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Glycosylated haemoglobin levels in healthy pregnant Nigerian women and in the cord blood of their new-born babies

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Summary

Glycohaemoglobin (GHb) levels were measured in 34 non-pregnant controls and 20 healthy pregnant Nigerian women in all the trimesters of pregnancy and the puerperium. In addition, cord blood GHb was determined in the new-born babies of the pregnant women. The mean (±s.d.) GHb levels, expressed as mmol fructose equivalents per gram of haemoglobin, for the controls and for the first, second and third trimesters and the puerperium respectively, were as follows: 89.9 (19.9), 82.1 (54.8), 88.2 (55.5), 85.7 (62.7), 96.7 (51.3). There were no significant differences between the values for the controls and the pregnant women and the results for the first, second and third trimesters were also not significantly different from each other. There was a significant positive correlation between the GHb levels in the second and third trimesters and the puerperium with the fasting blood glucose in the respective preceding trimester. The mean cord blood GHb value of 88.5 (46.7) was not significantly different from the maternal levels throughout pregnancy. There was a significant positive correlation between cord blood GHb and maternal GHb in the third trimester ($r = 0.64$, $P < 0.05$) as well as the maternal third trimester fasting blood glucose ($r = 0.69$, $P < 0.05$). However, there was no correlation between cord blood GHb and cord blood glucose or infant birth weight. The results of this study should prove useful in optimizing glycemic control in pregnant Nigerian diabetic women.

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Résumé

Les niveaux de glycohémoglobine (GHb) étaient mesurés chez 34 témoins non-enceints et 20 Nigérianes enceintes en bonne santé pendant tous les trimestres de la grossesse et de la période puerpérale. La GHb du sang de cordon ombilical était aussi déterminée chez les nouveau-nés des femmes enceintes. La moyenne (±s.d.) de niveaux de GHb, exprimée en mmol équivalents de fructose par gramme d'hémoglobine des témoins pendant la période du premier, du second, du troisième trimestre et puerpérale étaient respectivement comme suivante: 89.9 (19.9); 82.1 (54.8); 88.2 (55.5); 85.7 (62.7); 96.7 (51.3). Il n'y avaient pas de différences significatives entre les valeurs pour les témoins et celles-ci des femmes enceintes. Les résultats pour le premier, le second et le troisième trimestre n'ont pas été aussi significativement différents. Il y avait une significative correlation positive entre les niveaux de GHb pendant la seconde et la troisième période puerpérale avec la glycémie à jeun pendant le trimestre précédent respectif. La moyenne de la valeur de GHb dans le cordon était 88.5 (46.7) mais n'était pas significativement différente des niveaux maternelles pendant la grossesse. Il y avait une correlation significative positive entre la GHb du cordon et la GHb maternelle pendant le troisième trimestre ($r = 0.64$; $P < 0.05$) également la glycémie à jeun maternelle du troisième trimestre ($r = 0.69$; $P < 0.05$). Néanmoins, il n'y avait pas de correlation entre la GHb et le glucose du cordon ou le poids de naissance du bébé. Les résultats de cette étude seraient utiles dans la contrôle glycémique optimal chez les enceintes diabétiques nigérianes.

Introduction

Glycosylated haemoglobin (GHb) measurement is now well established as a useful tool for assessing glycemic control in all categories of diabetic patients, including pregnant women. One of its earliest applications in pregnant diabetic or potentially diabetic women was in assessing the degree of hyperglycemia in the early weeks of gestation. It was suggested by Mills *et al.* [1] that congenital malformation in infants of diabetic mothers occurred before the seventh week of gestation. Several investigators have also demonstrated that there is an association between abnormally raised first trimester glycohaemoglobin levels, which reflects pregestational and early gestational maternal hyperglycemia, and the occurrence of congenital abnormalities [2,3]. Many pregnant women will not attend the antenatal booking clinic until they are at least 12 weeks pregnant, or even 24 to 30 weeks in most developing countries. Consequently, GHb estimation is extremely useful in assessing the degree of maternal glycemia in the 6–8 weeks prior to booking. It was recently suggested that women with elevated first trimester GHb should have an antenatal ultrasound examination between 18 and 20 weeks, as some of the congenital anomalies can be so diagnosed very early [4].

Mintz and Skyler [5] recently concluded that the 'magic bullet' that has made metabolic normalization feasible and practical in diabetic patients has been the advent of self-monitoring of blood glucose at home. Unfortunately, this modality of management is not available for the majority of diabetic patients in most developing countries. Occasional or sporadic blood glucose estimation and unreliable urine testing are still all that is usually possible. In view of this prevailing reality, Famuyiwa *et al.* [6] have emphasized that GHb determination is highly suited for the management of diabetic patients in developing countries. They suggested that combining blood glucose estimation as frequently as possible with 3-monthly measurements of GHb, with every effort made to normalize the latter in particular, should result in an overall measure of control which is near optimal and which probably would not be achieved with the prevailing practice. It was also believed that such an approach would be cost effective. This suggestion applies even more to pregnant diabetic women who need to have normal blood glucose throughout pregnancy.

