GLYCOSYLATED HAEMOGLOBINS IN WOMEN WITH LOW RISK FOR DIABETES IN PREGNANCY

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ABSTRACT

Glycosylated haemoglobin levels (HbA_1) were determined in 489 normal pregnant Singaporean women, attending a diabetic screening programme using a 50g glucose challenge test. All subjects had no risk factors for diabetes mellitus nor a history of previous gestational diabetes. They were selected when the 1h 50g glucose challenge (GCT) is < 6.66 mmol/l or a 2h post-glucose level of < 7.77 mmol/l (a 75g OGTT is repeated within a week of an abnormal GCT). Another 18 subjects with normal OGTT but did not have a 50g glucose challenge done were also included in the study. Mean and normal range (2 SD) of HbA_1 levels in all subjects were 4.64% and 3.66-5.62%, respectively. Mean HbA_1 levels also varied with gestation and ethnic group. The HbA_1 appeared to be low at early gestation with nadir at 21-24 weeks and thereafter returned to initial levels at term. In Chinese, mean HbA_1 levels, random plasma glucose and 1h glucose challenge were significantly higher than those of the Malays but not the Indians. In referring to these levels, the variation within gestation and ethnic group must also be considered.

Keywords: *glycosylated haemoglobin, pregnancy*

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INTRODUCTION

Glycosylated haemoglobin (HbA_1) measurements have helped in the assessment of treatment of diabetic patients [1-3]. They have proved valuable as an additional parameter for follow-up of pregnant diabetics where optimal control is of importance. While HbA_1 levels have been determined in various pregnant populations, there is no standard reference range for comparison between populations. This is because different methods are used for measurement and also because standard reference materials are lacking.

In an attempt to define a reference range for our local population, we measured the HbA_1 levels of a group of normal pregnant women at different gestation periods undergoing a screening programme for diabetes mellitus using a 50g glucose challenge test (GCT)[4]. This paper reports the HbA_1 levels in women with a normal glucose challenge alone or with an abnormal GCT but a normal oral glucose tolerance test (75g OGTT) and we also compare the levels in different ethnic groups and gestation periods.

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MATERIALS AND METHODS

Subjects were recruited from the ante-natal clinic at the National University Hospital. These women did not have any historical or obstetrical risk factors for diabetes mellitus. On admission into the study, 2 venous blood samples (random samples) were drawn for glucose (KF coated tube) and HbA_1 (EDTA-coated tube) before a 50g glucose load was given to the patient. Another venous blood sample was collected one hour after the glucose load for glucose estimation. Plasma glucose was determined by the hexo-kinase method[5] on the Cobas Mira. Inter-assay precision was 2%. Samples for HbA_1 were haemolysed on the day of collection and stored at 4°C and analysed within 4 days by affinity chromatography[6] using the Glycogel (Pierce) Test Kit. Inter-assay precision was 5% at 5.3% HbA_1 level and 4% at 16% HbA_1 level.

Patients with a 1h glucose challenge ≥ 6.66 mmol/l were recalled within a week for a 75g glucose tolerance test. Only subjects with a normal GCT (1h glucose challenge < 6.66 mmol/l) or an abnormal GCT but normal OGTT (2h post-glucose level ≤ 7.77 mmol/l by WHO criteria[7]) were included in the study. An additional 18 subjects who were not screened by a glucose challenge but had a normal 75g OGTT response were also included in the study.

To investigate the variation of HbA_1 with duration of gestation, the subjects were grouped in smaller time intervals (Table I). As there were less subjects at early (1-12 weeks) and late (37-42 weeks) gestation, the time interval for these periods were longer to include a substantial number of subjects for each interval.

Laboratory values are reported as mean ± 1 standard deviation of the mean. Comparison of gestation periods between different ethnic groups was done by analysis of variance and Duncan test. To adjust for differences in gestation between ethnic groups, the data were compared by analysis of covariance with gestation as covariate. Similarly, comparison of data between different gestation groups was done by analysis of covariance with race as covariate. The data were also submitted to stepwise multiple regression analysis until all independent factors were significant at $p<0.05$ level. The statistical package, SAS, was used for analysis.

RESULTS

Table II shows the mean HbA_1 and glucose levels in normal pregnant women. Mean and range (2 SD limits) of HbA_1 levels

were 4.64% and 3.66% to 5.62% respectively.

