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γ–GLUTAMYLTRANSFERASE IN URINE AS AN EARLY INDEX OF KIDNEY INVOLVEMENT IN CADMIUM EXPOSURE

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A study has been undertaken to determine the relationship between the urinary γ-glutamyltransferase activity and cadmium body burden, as determined by the concentration of the metal in the urine. The cadmium concentration and enzyme activity in urine was determined in 59 subjects exposed to the metal and in 21 subjects without occupational exposure. The groups were subdivided as follows according to cadmium excretion in urine: lower than 3, between 3 and 6, between 6 and 10, between 10 and 20, and higher than 20 μg/g of creatinine. The correlation between the concentration of cadmium and γ-glutamyltransferase activity in urine in the total group (80 subjects) is significant ($r = 0.682$, $p < 0.001$), and γ-glutamyltransferase activity shows a significant increase even in workers with low cadmium exposure. The present study supports the hypothesis that the determination of brush border enzyme activities is an early index of kidney involvement during cadmium exposure, and it could be considered an index of "subcritical effect".

KEY WORDS: Cadmium, γ-glutamyltransferase, kidney

INTRODUCTION

γ-Glutamyltransferase (GGT, E.C. 2.3.2.2) is an enzyme expressed in all organs[1] but found in highest levels in the kidney with lesser amounts in the pancreas and the liver.

Kidney GGT activity has been shown in the proximal tubule brush border, and the excretion in urine is of renal origin; a contamination by genital gland secretion[2] is possible but thought unlikely. A significant sex-related difference[3], and an increase with age in children[4] but not in adult subjects have been shown.

An increase of urinary GGT activity was observed in acute tubular necrosis[5], during treatment with aminoglycosides[7], in poisoning with inorganic and organic mercuric compounds [5,7,8] and during occupational exposure to cadmium[9–12].

The aim of the present work is to study changes in GGT activity in urine as a consequence of cadmium body burden, to establish whether this could serve as an early test of effect of cadmium exposure.

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METHODS

Eighty (21 controls and 59 exposed) male subjects, living in the same area, with similar smoking habit and socio-economical status, were selected for the study. The exposed subjects were employed in different factories utilizing cadmium: cadmium-zinc metallurgy (n. 48), cadmium pigment (n. 5), and brazing (n. 6).

Cadmium in urine (CdU) and urinary GGT (GGTU) activity were determined immediately after the sampling in spot samples collected between 8 and 9 a.m.

CdU was measured according to the method of Ross and Gonzales[13] with an atomic absorption spectrophotometer, Perkin-Elmer 305 model, that employed a graphite furnace HGA 76 B and background corrector. The C.V. of the method used is ± 5% and accuracy 100% after two extractions

GGTU activity was determined with a commercial kit (Boehringer, Mannheim, FRG) at 30°C. The variation coefficient (C.V.) of the method is ±1.5%. For the analytical determination a spectrophotometer Perkin-Elmer 550 model was used.

The urinary values were referred to grams of creatinine (CR).

The subjects were subdivided in five groups according to CdU, considered as indicator of cadmium body burden and as an index of severity and length of the exposure, as follows: 1st group (controls), subjects with CdU lower than 3 μg/g CR; 2nd group(very low exposure), subjects with CdU ranged between 3 and 6 μg/g CR; 3rd group (low exposure), subjects with CdU ranged between 6 and 10 μg/g CR; 4th group (likely high exposure), subjects with CdU ranged between 10 and 20 μg/g CR; 5th group (very high exposure), subjects with CdU higher than 20μg/g CR.

Statistics: Student t test, Chi-square (Yates correction) and linear correlation coefficient were used for the statistical evaluations.

RESULTS

A good correlation between CdU and GGTU activity ($r = 0.682$, $p < 0.001$) was observed in all the subjects grouped together (Figure 1). No significant correlation was observed between age and GGTU activity in the subjects studied ($r = -0.163$, p NS).

