Analysis of Urinary Albumin, Transferrin, *N*-Acetyl- β -D-Glucosaminidase and β_2 -Microglobulin in Patients With Impaired Glucose Tolerance

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We investigated the changes in urinary albumin and urinary transferrin as glomerular proteins, and in urinary *N*-acetyl- β -D-glucosaminidase and urinary β_2 -microglobulin as tubular proteins, in patients with impaired glucose tolerance. We attempted to compare the proteins of normal subjects to those of diabetics with pre-nephropathy. Transferrin and *N*-acetyl- β -D-glucosaminidase levels were significantly increased in patients with impaired glucose tolerance, while albumin and β_2 -microglobulin levels were only slightly increased. In addition, there was no significant difference in transferrin levels between patients with impaired glucose tolerance and type 2 diabetics with pre-nephropathy.

In our observation, although albumin levels were only slightly increased in patients

with impaired glucose tolerance, a sharp increase in transferrin levels was reflected in patients with glomerular disorders. In addition, since *N*-acetyl- β -D-glucosaminidase levels varied markedly, tubular disorders were suspected. It should be stressed that increased parameters for both glomerular and tubular disorders in group C—patients who showed abnormal levels in three proteins—had already been observed in some patients with impaired glucose tolerance.

Therefore, the evaluation of the mutual relationships between various urinary protein components in patients with impaired glucose tolerance will become a more important assessment tool than that of single urinary protein components. *J. Clin. Lab. Anal.* 12:351–355, 1998. © 1998 Wiley-Liss, Inc.

Key words: urinary albumin; urinary transferrin; urinary *N*-acetyl- β -D-glucosaminidase, urinary β_2 -microglobulin, patients with impaired glucose tolerance

INTRODUCTION

According to the WHO classification parameters for the evaluation of glucose tolerance test results using 75 g glucose, there is an impaired glucose tolerance between diabetics and normal subjects. WHO defines impaired glucose tolerance as a condition in which fasting blood sugar levels in venous plasma drop to less than 140 mg/dl and two hours after 75g oral glucose administration are maintained between 140 and 200 mg/dl (1). Since impaired glucose tolerance represents the preliminary state of diabetes mellitus, the incidence of this condition is high in subjects demonstrating impaired glucose tolerance; 19–61% of such subjects develop diabetes mellitus within 5–10 years.

We conducted a comparative investigation of diabetics in the pre-nephropathy stage and diabetics in the early and overt nephropathy stage. The pre-nephropathy stage is the least significant state in the classification of diabetic nephropathy in which urinary albumin levels are maintained in the nor-

mal range (2). Since patients with impaired glucose tolerance have not been definitively diagnosed as having diabetes, only the routine glucose tolerance test was administered to check blood glucose levels, and detailed fluid components were not examined. Urinary protein components were not examined in patients with impaired glucose tolerance either. Thus, the development of diabetic nephropathy could not be estimated in patients with impaired glucose tolerance at all. Therefore, to investigate changes in various protein components in patients with impaired glucose tolerance, we attempted to compare the protein components of normal subjects and diabetics with pre-nephropathy.

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Among the various protein components, we selected albumin (3–5) and urinary transferrin (6–8) as glomerular proteins together with urinary *N*-acetyl- β -D-glucosaminidase (9–11) and urinary β_2 -microglobulin (12–15) as tubular proteins.

MATERIALS AND METHODS

Subjects

Untimed urine specimens were collected from 47 healthy staff members (56.2 ± 3.7 years old, mean $\pm$ 1SD), 44 (56.2 ± 3.7 years old) and 75 internal medicine outpatients (Tokyo Medical and Dental University Hospital) (58.9 ± 9.8 years old) with non-insulin dependent diabetes mellitus (NIDDM) in the pre-nephropathy stage. We divided the diabetic nephropathy cases into three groups by albumin values (mg/g $\cdot$ Cre.) according to the criteria proposed by Shigeta (16) as follows: albumin values under 30mg/g $\cdot$ Cre. (normo-albuminuria), albumin values between 30mg/g $\cdot$ Cre. and 300mg/g $\cdot$ Cre. (micro-albuminuria) and albumin values over 300mg/g $\cdot$ Cre. (macro-albuminuria) which were named pre-nephropathy, early nephropathy, and overt nephropathy, respectively.

