Correlation between Glycated Lipoproteins and Fructosamine Level in Serum

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The correlation between the level of fructosamine and glycated proteins, including glycated lipoproteins, in serum from diabetic and nondiabetic subjects was studied. Assay of glycated proteins in serum was performed using an agarose gel film electrophoresis with nitroblue tetrazolium coloration. Glycated albumin correlated well with the fructosamine level in the diabetics ($r=0.83-0.92$, $p<0.01$) but showed no correlation with the nondiabetics ($r=0.25-0.26$). Also, a high correlation between the glycated β -lipoprotein and fructosamine levels was observed in diabetic patients with hyperglycemia and in nondiabetic subjects with a high risk of atherogenesis (atherogenic index, low-density lipoprotein-cholesterol/high-density lipoprotein-cholesterol >2.8) ($r=0.51-0.66$, $p<0.01$). Nondiabetics with a high level of β -lipoprotein, which is well known to cause high atherogenesis, showed a high level of glycated β -lipoprotein similar to that in the diabetic groups with hyperglycemia; therefore, the high level of glycated β -lipoprotein seems to be attributable not only to the hyperglycemia-accelerated glycation of β -lipoprotein but also to an increase in the level of β -lipoprotein in serum.

Consequently, the present results show that the fructosamine level in serum reflects not only the glycation of albumin but also that of lipoproteins which are known to increase in diabetes mellitus.

Keywords fructosamine; glycated albumin; glycated lipoprotein; glycation; diabetes mellitus; hyperglycemia; atherogenic index

Fructosamine and glycated hemoglobin are widely accepted as reliable indicators of metabolic control in diabetes mellitus.^{1,2)} However, in some diseases, such as renal insufficiency with hypoproteinemia, the fructosamine value varies widely without changes in the level of glycated albumin (glc albumin).³⁾ In addition, not only albumin but also apolipoprotein B in pre β - and β -lipoproteins are nonenzymatically glycated, and in diabetic hyperglycemia, the glycation is accelerated.^{4,5)} The fructosamine value represents the sum of glycated proteins in serum such as glc albumin and glycated lipoproteins (glc Lps),⁴⁾ but it has been considered to reflect the glycation of albumin since glc albumin is a major component of the glycated proteins in serum.⁶⁾

We observed a discrepancy in the correlation between glc albumin and fructosamine levels in serum from diabetic and nondiabetic subjects,⁴⁾ suggesting that a different protein from albumin may contribute to changes in the fructosamine value.⁷⁾ If the glycation of different proteins from albumin, e.g., lipoproteins, affect the fructosamine value, it may become useful not only as an indicator of glycemic control but also of the extent of glycation of lipoproteins related to the risk of atherogenesis based on the abnormal metabolism of lipoproteins.⁸⁾

In the present study, correlation between the value of fructosamine and other proteins, such as α -, pre β - and β -lipoproteins, in serum were examined using sera from diabetic and nondiabetic populations, and the clinical significance of fructosamine was evaluated.

Materials and Methods

Subjects The nondiabetic groups (I, AI: atherogenic index, low-density lipoprotein-cholesterol/high-density lipoprotein-cholesterol ≤ 2.8 , $n=31$; II, AI >2.8 , $n=31$) consisted of a population (aged 33 to 74 years) certified as being healthy in a group medical examination. The diabetic groups (III, AI ≤ 2.8 , $n=20$; IV, AI >2.8 , $n=63$) consisted of inpatients with or without complications (aged 21 to 76 years) who were undergoing medical treatment at Hyogo College of Medicine Hospital and had been classified as having diabetes mellitus in a glucose tolerance test (75-g glucose load) on the basis of World Health Organization (WHO) criteria.⁹⁾ Blood was sampl-

ed preprandially without an anticoagulant. After the blood had clotted, the tube was centrifuged at $1400 \times g$ for 10 min and the supernatant serum was separated and stored at 4°C until use.

Fructosamine Assay The fructosamine was essentially assayed by the method of Johnson *et al.* based on the nitroblue tetrazolium (NBT) reducing method.²⁾ The fructosamine value (mm) was expressed in terms of 1-deoxy-1-morpholino fructose (DMF).

Other Measurements Glc α -Lp, glc pre β -Lp, glc β -Lp, and glc albumin were quantified by the electrophoretic method using agarose gel film.⁴⁾ Concentrations of albumin, total protein, β -Lp, total cholesterol and high-density lipoprotein-cholesterol (HDL-cholesterol) were measured with an automated clinical analyzer (JCA RX-30; Japan Electronic Optical Laboratory Ltd., Tokyo, Japan) in our clinical laboratory.

