ORIGINAL ARTICLES

Analysis of Serum and Urinary β_2 -Microglobulin in Japanese Subjects

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(Received July 5, 1983 and revised form October 4, 1983)

Abstract: The correlation between age and serum β_2 -MG levels and between age and urinary β_2 -MG levels of Japanese was studied, and the results were as follows.

1. The correlation coefficients between age and β_2 -MG level in the serum were +0.240 for males, +0.448 for females and +0.303 for both sexes.
2. The correlation coefficients between age and actual β_2 -MG level in the urine were +0.321 for males, +0.294 for females and 0.288 for both sexes.
3. The correlation coefficients between age and β_2 -MG level corrected for creatinine in the urine were +0.265 for males, +0.423 for females and +0.345 for both sexes.
4. The correlation coefficients between age and β_2 -MG level corrected for density in the urine were +0.278 for males, +0.315 for females and +0.298 for both sexes.
5. The correlation coefficients between cadmium level and β_2 -MG level in the urine were +0.075 for males, +0.618 for females and +0.441 for both sexes.
6. The correlation coefficients for items (1) to (5) were significantly different from zero ($p < 0.05$).

Key words: β_2 -Microglobulin—Serum—Urine—Cadmium—Normal value—Correlation

INTRODUCTION

Human β_2 -microglobulin (β_2 -MG) is a small protein (mol. wt. 11,800) originally isolated from the urine of patients with renal tubular disorders.¹⁾ It has been shown to be present on the surface of human lymphocytes^{2,3)} and to be synthesized and secreted by lymphocytes in culture.^{4,5)} Enhancement of β_2 -MG production was induced by phytohemagglutinin and mercuric ion in cultured lymphocytes,⁶⁾ and β_2 -MG production was also found to be slightly enhanced by phytohemagglutinin and cadmium ion.⁷⁾ An increase in serum levels has also

been reported in patients with malignant disease.⁸⁻¹⁰⁾

The normal level of serum β_2 -MG in Caucasians was determined by Evrin *et al.*^{11,12)} and Teasdale *et al.*¹³⁾, and that in Japanese by Kimura and Uehara,¹⁴⁾ Takasugi *et al.*¹⁵⁾, Ikekubo *et al.*¹⁶⁾, Harada *et al.*¹⁷⁾, Tatsumi *et al.*¹⁸⁾, Kanai *et al.*¹⁹⁾ and Kuro *et al.*²⁰⁾ However, there has been no report on the normal level of serum β_2 -MG in Japanese classified precisely by age and sex. In a previous study,²¹⁾ the normal level of urinary β_2 -MG was assessed.

Urinary excretion of β_2 -MG increases in chronic cadmium poisoning and other conditions leading to renal tubular malfunction.²²⁾ To simplify the determination of the urinary level of β_2 -MG, the β_2 -MG concentration in spot-sampled urine from normal persons instead of 24-hour urine specimens was measured. Evrin *et al.*¹¹⁾ reported the normal levels of urinary β_2 -MG in 24-hour urine specimens in different age groups in Caucasians. Kimura and Uehara¹⁴⁾ reported the urinary β_2 -MG concentration in normal adult men and Nomiyama *et al.*²³⁾ reported the urinary β_2 -MG level in normal Japanese over 60 years old. However, there is no report of normal levels in spot urine specimens, precisely classified by age and sex.

In previous studies, normal levels of urinary β_2 -MG^{21,24)} and serum β_2 -MG^{25,26)} in Japanese healthy subjects classified by sex and age were determined.

In this study, the relation between age and various type of β_2 -MG levels in individuals over 20 years old classified by sex was analyzed by correlation analysis. The relation between actual cadmium level and actual β_2 -MG level in the urine was also analyzed by correlation analysis.

MATERIALS AND METHODS

One group of subjects, composed of 285 males and 114 females aged from 20 to 69 years old, was examined for serum β_2 -MG. The other group, composed of 61 males and 85 females aged from 20 to 92 years old, was examined for urinary β_2 -MG. During the preceding month, all subjects had passed a public health examination. They also passed laboratory test examinations for serum urea nitrogen, serum creatinine, and Fishberg-concentration tests or phenolsulfophthalein tests, indicating values in the normal range. The serum β_2 -MG levels were deter-

Table 1. Correlation between age

Sex	Number of data	Correlation coefficient	Null hypothesis (5% level)	Regression equation
Male	285	0.240	reject	$\hat{Y}=0.0078X+1.035$
Female	114	0.448	reject	$\hat{Y}=0.0139X+0.662$
Total	399	0.303	reject	$\hat{Y}=0.0098X+0.914$

mined with a Phadebas β_2 -micro Test Kit (Shionogi Co.). Urinary β_2 -MG levels were determined with a Phadebas β_2 -micro Test Kit (Shionogi Co.). Urinary cadmium was measured with a flameless atomic absorption spectrophotometer (Perkin Elmer type 560 with an HGA 2100 flameless atomizer) after cadmium was extracted by the APDC-MIBK method. The urine samples were obtained from healthy persons (20~92 years old) at periodic health examinations. The urinary β_2 -MG levels in healthy persons were expressed as the concentration, the concentration corrected to urinary specific gravity of 1.024 and the ratio of urinary β_2 -MG to creatinine.

