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Glycosylated Haemoglobin in Cord Blood Following Normal and Diabetic Pregnancies

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Summary. Cord and maternal blood samples were obtained at delivery in 25 normal and 14 diabetic pregnancies (13 insulin-dependent, one gestational). Total glycosylated haemoglobin, measured by the colorimetric thiobarbiturate method (mmol hydroxymethylfurfural/mol haemoglobin), was lower in cord than maternal blood (mean 18.7 ± 1.7 versus 26.5 ± 2.1, mean $\pm$ SD, $p < 0.001$). Glycosylated haemoglobin was higher following diabetic pregnancies, both in cord (diabetic 19.9 ± 1.6 versus normal 17.9 ± 1.4, $p < 0.001$) and maternal samples (diabetic 27.7 ± 1.5 versus normal 25.6 ± 2.1, $p < 0.005$). Cord and maternal glycosylated haemoglobin correlated in the normal ($r = 0.60$, $p < 0.01$) but not in the diabetic group ($r = 0.02$, NS). Birth weight ratio was higher in infants of diabetic than of normal mothers (1.10 ± 0.16 versus 0.99 ± 0.13, $p < 0.05$) but failed to correlate with cord or maternal glycosylated haemoglobin or, in the diabetic group, with mean blood glucose.

Key words: Glycosylated haemoglobin, cord blood, diabetic pregnancy, normal pregnancy, diabetes, pregnancy, diabetic control.

Many studies have demonstrated an elevation of maternal glycosylated haemoglobin, and hence blood glucose levels, in insulin-dependent and gestational diabetic compared with non-diabetic mothers [1–6]. Attempts to relate these higher maternal levels to indices of fetal morbidity and mortality have shown variable results [1, 5, 7–15]. A more logical approach would be to obtain measurements of fetal blood glucose in utero. Direct sampling is impossible, except in labour, but measurement of glycosylated haemoglobin in cord blood should provide a retrospective measurement of fetal glycaemia in the final weeks of pregnancy. Unfortunately, the presence of fetal haemoglobin [16], part of which is acetylated [17, 18], effectively invalidates the commonly used 'rapid' cation-exchange column assays [19, 20]. Column techniques designed to overcome these problems are not suitable for routine use [13].

The colorimetric determination of cord glycosylated haemoglobin provides an alternative which is cheap, easy to perform and free of interference either from fetal haemoglobin, because it measures total glycosylated haemoglobins [21, 22], or from 'fast' glycosylation [23]. This method was therefore used to measure glycosylated haemoglobin in cord blood following normal and diabetic pregnancies. The results were related to established indices of maternal diabetic control and fetal morbidity.

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Patients and Methods

Data were obtained from 39 pregnancies (25 normal and 14 diabetic). All individuals gave informed consent to the investigation. The normal women had no family history of diabetes and were free of glycosuria during pregnancy. In the diabetic group, one had gestational diabetes, the remainder being insulin-dependent before the onset of pregnancy.

All mothers received routine antenatal obstetric care, including ultrasound measurements to assess the stage of gestation. Diabetic mothers were seen weekly in the latter part of pregnancy when strenuous efforts were made to optimise blood glucose control, including the use of home blood glucose monitoring. Prior to each clinic visit, diabetic patients collected a series of four pre-prandial capillary samples for subsequent analysis of blood glucose using a glucose oxidase method. All pregnancies resulted in healthy infants and no problems occurred with neonatal hypoglycaemia. The normal mothers produced 15 male and 10 female infants while the diabetic mothers gave birth to 8 male and 6 female infants.

At delivery, cord blood was collected for measurement of glycosylated haemoglobin and blood glucose. Maternal samples for glycosylated haemoglobin were obtained from mothers within 24 h of delivery. Maternal samples were not available on seven normal patients and the sample from one diabetic mother was unsatisfactory for assay.

Glycosylated Haemoglobin Analysis

Samples were taken into glass lithium heparin containers and stored at 4 °C. The plasma was discarded and the red blood cells washed twice in an equal volume of 0.154 mol/l saline. The cells were haemolysed by the addition of one to two volumes of distilled water and 0.25 volumes of carbon tetrachloride. Following vigorous shaking for 2 min, the samples were centrifuged at 1500 *g* for 20 min when the clear haemolysate was carefully transferred into a fresh tube. Haemolysate haemoglobin (Hb) concentration was determined by the method of Van Kampen and Zijlstra [24] and samples were adjusted accurately to 50 g/l total Hb by addition of distilled water. One ml of 0.3 mol/l oxalic acid was added to 2 ml of adjusted haemolysate, mixed and immediately placed in a boiling water bath for *exactly* 60 min. Evaporation was minimised by placing glass marbles on each test tube. After incubation the samples were put into cold water for 2 min and deproteinisation carried out by addition of 1.0 ml of trichloracetic acid (40%). Following 'Vortexing' the deproteinised haemolysates were centrifuged for 10 min at 4 °C at 1500 *g*. Two ml of clear supernatant were carefully transferred to a clean test tube and 0.5 ml of 0.05 mol/l thiobarbituric acid reagent added. Samples were mixed and incubated for 60 min at 40 °C. The colour developed was read spectrophotometrically at 443 nm.