When subjects with normal glucose response were analysed according to ethnic groups, the mean gestation of the Malays was significantly higher than that of the Chinese or Indians (Table III). Before adjustment for differences in gestation, there was no significant differences in mean HbA_1 levels between different ethnic groups. However, after adjustment for differences in gestation, the Chinese were found to have significantly higher mean HbA_1 levels than the Malays (Table III). There were no significant differences in fasting plasma glucose (0h GTT) and glucose tolerance test levels between all groups. However, random plasma glucose and 1h post-glucose challenge levels were significantly higher in the Chinese than the Malays or Indians ($p<0.01$).

Table I - Adjusted* mean HbA_1 (±SD) levels in low risk pregnant women at various gestation.

Gestation Group No	Gestation Weeks	No of Subjects	HbA_1 (%) Normal Group
1	1-12	19	4.74 ± 0.48
2	13-16	74	4.67 ± 0.48
3	17-20	97	4.62 ± 0.48
4	21-24	91	4.45 ± 0.48[a]
5	25-28	63	4.65 ± 0.48
6	29-32	61	4.75 ± 0.47
7	33-36	70	4.76 ± 0.48
8	37-42	14	4.76 ± 0.48

*Means adjusted for differences in race
[a]$p<0.05$ vs all Gestation Groups.

Table II - Mean (±SD) glucose and HbA_1 levels in low risk pregnant women with normal glucose response.

Parameter	Mean ± SD	No.
Gestation (weeks)	23.7 ± 7.5	489
Random plasma glucose (mmol/l)	5.30 ± 1.38	471
1h glucose challenge (mmol/l)	6.75 ± 1.38	471
0h GTT (mmol/l)	4.29 ± 0.39	219
1h GTT (mmol/l)	7.46 ± 1.42	219
2h GTT (mmol/l)	6.10 ± 1.01	220
HbA_1 (%)	4.64 ± 0.49	489

Table III - Adjusted* mean (±SD) glucose and HbA_1 levels in low risk pregnant women of different races with normal glucose response.

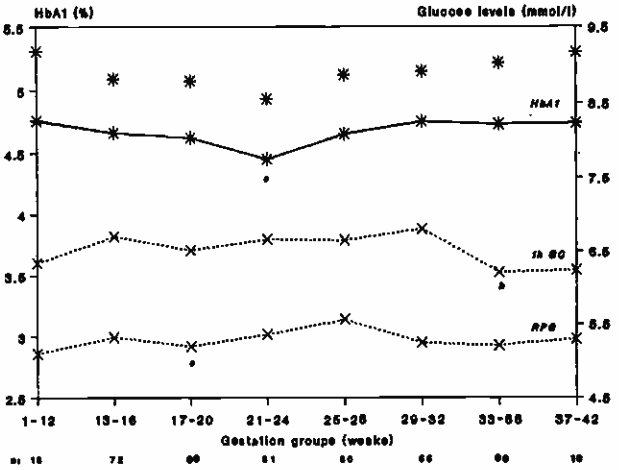
Parameter	Chinese	Malays	Indians
Gestation (weeks)	23.1 ± 7.2 (306)	25.6 ± 8.0 (137)[a]	21.9 ± 7.2 (46)
Random plasma glucose (mmol/l)	5.46 ± 1.00 (295)[b]	5.03 ± 1.01 (132)	5.02 ± 1.00 (44)
1h glucose challenge (mmol/l)	6.78 ± 1.36 (295)[c]	6.28 ± 1.38 (132)	6.02 ± 1.36 (44)
0h GTT (mmol/l)	4.29 ± 0.39 (158)	4.28 ± 0.39 (48)	4.27 ± 0.39 (13)
1h GTT (mmol/l)	7.42 ± 1.38 (158)	7.66 ± 1.38 (47)	9.28 ± 1.38 (13)
2h GTT (mmol/l)	6.15 ± 0.99 (158)	6.05 ± 0.99 (48)	5.77 ± 0.99 (13)
HbA_1 (%)	4.68 ± 0.48 (306)[d]	4.57 ± 0.49 (137)	4.60 ± 0.48 (46)

*Means adjusted for differences in gestation. Numbers in brackets denote number of results for the particular parameter. [a]$p<0.05$ vs Chinese or Indians; [b]$p<0.01$ vs Indians and $p<0.001$ vs Malays; [c]$p<0.001$ vs Malays or Indians; [d]$p<0.05$ vs Malays.