Methods

Urinary albumin, transferrin, *N*-acetyl- β -D-glucosaminidase, and β_2 -microglobulin were determined by Hitachi 7150 (Hitachi Medico, Japan) base on turbidimetric assay using *N*-assay TIA Microalbumin (Nittobo Medical, Japan), superior microtransferrin (Nihon DPC, Japan), *N*-acetyl- β -D-glucosaminidase Rate Shionogi (Shionogi, Japan), and superior micro β_2 -microglobulin (Nihon DPC, Japan), respectively. Albumin, transferrin, *N*-acetyl- β -D-glucosaminidase and β_2 -microglobulin values were expressed as values per creatinine (Cre.) value (g) in urine (mg/g $\cdot$ Cre. on albumin and transferrin, μ g/g $\cdot$ Cre. on β_2 -microglobulin, and U/g $\cdot$ Cre. on *N*-acetyl- β -D-glucosaminidase.

Statistical Analysis

Significant differences were determined by Wilcoxon's test and the Mann-Whitney U test.

RESULTS

Concentration of Albumin, Transferrin, *N*-Acetyl- β -D-Glucosaminidase, and β_2 -Microglobulin

We determined the mean $\pm$ 1SD of albumin, transferrin, *N*-acetyl- β -D-glucosaminidase, and β_2 -microglobulin levels in

three groups including normal subjects, patients with impaired glucose tolerance, and diabetics with pre-nephropathy. As shown in Table 1, there was little difference in albumin levels between normal subjects and patients with impaired glucose tolerance. However, transferrin and *N*-acetyl- β -D-glucosaminidase levels were significantly increased in patients with impaired glucose tolerance, while albumin and β_2 -microglobulin levels were only slightly increased. There was no significant difference in protein levels between patients with impaired glucose tolerance and diabetics with pre-nephropathy.

Correlation Between Levels of Albumin and Those of Transferrin, *N*-Acetyl- β -D-Glucosaminidase, and β_2 -Microglobulin

The correlation between albumin and other protein levels was examined and the coefficients obtained were compiled shown in Table 2. There was little correlation between albumin and transferrin levels in normal subjects as well as patients with impaired glucose tolerance. (The correlation coefficient between albumin and transferrin levels in normal subjects was 0.154, while that in patients with impaired glucose tolerance was 0.377.) However, there was an excellent correlation between albumin and transferrin levels in diabetics with pre-nephropathy (correlation coefficient of 0.860). The correlation between albumin and *N*-acetyl- β -D-glucosaminidase levels in patients with impaired glucose tolerance (correlation coefficient of 0.334) was poorer than that in normal subjects or diabetics with pre-nephropathy. There was no correlation between albumin and β_2 -microglobulin levels in any of the three groups.

Number of Patients With Impaired Glucose Tolerance or Diabetics With Pre-Nephropathy who Showed Levels of Various Proteins Exceeding the Upper Limit of the Normal Range

Normal ranges of albumin, transferrin, *N*-acetyl- β -D-glucosaminidase, and β_2 -microglobulin (mean $\pm$ 1SD) were obtained in normal subjects by the parametric method. The analysis of the same proteins in patients with impaired glucose tolerance revealed higher protein levels than the normal range's upper limit in some cases. The results were classified as follows: group A, patients who showed an abnormal level in one protein; group B, patients who showed abnormal lev-

