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CLINICAL & BASIC RESEARCH

Establishing Trimester-Specific Haemoglobin A1c Reference Intervals in Pregnant Women

A retrospective study of healthy South Asian women with normal pregnancy outcomes

*John Punnose,[1] Rajeev K. Malhotra,[5] Komal Sukhija,[1] Rashika M. Rijhwani,[1] Asha Sharma,[2] Naimaa Choudhary,[2] Prassan Vij,[3] Renuka Joseph[4]

abstract: ***Objectives:*** This study aimed to define trimester-specific haemoglobin A1c (HbA1c) reference intervals in healthy, pregnant South Asian women. ***Methods:*** This retrospective study was conducted at St. Stephen's Hospital, Delhi, India, between January 2011 and December 2016. Healthy pregnant women were compared to a control group of healthy non-pregnant women. Pregnant participants had term deliveries of babies with appropriate gestational weights. The HbA1c levels were calculated in terms of non-parametric 2.5 and 97.5 percentiles for women in first (T1), second (T2) and third (T3) trimester groups. Statistical tests were used to obtain the normal HbA1c reference values and were considered significant when P <0.05. ***Results:*** This study included a total of 1,357 healthy pregnant women and a control group of 67 healthy, non-pregnant women. Pregnant women had a median HbA1c of 4.8% (4–5.5%) or 32 mmol/mol (20–39 mmol/mol); non-pregnant women had a median HbA1c of 5.1% (4–5.7%) or 29 mmol/mol (20–37 mmol/mol; P <0.001). The HbA1c levels for the T1, T2 and T3 groups were 4.9% (4.1–5.5%) or 30 mmol/mol (21–37 mmol/mol), 4.8% (4.5–5.3%) or 29 mmol/mol (20–34 mmol/mol) and 4.8% (3.9–5.6%) or 29 mmol/mol (19–38 mmol/mol), respectively. The HbA1c values were significant when comparing T1 versus T2 (P <0.001), T1 versus T3 (P = 0.002) and T1 versus the non-pregnant group (P = 0.001). However, T2 versus T3 was not significant (P = 0.111). ***Conclusion:*** Compared to non-pregnant women, HbA1c levels were lower in pregnant women, despite women in the T2 and T3 groups having a higher body mass index than the women in the T1 and non-pregnant groups. Further research is recommended to understand the factors responsible and validate these findings.

Keywords: Asian; Gestational Diabetes; HbA1c; Pregnancy Trimesters; Reference Values; India.

Advances in Knowledge

- *There is a significant decrease in haemoglobin A1c (HbA1c) levels during pregnancy in South Asian women; this decrease is most apparent in the early pregnancy stage.*
- *Among healthy South Asian women, the suggested upper reference limits of HbA1c in the first, second and third trimesters are 37, 34 and 38 mmol/mol, respectively.*

Application to Patient Care

- *The proposed upper trimester-specific HbA1c reference values may be used as threshold values to identify women prone to gestational diabetes mellitus (GDM) and other adverse pregnancy outcomes.*
- *Early identification of these high risk women will provide an opportunity to introduce preventive strategies.*
- *These HbA1c reference values can help when designing further prospective studies involving pregnant South Asian women, developing alternate tests to the oral glucose tolerance test for GDM diagnosis or establishing glycaemic targets in pregnancies complicated by diabetes.*

Although glycated haemoglobin A1c (HbA1c) is widely used as a standard biomarker for glycaemic control during management of diabetes mellitus in the general population, its role in glycaemic assessment during pregnancy is uncertain.[1] The accuracy of HbA1c estimation during pregnancy is affected by several physiological changes, such as increase in red blood cell (RBC) production, younger RBC age distribution and reduced RBC life span.[2] Moreover, the high prevalence of iron deficiency and the widespread use of iron supplementation in pregnant women (especially in developing countries) can influence HbA1c estimation during pregnancy.[3] Despite these limitations, several prestigious organisations have recommended HbA1c estimation during pregnancy for various reasons. The World Health Organization advocates HbA1c estimation at the first antenatal visit to identify women with 'diabetes during pregnancy' (HbA1c >6.5% or >48 mmol/mol).[4]

