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Glycosylation of nail in diabetics: possible marker of long-term hyperglycemia

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Summary

Fingernail samples from 32 patients with diabetes mellitus and from 26 non-diabetics were analyzed in order to determine the protein glycosylation rate in nail. Nail glycosylation was assayed by the thiobarbituric acid reaction. Blood was taken from both diabetics and non-diabetics at the same time for measurement of hemoglobin glycosylation. In non-diabetics, the protein glycosylation in nail and glycosylated hemoglobin were found to be 8.35 ± 2.7 nmol fructosamine/mg nail and 2.24 ± 0.45 μmol fructosamine/g hemoglobin, respectively. In diabetics, however, there was an extremely high glycosylation in both nail protein and hemoglobin: 16.0 ± 7.35 nmol fructosamine/mg nail and 5.17 ± 1.17 μmol fructosamine/g hemoglobin ($p < 0.001$ for both). A significant correlation was found between nail glycosylation and glycosylated hemoglobin in diabetics ($r = 0.923$, $p < 0.001$). Also, there was a correlation between diabetic fasting blood glucose and protein glycosylation in nail ($r = 0.947$, $p < 0.001$). Our findings show that it might be useful in the investigation of microvascular complications of diabetes mellitus to evaluate the possibility of nail glycosylation providing a stable long-term measure of tissue glycosylation.

Introduction

Glycosylated proteins, especially hemoglobins, have gained acceptance as an accurate index of long-term blood glucose control in diabetic patients [1–4]. The discovery of increased non-enzymatically glycosylated hemoglobin concentrations in disease has led to intensive research into similar excess glycosylation of other tissue

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proteins [5–9], because there may be a link between glycosylation and chronic complications of diabetes mellitus. In assessing diabetic control, measurement of percentage concentration of glycosylated hemoglobin has proved useful, but tissue collagen, being susceptible to functional changes from excessive glycosylation, has a much slower turnover rate than hemoglobin. We have conducted such a study because it is of interest to investigate possible longer term measures of chronic hyperglycemia other than hemoglobin.

Subjects and methods

The patients comprised 32 insulin-dependent diabetics (age range 20–76 yr, 21 females). Twenty-six non-diabetic subjects (age range 17–50 yr, 10 females) served as controls. Blood samples were taken from both groups for measurement of glycosylated hemoglobin, whole blood hemoglobin, and fasting blood glucose concentrations at the time of nail sampling. Fingernail samples, about 20 mg, were obtained from healthy ones by paring away finely at the surface, but not the tip, of them. We avoided dirt on the nails.

Hemoglobin was assayed by the cyanmethemoglobin method, and glucose by the glucose oxidase method (Boehringer, GOD perid method).

Glycosylated proteins were measured by the colorimetric method of Parker and co-workers [10]. The method was modified in accordance with our experimental conditions. All assays were duplicated and conducted in one series. Another series was undertaken to determine the interassay coefficient of variation.

Measurement of nail glycosylation

Fingernail samples pared away finely were carefully weighed out into 5 mg portions and incubated for two hours at 120°C in an autoclave (Marsh Inst. Co., Leave, Spacey, 0–150°C, 0–4 lbs/in^2) with 2 ml of oxalic acid solution (0.5 mol/l). When cool 2 ml of 40% trichloroacetic acid was added to each tube, the tubes centrifuged, and two 1.5-ml samples of supernatant taken. One was incubated with 0.5 ml of thiobarbituric acid solution (0.05 mol/l), and the other with 0.5 ml distilled water for 30 min at 40°C. After cooling to room temperature, the color produced was measured by spectrophotometry at 443 nm, the distilled water incubate serving as a sample blank. Serial dilutions of 200 μmol fructose and 5-hydroxymethylfurfural (5-HMF) per liter to 10–80 μmol/l were tested to obtain a standard curve for each assay series (Fig. 1). The intra-assay coefficient of variation was found to be 2.8% for control and 4.3% for diabetic samples, and that of interassay 4.9% and 8.2%, respectively.

Statistics

Results were evaluated using least mean squares regression analysis for correlations, with analysis of variance and paired and unpaired t tests for difference between groups; for the difference between glycosylation of normal and diabetic nail, an unpaired t test allowing for dissimilar variances was used.