In view of its immense potential for the management of diabetic pregnancy in developing countries, we have measured the GHb levels in a group of healthy non-diabetic pregnant Nigerian women in all three trimesters of pregnancy and the puerperium in order to derive normal reference ranges. Cord blood GHb was also determined in the new-born babies of these women.

Subjects and methods

Pregnant subjects

Forty healthy pregnant Nigerian women who were sure about their menstrual dates were recruited in the first trimester. They were among women attending the antenatal clinic of the University College Hospital, Ibadan, Nigeria. Excluded from the study were those with any history of risk factors for diabetes in pregnancy, such as history of unexplained intrauterine death or stillbirth, large babies (weight $\geq$4 kg), strong family history of diabetes, gross obesity (weight $\geq$90 kg) or glycosuria in current pregnancy. Also excluded were those with obstetric problems such as hydramnios or antepartum haemorrhage and those with significant medical disorders such as anaemia (packed cell volume $\leq$30%), hypertension in pregnancy (blood pressure $\geq$140/90), systemic infections, hepatitis, haemoglobinopathy, etc.

Each of the participants was studied in each of the three trimesters and the puerperium. In addition, their new-born infants were also evaluated. Only 20 of the 40 subjects recruited completed the study. Their ages ranged from 19 to 38 yr with a mean of 26.2 yr. Their mean parity was 2.1.

Non-pregnant controls

Thirty-four non-pregnant women attending the Gynaecology clinic for minor gynaecological problems were recruited as controls. They were matched with the pregnant subjects as well as possible for age and parity. None of them had any past history to suggest diabetes mellitus. Their mean age was 28.1 yr and mean parity 1.7.

Glycosylated haemoglobin (HbA1) estimation

Fasting blood for GHb estimation was drawn from each of the pregnant subjects in the first trimester (8–14 weeks), second trimester (24–28 weeks), third trimester (32–36 weeks) and the puerperium — about 2 weeks after delivery. The fasting blood for GHb was followed by an oral glucose tolerance test (the results of this will be reported separately).

Cord blood samples for glucose and GHb were also obtained from the babies of the women who completed the study. The controls were studied similarly but only once.

The colorimetric method of Fluckiger and Winterhaulter [7], as modified by Fisher *et al.* [8], was used for GHb estimation. The results were derived by estimating the amount of carbohydrate (fructose) equivalents of GHb from a fructose standard curve and expressed as mmol fructose per gram of haemoglobin.

Student's *t*-test was used to determine the statistical difference between the means and Spearman's correlation coefficient and linear regression analysis were used to assess the relationship between GHb and several variables.

Results

Only the results of the 20 pregnant women who completed the study are presented. The GHb levels (mmol fructose per gram haemoglobin) for the controls and for the pregnant women in the three trimesters and the puerperium are shown in Table 1. There were no significant differences between the values for the pregnant subjects and those for the controls. However, when the GHb values for the different stages of pregnancy were compared with each other, the mean results for the first trimester and second trimester were significantly lower then those for the puerperium ($P < 0.05$). There were no differences between the values for the first, second and third trimesters.

Analysis of the correlation of GHb levels in the second trimester, third trimester and the puerperium with the fasting blood glucose in the respective preceding trimester demonstrated statistically significant positive correlations (Table 2).

Table 3 shows the data on infant birth weight, cord blood glucose and cord blood GHb levels in the 20 singleton births from the pregnant subjects. The mean cord blood GHb was not different from the maternal GHb at any stage of pregnancy. None of the babies was hypoglycemic. There was a significant positive correlation between the cord blood GHb and maternal GHb in the third trimester ($r = 0.64$, $P < 0.05$). There was a similar relationship between cord blood GHb and maternal fasting blood glucose in the third trimester ($r = 0.60$, $P < 0.05$) (Fig. 1). However, there was no significant correlation between cord blood GHb and cord blood glucose ($r = 0.05$, $P > 0.05$) or between cord blood GHb and infant birth weight ($r = 0.19$, $P > 0.05$).

Discussion

In this study, the GHb levels observed in healthy pregnant Nigerian women during the three trimesters and the puerperium were not

Table 1. Glycohaemoglobin (GHb) values in pregnant subjects and controls

	Controls (mmol fructose/g haemoglobin)	Pregnant subjects (mmol fructose/g haemoglobin)			
		T1*	T2*	T3	pp
n	34	20	20	20	20
Range	57.30–139.20	61.20–104.30	62.60–105.20	61.20–111.20	70.20–114.40
Mean	89.90	82.06	88.21	85.67	96.70
s.d.	19.90	54.87	55.55	62.72	51.28

T1, first trimester; T2, second trimester; T3, third trimester; pp = puerperium.
*$P < 0.05$ compared to value in puerperium.

