Relationship Between Glycosylation of Haemoglobin and the Duration of Diabetes: A Study During the Third Trimester of Pregnancy

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Summary. The relationship between blood glucose and glycosylated haemoglobin (HbA_{1c}) has been investigated during an 8 week period in 53 Type 1 (insulin-dependent) diabetic women studied during the third trimester of pregnancy. Blood glucose estimations (fasting and 2 h post-prandially) were made an average of 41 times in each patient during this period and HbA_{1c} was determined once at the end of the study. There was a significant correlation between both the mean blood glucose over the preceding 8 weeks and the standard deviation of the fasting blood glucose with HbA_{1c} ($r = 0.69$, $p < 0.001$; $r = 0.46$, $p < 0.001$, respectively). A 'glycosylation index' was calculated for each patient (HbA_{1c} divided by the mean blood glucose value). There was a significant correlation between the 'glycosylation index' and duration of diabetes ($r = 0.68$, $p < 0.001$). In contrast, there was no correlation between red cell 2,3-diphosphoglycerate and HbA_{1c} or 'glycosylation index'. These findings suggest that increasing duration of diabetes influences the post-translational formation of HbA_{1c} and that isolated HbA_{1c} values need to be interpreted with caution in the pregnant diabetic.

Key words: Haemoglobin A_{1c}, duration of diabetes, diabetic pregnancy, glycosylation index, 2,3-diphosphoglycerate.

Glycosylation of haemoglobin, leading to the formation of haemoglobin A_{1c} (HbA_{1c}), is increased in diabetic patients [1]. It has been shown that HbA_{1c} is formed by a non-enzymatic, post-translational reaction during the 120-day life span of the erythrocyte [2]. The mean blood glucose concentration of the preceding 1–2 months is the major known factor influencing the glycosylation of haemoglobin in vivo in both non-

pregnant [3, 4] and pregnant diabetics [5]. Thus, glycosylated haemoglobin seems to be an indicator of blood glucose regulation of the preceding 1–2 months in diabetes. Studies in vitro have shown that the glycosylation of haemoglobin is impaired by carbon monoxide and 2,3-diphosphoglycerate (2,3-DPG) [6]. At present, no other factors are known to influence the glycosylation of haemoglobin. Recently, however, we found in a study of pregnant diabetics, that the same average blood glucose levels over the preceding 8 weeks could lead to quite variable levels of HbA_{1c} in different patients [7].

The purpose of the present study was to evaluate the possible effect of red cell 2,3-DPG and duration of diabetes on the glycosylation of haemoglobin.

Subjects and Methods

The subjects studied were 53 pregnant patients with well established Type 1 (insulin-dependent) diabetes before this pregnancy. The clinical features of the patients studied are shown in Table 1. The patients were classified according to White [8]. None of the patients had severe anaemia or suffered from renal insufficiency. In the third trimester venous blood samples for determinations of HbA_{1c} and red cell 2,3-DPG were taken in the morning after the usual morning insulin dose and a light breakfast. These determinations were preceded by frequent capillary blood glucose determinations (mean 41, range 21–60 mmol/l), taken fasting at 0700 h and 2 h postprandially at 1430 h for a period of 8 weeks. All blood samples were taken in hospital. The diabetes was treated according to Pedersen [9]. The patients were given five daily meals. Forty-seven received two insulin injections and six one injections per day, usually of a small amount of crystalline insulin mixed with NPH.

The levels of HbA_{1c} were determined by the macrocolumn chromatographic method of Trivelli et al. [10] as modified by Schwartz et al. [11]. The haemolysates were dialyzed for 18 h at 4 °C against 1000 volumes of glucose-free 42 mmol phosphate buffer (pH 6.70) which was also used to elute HbA_{1c} during chromatography. Biorex-70 was used as the ion exchange resin. The coefficient of variation by this method in our laboratory is 2.5%. Normal values for pregnant women are $4.0 \pm 0.7\%$ (mean $\pm$ SD). Capillary blood