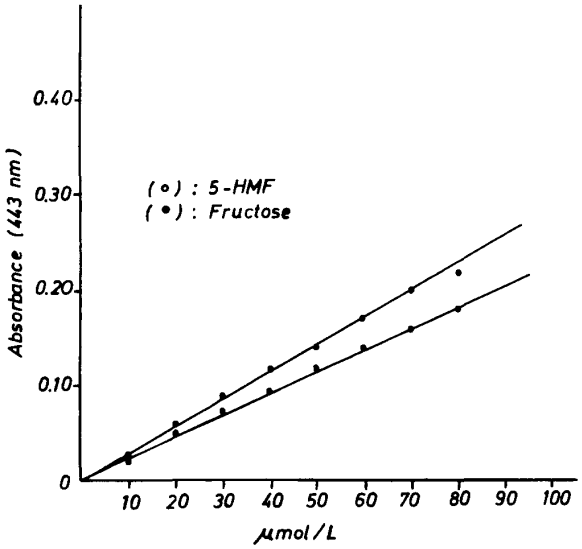

Fig. 1. Standard curves obtained from autoclaved fructose and 5-HMF.

Results

The ranges of glycosylation of nail and hemoglobin, fasting blood glucose, and hemoglobin are shown in Table I. The mean nail glycosylation, as well as glycosylated hemoglobin in the diabetics, was significantly higher than in the control subjects ($p < 0.001$). There was no alteration in the range of nail glycosylation with age and sex in either group of subjects. Nail from diabetics was found to be glycosylated in proportion to the hemoglobin glycosylation, that is, there was a good correlation ($r = 0.923$, $p < 0.001$) (Fig. 2). Hemoglobin values in diabetics and controls had no correlation with nail and hemoglobin glycosylation, while the

TABLE I

Mean values of parameters in non-diabetics and diabetics

	Non-diabetics ($n = 26$)	Diabetics ($n = 32$)	p values
Hemoglobin (g/dl)	14.5 ±1.15	14.1 ± 1.26	ns
Fasting blood glucose (mg/dl)	79.6 ±8.4	222.2 ±34.7	< 0.001
Glycosylated hemoglobin (μmol fructosamine/g Hb)	2.24±0.45	5.71± 1.17	< 0.001
Glycosylated nail (nmol fructosamine/mg nail)	8.35±2.7	16.0 ± 7.35	< 0.001

ns, Not significant.

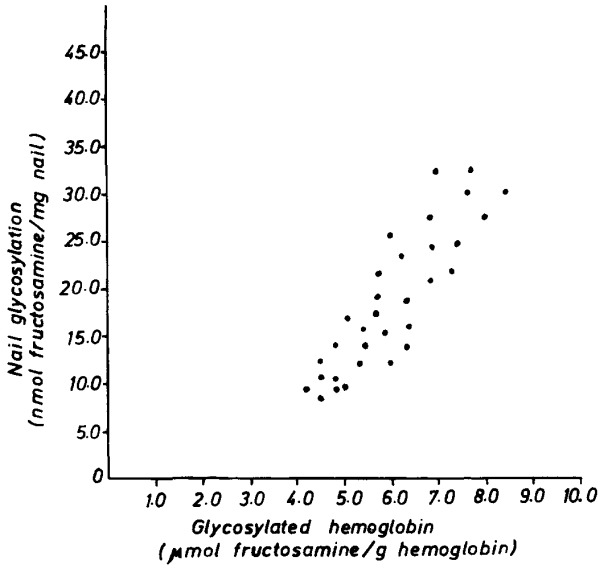

Fig. 2. Relation between glycosylation of nail and glycosylated hemoglobin values in diabetics ($n = 32$, $r = 0.923$, $y = -16.8 + 57.6x$).

correlation of the fasting blood glucose of diabetic group with nail and hemoglobin glycosylation was $p < 0.001$ for both.

The simultaneous test of both standards, fructose and 5-HMF, showed that the latter was destroyed during autoclave incubation at a rate of about 12% and that fructose was to be used as a standard in order to obtain a standard curve. We evaluated our data according to the fructose curve.

Discussion

The general nature of the reaction between aldose sugars and protein amino groups has led to studies involving the non-enzymatic glycosylation of proteins other than hemoglobin. Whether a causal relationship can be unravelled between the glycosylation of structural proteins and the development of macro- or microvascular complications of diabetes remains to be elucidated. Our findings show that glycosylation of nail proteins occurs and is increased in diabetics when compared with non-diabetics. There was a significant correlation between nail glycosylation and simultaneously measured glycosylated hemoglobin values. This closely related to high blood glucose concentrations in the preceding several weeks. However, it seems likely that nail proteins are glycosylated with different dynamics from hemoglobin, an important factor when evaluating possible links between tissue glycosylation and diabetic microvascular complications.

Further investigation into the chemical nature of the glycosylation in nail is needed. If more studies confirm our findings, the test may be of use forensically, because of the stability of fructosamine in nail. Such a test could also be of value in population studies, where a large number of samples might be taken quickly and stored easily.

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