Statistical Analysis The results were recorded with a Lotus 1-2-3 version R2-1J plus (Lotus Development Corp., Cambridge, MA, U.S.A.), which we programmed to perform statistical calculations. Statistical significance was assessed by the Student's *t*-test for paired or unpaired data, as appropriate.

Results and Discussion

Figure 1 illustrates the distribution of fructosamine levels in sera from the nondiabetic (I and II) and diabetic populations (III and IV). All histograms showed typical Gaussian distribution, similar to the profile demonstrated by Lim and Staley.¹⁰⁾ The mean fructosamine levels (mm) in groups III and IV were significantly higher than those in groups I and II. The nondiabetic groups I and II showed a slim bell-type curve with a small standard deviation (S.D.), whereas the diabetic groups III and IV showed a flatter bell-type curve with a large S.D., suggesting the existence of various complicating factors which influence the fructosamine value of diabetic patients.

The levels of AI, fructosamine, glc albumin, glc Lps and other measures of atherogenesis risk in the diabetic and nondiabetic groups are given in Table I. The fructosamine levels in diabetic groups III and IV were about 1.2-fold higher (3.00–3.09 mm) than those in the nondiabetic groups I and II (2.49–2.52 mm). Of the nondiabetic groups, group II showed a 3.5-fold higher level (0.45 mm) of glc pre β -Lp than group I (0.13 mm). The diabetic groups III and IV showed a 3.3–4.2-fold higher level of glc pre β -Lp than

Fig. 1. Frequency Distribution of Fructosamine Levels in Diabetic and Nondiabetic Groups

Mean value of fructosamine (mm, mean $\pm$ S.D.): a, 2.48 ± 0.27 (I, $n=31$); b, 2.52 ± 0.14 (II, $n=29$); c, 2.82 ± 0.68 (III, $n=18$); d, 3.09 ± 0.59 (IV, $n=61$).

TABLE I. Fructosamine, Glc Lps Levels and Other Measures of Atherosclerosis Risk in Diabetic and Nondiabetic Subjects

Analyte	Found (mean $\pm$ S.D.)				<i>p</i> (group-group)
	I ($n=31$)	II ($n=31$)	III ($n=20$)	IV ($n=63$)	
AI	1.95 ± 0.40	3.37 ± 0.47	2.19 ± 0.40	5.00 ± 1.92	<0.001 (I-II), (III-IV)
Fructosamine (mm)	2.49 ± 0.25	2.52 ± 0.17	3.00 ± 0.76	3.09 ± 0.51	<0.001 (II-III); <0.6 (I-II), (III-IV)
Glc α -LP (mm)	0.26 ± 0.09	0.27 ± 0.04	0.37 ± 0.10	0.35 ± 0.09	<0.001 (II-III)
Glc pre β -LP (mm)	0.13 ± 0.08	0.45 ± 0.15	0.54 ± 0.17	0.43 ± 0.16	<0.001 (I-II), (I-IV)
Glc β -LP (mm)	0.39 ± 0.17	0.69 ± 0.19	0.63 ± 0.24	0.82 ± 0.26	<0.001 (I-II), (III-IV)
Glc albumin (mm)	1.65 ± 0.25	1.12 ± 0.22	1.47 ± 0.48	1.45 ± 0.39	<0.001 (I-II, III, IV)
β -LP (g/l)	4.10 ± 0.75	4.63 ± 0.68	3.70 ± 0.75	4.87 ± 1.08	<0.001 (I-II), (III-IV)
Total protein (g/l)	78 ± 7	69 ± 4	67 ± 5	66 ± 5	<0.05 (II-III); <0.005 (II-IV)
Albumin (g/l)	45 ± 4	40 ± 2	38 ± 3	38 ± 3	<0.01 (II-III)