Table 1 shows the mean values (± standard deviations, SD) of age, CdU and GGTU activity in the five groups studied. GGTU activity shows a significant increase from the 3rd group (low exposure).

Table 2 shows the prevalence of an increase in GGTU activity in the different range values of CdU. GGTU activity was also found to show an increase in subjects with low cadmium excretion in urine.

Table 3 shows data on describing the validity and predictive values of GGTU activity, at 95th percentile of controls, in the different CdU ranges: validity increases with CdU and shows a good sensitivity (Se) for CdU ranging between 6 and 10 μg/g CR.

DISCUSSION

A level of urinary cadmium of 10 μg/g CR is considered critical in the exposed workers[14,15]. This corresponds[16] to a threshold concentration of cadmium in the

Table 1 Mean values ±SD of age, CdU and GGTU activity in the five groups

Group	*N of subjects*	*Age (years)*	*CdU* μg/g CR	GGTU U/g CR
1st	21	34.7± 6.5	1.46± 0.90	19.97±2.84
2nd	21	37.0± 7.2	4.26± 0.87*	21.93±3.59
3rd	18	39.2±11.2	7.23± 0.94*	26.71±4.60*
4th	15	48.0± 8.9*	13.91± 2.74*	29.92±8.19*
5th	5	43.2± 7.5*	37.00±15.30*	39.54±7.96*

* $p<0.05$ or more with the respect of the 1st group (Controls).

Table 2 Prevalence of GGTU activity increase for different excretions of cadmium in urine at 95th percentile of the control group (23.4 U/g CR)

Group	*CdU μg/g CR*	*prevalence*	%
1st	<3	1/21	4.8
2nd	3– 6	5/21	23.8
3rd	6–10	11/18	61.1*
4th	10–20	11/15	73.3*
5th	>20	5/5	100.0*

* Chi-square test (Yates correction) with respect to the controls is significant for $p<0.001$.

Table 3 Sensitivity (Se), Specificity (Sp), Validity (Va=Se+Sp), Positive predictive value (P+), Negative predictive value (P−), and Risk ratio (rr=P+/P−) of GGTU activity in different CdU range at 95th percentile of the control group

CdU range μg/g CR	*Se*	*Sp*	*Va*	*P+*	*P−*	*rr*
3–6	0.24	0.95	1.19	0.83	0.44	1.89
6–10	0.61	0.95	1.56	0.92	0.26	3.54
10–20	0.73	0.95	1.68	0.92	0.17	5.41
> 20	1.00	0.95	1.95	0.83	0.00	∞

kidney cortex of about 200 μg/g wet weight[17]. Below this level, signs of renal impairment are usually absent, but at this level some subjects (5–10%) may have signs of kidney damage.

One of the most relevant questions in occupational medicine is to discover tests which detect early and reversible alterations in target organs as a consequence of exposure to xenobiotics. Such tests improve the chances of preventing occupational diseases. In this context, the aim of the present work was to assess whether early indices of kidney impairment by cadmium were available.

The present study shows that the GGTU activity is increased in subjects with low cadmium exposure. In addition GGTU activity shows a good predictive value, enabling the toxic effect of cadmium to be monitored.

GGTU activity increases with the levels of CdU and correlates with this exposure index. A remaining problem is that it is not know whether the groups with low cadmium excretion, when removed from exposure, show a decrease of enzyme release in the urine.

Although our findings show that GGTU activity is a good and early index of kidney involvement during cadmium exposure it is also possible that the increase of enzymuria during low cadmium body burden does not reflect damage but is only a sign of increased protein synthesis induced by cadmium in the kidney[18].

In conclusion, measurement of GGTU activity in urine during cadmium exposure appears to represent an early and sensitive index of kidney impairment by cadmium but it is possible that enzymuria is an index of a "subcritical effect" of cadmium on the kidney.