TABLE 1. Concentration of Urinary Albumin, Transferrin, *N*-Acetyl- β -D-Glucosaminidase and β_2 -Microglobulin

	n	Albumin (mg/g-Cre)	Transferrin (mg/g-Cre)	<i>N</i> -acetyl- β -D- glucosaminidase (U/g-Cre)	β_2 -microglobulin (μ g/g-Cre)
Healthy subjects	47	11.92 $\pm$ 5.78	0.63 $\pm$ 0.41*	6.22 $\pm$ 3.36*	82.18 $\pm$ 45.70
Patients with impaired glucose tolerance	44	13.15 $\pm$ 16.45	1.31 $\pm$ 1.45*	9.03 $\pm$ 6.47*	110.83 $\pm$ 86.91
Diabetics with pre-nephropathy	30	12.83 $\pm$ 7.33	1.23 $\pm$ 0.83	11.09 $\pm$ 6.58	107.21 $\pm$ 99.24

*: $P < 0.05$.

TABLE 2. Correlation Coefficient Between Levels of Albumin and Those of Transferrin, N-Acetyl- β -D-Glucosaminidase and β_2 -Microglobulin

	Albumin transferrin	Albumin N-acetyl- β -D- glucosaminidase	Albumin β_2 -microglobulin
Healthy subjects	0.154	0.682	0.282
Patients with impaired glucose tolerance	0.377	0.334	0.134
Diabetics with pre-nephropathy	0.860	0.529	0.201

els in two proteins; and group C, patients who showed abnormal levels in three proteins. As shown in Table 3, three patients with impaired glucose tolerance showed abnormal albumin levels, six patients showed abnormal transferrin levels, and four patients showed abnormal *N*-acetyl- β -D-glucosaminidase levels, but none of the patients with impaired glucose tolerance showed abnormal β_2 -microglobulin levels. When two proteins were evaluated, one patient with impaired glucose tolerance showed abnormal albumin and transferrin levels, another patient showed abnormal *N*-acetyl- β -D-glucosaminidase and β_2 -microglobulin, and two patients showed

abnormal transferrin and β_2 -microglobulin levels. When three proteins were evaluated, one patient with impaired glucose tolerance showed abnormal albumin, transferrin, and β_2 -microglobulin levels as well as abnormal albumin, transferrin, and *N*-acetyl- β -D-glucosaminidase levels. Abnormal protein levels were observed in 19 of 44 patients with impaired glucose tolerance (43.2%). The evaluation of each protein showed that transferrin levels increased in 11 patients, accounting for a greater proportion of patients with impaired glucose tolerance (25.0%), while albumin levels increased in six patients. These findings suggest that transferrin levels became abnormal before the increase in albumin levels occurred in patients with impaired glucose tolerance.

We also studied diabetics with pre-nephropathy. One patient showed abnormal albumin levels, two patients showed abnormal transferrin levels, another patient showed abnormal *N*-acetyl- β -D-glucosaminidase, and three patients showed abnormal β_2 -microglobulin. When two proteins were evaluated, one patient showed abnormal transferrin, *N*-acetyl- β -D-glucosaminidase, transferrin, and β_2 -microglobulin levels. When three proteins were evaluated, three patients showed abnormal levels of albumin, transferrin, and *N*-acetyl- β -D-glucosaminidase, while one patient showed abnormal *N*-

TABLE 3. Number of Patients With Impaired Glucose Tolerance or Diabetics With Pre-nephropathy who Showed Levels of Various Proteins Exceeding the Upper Limit of the Normal Range

