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Originals

Increased prevalence of fetal haemoglobin in Type 1 (insulin-dependent) diabetes mellitus

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Summary. Fetal haemoglobin levels were measured in 106 patients with Type 1 (insulin-dependent) diabetes mellitus during a period of two to three years. In 15 patients (14.1%) increased fetal haemoglobin levels (>0.5%), determined by high pressure liquid chromatography, were found in contrast to 3% in a healthy control group (n: 100) of equal age distribution. In children aged over 6 years, elevated fetal haemoglobin levels were measured in 13 diabetic patients (13.3%) in contrast to none of the control group. There was no correlation between fetal haemoglobin levels and duration of diabetes, diabetic control (glycated haemoglobin) and dosage of insulin ($U \cdot kg^{-1}$, day^{-1}). The 15 patients had a younger mean age at onset of diabetes (5.6 years) than a sex and age matched control group of diabetic patients without increased fetal haemoglobin levels (7.4 years, $p<0.05$). Longitudinal assessment revealed a significant decline of fetal haemoglobin levels with age ($p<0.005$) but a further increase in fetal haemoglobin levels were found in adolescent patients (*n*: 2). These data indicate a possible effect of insulin-treatment on delaying transition from fetal to adult haemoglobin synthesis or on reactivation of fetal haemoglobin production.

Key words: diabetes control, diabetes mellitus, fetal haemoglobin, glycated haemoglobin, insulin.

The risk of late diabetic complications is presumably reduced by good glycaemic control. Measurements of glycated haemoglobin (Hb A_1) are now widely used to provide an index of average glucose control over the previous period of about 6 weeks [1]. Depending on the method used, fetal haemoglobin (Hb F) comigrates with Hb A_1 and when present can falsely elevate the measured Hb A_1 [2].

To determine the prevalence of Hb F in our paediatric diabetic clinic we have conducted a longitudinal study.

Patients and methods

Patients

During a 3 year period (May 1985-May 1988) 111 patients with Type 1 (insulin dependent) diabetes mellitus were followed at 3 or 6 monthly intervals at the Policlinic of the Paediatric Department of the University of Bern. At each visit metabolic control was assessed clinically and by laboratory means. Blood was drawn for measurement of Hb F, Hb A_1, total haemoglobin and erythrocyte indices. Treatment consisted of an age-adapted diet, twice-daily injections of a combination of short-acting insulin (Actrapid HM; Novo Industri A/S, Copenhagen, Denmark; or Velosulin H or porcine, Nordisk Gentofte A/S, Gentofte, Denmark) and intermediate-acting insulin (Protaphan HM, Novo Industri A/S; or Insulatard H or porcine, Nordisk Gentofte).

Hereditary persistence of Hb F, abnormalities of beta-chain synthesis such as thalassaemia and sickle cell disease, and several types of acute and chronic anaemia were excluded by haematologic control and by determination of Hb F levels in the parents. Duration of diabetes, diabetes control (Hb A_1), dosage of insulin ($U \cdot kg^{-1} \cdot day^{-1}$) and age at onset of diabetes in every patient with an increased Hb F (>0.5%) were compared to two sex and age matched diabetic patients with normal Hb F (<0.5%). For normal values of Hb F and Hb A_1 100 healthy control subjects with a comparable age-range were examined (Table 2).

Methods

Samples of Hb A_1 and Hb F were collected during a morning routine clinic visit in NH4-heparin-monovettes (Sarstedt AG, Sevelen, Switzerland), kept at 4°C and analysed within the next two days.