HbA1c is recommended as a screening and diagnostic test for gestational diabetes mellitus (GDM).[2,5] In 2011, the California State Diabetes and Pregnancy Program 'Sweet Success' adopted a

Departments of [1]Endocrinology, [2]Obstetrics & Gynecology, [3]Reproductive Medicine and [4]Biochemistry, St. Stephen's Hospital, New Delhi, India; [5]All India Institute of Medical Science, Ansari Nager, New Delhi, India

**Corresponding Author's e-mail: drpunnose@ststephenshospital.org*

new algorithm for the diagnosis and treatment of hyperglycaemia during pregnancy.[6] All women with HbA1c values of 5.7–6.4% (39–46 mmol/mol) in early pregnancy are advised to undergo GDM treatment without further confirmatory oral glucose tolerance testing (OGTT). The American Diabetes Association suggests periodic HbA1c estimations during pregnancy as a secondary measure of glycaemic control after glucose self-monitoring.[7] The National Institute for Health and Care Excellence in the United Kingdom proposes a guideline for using HbA1c estimations during pregnancy as a reliable tool for risk stratification and pregnancy outcome predictions.[8] This guideline recommends HbA1c testing at booking and in the second and third trimesters to ensure that the targets are achieved. In many population groups, the HbA1c level in the first trimester is recognised as a predictor of GDM later in pregnancy, as well as of adverse pregnancy outcomes.[2,9,10] The HbA1c levels in the second and third trimesters are predictive of several obstetric complications: macrosomia, gestational hypertension, preeclampsia, abnormal liquor volume, prematurity and neonatal deaths.[2,11]

However, many of these recommendations have not gained universal acceptance due to a lack of strong research evidence in the obstetric population. The HbA1c cut-off points for the diagnosis of 'diabetes during pregnancy' (>6.5%, >48 mmol/mol) and GDM in the 'Sweet Success' programme (5.7–6.4%, 39–48 mmol/mol) are guided by the HbA1c values for the diagnosis of diabetes and prediabetes in a non-obstetric population, respectively. However, HbA1c levels in pregnancy are lower than in the non-obstetric population and they show physiological variations between trimesters.[12] There are significant racial and ethnic differences in the glycation of haemoglobin for a given level of glycaemia.[13] Thus, there is a need to define ethnic- and trimester-specific HbA1c reference levels for women with normal pregnancies before they are recommended for GDM screening and diagnosis, risk stratification and measurement of metabolic control.

In the Middle East and South Asia, the incidence of type 2 diabetes is increasing, affecting both pregnant women and the general population. India is the leading country in the world in this regard; it has 5.7 million pregnant women with hyperglycaemia.[14] However, to the best of the authors' knowledge, HbA1c levels among healthy, pregnant South Asian women are not yet defined. This study aimed to identify trimester-specific HbA1c levels in healthy, non-diabetic, pregnant South Asian women who delivered babies with age-appropriate weights.

Methods

This retrospective study involved pregnant women who visited the antenatal clinic at St. Stephen's Hospital, a tertiary care hospital in New Delhi, India, between January 2011 and December 2016. The centre followed a universal thalassaemia screening strategy for pregnant women at the first antenatal visit. The protocol included the estimation of HbA, HbA2 and HbF through haemoglobin electrophoresis, with concurrent estimates of HbA1c. All women with HbA1c ≥6.5% (≥48 mmol/mol) were diagnosed with overt diabetes, while those with a value of <6.5% (<48 mmol/mol) were screened for GDM through a universal one-step 75g OGTT between 24 and 28 gestational weeks, or earlier if they had high GDM risk factors. The GDM diagnosis was made per the recommendations of the International Association of Diabetes and Pregnancy Study Group (IADPSG).[15] All pregnant women were on iron and folic acid supplementation.

