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Glycosylated haemoglobin in normal pregnancy: a longitudinal study with two independent methods

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Summary. Twenty-one women completed a longitudinal study of glycosylated haemoglobin in normal pregnancy. Glycosylated haemoglobin levels were measured using two independent techniques (ion-exchange column and colorimetric). Concurrent serial oral glucose tolerance tests (75-g glucose load) and erythrocyte indices were obtained. Changes in mean glycosylated haemoglobin were similar with both techniques with a nadir at 17 weeks, a peak at delivery ($p < 0.002$ versus 17 weeks) and a fall post-partum. Glycosylated haemoglobin levels in abnormal pregnancies, e.g. diabetic, should be interpreted in the knowledge of these physiological changes.

Key words: Normal pregnancy, diabetic pregnancy, glycosylated haemoglobin, glycosylated haemoglobin A, blood glucose, glucose tolerance, erythrocyte volume.

Good control of blood glucose levels is one important factor in obtaining a successful outcome of pregnancy in the diabetic woman [1–3]. Measurement of glycosylated haemoglobin can provide an additional index of the overall control achieved, for example around conception [4–6] and for subsequent audit of diabetic management [7–16] but the interpretation of results requires a knowledge of physiological levels during normal pregnancy [17–18]. Unfortunately most studies of this situation have been cross-sectional in design and have showed no change in glycosylated haemoglobin levels compared with the non-pregnant state [7–11], although there is one report of a fall [17] and one of a rise in levels [12] associated with pregnancy. Two sequential studies have been published but the results from these are inconsistent with each other [13, 14]. The conflicting information in the literature may be due partly to differences in study design but also to methodological difficulties [19–23].

In view of this confusion, and in the absence of longitudinal data from normal pregnancy incorporating two independent methods of measuring glycosylated haemoglobin under standard conditions, we designed a study to try to clarify the situation. Serial 75-g oral glucose tolerance tests and erythrocyte indices were obtained concurrently.

Subjects and methods

Subjects

Forty-five normal pregnant women were recruited into the study at their first attendance at the ante-natal clinic. None had a previous history of diabetes (personal or family), large babies (> 4.5 kg birth weight) or stillbirth. Subsequently 24 women were unable, for various social reasons, to complete the demanding protocol and results are therefore presented for the 21 subjects who completed all three glucose tolerance tests (see below). These women consisted of 14 primigravidae with six in their second and one in her third pregnancy. The mean ± SD age was 25 ± 4 years (range 18–35 years) and the mean ± SD body mass index was 22.3 ± 2.9 kg/m^2 (range 19.1–29.8 kg/m^2). Each pregnancy resulted in a healthy infant. The mean birth weight was 3404 ± 424 g (range 2552–4253 g) and the mean length of gestation was 39.8 ± 1.4 weeks (range 36–42 weeks). Blood loss following delivery was not excessive and no subject required transfusion. All women were recommended to take oral iron throughout gestation and gave informed consent to the study, which was approved by the Local Ethical Committee.

Study design

Samples for glycosylated haemoglobin were obtained at the first visit (before 12 weeks gestation), at 17, 24 and 34 weeks gestation, at delivery and between 6 and 15 weeks post-partum (mean 10 ± 2 weeks). Oral glucose tolerance tests (GTT) were performed at 17.2 ± 0.6 weeks

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(range 16–18), 33.7 ± 1.2 weeks (range 32–36) and at 10.0 ± 2.0 weeks post-partum (range 6–15). Tests were performed after a 10-h overnight fast and the 75-g glucose load was administered over 5 min as a lightly carbonated glucose syrup drink (Lucozade, Beecham Pharmaceuticals, London, UK) which was well tolerated. Venous samples were drawn from an indwelling cannula. Samples for glycosylated haemoglobin were taken fasting, anticoagulated in lithium heparin and stored at 4 °C for between 24 and 72 h before analysis. In view of the longitudinal nature of the study and the long-term instability of glycosylated haemoglobin samples, it was not possible to assay all samples from the same subject on the same assay run. Specimens for glucose were obtained fasting and at 30, 60, 90 and 120 min after the glucose load and preserved in fluoride oxalate.

Chemical methods

Colorimetric (thiobarbituric acid) estimation of glycosylated haemoglobin was performed with a modification of the method of Fluckiger and Winterhalter [24, 25]. Each assay included a set of five aqueous standards of 5-hydroxymethylfurfural (HMF) which were treated in the same way as sample haemolysates while quality control was checked by including two pooled haemolysates stored at −20 °C. Column determination employed a commercially available method (Bio-Rad Hemoglobin A_1 by column test, Bio-Rad Laboratories, Richmond, California, USA). All column assays were run in a waterbath maintained at 23 °C and quality control was assessed by the variability of fresh normal samples. The correlation obtained between the colorimetric and the column techniques was good ($y = 0.2 \times + 0.5$, $n = 133$, $r = 0.92$, $p < 0.001$) and the between assay coefficients of variation over a 2-year-period at normal levels of glycosylated haemoglobin were 5% and 3%, respectively. The normal ranges for non-pregnant women of similar age were 30–40 mmol HMF/mol haemoglobin and 6.4–8.5% for colorimetric and column methods, respectively. A number of samples clotted after collection and were therefore unsuitable for assay.