Stable Hb A_1 was measured chromatographically by a strictly thermostabilised (23°C) microcolumn method using a commercial kit (Boehringer Mannheim, Mannheim, FRG) [3]. The labile Hb A_1 was eliminated by a borate buffer. 50 µl of heparinised blood was haemolysed with 200 µl haemolysing reagent containing the eliminator of the labile Hb A_1. 50 µl of the haemolysate was applied to a microcolumn. After addition of the eluant for Hb A_1 and other haemoglobins, the Hb A_1 values were calculated by measuring the different absorbances in the eluants at 415 nm against water, by a spectrophotometer SP 6-550 UV/VIS (Pye Uni-cam, Philips, Zurich, Switzerland). Hb A_1 in-

Table 1. Characteristics of the diabetic patients

n	sex f/m	age (years)[a] range (mean)	Hb F(%)[a] range	Hb F(%)[a] $n>0.5\%$	Hb A_1 (%) mean ± SD (range)[b] cation-exchange[c] chromatography		Hb A_1 (%) mean ± SD (range)[b] HPLC
8	2/ 6	2.9–6 (3.95)	<0.5–0.8	2	7.9 ± 1.1 (6.4 – 9.6)		6.7 ± 0.9 (5.5 – 8.1)
26	13/13	6 –12 (9.7)	<0.5–0.8	6	9.6 ± 1.2 (8.0 – 12.0)		8.2 ± 1.2 (6.0 – 11.0)
32	17/15	12 –16 (14.0)	<0.5–2.3	5	10.8 ± 2.1 (6.9 – 16.0)		9.4 ± 2.1 (5.6 – 15.1)
40	19/21	16 –21 (17.2)	<0.5–1	2	10.1 ± 1.5 (7.7 – 13.9)		8.9 ± 1.7 (6.0 – 12.9)
106	51/55				10.0 ± 1.8 (6.4 – 16)	$p<0.001$	8.7 ± 1.8 (5.5 – 15)

[a] data result from the most recent clinic visit between May and July 1988
[b] the Hb A_1 values are the arithmetic means of five to ten measurements over the period of observation
[c] corrected for the presence of Hb F

Table 2. Characteristics of the control group

n	sex f/m	age (years) range (mean)	Hb F (%) range	Hb F (%) $n>0.5\%$	Hb A_1 (%) mean ± SD (range) cation-exchange chromatography		Hb A_1 (%) mean ± SD (range) HPLC
6	3/ 3	2– 6 (3.4)	<0.5–1.7	3	6.8 ±1.0 (5.5 –8.0)		5.8 ± 1.9 (3.1 – 8.8)
40	20/20	6–12 (9.5)	<0.5	0	6.35 ± 0.6 (5.1 –7.5)		5.9 ± 0.65 (4.9 – 7.3)
35	18/17	12–16 (14.1)	<0.5	0	6.4 ±0.5 (5.3 –7.7)		5.9 ± 0.9 (4.2 – 8.0)
19	7/12	16–20 (17.6)	<0.5	0	6.2 ±0.5 (5.2 –7.6)		5.8 ± 0.6 (4.2 – 6.7)
100	48/52				6.4 ±0.6 (5.1 –7.8)	$p<0.001$	5.8 ± 0.9 (3.1 – 8.8)

traassay CV was 2.6% at 9.8%, interassay CV were 4.6%, 3.4% and 2.6% at Hb A_1 of 7%, 10% and 16% respectively. Hb F and Hb A_1 were determined by high pressure liquid chromatography (HPLC) on a Kyoto Daiichi analyser (Kyoto Daiichi, Kyoto, Japan) [4]. The Haemoglobin A_{1c} analyser, HA-8110, is a highly reliable automated device consisting of an auto-sampler and an analysis section with simultaneous quantitative and entirely specific graduation of stable Hb A_1, Hb A_{1c} and Hb F fraction. The procedure is as follows: anticoagulated blood (NH4-heparin) is mixed with haemolysis reagent (phosphate-buffer, pH 5) to form haemolysate. The haemolysate is then applied to HA-8110 equipped with a column packed with Micropearl SF-W-A_{1c}. This filler is a hard porous polymer gel composed of methacrylic acid and methacrylate co-polymer, containing appropriate amounts of hydrophobic and hydrophilic function groups. The differences in affinity between these groups and haemoglobin components separate the different fractions. The eluated fraction from the column is detected by a dual wavelength photometer and processed by the built-in microcomputer to identify and calculate the area of the peaks. Hb F intraassay CV was 4.7% at 1.2%, interassay CV 9.8% at 1.2%, Hb A_1 intraassay CV 3.6% at 6.2% and interassay CV 4.2% at 5%.