RESULTS

1) Correlation between age and β_2 -MG level in the serum

The correlation coefficients between age and β_2 -MG level in the serum were +0.24 for males, +0.448 for females and +0.303 for both sexes; the correlation coefficient for females was higher than for males. The null hypothesis for the correlation coefficient was rejected at the 5% level in each group.

Linear regression equations were $Y = 0.0078X + 1.035$ for males, $Y = 0.0139X + 0.662$ for females and $Y = 0.0098X + 0.914$ for both sexes, where $Y = \beta_2$ -MG level in the serum and $X = \text{age}$. Calculated from the regression equations, serum β_2 -MG increased 0.078 mg/l for males, 0.139 mg/l for females and 0.098 mg/l for both sexes per decade as shown in Table 1. The regression line and 95% fiducial region for females, as an example, are shown in Fig. 1.

2) Correlation between age and β_2 -MG level in the urine

A) Correlation between age and actual β_2 -MG level in the urine

The correlation coefficients between age and actual β_2 -MG level in the urine were +0.321 for males, +0.294 for females and +0.288 for both sexes. The null hypothesis for the correlation coefficient was rejected at the 5% level in each group.

Linear regression equations were $Y = 6.6172X - 166.65$ for males, $Y = 10.578X - 304.85$ for females and $Y = 8.685X - 238.86$ for both sexes, where $Y = \text{actual}$

and serum β_2 -MG classified by sex

Sum of squares		Cross product of X & Y S(XY)	at Mean of X (Age)			
S(XX)	S(YY)		Estimated standard error of $\bar{Y}$	Standard error of the prediction	95% fiducial region	95% confidence interval of individual Y
0.332E+5	34.96	259.0	0.02021	0.3418	1.359~1.438	0.726~2.071
0.115E+5	11.20	160.8	0.02648	0.2840	1.226~1.331	0.715~1.841
0.452E+5	47.34	443.8	0.01647	0.3295	1.332~1.397	0.716~2.012

β_2 -MG level in the urine and X =age. Calculated from the regression equations, actual β_2 -MG increased $66.172 \mu\text{g/l}$ for males, $105.78 \mu\text{g/l}$ for females and $86.85 \mu\text{g/l}$ for both sexes per decade as shown in Table 2, A.

B) Correlation between age and β_2 -MG level corrected for creatinine in the urine
The correlation coefficients between age and β_2 -MG level correlated for

Fig. 1. Correlation of age (abscissa) with β_2 -microglobulin concentration (ordinate) in female serum (over 20 years old)

Table 2. Correlation between age and

Subject	Sex	Number of data	Correlation coefficient	Null hypothesis (5% level)	Regression equation
A Actual value	Male	61	0.321	reject	$\hat{Y} = 6.6172X - 166.65$
	Female	85	0.294	reject	$\hat{Y} = 10.578X - 304.85$
	Total	146	0.288	reject	$\hat{Y} = 8.685X - 238.86$
B Concentration vs creatinin	Male	61	0.265	reject	$\hat{Y} = 10.163X - 294.37$
	Female	85	0.423	reject	$\hat{Y} = 12.630X - 376.99$
	Total	146	0.345	reject	$\hat{Y} = 11.588X - 342.78$
C Corrected concentration*	Male	61	0.278	reject	$\hat{Y} = 10.177X - 299.13$
	Female	85	0.315	reject	$\hat{Y} = 12.279X - 371.65$
	Total	146	0.298	reject	$\hat{Y} = 11.276X - 337.10$

creatinine in the urine were $+0.265$ for males, $+0.423$ for females and $+0.345$ for both sexes; the correlation coefficient for females was higher than that for males. The null hypothesis was rejected at the 5% level in each group.

Fig. 2. Correlation of age (abscissa) with β_2 -microglobulin concentration corrected for creatinine (ordinate) in female urine (over 20 years old)

urinary β_2 -MG concentration classified by sex

Sum of squares		Cross product of X & Y S(XY)	at Mean of X (Age)			
S(XX)	S(YY)		Estimated standard error of $\bar{Y}$	Standard error of the prediction	95% fiducial region	95% confidence interval of individual Y
0.209E+5	0.888E+7	0.138E+6	47.036	370.36	80.39~268.62	-566.58~915.60
0.272E+5	0.353E+8	0.288E+6	67.642	627.28	66.00~335.08	-1047.10~1448.18
0.487E+5	0.442E+8	0.423E+6	43.919	532.49	102.85~276.47	-862.85~1242.18
0.209E+5	0.308E+8	0.213E+6	89.174	702.159	50.82~407.69	-1175.76~1634.27
0.272E+5	0.243E+8	0.344E+6	53.175	493.12	120.66~332.20	-754.37~1207.24
0.487E+5	0.550E+8	0.565E+6	48.028	582.31	134.05~323.91	-922.00~1379.96
0.209E+5	0.282E+8	0.214E+6	84.987	669.19	55.19~395.31	-1113.80~1564.30
0.272E+5	0.414E+8	0.334E+6	72.695	674.15	70.40~359.57	-1125.87~1555.83
0.487E+5	0.696E+8	0.549E+6	54.900	665.63	110.76~327.79	-1096.39~1534.94