Table 1. Clinical features and results of the patients studied

	Age (years)	Duration of diabetes (years)	Insulin dose (U)	HbA _{1c} (%)	Blood glucose (mmol/l)			Standard deviation of fasting blood glucose (mmol/l)	2,3-DPG (μ mol/g Hb)
					Fasting (f)	Post-prandial (p)	Average (f + p)		
Mean	25.5	9.6	54.6	7.1	6.2	7.4	6.8	1.9	17.1
$\pm$ SD	4.6	5.6	22.4	1.2	1.7	2.0	1.3	1.0	1.3
Range	18–42	1–23	8–112	4.9–11.0	3.1–11.0	2.9–12.4	4.3–10.5	0.3–5.4	14.7–20.4

White classification					Retinopathy		
	White B	White C	White D	White F	Absent	Background	Proliferative
Number	14	12	22	5	26	22	5

Fig. 1. Relationship of HbA_{1c} to mean blood glucose levels in the preceding 8 weeks in 53 pregnant diabetic subjects ($y = 0.61x + 2.92$, $r = 0.69$, $p < 0.001$)

glucose was measured with a glucose dehydrogenase procedure using Technicon autoanalyzer [12].

Red cell 2,3-DPG concentrations were determined by an enzymatic method according to Ericsson and deVerdier [13]. The coefficient of variation is 2.1% and normal values of pregnant women are $16.0 \pm 1.7 \mu\text{mol/g}$ haemoglobin.

One possible method for detecting other influences on the formation of HbA_{1c} apart from blood glucose per se, is to calculate a 'glycosylation index' by dividing HbA_{1c} by the mean blood glucose level of the preceding 8 weeks.

Statistical correlations were performed by linear regression analysis.

Results

Table 1 presents the mean $\pm$ SD values for HbA_{1c}, blood glucose (fasting and 2 h post-prandially), standard deviation of fasting blood glucose and 2,3-DPG.

There was a significant correlation between HbA_{1c} and mean blood glucose of the preceding 8 weeks ($r = 0.69$, $p < 0.001$; Fig. 1). In addition, the standard deviation of fasting blood glucose (in absolute units) correlated significantly with HbA_{1c} ($r = 0.46$, $p < 0.001$) and the mean blood glucose ($r = 0.48$, $p < 0.001$). The correlation between standard deviation of fasting blood glucose and HbA_{1c} persisted when the standard deviation was expressed as a percentage of the mean fasting blood glucose (i.e. 'correcting' for the level of glycaemia), although this correlation was weak ($r = 0.35$, $p < 0.01$). From Figure 1 it can also be observed, that HbA_{1c} values may vary by approximately 50% for the same mean blood glucose concentration in different patients.

Red cell 2,3-DPG was not correlated to HbA_{1c} ($r = 0.07$, NS) or to 'glycosylation index' ($r = 0.04$, NS). No correlation was found between the duration of diabetes and the actual HbA_{1c} level ($r = 0.02$, NS). However, a significant correlation was present between the duration of diabetes and 'glycosylation index' ($r = 0.68$, $p < 0.001$; Fig. 2).

The subjects were divided into two groups; group 1 consisting of 23 patients with duration of diabetes less than 10 years and group 2 of 30 patients with duration of diabetes of 10 years or more. The correlation between HbA_{1c} and mean blood glucose for each group was higher than for the collective material (group 1: $r = 0.80$, $p < 0.001$; group 2: $r = 0.79$, $p < 0.001$; Fig. 3). The regression line for the group with longer duration of diabetes was placed significantly higher than that of the group with shorter duration of the disease ($p < 0.001$). There was no difference between the slopes of the regression lines.

The standard deviation of fasting blood glucose did not correlate with the duration of diabetes, neither when the standard deviation was expressed in absolute units ($r = 0.06$, NS), nor as a percentage of the mean fasting blood glucose ($r = 0.22$, NS).