Included in each assay was a sample blank, using distilled water instead of haemolysate, aqueous standard of hydroxymethylfurfural (HMF) at concentrations of 10, 30 and 50 mmol/l, together with aliquots from normal and diabetic pooled samples previously stored at −70 °C. Results are expressed as mmol HMF/mol Hb.

Recovery of added HMF (mean ± SD) was 101.8 ± 4.0% ($n=5$). Between assay variation was < 4%.

Statistical Analysis

Results were analysed using Student's unpaired t-test and linear regression analysis. Values are expressed as mean ± SD. The birth weight ratio was obtained from the ratio between actual birth weight and the expected birth weight (fiftieth centile) for sex and gestational age of delivery, according to published tables [25].

Results

Cord blood glycosylated haemoglobin was significantly higher after diabetic (19.9 ± 1.6 mmol HMF/mol Hb) than after normal pregnancies (17.9 ± 1.4, $p<0.001$; Table 1). Cord blood glucose at delivery was similar in diabetic (4.5 ± 1.6 mmol/l) and normal pregnancies (3.9 ± 0.9 mmol/l, NS; Table 1).

Significant differences between diabetic and normal women were also observed for maternal glycosylated haemoglobin (diabetic: 27.7 ± 1.5 versus normal: 25.6 ± 2.1 mmol HMF/mol Hb, $p<0.005$; Table 1) although all but three diabetic patients had a glycosylated haemoglobin within our normal non-pregnant range (21.4–29.0 mmol HMF/mol Hb; Fig. 1).

In all pregnancies cord glycosylated haemoglobin ($n=39$, 18.7 ± 1.7 mmol HMF/mol Hb) was significantly lower than maternal glycosylated haemoglobin ($n=31$, 26.5 ± 2.1, $p<0.001$; Fig. 1). A correlation existed between cord and maternal glycosylated haemoglobin in the group overall ($r=0.51$, $p<0.01$) and also in the normal group ($r=0.60$, $p<0.01$) but not in the diabetic group alone ($r=0.02$, NS; Fig. 2).

Table 1. Fetal and maternal glycosylated haemoglobin and cord blood glucose values, gestational age and birth weight in normal and diabetic subjects

	Normal subjects		Diabetic subjects		p
	n		n		
Cord glycosylated haemoglobin (mmol HMF/mol Hb)	25	17.9 ± 1.4	14	19.9 ± 1.6	< 0.001
Cord blood glucose (mmol/l)	22	3.9 ± 0.9	14	4.5 ± 1.6	NS
Maternal glycosylated haemoglobin (mmol HMF/mol Hb)	18	25.6 ± 2.1	13	27.7 ± 1.5	< 0.005
Gestational age at delivery (weeks)	25	39.8 ± 1.2	13*	37.7 ± 0.8	< 0.001
Birth weight ratio	25	0.99 ± 0.13	13*	1.10 ± 0.16	< 0.05

Results expressed as mean ± SD
* Excludes one premature labour – see text

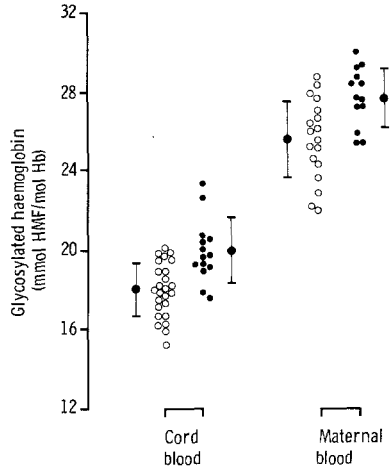

Fig. 1. Glycosylated haemoglobin levels in cord and maternal blood following normal (○) and diabetic (●) pregnancies. Bars indicate mean ± SD

One diabetic mother delivered a 1460 g infant prematurely at 30 weeks. After excluding this patient, gestational age at delivery was still lower in diabetic (37.7 ± 0.8 weeks) than in non-diabetic pregnancies (39.8 ± 1.2 weeks, $p<0.001$; Table 1) and reflected the policy of induction of labour in diabetic pregnancy around 38 weeks of gestation.

Birth weight ratio was higher in the diabetic (1.10 ± 0.16) than in the normal group (0.99 ± 0.13, $p<0.05$; Table 1) but considerable overlap occurred between the populations. The correlation between birth weight ratio and either cord (Fig. 3) or maternal glycosylated haemoglobin was not significant for any group.

