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Critical evaluation of α_1- and β_2-microglobulins in urine as markers of cadmium-induced tubular dysfunction

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Abstract

The purpose of the study was to examine the validity of α_1-microglobulin (α_1-MG) in comparison with popularly used β_2-microglobulin (β_2-MG). A database was revisited to select ca. 7,500 spot urine samples (of adequate urine density) from non-pregnant, non-lactating and never-smoking adult women. The validity of the MGs was examined in terms of stability of the MG-uria prevalence in urine samples of various creatinine (CR or cr) concentration or specific gravity (SG or sg). Comparisons were made for MGs as observed (e.g., α_1-MG_{ob}), as corrected for CR (e.g., α_1-MG_{cr}) and as corrected for SG of 1.016 (e.g., α_1-MG_{sg}). A cut-off value of 5.7 mg/g cr (or mg/l) for α_1-MG was deduced from a cut-off value of 400 μg/g cr (or μg/l) forβ_2-MG, because the correlation between α_1-MG_{cr} and β_2-MG_{cr} was statistically significant. The prevalence of a 1-MG_{sg}-uria was essentially unchanged (i.e., from a low of 13.6% to a high of 17.0%, or 1.2 times) except for in very dense or very thin urine samples, in contrast, β_2-MG_{cr}-uria showed a substantial increase (from 0.0% to 2.8% with an infinite rate) as a reverse function of a decrease in CR in urine. The prevalence of uncorrected markers, i.e., α_1-MG_{ob}-uria and β_2-MG_{ob}-uria, showed even greater CR- or SG-dependent changes. Thus, it appeared prudent to consider a 1-MG_{sg} rather than β_2-MG_{cr} as a marker of tubular dysfunction among a general population with various urine density.

Introduction

Cadmium is an environmental pollutant that can induce renal tubular dysfuction in human subjects after long-term exposure even at low levels (International Programme on Chemical Safety 1992a and b). β_2-microglobulin in urine (β_2-MG) has been most commonly used for the monitoring of the effect, whereas increasing attention has been paid to urinary α_1-microglobulin (α_1-MG) in recent years.

It is also known that biases in the dysfunction evaluation may be induced when β_2-MG was corrected for creatinine (CR or cr; thus β_2-MG_{cr}). Thus, this study was initiated to compare the two markers of α_1-MG and β_2-MG from the view point of stability of microglobulinuria (MG-uria) prevalence over groups of urine samples of various urine density.

Materials and methods

A previously established data base (Ezaki *et al.* 2003) was revisited. The database was constructed on the analyses of spot urine samples obtained from over 10,000 adult women in 10 areas in Japan, who agreed to participate in the study and provided written informed consent. From the total cases, never-smokers were selected to exclude the effects of smoking, a known non-dietary source of cadmium. Pregnant or lactating women were excluded. Selection of cases with adequate urine density [0.5 g/l$<$CR$<$3.0 g/l, and

Table 1. Prevalence of α_1-MG-uria as classified in terms of specific gravity

Factor G[c] range	No. of cases	α_1-MG (%) in excess of 5.7 mg/g cr or 5.7 mg/l[a]					
		OB[b]	(%)	CR[b]	(%)	SG[b]	(%)
10 to < 15	1692	51	3,0%	250	14,8%	279	16,5%
15 to < 20	2344	188	8,0%	288	12,3%	399	17,0%
20 to < 25	2469	420	17,0%	250	10,1%	404	16,4%
25 to < 30	1091	274	25,1%	75	6,9%	148	13,6%

[a]mg/g cr for α_1-MG_{cr}, and in mg/l for α_1-MG_{ob} and α_1-MG_{sg}.
[b]CR or cr; creatinine; SG or sg, specific gravity (1.016); OB or ob, as observed.
[c]Factor G = (specific gravity - 1.000)×1,000.

Table 2. Prevalence of β_2-MG-uria as classified in terms of creatinine concentration

CR[b] range (g/l)	No. of cases	β_2-MG (%) in excess of 400 μg/g cr or 400 μg/l					
		OB[b]	(%)	CR[b]	(%)	SG[b]	(%)
0.5 to < 1.0	3251	46	1.4%	92	2.8%	43	1.3%
1.0 to < 1.5	2405	39	1.6%	16	0.7%	13	0.5%
1.5 to < 2.0	1163	23	2.0%	6	0.5%	10	0.9%
2.0 to < 2.5	387	11	2.8%	2	0.5%	2	0.5%
2.5 to < 3.0	106	4	3.8%	0	0.0%	1	0.9%

[a]μg/g cr for β_2-MG_{cr}, and in μg/l for β_2-M_{ob} and β_2-MG_{sg}.
[b]CR; creatinine, SG; specific gravity (1.016), OB; as observed.

1.010< specific gravity (SG or sg) <1.030] was further conducted after Alessio *et al.* 1985. In practice, the data were cited from two recent publications from this study group (Ikeda *et al.* 2003; Moriguchi *et al.* 2003).