	Cases	Albumin	Transferrin	N-acetyl- β -D- glucosaminidase	β_2 -Microglobulin
(1) Patients With Impaired Glucose Tolerance					
Albumin	3	3			
A Transferrin	6		6		
N-acetyl- β -D-glucosaminidase	4			4	
Albumin, Transferrin	1	1	1		
B N-acetyl- β -D-glucosaminidase, β_2 -microglobulin	1			1	1
Transferrin, β_2 -microglobulin	2		2		2
Albumin, Transferrin, C β_2 -microglobulin	1	1	1		1
Albumin, Transferrin, N-acetyl- β -D-glucosaminidase	1	1	1	1	
Total	19	(6)	(11)	(6)	(4)
(2) Diabetics With Pre-Nephropathy					
Albumin	1	1			
A Transferrin	2		2		
N-acetyl- β -D-glucosaminidase	1			1	
β_2 -microglobulin	3				3
Transferrin, B N-acetyl- β -D-glucosaminidase	1		1	1	
Transferrin, C β_2 -microglobulin	1		1		1
Albumin, Transferrin, N-acetyl- β -D-glucosaminidase	3	3	3	3	
N-acetyl- β -D-glucosaminidase, Transferrin, β_2 -microglobulin	3		3	3	3
Total	15	(4)	(10)	(8)	(7)

acetyl- β -D-glucosaminidase, transferrin, and β_2 -microglobulin levels. Abnormal protein levels were observed in 15 of 30 diabetics with pre-nephropathy (50%). The evaluation of each protein level showed abnormal increases in transferrin levels in ten patients, and abnormal albumin levels in four patients. None of the patients with impaired glucose tolerance or diabetics with pre-nephropathy showed abnormal levels in all of the four proteins at once.

Comparison Between Patients With Impaired Glucose Tolerance and Diabetics With Pre-Nephropathy With Abnormal Protein Levels

Patients with abnormal protein levels were classified into the following four groups for comparison: Group A, patients who showed abnormal levels of either albumin or transferrin (or both) as indices of glomerular disorder; group B, patients who showed abnormal levels of either *N*-acetyl- β -D-glucosaminidase or β_2 -microglobulin or both as indices of tubular disorder; group C, patients who showed abnormal levels of either albumin or transferrin or both *and* either *N*-acetyl- β -D-glucosaminidase or β_2 -microglobulin or both as indices of glomerular and tubular disorders; group D, patients who showed no abnormality in protein levels. As shown in Table 4, ten patients (22.7%) fall into group A, five (11.4%) into group B, four (9.1%) into group C, and 25 (56.8%) into group D.

To clarify the causative factors for patients in groups C and D, we compared fasting blood sugar levels. As shown in Figure 1, the mean fasting blood sugar level in group C was 109.0 ± 6.8 mg/dl, while that in group D was 109.5 ± 9.8 mg/dl, revealing no significant difference between the two groups.

When HbA_{1c} levels were compared between groups C and D, as shown in Figure 2, the HbA_{1c} level in group C was 5.53 ± 0.47 % (mean $\pm$ 1SD), while that in group D was 5.41 ± 0.35 %, showing no significant difference between the two groups.

We also classified diabetics with pre-nephropathy into the former four groups to clarify which group of patients with impaired glucose tolerance in the preliminary stage of diabetes mellitus was at greater risk when the disease progressed to the next stage, the state of diabetic with pre-nephropathy. Then, we evaluated the rate of progression from impaired

TABLE 4. Comparison Between Patients With Impaired Glucose Tolerance and Diabetics With Pre-Nephropathy With Abnormal Protein Levels

	Patients with impaired glucose tolerance		Diabetics with pre-nephropathy		Progression rates
	Cases	%	Cases	%	
Group A	10	22.7	3	10.0	0.44
Group B	5	11.4	4	13.3	1.16
Group C	4	9.1	8	26.7	2.93
Total	19	43.2	15	50.0	
Group D	25	56.8	15	50.0	0.88
Total	44	100.0	30	100.0	

Fig. 1. The appearance of rate fasting blood sugar in patients from groups C and D. Group C, patients who showed abnormal levels of either albumin or transferrin (or both) and either *N*-acetyl- β -D-glucosaminidase or β_2 -microglobulin; group D, patients who showed no abnormality in protein levels.

glucose tolerance to diabetic with pre-nephropathy. Our results indicate that three patients (10.0%) fell into group A, four (13.3%) into group B, eight (26.7%) into group C, and 15 (50.0%) into group D.