group I. Group IV, which seems to have a highly abnormal metabolism of lipoprotein showed a lower level of glc pre β -LP than group III, in compensation for the glc β -LP level. However, we cannot explain this finding in the present study. Group II had 0.69 mm of glc β -LP, which was 1.8-fold more than that of group I (0.39 mm). Group IV showed the highest level of glc β -LP (0.82 mm), which was 1.3-fold higher than that of group III (0.63 mm). Groups II and IV, which had high values of AI (II, 3.37; IV, 5.00), showed higher levels of β -LP (II, 4.63 g/l; IV, 4.87 g/l) than groups I and III (I, 4.10 g/l; III, 3.70 g/l). For group III, the levels of glc β -LP and β -LP were 0.63 mm and 3.70 g/l, respectively, suggesting that the glycation of β -LP was accelerated by diabetic hyperglycemia. The glc β -LP level was the highest (0.82 mm) in group IV, which showed a high level of β -LP and hyperglycemia. Although the level of β -LP in group III was lower than that of group IV, the ratio of glc β -LP/ β -LP (0.17 mmol/g) of group III was the same as that of group IV. Therefore, this result suggests that the high level of glc β -LP in group IV is attributable not only to an increase in the level of β -LP but also to the accelerated glycation of β -LP in diabetic groups III and IV.

Neither the level of glc albumin nor total albumin par-

TABLE II. Correlation between Glc Lps and Fructosamine

Analyte	Correlation (<i>r</i>)			
	I	II	III	IV
Glc α -LP	0.68 ^{a)}	0.27	0.40	0.13
Glc pre β -LP	0.25	0.03	0.80 ^{a)}	0.23
Glc β -LP	0.20	0.51 ^{a)}	0.63 ^{a)}	0.66 ^{a)}
Glc albumin	0.25	0.26	0.92 ^{a)}	0.83 ^{a)}
β -LP	0.12	0.31	0.43 ^{b)}	0.46 ^{a)}

a) $p < 0.01$, b) $p < 0.05$.

alleled the changes in the fructosamine level in this study. The fructosamine level was around 2.5 mm in the nondiabetic groups I and II, and around 3.0 mm in the diabetic groups III and IV. The levels of total protein and albumin in the diabetic groups were slightly lower ($\sim 15\%$) than those in the nondiabetic groups. Of the nondiabetic groups showing similar fructosamine levels (2.49–2.52 mm), group II, with the high risk of atherogenesis (AI > 2.8), showed higher levels of glc pre β -LP, glc β -LP and β -LP than group I, which had a low AI value (AI ≤ 2.8). On the other hand, the level of glc albumin seems to decrease in

Fig. 2. Correlations between Glc Albumin and Fructosamine Levels in Diabetic and Nondiabetic Subjects

a, nondiabetic groups I ($\circ$, $y = 4.00x + 4.12$, $r = 0.25$, $n = 31$), II ($\triangle$, $y = 2.86x + 0.45$, $r = 0.26$, $n = 31$); b, diabetic groups III ($\bullet$, $y = 1.72x + 0.45$, $r = 0.92$, $n = 20$), IV ($\blacktriangle$, $y = 1.59x + 0.75$, $r = 0.83$, $n = 61$).

compensation for the glc Lps levels in group II.

Table II shows the correlations between the fructosamine level and the glc Lps, glc albumin and β -Lp levels in the diabetic and nondiabetic groups. In group I, only the glc α -Lp level correlated with the fructosamine level. Although the level of α -Lp was not measured, the serum from group I probably contained a high level of α -Lp because the AI value was small and the β -Lp level was not particularly low.

Correlations with fructosamine were observed for the four parameters other than glc α -Lp in group III, and glc β -Lp and glc albumin in group IV. Glc albumin and glc β -Lp were also correlated with the fructosamine level only in groups III and IV; therefore, these parameters seem to reflect diabetic hyperglycemia. High correlation between the glc β -Lp and fructosamine levels was also found in group II, suggesting that an increase in the level of glc β -Lp reflects hyperglycemia or a high level of β -Lp in serum.

Figure 2 shows profiles of the correlation between glc albumin and fructosamine levels in the diabetic and nondiabetic populations. There was a significant difference between the correlation with the fructosamine level in the diabetic ($r = 0.83$ – 0.92 , $p < 0.01$) and nondiabetic subjects ($r = 0.25$ – 0.26).

As shown in Fig. 2, the level of glc albumin of group I was distributed in a slightly higher range (around 1.7 mM) than in group II (around 1.1 mM). In spite of the glc albumin level not having increased in group II, the fructosamine level changed by 2.0 to 3.0 mM. On the other hand, although

the range of the glc albumin level was similar to that of the nondiabetics, the fructosamine level increased significantly, up to around 4.0 mM, in the diabetic groups.

In conclusion, the present results suggest that the fructosamine level in serum reflects not only the glycation of proteins, especially albumin, but also that of lipoproteins such as pre β - or β -LPs related to the risk factor of atherogenesis based on abnormal metabolism of lipoproteins.

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