The methods of chemical analyses and quality assurance programs were previously described (Ikeda *et al.* 2003; Moriguchi *et al.* 2003). Urinary levels of Cd, α_1-MG and β_2-MG were presented after correction for CR (thus Cd_{cr}, α_1-MG_{cr} andβ_2-MG_{cr}), or a specific gravity (SG or sg) of 1.016 (Cd_{sg}, α_1-MG_{sg} and β_2-MG_{sg}), in addition to observed values (α_1-MG_{ob} and β_2-MG_{ob}). Log-normality was assumed for Cd, α_1-MG and β_2-MG, so that GM and GSD were calculated as distribution parameters. A cut-off value of 400 μg/g cr (or/l) was employed for the definition of β_2-MG-uria after rounding of the figure proposed by Yamanaka *et al.* 1998; the average CR and SG levels were about 1 g/l and close to 1.016, respectively. A cut-off value for α_1-MG-uria of 5.7 mg/g cr (or/l) was estimated from a significant correlation ($p<0.01$) between α_1-MG_{cr} and β_2-MG_{cr}.

Results and discussion

Selection for urine samples with adequate density gave 7,312 cases of adequate CR concentration (Cd_{cr} = 1.23 μg/g cr as GM and 2.135 as GSD) and 7,596 cases of adequate SG [Cd_{sg} = 1.09 mg/l (after correction for 1.016) as GM and 2.139 as GSD] for the present analyses.

When the cases were classified in terms of SG and prevalence of MG-uria was calculated fora 1-MG_{ob}, α_1-MG_{cr}, or α_1-MG_{sg}, the analysis with α_1-α_1-MG_{ob} showed a marked SG-dependent increase. In contrast, the analysis withα_1-MG_{sg} showed rather constant prevalence, and it was also essentially the case with α_1-MG_{cr} (Table 1). The results were basically reproducible when cases were classified in terms of CR (data not shown).

The results of analyses for β_2-MG-uria prevalence as classified in terms of CR are summarized in Table 2. β_2-MG_{ob} showed a CR-dependent increase in the prevalence, and β_2-Mcr showed a decrease. A weak decreasing trend was observed with β_2-MG_{sg} at higher CR levels. The results were essentially the same when cases were classified in terms of SG (data not shown).

Table 3 summarizes the observation on the MG-uria prevalence in relation to urine density. Namely,

Table 3. Changes in MG-uria prevalence by urine density

Urine density	Changes in MG-uria prevalence (%) (from low to high urine density)[a]		
	α_1-MG_{ob}-uria	α_1-MG_{cr}-uria	α_1-MG_{sg}-uria
CR	6, 3% → 46, 2%(×7.3)	14, 4% → 2, 8%(×5.1)	16, 1% → 23, 6%(×1.5)
SG	3, 0% → 25, 1%(×8.4)	14, 8% → 6, 9%(×2.1)	13, 6% → 17, 0%(×1.2)
	β_2-MG_{ob}-uria	β_2-MG_{cr}-uria	β_2-MG_{sg}-uria
CR	1, 4% → 3, 8%(×2.7)	2, 8% → 0, 0%(×??)	1, 3% → 0, 9%(×1.5)
SG	1, 1% → 2, 5%(×2.3)	4, 7% → 0, 6%(×7.8)	1, 8% → 0, 7%(×2.6)

[a]Cases of 0.5 g/l <CR<3.0 g/l, or 1.010 <SG<1.030 were selected.

when the changes in α_1-MG_{ob}-uria prevalence were examined after classification of the cases in terms of CR, there was a $>$ 7 times difference from a low of 6.3% in the thin urine group to a high of 46.2% in the dense urine group. A similar calculation after classification of cases in terms of SG showed even wider variation (i.e., $>$ 8 times). Analyses for α_1-MG_{cr}-uria gave smaller variations, and the variation was smallest when α_1-MG_{sg}-uria prevalence was examined.

The analyses for β_2-MG-uria gave more or less similar results. It should be noted, however, that the variation was not small when β_2-MG_{cr} was subjected to the analysis. Namely, the rate of the highest prevalence over the lowest prevalence was infinite when classified by CR, because there was no case of β_2-MG-uria in dense urine samples with 2.5 g CR/l or above (thus the denominator was 0%). Calculation taking 0.5% (for 2.0 to $<$ 2.5 g/l CR group) as a surrogate denominator (Table 2) gave 5.6 times. Variation in β_2-MG_{ob}-uria was between 2 and 3 times. Similarly, variation was $<$ 3 times for β_2-MG_{sg}, but the rate was either equal (both 1.5 times) to or greater (2.6 times vs. 1.2 times) than the counterpart rate for α_1-MG_{sg}, when classified in terms of CR and SG, respectively.

Conclusion

It appeared prudent to consider α_1-MG_{sg} rather than β_2-MG_{cr} as a marker of tubular dysfunction among a general population of various urine density, although it is rather contrary to the common practice in epidemiological studies on cadmium toxicity.

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