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Fetal haemoglobin levels in adult Type 1 (insulin-dependent) diabetic patients

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Summary. Glycated haemoglobin levels (HbA_1 and HbA_{1c}) are established parameters of long-term glycaemic control in diabetic patients. Depending on the method used, fetal haemoglobin interferes with the assays for glycated haemoglobin. If present in high amounts, fetal haemoglobin may lead to overestimation of glycated haemoglobin levels, and therefore, of average blood glucose concentration in diabetic patients. Glycated (HbA_{1c}) and fetal haemoglobin levels were measured by high pressure liquid chromatography in 60 (30 female) adult Type 1 (insulin-dependent) diabetic patients of Swiss descent, and were compared with levels obtained from 60 normal, non-diabetic control subjects matched for age and sex. Fetal haemoglobin levels were significantly higher in the diabetic patients ($0.6 \pm 0.1\%$, mean ± SEM; range: 0–3.6 %) than in the control subjects ($0.4 \pm 0.1\%$, $p < 0.001$). Elevated fetal haemoglobin levels ($\geq 0.6\%$) were found in 23 of 60 diabetic patients (38 %) compared to 9 of 60 control subjects (15 %; $\chi^2 = 8.35$, $p < 0.01$). In addition, fetal haemoglobin levels in diabetic patients are weakly correlated with glycated haemoglobin (HbA_{1c}) ($r = 0.38$, $p < 0.01$). Fetal haemoglobin results were confirmed with the alkali denaturation procedure, and by immunocytochemistry using a polyclonal rabbit anti-fetal haemoglobin antibody. A significant proportion of adult patients with Type 1 diabetes has elevated fetal haemoglobin levels. In certain patients this may lead to a substantial overestimation of glycated haemoglobin levels, and consequently of estimated, average blood glucose levels. The reason for this increased prevalence of elevated fetal haemoglobin remains unclear, but it may be associated with poor glycaemic control.

Key words: Type 1 (insulin-dependent) diabetes mellitus, glycaemic control, HbA_{1c}, fetal haemoglobin.

Glycated haemoglobin levels (HbA_1 and HbA_{1c}) are established parameters of long-term glycaemic control [1–4]. Fetal haemoglobin (HbF) co-migrates with HbA_{1c}, when glycated haemoglobin is determined using ion-exchange based microcolumns or electrophoretic methods. If present in high amounts, HbF may lead to overestimation of glycated haemoglobin levels, and consequently to overestimation of average blood glucose concentration [5]. In humans, HbF represents the major fetal haemoglobin the level of which begins to decrease late in gestation, and further declines rapidly in the neonatal period. By 6 months of age, HbF accounts for less than 5 % of the total haemoglobin. In adults HbF levels are usually below 0.5 %. However, elevated HbF levels have been reported in diabetic children with the elevation appearing to decline with age [6].

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The present study was initiated to determine whether elevated HbF levels are also found in adult Type 1 (insulin-dependent) diabetic patients.

Subjects and methods

Subjects

Sixty adult Type 1 diabetic patients of Swiss descent, and 60 normal, non-diabetic control subjects of Swiss descent matched for age and sex (Table 1) were studied. Control subjects were chosen from among hospital staff. Since HbF continues to decline throughout adult life [7], particular care was taken to match patients and control subjects for age. Pairs of diabetic and control subjects were formed with less than 24 months age difference within pairs. Mean age was 33 ± 1 years (mean ± SEM) in both groups (range 18–59 years in diabetic patients and 19–59 years in control subjects). None of the diabetic patients were anaemic or had nephropathy with increased creatinine levels or macroproteinuria, as these are conditions that might affect erythrocyte survival time and, therefore, glycated haemoglobin levels.

Table 1. Clinical characteristics of adult Type 1 diabetic patients and normal, non-diabetic control subjects

	Type 1 diabetic patients	Control subjects
n	60	60
Age (years)	33 ± 1	33 ± 1
Sex (female/male)	30/30	30/30
HbA_{1c} (%)	8.4 ± 0.2[a]	5.1 ± 0.1

[a] $p < 0.001$ vs control subjects.
Values shown are mean ± SEM

Table 2. Prevalence of increased fetal haemoglobin (HbF) levels in adult Type 1 diabetic patients and in normal control subjects matched for age and sex

