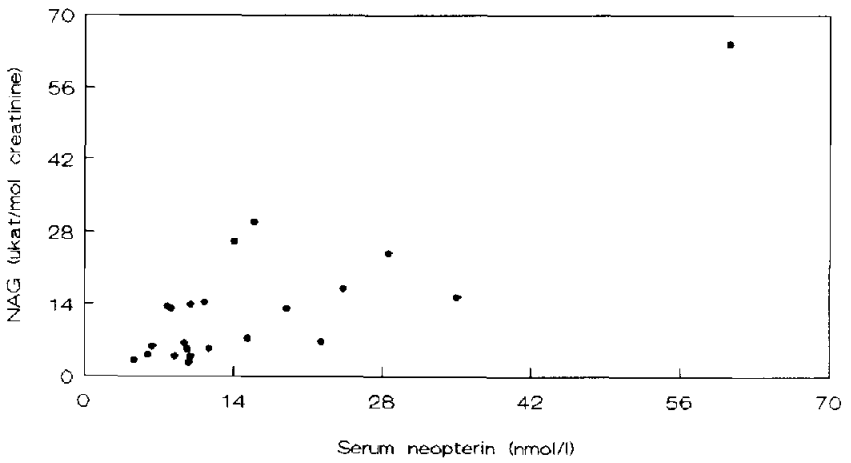

Figure 1. Correlation between serum neopterin and urine NAG ($rs = 0.6761$, $P < 0.002$).

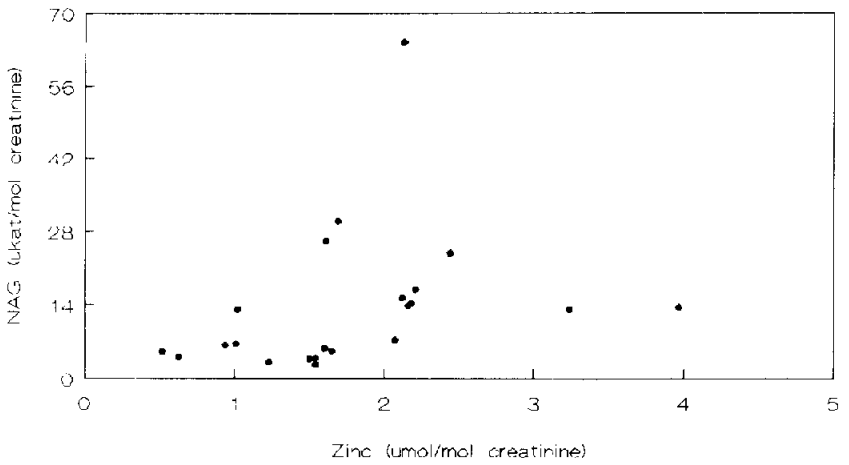

Figure 2. Correlation between urinary zinc and NAG (rs = 0.6348, $P < 0.002$).

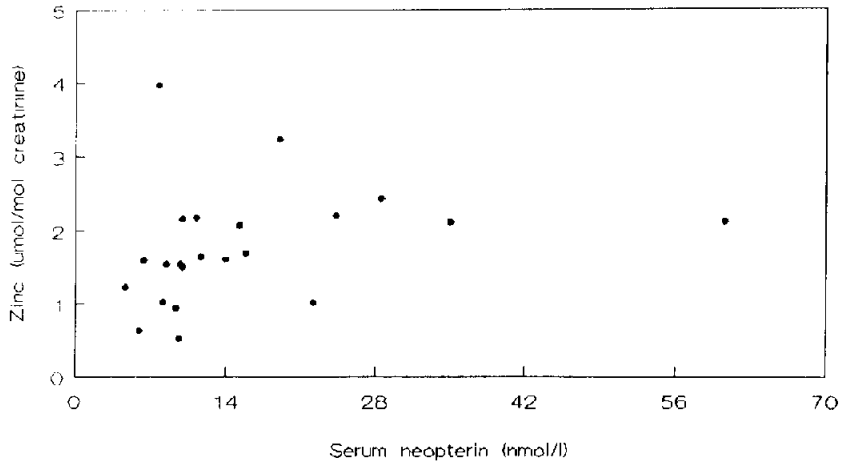

Figure 3. Correlation between serum neopterin and urinary zinc (rs = 0.5119, $P < 0.02$).

activation in cancer patients (Wachter *et al.* 1989, Reibnegger *et al.* 1991) and to result from the action of interferon-γ on macrophages (Huber *et al.* 1984), the significance of hyperzincuria is less clear. In addition to cancer, increased urinary zinc excretion is found in a variety of conditions, including diabetes mellitus (Honnorat *et al.* 1992), renal transplantation (Mahajan *et al.* 1984) or burns (Boosalis *et al.* 1991). Both an increase in primary urine zinc concentration resulting from skeletal muscle catabolism with subsequent release of zinc bound to low molecular weight ligands (Fell *et al.* 1973) and renal tubular cell dysfunction (Boosalis *et al.* 1991, Yuzbasiyan-Gurkan *et al.* 1989) have been invoked to explain this phenomenon. Hyperzincuria was observed in experi-mental renal tubular cell necrosis (Chmielnicka *et al.* 1992) and could be produced by injection of killed bacteria in experimental animals (Klaiman *et al.* 1981, Kirby *et al.* 1982).

The finding of a correlation between urinary zinc and serum neopterin confirms the results of a previous study (Melichar *et al.* 1993). In addition, urinary zinc was shown to correlate with NAG in cancer patients. However, urine NAG levels showed considerable variation and were similar in the cancer patients and the reference group. NAG activity in urine is thought to result from the enzyme released from the lysosomes of the damaged renal tubular cells (Price 1992). The measurement of NAG has been of use in various clinical situations (Guder & Hofmann 1992, Price 1992), which may be, however, confounded by the marked variation of values even in apparently healthy subjects. The association between urine zinc and NAG indicates that renal tubular cell dysfunction may, indeed, be involved in hyperzincuria in cancer patients. Even more interesting is the observation of a correlation between serum neopterin and urinary NAG. There have been observations of an association between immune activation and renal tubular cell disorders. Interleukin-1 administration was, for example, shown to increase natriuresis (Caversio *et al.* 1987). There seems to be a link between immune activation and renal tubular cell dysfunction which may have hyperzincuria as one of the manifestations (Melichar 1993).

In conclusion, hyperzincuria in cancer patients appears to be linked to both immune activation and tubular dysfunction. In addition, tubular dysfunction itself may be associated with immune activation.

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