Figure 4 shows the distribution of mean maternal blood glucose levels in the final month and final week of diabetic pregnancies. In all but one diabetic mother levels were below 5.5 mmol/l. No correlation was ob-

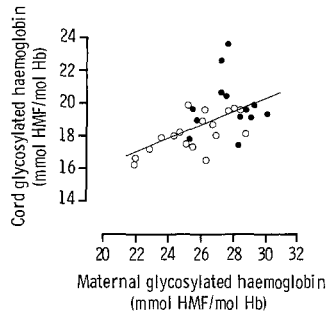

Fig. 2. Correlation between cord and maternal glycosylated haemoglobins. Regression line $y = 0.4 + 8.3$, $r = 0.51$, $p < 0.01$ (for combined groups). Normal (○) and diabetic (●) pregnancies

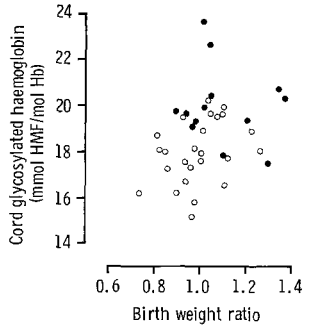

Fig. 3. Correlation between cord glycosylated haemoglobin and birth weight ratio ($r = 0.29$, $p < 0.1$, NS). Normal (○) and diabetic (●) pregnancies

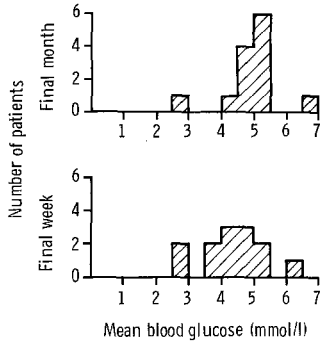

Fig. 4. Distribution of mean pre-prandial blood glucose in diabetic mothers in the final month and the final week of pregnancy

tained between mean blood glucose in the final month of pregnancy and maternal glycosylated haemoglobin or birth weight ratio. Cord glycosylated haemoglobin correlated inversely with mean maternal blood glucose in the final month ($r = -0.57$, $p < 0.05$) but not in the final week of pregnancy ($r < 0.005$, N.S.).

Discussion

Most previous clinical studies of glycosylated haemoglobin in cord blood samples have used either cation-exchange resin chromatography [13] or isoelectric focussing techniques [14]. The present study demonstrates that the thiobarbiturate colorimetric assay of glycosylated haemoglobin can be applied to cord blood samples without the need for any special modifications to the method.

Colorimetric analysis has shown considerably lower levels of glycosylation of fetal compared with maternal haemoglobin which would be expected for the following reasons; fetal erythrocytes are produced at a higher rate [26], survive for a shorter time [26] and are exposed to lower levels of blood glucose [27–29] than maternal cells, all factors tending to reduce glycosylation. Also glycosylation of fetal haemoglobin is prevented in part by acetylation [17, 18] although this does not affect the dynamics of glycosylation at other sites in vitro [30, 31].

The failure to show a correlation between cord and maternal glycosylated haemoglobin in the diabetic group is explicable partly by the fact that the diabetic mothers were well controlled. Thus only three of 13 diabetic mothers had maternal glycosylated haemoglobin values at delivery above the upper limit of the normal non-pregnant range and all except one had mean pre-prandial blood glucose levels below 5.5 mmol/l in the last month of pregnancy. However, this cannot be the only reason since a correlation was obtained within the similarly tightly grouped non-diabetic subjects. The present results are similar to those of Fadel et al. [13], using ion-exchange chromatography, who showed higher glycosylated haemoglobin levels in infants of diabetic than normal mothers. Poon et al. [14], using isoelectric focussing, showed a correlation between cord and maternal glycosylated haemoglobin for a combined group of diabetic and normal pregnancies.

Birth weight ratio was higher in diabetic patients but the lack of correlation between this and any of cord or maternal glycosylated haemoglobins or mean blood glucose values was probably due to the relatively 'tight' control of maternal diabetes and to the multifactorial nature of determinants of birth weight [32]. Using different methods other workers [13, 14] have likewise failed to correlate cord glycosylated haemoglobin and birth weight ratio. Most previous authors have also failed to obtain a correlation between maternal glycosylated haemoglobin and birth weight ratio [7, 10–14], although some have succeeded [1, 5, 8, 9, 15]. As would be expected, the poorer the control of maternal diabetes the easier it becomes to obtain such a correlation [1, 5, 8, 9, 15].

We are doubtful of the significance of the surprising inverse correlation obtained between cord glycosylated haemoglobin and maternal mean blood glucose in the final month of pregnancy in view of the small numbers, the low level of statistical significance and the absence of any similar correlation with maternal mean blood glucose in the final *week* of pregnancy. The lack of correlation between maternal glycosylated haemoglobin and mean blood glucose was probably due to the relatively tight grouping of results and the absence of post-prandial blood glucose samples.

In a recent report Sosenko et al. using the thiobarbiturate colorimetric assay, found similar differences in glycosylation between cord and maternal blood and between normal and diabetic pregnancies [33]. While they also were unable to relate glycosylated haemoglobins to macrosomia they did obtain a correlation between glycosylation in cord and maternal samples in diabetic pregnancies, probably as a result of the larger number of patients studied.

In conclusion, use of the colorimetric assay has demonstrated major differences between the levels of glycosylation of cord and maternal haemoglobins. Despite apparently good control in diabetic pregnancies, fetal and maternal glycosylated haemoglobins were elevated compared with non-diabetic pregnancies, but the elevation could not be related directly to macrosomia.

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