Table 4 shows that the highest progression rate for group C was 2.93. The progression rate from impaired glucose tolerance to diabetic with pre-nephropathy increased 0.44 times in group A and 1.16 times in group B, but in group C a marked increase by 2.93 times was observed. Based on these results, it was considered that closer attention should be paid to group C in patients with impaired glucose tolerance as a preliminary group of diabetic nephropathy.

Fig. 2. The appearance of HbA_{1c} in patients from groups C and D. Group C, patients who showed abnormal levels of either albumin or transferrin (or both) and either *N*-acetyl- β -D-glucosaminidase or β_2 -microglobulin; group D, patients who showed no abnormality in protein levels.

DISCUSSION

It was reported that urinary transferrin levels and the transferrin/albumin ratio are higher in diabetic patients than in normal subjects because the barrier of the renal basement membrane is already damaged at the stage of diabetic with pre-nephropathy (17). Our study revealed that transferrin levels in patients with impaired glucose tolerance were significantly higher than in normal subjects. Our study further revealed that there was no significant difference in transferrin levels between patients with impaired glucose tolerance and diabetics with pre-nephropathy. Therefore, it was speculated that nephropathy could develop earlier than usual in patients with impaired glucose tolerance. This speculation was supported by observations in patients with impaired glucose tolerance or diabetics with pre-nephropathy who showed abnormal protein levels including albumin, transferrin, *N*-acetyl- β -D-glucosaminidase, or β_2 -microglobulin, exceeding the upper limit of normal ranges; in the group of patients with impaired glucose tolerance, albumin was increased in six patients, transferrin was increased in 11 patients, *N*-acetyl- β -D-glucosaminidase was increased in six patients, and β_2 -microglobulin was increased in four patients, while in the diabetic with pre-nephropathy group, albumin was increased in four patients, transferrin was increased in ten patients, *N*-acetyl- β -D-glucosaminidase was increased in eight patients and β_2 -microglobulin was increased in seven patients. When fasting blood sugar and HbA_{1c} levels were compared between group C and D, no significant difference between the two groups was found. It is interesting that fasting blood sugar and HbA_{1c} levels (diabetes mellitus markers) didn't show abnormality, but some protein levels showed abnormality.

Sayegh (18) reported that probability of complications was small in patients with impaired glucose tolerance, while Fuller et al. (19) reported that the possibility of development of arteriosclerotic lesions was markedly higher in patients with impaired glucose tolerance than in normal subjects. However, we believe that greater attention should be paid to the state of impaired glucose tolerance.

Hotta (20) reported that increased urinary albumin levels observed in patients with impaired glucose tolerance resulted from increased blood stream, and that urinary β_2 -microglobulin levels were invariable.

Our observations showed that although albumin levels were only slightly increased in patients with impaired glucose tolerance, sharp increases in transferrin levels reflected certain disorders in the glomeruli. In addition, since *N*-acetyl- β -D-glucosaminidase levels varied markedly, tubular disorders were suspected. Moreover, increased parameters for both glomerular and tubular disorders were already observed in some patients with impaired glucose tolerance.

Therefore, the evaluation of the mutual relationships between various urinary protein components in patients with impaired glucose tolerance is bound to become a more sig-

nificant assessment tool than the evaluation single urinary protein components.