Table 2. Correlation of glycohaemoglobin (GHb) levels with the fasting blood sugar in the preceding trimester

	GHb concentration (mmol fructose/g haemoglobin)					
	FBS (T1)	GHb (T2)	FBS (T2)	GHb (T3)	FBS (T3)	GHb (pp)
Mean	70 75	88.21	66.10	85.67	66.80	96.70
s.d	9.97	55.55	12.61	62.72	13.83	51.80
n	20	20	20	20	20	20
r	0 60		0.49		0.71	
P	< 0.05		< 0 05		< 0.05	

T1, first trimester, T2, second trimester; T3, third trimester; pp, puerperium.
FBS, fasting blood sugar
r, correlation coefficient.

Table 3. Birth weights, cord blood glucose and cord blood glycohaemoglobin (GHb) levels in newborn Nigerian babies

	Infant birth weight (kg)	Cord blood sugar (mg/100 ml)	Cord blood GHb (mmol fructose equivalents)
n	20	20	20
Mean	3.2	59.8	88.5
s.d.	1.3	34.5	46.7

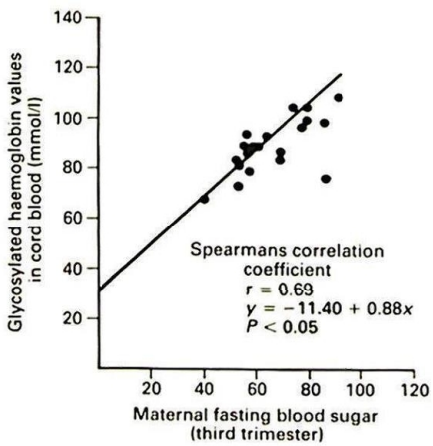

Fig. 1. Correlation of glycohaemoglobin values in cord blood with maternal third trimester fasting blood sugar.

different from those in controls. The GHb values in this study were also similar to those reported by Kuti *et al.* [9] and Erasmus *et al.* [10] in Nigerian women using the colorimetric method. However, this is the first time that the same group of women have been followed throughout pregnancy. The results indicate that there is little change in GHb levels throughout pregnancy in healthy Nigerian women. These findings are similar to the observations of Leslie *et al.* [2] and Paulsen and Koury [11] who also did not find any significant changes in GHb levels during normal pregnancy. It is in contrast, however, with the results of Schwartz *et al.* [12] who observed a significant increase in the third trimester.

The observation of a significant correlation between maternal GHb levels in the second

trimester through the puerperium and the fasting blood glucose in the preceding trimesters confirmed its potential clinical usefulness in monitoring glycemic control for the preceding weeks. This is in agreement with the observations of other workers [2,13].

Cord blood GHb levels from Nigerian newborn babies are being reported for the first time in this study. There was no difference between cord blood GHb and maternal GHb throughout pregnancy. This contrasts with the observations of Zeller *et al.* [14] who reported that cord blood GHb was only 60% of that of the mother, whether maternal diabetes was present or not. However, Fadel *et al.* [15] demonstrated an increase in glycosylated haemoglobin in the cord blood of infants of diabetic mothers. It must be noted that cord blood GHb is a mixture of HbFIc and the HbA1 usually found in adults. HbFIc itself is believed to be a combination of acetylated and glycosylated components [16,17].

There was a strong positive correlation between cord blood GHb and both maternal GHb and fasting blood glucose in the third trimester. This indicates that cord blood GHb level is most likely a direct function of maternal glycemia in the preceding trimesters. However, there was no correlation between cord blood GHb and infant birth weight. Similarly, Fadel *et al.* [15] did not find any correlation between cord blood glycosylated haemoglobins and the birth weight ratio which is a more sensitive indicator of relative changes in the birth weight. Although their study included diabetic pregnant women, they were all well controlled. Studies which include late-presenting, poorly controlled diabetic women with large babies may demonstrate a relationship between cord blood GHb and birth weight.

GHb was measured in this study using the colorimetric method of Fluckiger and Winterhaulter [7] as modified by Fisher *et al.* [8]. This method was also used by Kuti *et al.* [9] and Erasmus *et al.* [10] in their study of Nigerians. It has also been shown previously that the prevailing high environmental temperature and humidity in a tropical developing country like Nigeria did not make the micro-column cation-exchange chromatographic technique suitable for use [6].