Statistical analysis

For statistical analysis, the paired and unpaired Student t-test was used for parametric, and the Wilcoxon test for non-parametric data. Linear correlations were estimated using the method of least square.

Results

111 patients were studied longitudinally. Excluded from the statistical analysis were five diabetic patients with increased Hb F levels (0.6–1.0%) because of increased Hb F values in their parents (*n*: 3, 0.5–0.8%), all those of mediterranean origin, as well as patients with one of their parents suffering from diabetes bearing elevated Hb F levels ($n=2$, 0.6–0.8%). Therefore, 106 patients without haematological abnormalities (51 girls/55 boys) were included in the study (Table 1).

Hb F was detected in none of the control group aged 6 to 20 years (*n:* 94, 45 girls, 49 boys). In the younger age-group 3 children with elevated Hb F values (0.7–1.7%) without diabetes were identified (Table 2). In the diabetic patients an Hb F prevalence of 14.1% (*n:* 15) over the whole age range (2.9–21 years) and 13.3% (*n:* 13) in those under 6 years of age was observed (Table 1). The longitudinal assessment of the increased Hb F levels in 15 diabetic patients is depicted in Figure 1. There was a significant decrease in Hb F from the first (mean: 1.4%, range: 0.8–2.7%) to the last value (mean: 0.8%, range: 0.6–2.3%) ($p<0.005$).

Table 3. Comparison of data in diabetic patients with elevated Hb F and in a control group (mean ± SD, mean and range)

	children with Type 1 (insulin-dependent) diabetes mellitus	
	Elevated Hb F	Control subjects
n	15	30
sex f/m	10/5	20/10
age (years)	11.3 (3.8-17.3)	11.3 (3.5-18.7)
duration of diabetes (years)	5.7 (2.0-14.0)	4.2 (2.0-10.0)
Hb A_1 (%)[a]	9.8 (8.4-11.4)	10.2 (8.0-15.4)
dosage of insulin $U \cdot kg^{-1} \cdot day^{-1}$	0.98 (0.65- 1.5)	0.9 (0.53- 1.2)
age at onset of diabetes[b]	5.3 ± 2.1	7.4 ± 2.1
(years)	(1.7 -13.2)	(2.0 -13.5)

[a] Cation-exchange microcolumn chromatography
[b] $p < 0.05$

In Table 3 data from the patients with elevated Hb F levels are compared with an age and sex matched diabetic control group (*n*: 30). The Hb A_1 values are the arithmetic means of 5 to 10 determinations over the last two years. Patients with increased Hb F levels were found to be significantly younger at the onset of diabetes mellitus ($p < 0.05$).

Comparing the two measuring methods of Hb A_1 (values measured by the Boehringer kit are corrected for the presence of Hb F) there was no age-dependency of glycated haemoglobin in control subjects either by cation-exchange column-chromatography (Boehringer kit) or HPLC estimation (Table 2) ($r = 0.9$) in diabetic patients. Highly significant lower Hb A_1 values ($p < 0.001$) measured by HPLC were found in control subjects as well as in diabetic patients.

Discussion

The transition from fetal haemoglobin (alpha 2/gamma 2) to adult haemoglobin (alpha 2/beta 2) begins at 32 to 36 weeks postconception [5]. The transition is gradual with a half-life of 16 to 18 weeks and there is considerable individual variation [5]. Betke pointed out that the adult distribution of Hb F is not reached until puberty, or at least until 6 years of age [6].

None of our control group aged 6 to 20 years (*n*: 94) reached a Hb F level of 0.5% by HPLC. The Hb F estimation by the HPLC method carried out at pH 5.0 giving a typical pattern, is regarded as entirely specific. In the age-group up to 6 years a small elevation of Hb F in an individual child is quite possible and normal without attributing to a particular disease-state.