Mean HbA_1 levels decreased from early gestation to a minimum at 21-24 weeks and increased to initial levels at term (Table I). Mean HbA_1 level at 21-24 weeks was significantly lower ($p<0.05$) than levels at other gestation periods.

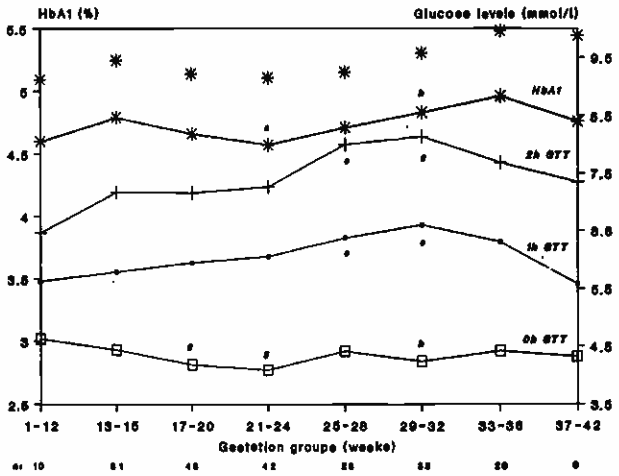
Fig 1 shows the trend in mean HbA_1, random plasma glucose and 1h post-glucose challenge levels in all subjects (471) with a glucose challenge done at various gestation periods. Mean random plasma glucose level was significantly lower at 17-20 weeks than 25-28 weeks ($p<0.05$) while 1h post-glucose challenge levels at 33-36 weeks were significantly lower than those at 13-16 or 29-32 weeks ($p<0.05$).

Fig 1 - Mean (±SD) HbA_1, mean random plasma glucose (RPG) and 1h glucose challenge (1h GC) levels in low risk pregnant women with normal glucose response.



Footnote:
n: number of subjects per gestation group; a:$p<0.05$ vs all groups; b:$p<0.05$ vs 13-16 & 29-32 weeks gestation groups; c:$p<0.05$ vs 25-28 weeks gestation group.

Fig 2 - Mean (±SD) HbA_1, mean fasting plasma glucose (0h), 1h and 2h post glucose loading (1h and 2h GTT) levels in low risk pregnant women with normal glucose response.



Footnote:
n: number of subjects per gestation group;
a:$p<0.05$ vs 13-16, 29-32 & 33-36 weeks gestation groups;
b:$p<0.05$ vs 17-20 & 21-24 weeks gestation groups;
c:$p<0.05$ vs 1-12, 13-16 & 37-42 weeks gestation groups;
d:$p<0.05$ vs 1-12 to 21-24 & 37-42 weeks gestation groups;
e:$p<0.05$ vs 1-12 to 21-24 weeks gestation groups;
f:$p<0.05$ vs 1-12 weeks gestation group;
g:$p<0.05$ vs 1-12, 13-16, 25-28 & 33-36 weeks gestation groups;
h:$p<0.05$ vs 1-12 & 13-16 weeks gestation groups.

Fig 2 shows the trend in mean HbA_1, 0h, 1h and 2h GTT levels in subjects (221) with a GTT done at various gestation periods. Mean HbA_1 at 21-24 weeks was significantly lower than levels at 13-16, 29-32 and 33-36 weeks gestation. Mean fasting glucose levels, 0h GTT, at 17-20 and 21-24 weeks were significantly lower than levels at 1-12, 13-16, 25-28 and 33-36 weeks gestation. Mean 1h GTT levels at 25-28 and 29-32 weeks were significantly higher than those at 1-12 to 21-24 weeks gestation periods. Similarly, mean 2h GTT level at 25-28 weeks was significantly higher than those at 1-12, 13-16 and 37-42 weeks gestation periods and that at 29-32 weeks was significantly higher than those at 1-12 to 21-24 and 37-42 weeks gestation periods.

The results of multiple regression analysis of HbA_1 with gestation, race, random plasma glucose and 1h glucose challenge are given in Table IV. Only gestation and 1h glucose challenge were significantly correlated with HbA_1.

DISCUSSION

The mean and range of HbA_1 levels in this study group with normal 1h glucose challenge or a normal 75g OGTT were 4.64% and 3.66%-5.62%, respectively. In an earlier study[8], we used an electro-phoretic method to measure the HbA_1 levels in a smaller group of 33 pregnant women with normal 50g OGTT response. These subjects were different from the low risk patients in our current study in that they had risk factors for diabetes mellitus. The mean and normal range of HbA_1 levels then were 6.34% and 4.96% to 8.11% respectively. The reason for higher levels of HbA_1 obtained in the previous study is probably due to difference in the method of estimation of HbA_1.

