Original Article

Influence of Serum Protein Levels on Serum Fructosamine Levels

Munetada OIMOMI, Shogo MASUTA, Makoto SAKAI, Takeshi OHARA,
Fumihiko HATA and Shigeaki BABA

The serum fructosamine level is influenced by the serum protein level. Therefore, we investigated the relationship between serum fructosamine levels and serum total protein and albumin levels measured simultaneously in non-diabetic and diabetic patients. Significant positive correlations were found between them. A correction formula was constructed to express the serum fructosamine level when the serum total protein level was 7 g/dl or when the serum albumin level was 4 g/dl: corrected fructosamine (mmol/l) = measured fructosamine (mmol/l) + A (7 (g/dl) or 4 (g/dl) — serum total protein (g/dl) or serum albumin (g/dl)) where A is 0.28 for serum total protein and 0.30 for serum albumin in non-diabetic subjects; 0.29 for serum total protein and 0.35 for serum albumin in diabetic patients; and 0.29 for serum total protein and 0.31 for serum albumin in non-diabetic and diabetic subjects combined.

Key words: Glycation, Glycated serum protein, Diabetes mellitus, Blood glucose control

Serum fructosamine has been clinically used as an indicator of blood glucose control in the previous two weeks. The fructosamine level is measured by colorimetry (1), taking advantage of the fact that fructose-lysine, which consists of glucose bound to a lysine residue of serum protein, is reduced under alkaline conditions and, therefore, the level may be affected by the level of serum proteins (2, 3). Thus, the serum fructosamine level should be expressed after correction for serum protein level (4). Howey et al. (5) have already constructed a correction formula for calculating the serum fructosamine level, taking the serum protein levels into consideration. We have undertaken studies in vitro and in vivo to construct a correction formula for calculating the serum fructosamine level in a similar manner.

MATERIALS AND METHODS

The study was conducted with 175 non-diabetic subjects with fasting plasma glucose $\leqq$ 100 mg/dl and 155 diabetic patients with fasting blood glucose $\geqq$ 120 mg/dl and serum fructosamine levels $<$ 4.0 mmol/l. The serum total protein, and serum albumin levels were measured in the samples which were used for the measurement of serum fructosamine.

For studies in vitro, glucose at concentrations of 100 mg/dl, 200 mg/dl, and 300 mg/dl was added to human serum albumin at various concentrations under sterile conditions. The mixture was then incubated in 0.5 M sodium phosphate with 3 mM of sodium azide, pH 7.45, at 37°C for 2 weeks.

Serum samples were used for the measurement of fructosamine. Fructosamine was measured according to the method of Johnson et al. (1) by the fructosamine test (Hoffman La Roche, Basel). As a primary standard, 1-deoxy-1-morpholino-fructose was used. Twenty microliters of serum were colored with 0.25 mmol/l of nitro-blue tetrazolium at 37°C, at pH 10.35, and absorbance was measured at 550 nm using an automated analyzer (Cobas Mira, Japan Roche, Tokyo).

Serum glucose was measured by the glucose oxidase method and HbA_{1c} was measured by high-

From The Second Department of Internal Medicine, Kobe University School of Medicine, Kobe, Japan
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Reprint request to: Munetada Oimomi MD, The Second Department of Internal Medicine,
Kobe University School of Medicine, 5-1, 7-chome, Kusunoki-cho, Chuo-ku, Kobe, 650, Japan

performance liquid chromatography (HPLC) using the column chromatography system Auto A_{1c} (Kyoto Daiichi Co., Ltd., Kyoto, Japan).

Serum total protein and serum albumin were measured by the Biuret method and by the brom-cresol-purple method (TBS-80S; Toshiba Co., Tokyo), respectively.

To test differences between correlation coefficients, normal approximation after z-transformation of Student's t test was used.

RESULTS

In non-diabetic subjects, the serum total protein level and serum albumin level were significantly and positively correlated with the serum fructosamine level ($Y = 0.285X + 0.850$ and $Y = 0.304X + 1.598$, respectively, where Y = serum fructosamine level, X = serum total protein level or serum albumin level (Fig. 1). Therefore, the corrected value of serum fructosamine can be expressed as follows: corrected value (mmol/l) = measured value (mmol/l) — A (7 (g/dl) or 4 (g/dl) — serum total protein (g/dl) or albumin (g/dl)) where A is the gradient of the regression line, and 7 and 4 are the target values of the corrected serum total protein and