The thalassaemia screening was done at the first antenatal visit. All pregnant women who had Hb electrophoresis (HbA1c estimation) were evaluated for inclusion in this study. Any patient with unclear dates of their last menstrual period, deliveries outside the hospital, the diagnosis of diabetes and gestational diabetes, GDM risk factors, anaemia, systemic diseases and delivery of babies small or large for gestational age (LGA) were excluded.[16,17] The remaining women were sub-categorised into three groups based on the gestational age of HbA1c estimation: (a) first trimester (<14 weeks [T1]); (b) second trimester (14–26 weeks [T2]); and (c) third trimester (27–41 weeks [T3]). Body mass index (BMI) was calculated from the height and weight recorded at the first antenatal visit. The serum thyroid stimulating hormone (TSH) was estimated at the first antenatal visit in all women and, if elevated, was corrected with oral L-thyroxine therapy (target serum TSH level below 2.5, 3 and 3 mIU/L in the first, second and third trimesters, respectively).

A control group was recruited from healthy, non-pregnant women who visited the pre-pregnancy counselling clinic of the hospital during the study period. All control group women had a fasting plasma glucose (FPG) of <5.5 mmol/l (<100 mg/dL) or random plasma glucose of <7 mmol/L (126 mg/dL), Hb >11 g/dL, normal HbA2 and HbF levels, no prior history of gestational diabetes or abortion, no family history of diabetes in first degree relatives and no systemic disease. The women in all trimesters were age-matched with the controls. The HbA1c levels of the control group were compared with the HbA1c levels from the first antenatal visits. The reference intervals

of HbA1c levels in each trimester were estimated and compared for any differences.

The authors' laboratory is certified by the National Accreditation Board for Testing and Calibration Laboratories and uses Bio-Rad laboratories for proficiency testing. The complete blood count was done on ethylenediaminetetraacetic acid (EDTA) anticoagulated blood using a Beckman Coulter LH 750/780 analyzer (Beckman Coulter Inc., Brea, California, USA) using volume, conductivity, light scatter (VCS) technology. Standard protocol was used for the OGTT (i.e. ingestion of 75g anhydrous D-glucose dissolved in 250 mL distilled water). The sample for plasma glucose estimation was collected in EDTA and sodium fluoride (grey top) Vacuette tubes (Vitrex medical A/S, Vasekaer, Denmark). The glucose estimation was done using the hexokinase method and a Beckman AU680/480 clinical chemistry analyzer (Beckman Coulter Inc.). Two levels of plasma glucose controls (at Bio-Rad) were run daily: Level 1 at 4.53 mmol/L (81.50 mg/dL) and Level 2 at 15.57 mmol/L (280.2 mg/dL). The monthly coefficients of variation (CV) percentages calculated for the Level 1 and Level 2 controls were 1.7% and 1.4%, respectively. The blood for HbA1c estimation were non-fasting samples collected in EDTA vials. Estimation was done within two hours of sampling using the ion-exchange high-performance liquid chromatography method with a Bio-Rad D10TM machine (Bio-Rad Laboratories, Hercules, California, USA). The estimation was traceable to the reference methods of both the National Glycohemoglobin Standardization Program and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). The inter-assay CV was 1.3% and 1.5% for low control (mean HbA1c = 5.45%, 37 mmol/mol) and high control (mean HbA1c = 9.95%, 86 mmol/mol), respectively. The laboratory participated in an external quality assurance scheme for both glucose and HbA1c estimations. The Z-score for glucose was 0.60 and 0.65 for HbA1c.

All study groups had a minimum of 40 subjects as mandated by the IFCC for the identification of reference intervals.[18] The data analysis was performed using the Statistical Package for the Social Sciences (SPSS), Version 16 (IBM Corp., Armonk, New York, USA), and the R software, Version 4.0.2 (R Foundation, Vienna, Austria). Continuous variables were presented as mean ± standard deviation. An unpaired student's t-test was used to compare the means of these variables (age, BMI, gestational age of delivery, birthweight, red blood cell indices) of the pregnant and non-pregnant groups .The homogeneity of variances was checked using Leven's test. A one-way analysis of variance followed by a post-hoc Tukey's test was applied to compare the means of groups T1, T2 and T3. Five normality tests were used to obtain the normal reference value of HbA1c: Anderson-Darling, Cramer-von Mises, Kolmogorov-Smirnov, Shapiro-Francia and Pearson Chi-squared test. The mean plus two standard deviations were reported as reference values when the normality condition was fulfilled; otherwise, a non-parametric method and median with 2.5th and 97.5th percentiles were reported as the normal range. The 95% confidence intervals of these percentiles were determined using bootstrapping with 10,000 replications using the boot package of