The method will also not distinguish HbF from the minor adult fast haemoglobins. The micro-column affinity chromatography method was, however, found to be satisfactory in the hot tropical environment [18] but the difficulties associated with procuring kits from abroad have limited its applicability. The colorimetric method is highly suited for use in developing countries because all that is required is a colorimeter in good working condition and the procurement of reagents which are fairly standard. It measures total glycosylated haemoglobin and it has the advantages that it is affected little by sample handling, storage conditions, haemoglobinopathies or non-glucose adducts [19]. However, it has the disadvantage that the GHb assay units are reported differently from laboratory to laboratory [20]. This makes it difficult to compare the results from two different laboratories or with those done by other methods.

Notwithstanding the foregoing, this study has tried to provide some reference values for GHb in all the three trimesters of pregnancy and the puerperium in healthy Nigerian women. In addition, cord blood GHb levels in healthy newborn infants have been reported. It is hoped that these data will prove helpful in the management of pregnant diabetic Nigerian females. Similar data are needed for other tropical developing countries.

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References

1. Mills JL, Baker L, Goodman AS. Malformation in infants of diabetic mothers occur before the seventh gestational week. Diabetes 1979; 28:292–3.
2. Leslie RDG, Pyke DA, John PN, White JM. Hemoglobin A1 in diabetic pregnancy. Lancet 1978;ii:958–9.
3. Miller E, Hare JW, Cloherty JP, Dunn P, Gleason RE, Soeldner JS, Kitzmiller JL. Elevated maternal hemoglobin A1c in early pregnancy and major congenital anomalies in infants of diabetic mothers. New Engl J Med 1981;304:1331–4.

4. Stubbs SM, Doddridge MC, John PN, Steel JM, Wright AD. Hemoglobin A1 and congenital malformation. Diabetic Med 1987;4:156–9.
5. Mintz DH, Skyler JS. Diabetes and pregnancy — 1985. In: Syllabus Thirty-Seventh Annual Postgraduate Assembly of the Endocrine Society, 1985:62–88.
6. Famuyiwa OO, Ogunmekan GO, Osotimehin, BO. Hemoglobin A1 determination in Nigerian diabetics. Afr J Med Med Sci 1983;12:29–35.
7. Fluckiger R, Winterhaulter KH. In vitro synthesis of HbA1c. FEBS Lett 1976;71:356–60.
8. Fisher RW, De Jong C, Voight E, Berger W, Winterhaulter KH. The colorimetric determination of HbA1c in normal and diabetic subjects. Clin Lab Hematol 1980;2:129–38.
9. Kuti JA, Ohwovoriole AE, Johnson TO. Hemoglobin A1 levels in Nigerians. Nig Q J Hosp Med 1981;1:21–3.
10. Erasmus RT, Osotimehin BO, Ugbode C, Famuyiwa OO. HbA1c measured by a colorimetric method in normal and diabetic Nigerians. Afr J Med Med Sci 1983;12:177–82.
11. Paulsen EP, Koury M. Hemoglobin A1c levels in insulin-dependent and independent diabetes mellitus. Diabetes 1976;(Suppl. 2):890–6.
12. Schwartz HC, King KC, Schwartz AL, Edmunds D, Schwartz R. Effects of pregnancy on hemoglobin A1 in normal, gestational diabetic and diabetic women. Diabetes 1976; 25:1118–23.
13. Widness JA, Schwartz HC, Thompson D, King KC, Kahn CB, Oh W, Schwartz R. Glycohemoglobin (HbA1c): a predictor of birth weight in infants of diabetic mothers. J Pediatr 1978;92:8–12.
14. Zeller WP, Susa JB, Widness JA, Schwartz HC, Schwartz R. Glycosylation of hemoglobin in normal and diabetic mothers and their fetuses. Pediatr Res 1983;17:200–3.
15. Fadel HE, Reynolds A, Stallings M, Abraham EC. Minor (glycosylated) hemoglobins in cord blood of infants of normal and diabetic mothers. Am J Obstet Gynecol 1981;139:397–402.
16. Abraham EC, Cope ND, Braziel N, Huisman THJ. On the chromatographic heterogeneity of human fetal hemoglobin. Biochim Biophys Acta 1979;577:159–65.
17. Abraham EC. Glycosylated minor components of fetal hemoglobin. Biochim Biophys Acta 1981;667:168–74.
18. Famuyiwa OO, Akanji AO, Bella AF. Hemoglobin A1 levels in Nigerian diabetic patients using microcolumn affinity chromatography. Afr J Med Med Sci 1986;15:49–53.
19. Little AR, England JD, Wiedmeyer H, Goldstein DE. Effects of whole blood storage on results for glycosylated hemoglobins as measured by ion-exchange chromatography, affinity chromatography and colorimetry. Clin Chem 1983;29:1113–15.
20. Goldstein DE, Little RR, Weidmeyer H, England JD, McKenzie EM. Glycosylated hemoglobin: methodologies and clinical applications. Clin Chem 1986;32:864–70.

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