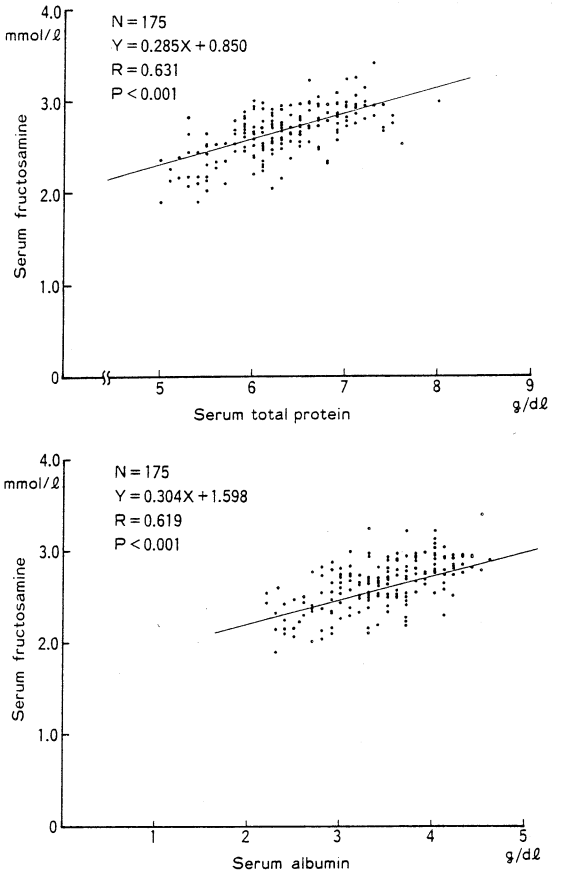

Fig. 1. Correlation between serum total protein and serum albumin concentrations and serum fructosamine concentration in non-diabetic subjects.
Upper panel: Correlation between serum total protein and serum fructosamine concentrations
Bottom panel: Correlation between serum albumin and serum fructosamine concentrations

Table 1. Fructosamine correction formula

Corrected fructosamine = {measured fructosamine + **A** (7.0 or 4.0−serum total protein or serum albumin concentration, g/dℓ)} mmol/ℓ

	A	
	serum total protein	serum albumin
Non-diabetic subjects	0.28	0.30
Diabetic patients (Fructosamine < 4.0)	0.29	0.35
All subjects (Fructosamine<4.0)	0.29	0.31

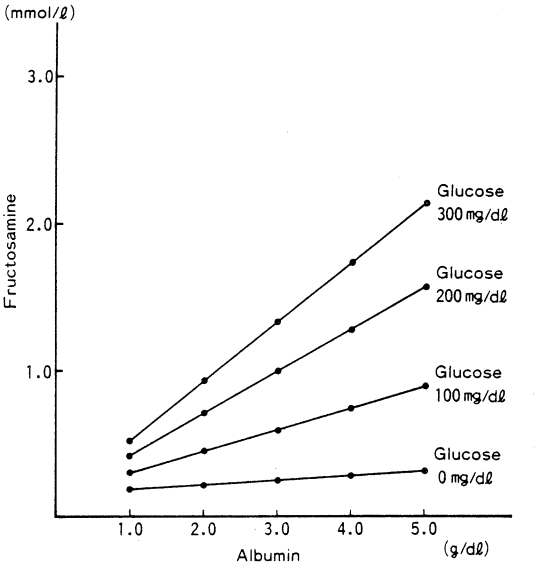

Fig. 2. Fructosamine levels after incubation of various concentrations of human serum albumin with glucose at 37°C for 2 weeks.

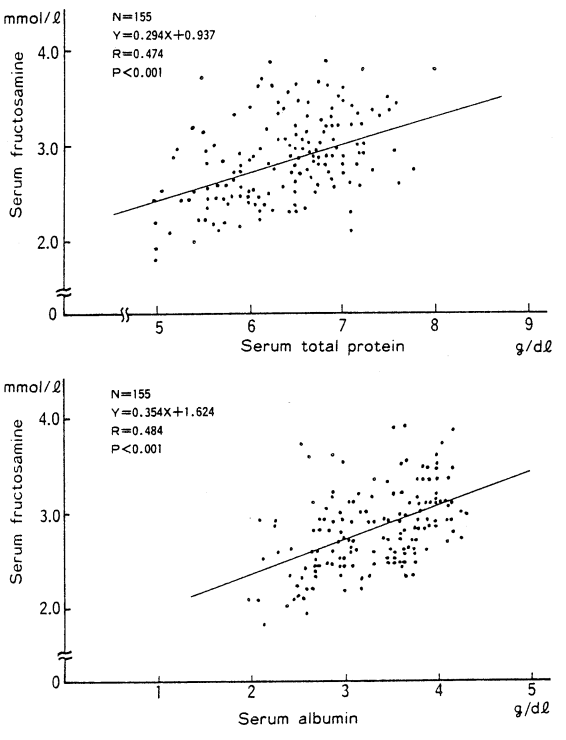

Fig. 3. Correlation between serum total protein and serum albumin concentrations and serum fructosamine concentration in diabetic patients.
Upper panel: Correlation between serum total protein and serum fructosamine concentrations
Bottom panel: Correlation between serum albumin and serum fructosamine concentrations