References

1. Braun, J.P., Rico, A.G., Benard, P. and Burgat-Sacaze, V. (1977) La gamma glutamyltransferase. De la pathologie humaine a la biologie animale. *Annales de Biologie Clinique*, **35**, 433–457
2. Albert, Z., Orlowski, M. and Szewczuk, A. (1961) Histochemical demonstration of gamma-glutamyltranspeptidase. *Nature*, **191**, 767–768
3. Marhun, D., Fuchs, I., Mues, G. and Bock, D. (1976) Normal limits of urinary excretion of eleven enzymes. *Clinical Chemistry*, **22**, 1567–1574
4. Plebani, M., Rizzoni, G., Tomain, I., Pinton, A., Di Terlizzi, A. and Ceriotti, G. (1980) Variazioni circadiane e livelli di riferimento di α-glucosidasi, γGT, ribonucleasi e β_2-microglobuline urinarie nell' eta' pediatrica. *Giornale Italiano di Chimica Clinica*, **5**, 275–286
5. Dierickx, P.J. (1981) Urinary gamma-glutamyl transferase as an indicator of acute nephrotoxicity in rats. *Archives of Toxicology*, **47**, 209–215
6. Beck, P.R., Thomsom, R.B. and Chandhuri, A.K.R. (1977) Aminoglycoside antibiotics and renal function: changes in urinary γ-glutamyltransferase excretion. *Journal of Clinical Pathology*, **30**, 432–437
7. Dierickx, P.J. (1980) Urinary gamma-glutamyl transferase as a specific marker for mercury after heavy metal treatment of rats. *Toxicology Letters*, **6**, 235–238
8. Burgat-Sacaze, V., Braun, J.-P., Rico, A., Benard, P. and Eghbali, B. (1980) Methoxymethylmercury nephrotoxicity: effects on enzymuria and kidney function. *Archives of Toxicology*, **43**, 227–231
9. Trevisan, A., Buzzo, A., Moretto, A., Lonardi, U. and Chiesura, P. (1981) Urinary enzymes (GGT and ACE) in chronic cadmium exposure. *Berichte der Osterreichischen Gesellschaft fur Klinische Chemie*, **4**, 163
10. Chiesura, P., Trevisan, A., Buzzo, and Moretto, A. (1983) Monitoraggio biologico dell' esposizione in addetti alla metallurgia del cadmio. *La Medicina del Lavoro*, **74**, 404–413
11. Chiesura, P., Trevisan, A., Gori, G.P., Buzzo,A. and Calzavara, V. (1984) Sul rischio di intossicazione da cadmio nella saldobrasatura. *La Medicina del Lavoro*, **75**, 300–305
12. Trevisan, A. and Bonadonna, A. (1984) Su di un caso di nefropatia conclamata da cadmio. *La Medicina del Lavoro*, **75**, 322-327
13. Ross, R.T. and Gonzales, J.G. (1974) Direct determination of cadmium in biologicl samples by selective volatilization and graphite tube reservoir atomic absorption spectrophotometer. *Analytical Chimica Acta*, **70**, 443–447
14. Lauwerys, R., Buchet, J.P., Roels, H., Brouwers, J. and Stanescu, D. (1974) Epidemiological survey of workers exposed to cadmium: effect on lung, kidney and several biological indices. *Archives of Environmental Health*, **28**, 145–148
15. Bernard, A., Buchet, J.P., Roels, H., Masson, P. and Lauwerys, R. (1979) Renal excretion of proteins and enzymes in workers exposed to cadmium. *European Journal of Clinical Investigation*, **9**, 11–22
16. Roels, H., Lauwerys, R., Buchet, J.P., Bernard, A., Chattle, D.R., Harvey, T.C. and Al-Haddad, I.K. (1981) *In vivo* measurement of liver and kidney cadmium in workers exposed to this metal: its significance with respect to cadmium in blood and urine. *Environmental Research*, **26**, 217–240
17. WHO (1977) WHO Environmental criteria for cadmium. *Ambio*, **6**, 287–290
18. Kuliszewski, M.J. and McEwen Nicholls, D. (1981) The effects of cadmium on kidney ribosomes. *Chemico-Biological Interactions*, **33**, 307–318