Standard automated methods were used for analysis of whole blood glucose (glucose oxidase) and erythrocyte indices.

Statistical methods

Results are quoted as mean ± SD. Analysis of variance was performed on the glycosylated haemoglobin data. Differences were sought by Student's paired t-test (two-tailed).

Results

Glycosylated haemoglobin

The variance ratio (F) for colorimetric values of glycosylated haemoglobin was 6.93 for 5 degrees of freedom (DF) between and 114 DF within groups ($p < 0.005$) while for column values the ratio was 7.23 for 5 DF between and 102 within groups ($p < 0.005$). This led to a rejection of the null hypothesis for both groups.

In comparison with results at delivery colorimetric levels of glycosylated haemoglobin were significantly lower ($p < 0.001$) at the first visit, 17 and 24 weeks, while column levels were lower at the first visit ($p < 0.02$) and at 17 weeks ($p < 0.002$; Table 1).

Levels in pregnancy were compared with post-partum values and were significantly lower with the colorimetric method at the first visit ($p < 0.02$), 17 ($p < 0.002$) and 24 weeks ($p < 0.002$) and with the column method at 17 ($p < 0.001$) and 24 weeks ($p < 0.01$, Table 1).

Table 1. Glycosylated haemoglobin: sequential changes during pregnancy and post-partum

Time of sample (weeks)	Glycosylated haemoglobin			
	Colorimetric (mmol HMF/mol Hb)		Column (%)	
First visit	34.9 ± 2.0[ag]	(20)	6.5 ± 0.4[e]	(18)
17	34.4 ± 1.9[cg]	(20)	6.0 ± 0.5[df]	(15)
24	34.9 ± 1.9[cg]	(21)	6.1 ± 0.7[b]	(18)
34	36.1 ± 2.4	(21)	6.7 ± 0.8	(20)
Delivery	37.4 ± 2.3	(19)	7.1 ± 0.7	(17)
Post-partum	36.9 ± 1.9	(19)	6.7 ± 0.5	(20)

Results expressed as mean ± SD with the number of samples in parentheses; the remainder were unsuitable for assay due to clotting. [a] $p < 0.02$, [b] $p < 0.01$, [c] $p < 0.002$, [d] $p < 0.001$ versus post-partum; [e] $p < 0.02$, [f] $p < 0.002$, [g] $p < 0.001$ versus delivery

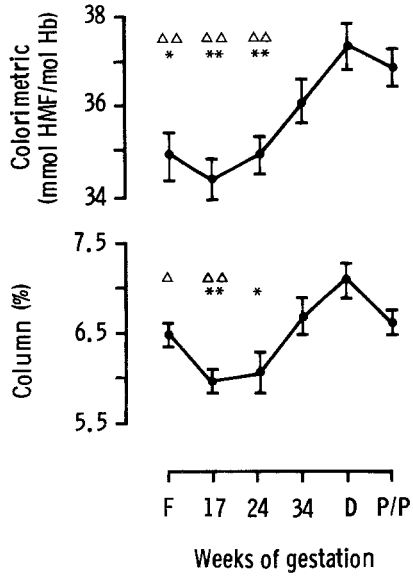

Fig. 1. Colorimetric and column results for glycosylated haemoglobin during pregnancy and post-partum. F: first visit; D: delivery; P/P: post-partum. Values shown as mean ± SEM. * $p < 0.02$, ** $p < 0.002$; significant differences versus post-partum. Δ $p < 0.02$, Δ Δ $p < 0.002$; significant differences versus delivery

The sequential changes in glycosylated haemoglobin showed a similar pattern with both methods showing a nadir at 17 weeks followed by a progressive rise with a peak at the time of delivery and a subsequent fall in the post-partum period (Fig. 1).

Blood glucose

At 17 weeks blood glucose levels were significantly lower than non-pregnant levels when fasting and at 30 min after the glucose load (Table 2). In contrast, values at 34 weeks gestation were significantly higher than non-pregnant levels at 60, 90 and 120 min after loading (Table 2).