Table 1: Clinical parameters of pregnant women in first, second and third trimesters and control group (N = 1,424)

Parameter	Group, n (%)				*P* value				
	Control (n = 67; A)	T1 (n = 513; B)	T2 (n = 550; C)	T3 (n = 294; D)	A vs B	B vs C	B vs D	C vs D	Overall
Mean age in years ± SD	26-6 ± 2.89	26.83 ± 3.50	26.69 ± 3.50	26.34 ± 3.57	0.957	0.914	0.220	0.507	0.2899
BMI in kg/m^2 ± SD*	24.43 ± 3.22	24.40 ± 4.29	25.13 ± 4.39	26.59 ± 3.99	1.00	0.0253	<0.001	<0.001	<0.001
Primigravida	NA	283 (55.2)	302 (54.9)	162 (55.1)	NA	-	-	-	0.998
Family history of DM	0	0	0	0	-	-	-	-	-
History of GDM	0	0	0	0	-	-	-	-	-
GA in weeks at delivery	NA	38.53 ± 1.02	38.56 ± 1.02	38.53 ± 1.00	NA	0.921	0.999	0.925	0.8670
Birth weight of baby in kg	NA	2.89 ± 0.29	2.87 ± 0.36	2.85 ± 0.28	NA	0.562	0.199	0.660	0.2180
Anaemia (Hb <11g)	0	0	0	0	-	-	-	-	-
Beta-thalassaemia trait (HbAF >0.8% or HbA2 >3%)	0	0	0	0	-	-	-	-	-

T1 = first trimester (0–13 weeks); T2 = second trimester (14–26 weeks); T3 = third trimester (27–41 weeks); SD = standard deviation; BMI = body mass index; NA = not applicable; DM = diabetes mellitus; GDM = gestational diabetes mellitus; GA = gestational age; Hb = haemoglobin.

**Calculated from height and weight of pregnant women at first antenatal visit in T1, T2 and T3 trimesters.*

Table 2: The median and percentiles of haemoglobin A1c levels in non-pregnant women and in pregnant women during the first, second and third trimester (N = 1,424)

Study group	Total	HbA1c in %				*P* value	Type of distribution¶
		Median (95% CI)	Percentile (95% CI)		Min-max		
			2.5th	97.5th			
Non-pregnant	67	5.1 (4.9–5.2)	4.0 (3.9–4.6)	5.7 (5.5–6.0)	3.9–6.0	<0.001*	Non-Gaussian (5)
Pregnant	1,357	4.8 (4.8–4.8)	4.0 (3.9–4.1)	5.5 (5.4–5.5)	3.2–5.9	<0.001*	Non-Gaussian (5)
T1	513	4.9 (4.8–4.9)	4.1 (4.0–4.2)	5.5 (5.4–5.5)	3.7–5.8	<0.001†	Non-Gaussian (5)
T2	550	4.8 (4.7–4.8)	4.0 (3.9–4.1)	5.3 (5.2–5.4)	3.2–5.5	<0.001‡	Non-Gaussian (5)
T3	294	4.8 (4.7–4.8)	3.9 (3.8–4.1)	5.6 (5.4–5.7)	3.6–5.9	0.002‡ 0.111§	Non-Gaussian (5)

CI = confidence interval; T1 = first trimester (0–13 weeks); T2 = second trimester (14–26 weeks); T3 = third trimester (27–41 weeks).