* Concentration corrected urinary density of 1.024

Table 3. Correlation between cadmium concentration

Sex	Number of data	Correlation coefficient	Null hypothesis (5% level)	Regression equation
Male	60	0.0749	accept	$\hat{Y} = 10.676X + 150.39$
Female	74	0.618	reject	$\hat{Y} = 151.78X - 193.28$
Total	134	0.441	reject	$\hat{Y} = 91.600X - 37.794$

Linear regression equations were $Y = 10.163X - 249.37$ for males, $Y = 12.630X - 376.99$ for females and $Y = 11.588X - 342.78$ for both sexes, where $Y = \beta_2$ -MG level corrected for creatinine in the urine and $X = \text{age}$. Calculated from the regression equations, the β_2 -MG level corrected for creatinine in the urine increased $101.63 \mu\text{g/g}$ for males, $126.30 \mu\text{g/g}$ for females and $115.88 \mu\text{g/g}$ for both sexes per decade as shown in Table 2, B. The regression line and 95% fiducial region for females, as an example, are shown in Fig. 2.

C) Correlation between age and β_2 -MG level corrected for density in the urine

The correlation coefficients between age and β_2 -MG level corrected for density in the urine were $+0.278$ for males, $+0.315$ for females and $+0.298$ for both sexes. The null hypothesis for the correlation coefficient was rejected at the 5% level in each group.

Linear regression equations were $Y = 10.177X - 299.13$ for males, $Y = 12.279X - 371.65$ for females and $Y = 11.276X - 337.10$ for both sexes, where $Y = \beta_2$ -MG level corrected for density in the urine and $X = \text{age}$. Calculated from the regression equations, the β_2 -MG level corrected for density in the urine increased $101.77 \mu\text{g/l}$ for males, $122.79 \mu\text{g/l}$ for females, $112.76 \mu\text{g/l}$ for both sexes per decade as shown in Table 2, C.

3) Correlation between cadmium level and β_2 -MG level in the urine

The correlation coefficients between cadmium level and β_2 -MG level in the urine were $+0.0749$ for males, $+0.618$ for females and $+0.441$ for both sexes; the correlation coefficient for females was clearly higher than that for males. The null hypothesis was accepted for males and rejected for females and both sexes at the 5% level.

Linear regression equations were $Y = 10.676X + 150.39$ for males, $Y = 151.78X - 193.28$ for females and $Y = 91.600X - 37.784$ for both sexes, where $Y = \text{cadmium level in urine}$ and $X = \beta_2$ -MG level in urine as shown in Table 3. The regression line and 95% fiducial region for females, as an example, are shown in Fig. 3.

and β_2 -MG concentration classified by sex

Sum of squares		Cross product of X & Y S(XY)	at Mean of X (Cadmium concentration)			
S(XX)	S(YY)		Estimated standard error of $\hat{Y}$	Standard error of the prediction	95% fiducial region	95% confidence interval of individual Y
435.90	0.886E+7	4653.8	50.327	393.07	1.98~350.88	-1186.06~1538.93
583.44	0.351E+8	8854.6	81.059	701.99	45.98~379.16	-1181.82~1616.96
1021.55	0.441E+8	93574	44.795	520.47	111.55~287.76	-830.38~1228.69

Fig. 3. Correlation of cadmium concentration (abscissa) with β_2 -microglobulin concentration (ordinate) in female urine (over 20 years old)

DISCUSSION

The correlation coefficient between age (X) and serum β_2 -MG level (Y) of Japanese was +0.240 for males and +0.448 for females respectively, and its regression equation in both sexes was $Y=0.0098X+0.914$. The correlation coefficient for males and females was a little lower than that reported by Evrin *et al.*¹²⁾, and the regression equation was similar to that obtained by Evrin *et al.*¹²⁾ ($Y=0.0115X+1.058$).

The correlation coefficient between age (X) and actual β_2 -MG level (Y) in the urine of both males and females was +0.288 and its regression equation was

$Y = 8.685X - 238.86$. The correlation coefficient obtained was similar to that reported by Kitamura²⁷⁾ (+0.259).

The correlation coefficient between cadmium level (X) and β_2 -MG level (Y) in the urine was +0.441 for both sexes and its regression equation was $Y = 91.600X - 37.794$. The correlation coefficient showed a higher value than that (+0.016) reported by Kitamura²⁷⁾ obtained from the inhabitants in an area not polluted with cadmium.

ACKNOWLEDGEMENT

The authors express their thanks to Assistant Professor H. Osaki of the Department of Industrial Science, School of Engineering, Okayama University, and Dr. M. Kimura of the National Institute of Industrial Health for correcting this manuscript.

This work was partially supported by a grant from the Ministry of Health and Welfare.

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