Table IV - Multiple regression analysis of HbA_1 with gestation, race, random plasma glucose and 1h glucose challenge

Variable	b	F	p value	r
Intercept	4.056	999.3	0.0001	
1h glucose challenge	0.006	17.3	0.0001	0.18
Gestation	0.004	4.8	0.0292	0.10
			R	0.21

r, R - partial and multiple correlation coefficient. Only significant partial correlation coefficients ($p<0.05$) obtained after elimination by stepwise multiple regression analysis are listed.

HbA_1 levels in our population of pregnant women were found to vary with ethnic groups. The Chinese had significantly higher levels of HbA_1, random plasma glucose and 1h post-glucose challenge glucose levels than the Malays. These differences could not be due to difference in gestation of the 2 groups since this variation had been taken into consideration in the analysis of the results. While 1h post-glucose challenge was observed to be significantly correlated to HbA_1 levels, the variation of HbA_1 cannot be satisfactorily explained by the variation in 1h glucose challenge since this factor only contributes about 3% of the variation ($r=0.18$, $r^2=0.03$). There maybe other factors including socio-economic status, weight for height gain, and dietary habits which affect the HbA_1 levels in Chinese and Malays.

Mean HbA_1 levels decreased initially from early gestation to a nadir at 21-24 weeks and returned to initial levels at the last trimester. Variable trends had been observed in other studies. Some have reported no change of HbA_1 with stage of gestation[9], while others have found a fall with increasing gestation[10,11] or a biphasic trend with a nadir level observed at mid-gestation[12-14]. These differences had been attributed to difference in methodology and study design. Many of the earlier studies were cross-sectional in design and used ion-exchange techniques which are sensitive to changes in temperature, acute changes in blood glucose concentrations and interference from minor haemoglobins[6]. In our study, HbA_1 was analysed using affinity chromatography, a method which specifically measures HbA_1 with minimal interference from labile fractions, pH and temperature changes[6,15-17]. Our results showed a similar trend with those of Morris et al[12] who also used the same methodology for analysing HbA_1 albeit in a longitudinal study. They observed that in their normal pregnant women, HbA_1 levels decreased progressively from 11-14 weeks, reaching a nadir of 5.2% at 23-26 weeks and returned to baseline concentration (5.6%) by 31-34 weeks. Worth et al[14] using 2 methods (a colorimetric and an ion-exchange method), measured HbA_1 levels sequentially at <12, 17, 24 and 34 weeks and found the nadir at 17 weeks. Phelps et al[13] estimating HbA_1 in a cross-sectional study on normal pregnancy, also observed a biphasic trend with a nadir level at 24 weeks while 1h post-glucose challenge levels followed a similar trend with a nadir level at 4 weeks earlier. They concluded that the changes in HbA_1 reflected with appropriate displacement in time, the biphasic alterations in mean blood glucose that characterised the sequential changes in glucoregulation during normal pregnancy. In our study, the lower mean random plasma glucose level at 17-20 weeks might probably reflect the nadir level of mean HbA_1 4 weeks later. However, 1h post-glucose challenge responses in our study did not show an obvious trend.

In cases where a GTT was done (221 cases), the variation of HbA_1 with gestation showed a similar trend with nadir at 21-24 weeks but a slight peak at 33-36 weeks (Fig 2). The difference towards the later stage of gestation may be due to the difference in case number for the gestation groups. The trend of glucose levels in 0h, 1h or 2h GTT did not appear to follow closely the trend of HbA_1 levels. However, the lower levels of 0h GTT at 17-20 and 21-24 weeks gestation periods may have contributed to the nadir level of HbA_1 at 17-20 weeks.

Theoretically HbA_1 values could be used as a simple and convenient screening test for gestational diabetes. However, investigators have not been able to find a clear cut-off point that will differentiate between normal and abnormal glucose tolerance in pregnancy. This overlap and the wide variation in the range of HbA_1 values between the 2 groups does not make HbA_1 an accurate screening test for gestational diabetes. HbA_1 may be used as an additional parameter for assessment of glucose control and a lower range (compared to non-pregnant women) shown here maybe used as a guideline for good control.

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