HbF	Type 1 diabetic patients	Control subjects
≤ 0.5 %	37	51
≥ 0.6 %	23	9

Type 1 diabetic patients vs control subjects: $\chi^2 = 8.35, p < 0.01$

Analytical methods

HbA_{1c} and HbF were determined by HPLC using a Diamat analyser (Bio-Rad, Glattbrugg, Switzerland). This automated device measures simultaneously (stable) HbA_{1c} and HbF. After pre-incubation with a haemolysing reagent containing a borate buffer allowing the simultaneous elimination of labile HbA_{1c}, the haemolysate is applied to cation-exchange analytical columns maintained at 23°C. The fractions are separated by step-gradient elution using phosphate buffers of increasing ionic strength and detected by a dual wavelength photometer at wave lengths of 415 nm and 690 nm. The data are then processed by a built-in data module to identify and calculate the areas under the peaks. With this method, the elution of the various zones is highly reproducible and the haemoglobin peaks are sharp. The HbF intraassay coefficient of variation (CV) was 4.5 % at 0.7 % HbF and the corresponding interassay CV was 9.8 %, for HbA_{1c}, CVs were below 2 %. In a subset of 10 diabetic patients with HbF levels of 0.5 % or higher, HbF was also determined using the alkali denaturation procedure [8]. In three patients blood smears which had been air dried, were fixed in methanol: acetone (1:6) for 10 min at room temperature. The slides were then stained using a polyclonal rabbit anti-human HbF-antibody (Calbiochem, Läufelfingen, Switzerland), followed by an alkaline phosphatase conjugated porcine anti-rabbit IgG-antibody (Dakopatts, Instrumenten-Gesellschaft, Zürich, Switzerland). The colour reaction was performed with a substrate solution containing Naphthol AS-BI-phosphate and Fast Red TR (Sigma Chemicals Inc., St. Louis, Mo., USA). Slides were then evaluated by light microscopy for the percentage of erythrocytes containing HbF stained red by immunocytochemistry.

Statistical analysis

Data are presented as mean ± SEM. Statistics for inter-group comparisons were performed by non-parametric analysis of variance (Kruskal-Wallis). Discrete variables were compared by chi-square (χ^2) analysis. Least-squares linear regression analysis, and Pearson correlation coefficients were used to assess bivariate interdependency. A p value of less than 0.05 was considered statistically significant.

Results

In the present series, we found HbF levels that were significantly higher in adult Type 1 diabetic patients (0.6 ± 0.1 %, mean ± SEM) than in the control subjects matched for age and sex (0.4 ± 0.1 %, $p > 0.001$). In individual Type 1 diabetic patients, HbF ranged from 0–3.6 %. HbF levels 0.6 % or more (> 80th percentile of normal control subjects) were found in 23 (15 female and 8 male) of the 60 Type 1 diabetic patients compared to 9 of the 60 control subjects ($\chi^2 = 8.35$, $p < 0.01$; Table 2).

To validate the HPLC findings, results obtained by HPLC were compared with HbF levels assayed by the alkali denaturation procedure in a subgroup of 10 Type 1 diabetic patients. As shown in Figure 1, there was an excellent correlation between HbF measured by the two techniques ($r = 0.97$, $p < 0.001$). Even if the patient with the highest HbF levels is excluded from the statistical analysis, the correlation remains highly significant ($r = 0.84$, $p < 0.01$). In addition, using a polyclonal, rabbit anti-HbF-antibody, intracellular HbF was confirmed in three patients. Most interestingly, the smears showed that HbF is not present in all erythrocytes, but rather confined to a subset of cells with 27 %, 21 %, and 17 %, respectively of erythrocytes containing HbF stained by immunocytochemistry (Fig. 2). Figure 3 shows that HbF levels in

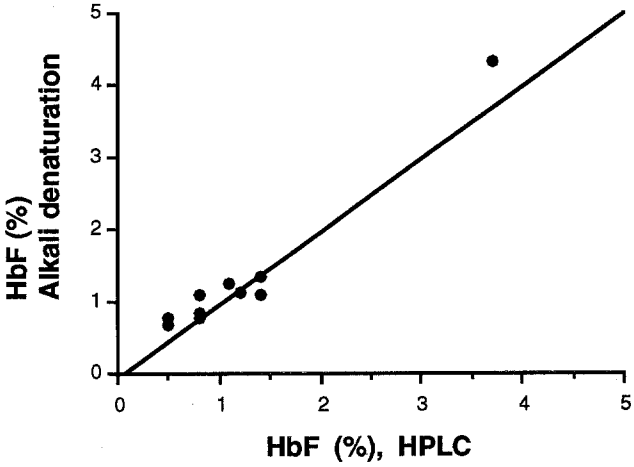

Fig. 1. Correlation between fetal haemoglobin (Hbf) measured by HPLC and HbF measured by alkali denaturation in 10 adult Type 1 diabetic patients with HbF levels of 0.5 % or more ($r = 0.97$)

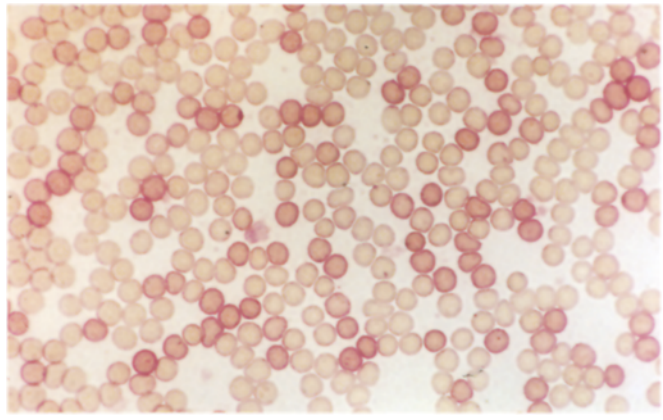

Fig. 2. Fetal haemoglobin (HbF) shown by immunocytochemistry using a polyclonal, rabbit anti-HbF-antibody. HbF-containing cells stain darker. The smear is taken from an adult Type 1 diabetic patient with HbF levels of 3.6 %