REFERENCES

1. WHO Expert Committee on Diabetes Mellitus Second Report. Technical Report Series, 646, 1980.
2. Hiratsuka N, Shiba K, Shinomura K, Hosaki S, Nishida K, Kobayashi S: Electrophoretic patterns of urinary proteins of diabetics in the pre-, early, and overt nephropathy stages. *Biol Pharm Bull* 20:651-655, 1997.
3. Mykkanen L, Haffner SM, Kuusisto J, Pyorala K, Laakso, M: Microalbuminuria precedes the development of NIDDM. *Diabetes* 43:552-557, 1994.
4. Metcalf PA, Baker JR, Scragg RK, Dryson E, Scott AJ, Wild CJ: Microalbuminuria in a middle-aged workforce: Effect of hyperglycemia and ethnicity. *Diabetes-Care* 16:1485-1493, 1993.
5. Collins VR, Dowse GK, Plehwe WE, Imo TT, Toelupe PM, Taylor HR: High prevalence of diabetic retinopathy and nephropathy in Polynesians of Western Samoa. *Diabetes-Care* 18:1140-1149, 1995.
6. Ishibashi H: Increased urinary transferrin excretion in non-insulin-dependent diabetes mellitus (NIDDM). *J Japan Diab Soc* 35:949-954, 1992.
7. Cheung CK, Cockram CS, Yeung VTF, Swaminathan R: Urinary excretion of transferrin by non-insulin-dependent diabetics: A marker for early complications. *Clin Chem* 35:1672-1674, 1989.
8. O' Donnell MJ, Watson J, Martin P, Chapman C, Barnett AH: Transferrinuria in type 2 diabetes: The effect of glycaemic control. *Ann Clin Biochem* 28:174-177, 1991.
9. Sanchez HMC, Mateo CJ, Zamora ME: Influence of glycemic blood glucose control and incipient diabetic nephropathy on the urinary excretion of *N*-acetyl- β -glucosaminidase (NAG) in diabetes mellitus. *An Med Interna* 12:216-220, 1995.
10. Ueta I, Takamatsu K, Hashimoto K: Urinary glycosaminoglycans in patients with incipient diabetic nephropathy. *Nippon Jinzo Gakkai Shi* 37:17-23, 1995.
11. Ishii N, Ogawa Z, Itoh H, Ikenaga H, Saruta T: Diagnostic significance of urinary enzymes for diabetes mellitus and hypertension. *Enzyme Protein* 48:174-182, 1994.
12. Viberti GC, Rickup JC, Phil DP, Keen RJ: Effect of control of blood glucose on urinary excretion of albumin and β_2 -microglobulin in insulin-dependent diabetes. *N Engl J Med* 300:638-649, 1979.
13. Peterson PA, Eurin PE, Berggard I: Differentiation of glomerular, tubular, and normal proteinuria: Determinations of urinary excretion of β_2 -microglobulin, albumin, and total protein. *J Clin Invest* 48:1189-1198, 1969.
14. Dance N, Price RG, Robinson D, Stirling JL: β -galactosidase, β -glucosidase, and *N*-acetyl- β -glucosaminidase in human kidney. *Clin Chim Acta* 24:189-197, 1969.
15. Gibery R, Dupond JL, Alber D, Floris RL, Henry JC: Predictive value of urinary *N*-acetyl- β -D-glucosaminidase (NAG), alanine-aminopeptidase (AAP), and β_2 -microglobulin (β_2 M) in evaluating nephrotoxicity of gentamicin. *Clin Chim Acta* 116:25-34, 1981.
16. Shigeta Y: Tounyoubyo, Shindan to Chiryo Inc: Tokyo, 1990 pp.194-203.
17. Misaki M, Shima T, Esaki A, Sumida Y, Shirayama K: Urinary transferrin and albumin excretion rates in type-2 diabetics. *J Japan Diab Soc* 36:909-915, 1993.
18. Sayegh AH, Jannet RJ: Oral glucose tolerance tests and the diagnosis of diabetes: The Whitehall study. *Lancet* 2(8140):431-432, 1979.
19. Fuller JH, Shipley MJ, Rose G, Torrett RJ, Keen H: Coronary heart disease risk and impaired glucose tolerance: The Whitehall study. *Lancet* 1(8183):1373-1376, 1980.
20. Hotta O, Taguma Y, Mitsuoka M, Takeshita K, Takahashi H: Urinary albumin excretion in patients with non-insulin-dependent diabetes mellitus in an early microalbuminuric stage. *Nephron* 58:23-26, 1991.