In contrast to the reference population, 15 of our diabetic patients had Hb F levels above 0.5%. Several variables were compared between Hb F bearing diabetic patients and age and sex matched diabetic control subjects (Table 3). Neither diabetic control (Hb A_1) nor dosage of insulin ($U \cdot kg^{-1} \cdot day^{-1}$) was related to the increased Hb F levels. Yet, the Hb F positive patients were significantly younger ($p < 0.05$) at the onset of diabetes. The occurrence of increased Hb F levels in diabetic patients and especially in patients with the onset of diabetes before the age of 6 years suggests that insulin-therapy or blood glucose derangements may delay transition from Hb F to Hb A synthesis or reactivate the Hb F synthesis as it has been described in pregnancy [7] and neonates with hyperinsulinism [8-10].

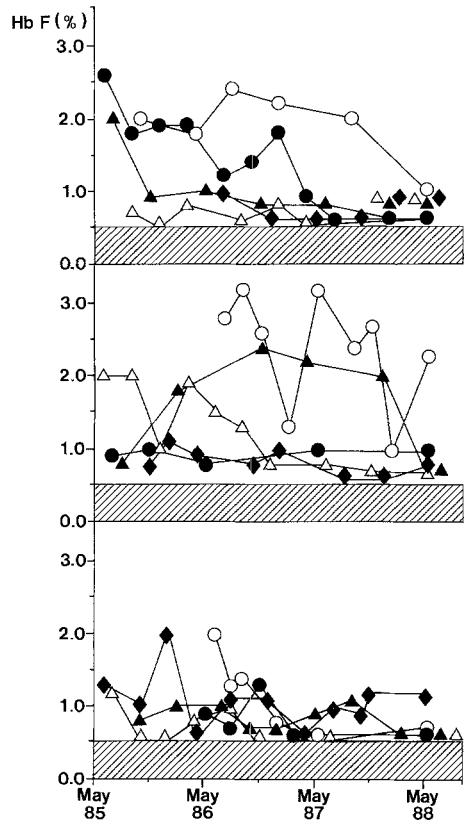

Fig. 1. Development of fetal haemoglobin (HbF) levels in diabetic patients (*n*: 15, age range 2.9-21 years). Hatched area: normal range using high pressure chromatography

Insulin has been shown to reactivate the expression of an herpes simplex thymidine kinase (Tk) gene in association with selective demethylation of DNA sequences surrounding the previously in active gene [11]. However, cellular and molecular studies showed that modulation of the methylation pattern linked to the process of differentiation of the erythroid stem cells might be the main mechanism for regulation of the expression of human globin genes during Hb switches [12]. Therefore, the elevation of Hb F in diabetes mellitus may represent an alteration of differentiation of erythroid stem cells and/or a burst promoting activity on less mature stem cells or progenitor population programmed for Hb F formation [13].

Although the Hb F values declined over the observation period (Fig. 1), a possible persistence of Hb F in adolescent diabetic patients has to be taken into con-

sideration. All the more so as in two of our diabetic adolescents aged 16 and 17 and in two diabetic parents elevated Hb F levels were found.

Finally, Hb F can be elevated in anaemia and other haematological disorders. This is unlikely to be the explanation in our patients as total haemoglobin and erythrocyte indices were regularly checked and no abnormalities detected.

In view of the general acceptance of the importance of strict glycaemic control, measurements of glycated haemoglobin are commonly used as an information feedback in the therapeutic management of the patient. The interference of Hb F on apparent Hb A_1 as measured by various methods has been well documented [2]. False elevation of Hb A_1 depending on the method used (ion-exchange column chromatography, electrophoretic methods) may lead to a misinterpretation of the metabolic control of the diabetic patients and is, therefore, of clinical importance. The problem may be resolved by using methods in which Hb F values do not interfere (eg the affinity chromatography, HPLC, thiobarbituric acid and isoelectric focusing method). In our study Hb A_1 values estimated by the Boehringer kit differed significantly from those estimated by the HPLC method although giving a good correlation. This finding has already been described in a comparative evaluation of glycated haemoglobin assays [14]. A variety of factors not analysed in our study can affect assay performance providing different normal values, for instance: temperature, pH, ionic strength, assay buffer and column capacity [15].

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