Fig. 2. Relationship of 'glycosylation index' to the duration of diabetes in 53 pregnant diabetic subjects ($y = 0.02x + 0.87$, $r = 0.68$, $p < 0.001$)

Fig. 3. Relationship of HbA_{1c} to mean blood glucose levels in the preceding 8 weeks for group 1 (○) ($y = 0.69x + 1.84$, $r = 0.80$, $p < 0.001$) and group 2 (●) ($y = 0.71x + 2.70$, $r = 0.79$, $p < 0.001$). (See text)

Discussion

This study confirms the results of others that HbA_{1c} is related to the mean blood glucose of the preceding 8 weeks [3–5]. However, as indicated in the present study and in those of others [3–5], HbA_{1c} may vary markedly (by approximately 50%) for similar mean blood glucose levels from one patient to another, suggesting that the glycosylation process of haemoglobin might also be influenced by factors other than preceding blood glucose level. These variations might be partly explained by the inherent inaccuracy of deter-

mining the actual variation in blood glucose through the 24 h period on the basis of a few separate measurements. The method for determining HbA_{1c} used in this study included 18 h of dialysis of the haemolysate against a glucose-free medium. This procedure minimizes the effect of short term changes in blood glucose on the level of HbA_{1c} [14, 15]. In a study of non-pregnant diabetic patients, we recently found that HbA_{1c} correlated significantly with the standard deviation of fasting blood glucose, suggesting that the more variable the blood glucose, the stronger its effect on the glycosylation of haemoglobin [16]. The same correlation between standard deviation of fasting blood glucose and HbA_{1c} was found in the present study of pregnant diabetic patients. A correlation of similar magnitude was also present between the standard deviation and mean blood glucose levels. Thus, it cannot be decided whether variations per se influence the glycosylation of haemoglobin. However, the slight correlation between HbA_{1c} and standard deviation of fasting blood glucose, expressed as a percentage of the mean fasting blood glucose, may indicate a weak influence of blood glucose variation on the glycosylation process.

From studies in vitro, it is known that 2,3-DPG impairs the glycosylation of haemoglobin [6]. In the present study such a negative relationship between red cell 2,3-DPG and the glycosylation process could not be demonstrated. However, as glycosylation occurs throughout the 120-day life span of the erythrocyte and 2,3-DPG was only determined once during this period, our study does not exclude an influence of 2,3-DPG on the formation of HbA_{1c}.

In the study of Trivelli et al. [10] HbA_{1c} was not found to be related to the duration of diabetes. In a recent study by Goldstein et al. [17] on 180 children and

adolescents with diabetes mellitus, a slight but significant correlation ($r = 0.34$) between HbA_{1c} and the duration of diabetes was found. In this study we did not find any such correlation. However, we found that the 'glycosylation index' was strongly related to the duration of diabetes, which may indicate that the rate of glycosylation increases with duration of the disease. The underlying reason for this finding is not known. In our study we made no correction for haemoglobin F, which co-elutes with HbA_{1c} on Biorex macrocolumns. However, haemoglobin F in normal pregnancy rarely exceeds 0.5% [18] and no evidence is available to indicate that haemoglobin F is increased in diabetic pregnancy [11]. Though haemoglobin F may theoretically increase with duration of diabetes, it is unlikely that this would explain the present finding. As we found no correlation between the variability of blood glucose and duration of diabetes, it is not likely either, that the duration effect can be explained by increasing variability of blood glucose with duration of diabetes. A possible biological explanation for our finding may be a decreasing ability of the individual to secrete insulin. To determine whether the duration effect on the glycosylation of haemoglobin in diabetes is an isolated phenomenon during pregnancy requires further investigation.

Pregnancy is a period in which blood glucose must be strictly regulated to obtain the best prognosis for the fetus and newborn infant [8, 9]. As HbA_{1c} is related to time-integrated blood glucose, HbA_{1c} has been suggested as a valuable tool in optimising blood glucose regulation. However, because the glycosylation of haemoglobin seems to vary from one patient to another, possibly due to the influence of the duration of the disease, a single HbA_{1c} determination cannot satisfactorily predict the degree of regulation. Our experience suggests that, besides frequent blood glucose determinations, HbA_{1c} should be determined once or twice-weekly throughout the pregnancy.

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