Table 2. Results of glucose tolerance tests at 17 weeks, 34 weeks and post-partum in 21 diabetic patients

Time of sample (min)	Blood glucose (mmol/l)		
	17 weeks	34 weeks	Post-partum
Fasting	3.2 ± 0.4^a	3.4 ± 0.5	3.6 ± 0.4
30	4.8 ± 0.7^b	5.7 ± 0.7	5.5 ± 0.9
60	4.5 ± 1.0	6.1 ± 1.3^b	4.5 ± 1.4
90	4.0 ± 0.9	5.1 ± 1.1^b	3.7 ± 0.8
120	3.3 ± 0.8	4.4 ± 0.8^a	3.5 ± 0.6

Results expressed as mean ± SD. $^a p < 0.002$, $^b p < 0.001$ versus post-partum

Table 3. Haemoglobin and erythrocyte volume

Time of sample (weeks)	Haemoglobin (g/dl)	Erythrocyte volume (fl)[a]
17	12.4 ± 1.1^b (19)	88.6 ± 4.3^c (19)
34	12.1 ± 0.9^b (20)	85.8 ± 5.5 (18)
Post-partum	13.6 ± 1.1 (20)	84.3 ± 4.6 (20)

Results expressed as mean ± SD with the number of samples in parentheses; the remainder were unsuitable for assay due to clotting.
[a] $1\ \text{fl} = 10^{-15}$ litre; $^b p < 0.001$, $^c p < 0.02$ versus post-partum

Erythrocyte indices

Mean total haemoglobin levels were lower at both 17 and 34 weeks compared with post-partum levels (Table 3). Mean erythrocyte volume fell slightly but significantly between 17 weeks and post-partum, although only one value (74 fl) at the latter time fell outside the normal non-pregnant range (77–93 fl) (Table 3).

Discussion

At present, no single method of measurement of glycosylated haemoglobin has found universal acceptance since problems exist with all techniques [19–23]. An important part of the design of the present study was the concurrent use of two established methods which measure different properties of glycosylated haemoglobin [21, 26–28]. The column technique depends on physical changes which alter the ionic charge on haemoglobin [19], whereas the colorimetric method depends on a chemical alteration of the molecule [21, 24, 26, 28]. The latter method is independent of changes in fetal haemoglobin [27, 29]. The present study has shown a similar pattern of change in glycosylated haemoglobin levels through pregnancy and the puerperium with two separate techniques. Although changes due to analytical variation do sometimes look like trends, it is highly unlikely that two independent methods would, by chance, show such similar patterns. In the two other longitudinal studies (both depending on single methods) Ylinen et al. [14] found similar, although non-significant, trends during pregnancy but found a rise in levels rather than a fall, post-partum whereas in contrast Widness et al. [13] described a fall in glycosylated haemoglobin with increasing gestation. The changes described in the present study are relatively small and the reason for the failure to observe these trends in previous reports could be due to their cross-sectional design or the use of imprecise column methods as a result of failure to control critical variables such as assay temperature [19, 20].

The lower fasting blood glucose level in pregnancy when compared with the non-pregnant state is well described in the literature [2, 18, 30, 31] and confirmed in this study. Likewise, the changes in glucose tolerance in late pregnancy, shown previously using 50-g glucose loads [2, 18, 30, 32], were confirmed using the 75-g load.

We believe that the post-partum samples were obtained sufficiently long after delivery (mean 10 weeks) to have allowed the fasting blood glucose and glucose tolerance to return to normal [33] but changes in glycosylated haemoglobin lag behind changes in glycaemia. It is, therefore, possible that the post-partum glycosylated haemoglobin level may not have fully returned to the non-pregnant baseline although, since all the changes are small in absolute terms, this seems unlikely. In addition, in view of the further fall in levels between the first visit and 17 weeks gestation, we feel that the lower levels in early pregnancy represent a genuine change.

Haemoglobin levels showed the expected changes with a rise following delivery. The slight but significant fall in erythrocyte volume may indicate the development of a minor degree of iron deficiency in our patients due to lack of compliance with iron therapy [34, 35]. Unfortunately, data on the iron status of the subjects are not available.

The changes in glycosylated haemoglobin may have been due to changes in carbohydrate metabolism or erythrocyte dynamics, or a combination of both factors. Thus, the lower levels of glycosylated haemoglobin in early pregnancy may have been due to the lower fasting blood glucose levels, while the steady rise with progression of pregnancy may have been attributable to the changes in glucose tolerance. Interpretation should be cautious, however, since it has been shown that the rise in mean diurnal plasma glucose with progression of normal pregnancy is small [36]. The data concerning erythrocyte dynamics in pregnancy are confusing [34] and thus any attempt to relate the observed changes to the changes in glycosylated haemoglobin described in this study would be speculative.

The use of any measurement in pregnancy should take account of pregnancy-specific normal ranges [17, 18]. Thus, results of glycosylated haemoglobin measurements in pregnancy, e. g. in the pregnant diabetic woman, should be interpreted in the knowledge of these demonstrable physiological changes.

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