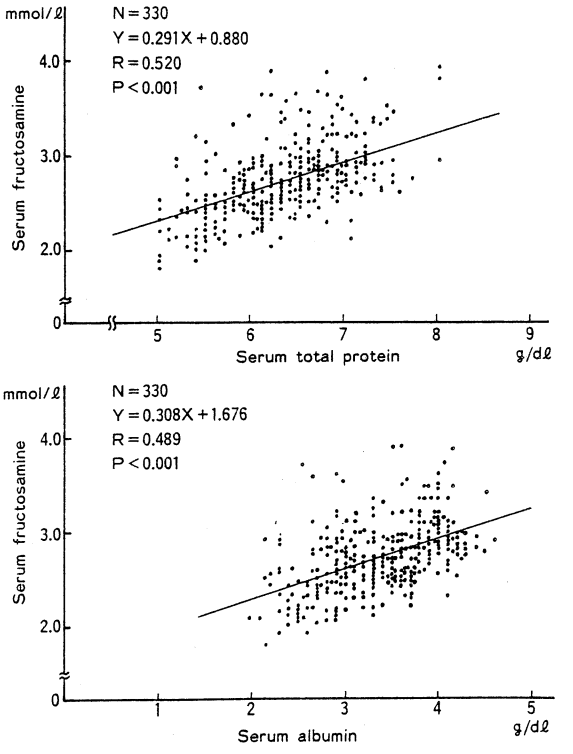

Fig. 4. Correlation between serum total protein and serum albumin concentrations and serum fructosamine concentration in non-diabetic and diabetic subjects.
Upper panel: Correlation between serum total protein and serum fructosamine concentrations
Bottom panel: Correlation between serum albumin and serum fructosamine concentrations

the corrected serum albumin, respectively (Table 1).

In vitro, incubation of various concentrations of human serum albumin with varying concentrations of glucose showed that the levels of fructosamine increased in proportion to the concentration of albumin (Fig. 2). At given concentrations of albumin, the level of fructosamine increased with the concentration of glucose. In particular, the production of fructosamine was accelerated with increasing concentration of glucose.

In diabetic patients with serum fructosamine levels < 4 mmol/l, the serum total protein and serum albumin levels were also significantly and positively correlated with the serum fructosamine level ($Y = 0.294X + 0.937$ and $Y = 0.354X + 1.624$, respectively) (Fig. 3) (Table 1).

In non-diabetic subjects and diabetic patients, the serum total protein and serum albumin levels were significantly and positively correlated with the serum fructosamine levels ($Y = 0.291X + 0.880$, $n = 330$, $r = 0.52$, $p < 0.001$ and $Y = 0.308X + 1.676$, $n = 330$, $r = 0.49$, $p < 0.001$, respectively) (Fig. 4) (Table 1).

DISCUSSION

In determining fructosamine level by the levels of serum protein, the proportion of albumin in serum protein, and the life span of serum protein may influence the measured value. Of these variables, serum protein levels are clinically the most important. The present studies in vivo and in vitro revealed that the serum protein level influences the fructosamine level. To correct for changes in serum

protein levels, a division of the fructosamine level by the total protein level may be considered. However, as seen from the relationship between the serum total protein or serum albumin level and the serum fructosamine level, if the serum fructosamine level is simply divided by the total protein or serum albumin level, the serum fructosamine level is high when total protein or serum albumin level is low and is low in the reverse case. We considered that, as Howey et al. (5) reported, a correction using fixed levels of total protein and albumin might be effective. As in our study in vitro, glycation might be accelerated by evaluating the glucose level and, therefore, we divided our subjects into non-diabetic and diabetic patients. The gradient of the linear regression line for levels of protein and fructosamine was greater for diabetic patients than for non-diabetic subjects. Diabetic patients were restricted to those who had serum fructosamine levels below 4 mmol/l. Since no remarkable difference in A, the gradient of the regression line, was found between in diabetic and non-diabetic groups, the coefficient A was estimated in non-diabetic subjects and diabetic patients having serum fructosamine levels of < 4 mmol/l and was found to be 0.29 for serum total protein and 0.31 for serum albumin. These values were considered to be of practical clinical use. The correction of serum fructosamine levels is required when screening for diabetes, investigating diabetic complications and comparing cases, whereas it is not always necessary for follow-up studies of each patient, including patients who have been subjected to immediate control of hyperglycemia such as is required after diabetic ketoacidosis. In patients with serum fructosamine level $\geqq 4$ mmol/l, some medical treatment is immediately given to lower blood glucose levels. Therefore, the correction is not always necessary in patients with fructosamine level $\geqq 4$ mmol/l. Furthermore, in patients with serum fructosamine $\geqq 4$ mmol/l, the coefficient A may be large. In contrast, if serum fructosamine is less than 4 mmol/l, the use of the same correction formula as in non-diabetic subjects seems to be appropriate.

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