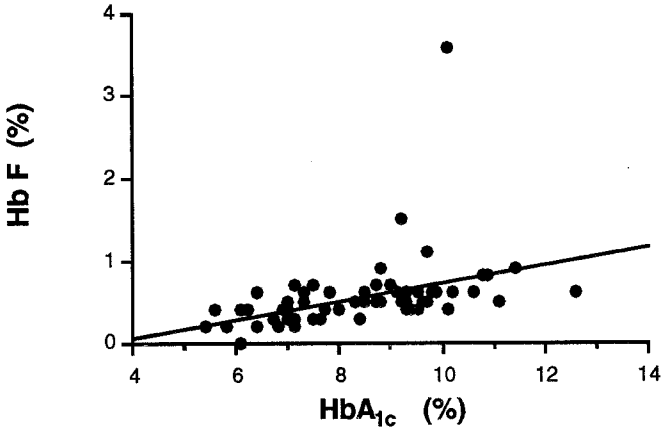

Fig. 3. Correlation between fetal haemoglobin (HbF) measured by HPLC and HbA_{1c} measured by HPLC in 60 adult Type 1 diabetic patients ($r = 0.38$)

Type 1 diabetic patients are related to the degree of metabolic control: there is in fact a weak but significant correlation between HbA_{1c} and HbF levels with $r = 0.38$ ($p < 0.01$). If the outlier with an HbF level of 3.6% were excluded from the analysis, the correlation coefficient would be $r = 0.52$ ($p < 0.0001$). In contrast, there were no significant correlations of HbF levels with age ($r = 0.17$) or diabetes duration ($r = 0.01$).

Discussion

We have found a small but clinically important elevation of HbF levels in adult Type 1 diabetic patients. This finding is in agreement with a previous report showing increased HbF levels in Type 1 diabetic children and adolescents [6]. The elevated HbF levels in diabetic patients are not a mere artefact of the HPLC method used, since the results could be confirmed by immunocytochemistry using a polyclonal rabbit anti-HbF-antibody, and with the alkali denaturation procedure. In individual patients, elevated HbF levels may have a considerable impact on the interpretation of glycated haemoglobin levels, and thus, on the management of the diabetic patient.

The elevation of HbF in Type 1 diabetic patients is difficult to explain. In contrast to HbA which is composed of two α-chains and two β-chains, HbF is composed of two α-chains and two γ-chains. In adults, the persisting amount of HbF is confined to a small erythrocyte population called F-cells. The mechanisms regulating γ-chain gene expression are poorly understood. Elevated levels of HbF have been reported in patients with haematological malignancies [9], β-thalassaemias [10] or sickle-cell disease [11]. Based on past medical history, normal haemoglobin concentration and normal blood smears, such conditions can be excluded in the patients included in this study. Interestingly, butyrate, and some butyric acid analogues have been shown to increase HbF levels [12, 13] and γ-chain gene expression [14, 15]. The effect of butyrates on HbF levels has been ascribed to localized modifications of the DNA, e.g. following therapy with butyric acid analogues, altered methylation of DNA has been proposed as a possible mechanism [16]. Based on these studies, treatment with butyrates has been proposed as a possible therapy of β-globin diseases [17]. The correlation between HbF and HbA_{1c} found in this study, together with the known delay in the fetal globin switch in infants of diabetic mothers [17, 18] suggests that metabolic derangements associated with Type 1 diabetes are able to activate HbF synthesis. Based on the preliminary finding that HbF levels are not elevated in Type 2 (non-insulin-dependent) diabetic patients [19], and the fact that certain butyrates enhance γ-chain gene expression in vitro and in vivo, one may speculate that in Type 1 diabetic patients, one or several factors associated with poor metabolic control (e.g. episodes of increased ketogenesis with elevated levels of β-hydroxy-butyrate) affect the switch from γ-chain to β-chain expression, and lead to increased γ-chain gene expression.

In conclusion, HbF levels are increased in some adult Type 1 diabetic patients. If glycated haemoglobin is measured by ion-exchange based microcolumns or by electrophoretic techniques HbA_{1c} (and also total HbA_1), levels may be falsely elevated leading to an overestimation of average blood glucose levels. Interference of HbF with HbA_{1c} determinations can be avoided by using more specific procedures (HPLC, affinity chromatography, thiobarbituric acid, or radioimmunoassay).

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