**Comparing non-pregnant with the pregnant groups. †Compared with the non-pregnant group. ‡Compared with the T1 group. §Comparing the T2 and T3 groups. ¶Values in parentheses indicate the number of tests for goodness of fit with P <0.05..*

Table 3: Clinical and laboratory parameters of the whole study population versus the control group and between first, second and third trimesters (N = 1,424)

Parameter	Mean ± SD								
	Whole study population			Women in different trimesters					
	Women all trimesters (n = 1,357)	Non-pregnant control group (n = 67)	*P* value*	T1 (n = 513)	T2 (n = 550)	T3 (n = 294)	*P* value† T1 vs T2	*P* value† T1 vs T3	*P* value† T2 vs T3
Age in years	26.67 ± 3.51	26.66 ± 2.89	0.9564	26.83 ± 3.50	26.69 ± 3.50	26.34 ± 3.57	0.790	0.135	0.351
GA at HbA1c estimation in weeks	-	-	-	9.53 ± 2.47	18.81 ± 3.62	31.23 ± 3.02	-	-	-
Haemoglobin in g/L	120.3 ± 7.4	122.8 ± 8.7	0.027	121.7 ± 7.5	119 ± 6.8	120.5 ± 8.0	<0.001	0.081	0.016
MCV in fl	88.36 ± 5.61	84.09 ± 7.86	<0.001	87.73 ± 5.84	88.70 ± 5.52	88.85 ± 5.22	0.016	0.025	0.937
MCH in pg	29.38 ± 2.41	28.18 ± 2.87	<0.001	29.06 ± 2.38	29.61 ± 2.23	29.53 ± 2.76	0.001	0.030	0.895
HCT in %	0.36 ± 0.03	0.37 ± 0.03	0.063	0.36 ± 0.03	0.36 ± 0.03	0.36 ± 0.03	0.366	0.974	0.375
MCHC in g/L	332.5 ± 10.2	328.5 ± 8.9	0.003	330.9 ± 9.7	333.6 ± 10.1	333.1 ± 11	<0.001	0.015	0.762
RDW in %	14.75 ± 2.16	14.76 ± 1.48	0.977	14.55 ± 1.68	14.74 ± 2.19	15.18 ± 2.75	0.337	<0.001	0.021
RBC × 10^{12} L	4.10 ± 0.40	4.43 ± 0.56	<0001	4.14 ± 0.41	4.07 ± 0.39	4.10 ± 0. 39	0.009	0.298	0.605
GA at delivery in weeks	-	-	-	38.53 ± 1.02	38.56 ± 1.02	38.53 ± 1.00	0.921	0.999	0.925
Birth weight in kg	-	-	-	2.89 ± 0.29	2.87 ± 0.36	2.85 ± 0.28	0.562	0.199	0.660

SD = standard deviation; T1 = first trimester (0–13 weeks); T2 = second trimester (14–26 weeks); T3 = third trimester (27–41 weeks); GA = gestational age; HbA1c = haemoglobin A1c; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; HCT = haematocrit; MCHC = mean corpuscular haemoglobin concentration; RDW = red blood cell diameter width; RBC = red blood cell.

**Using unpaired student's t-test to compare the mean value between the groups. †Using one-way analysis of variance, followed by a post-hoc Turkey's test..*

the R software (R Foundation). The Mann-Whitney U test was applied to compare the distribution of HbA1c between pregnant and non-pregnant women and between the trimesters; a *P* value of <0.008 was considered significant as per Bonferroni correction (0.05/number of comparisons). A *P* value of <0.05 was considered significant for other statistical tests.

This research protocol was approved by the ethics committee of St. Stephen's Hospital (No. SSHEC/R0136) with a waiver for the patient consent forms.

Results

A total of 9,388 pregnant women had Hb electrophoresis (HbA1c estimation) during the study period but 8,031 women were excluded. The final sample of 1,357 women in the study population were sub-categorised into T1 (n = 513), T2 (n = 550) and T3 (n = 294) [Figure 1]. A total of 67 healthy, non-pregnant women were recruited from 750 women. The age and BMI of the control group were comparable to those of the whole study population and the T1 pregnancy group [Table 1].

All five statistical tests to assess the normality of the HbA1c values showed a violation of normality. The median HbA1c value of 4.8% (29 mmol/mol) for the whole study population and 4.9% (30 mmol/mol) for the T1 group were lower than the median value of 5.1% (32 mmol/mol) for the control group (P <0.001 each). The HbA1c median values for the T1, T2 and T3 groups were 4.9%, 4.8% and 4.8% (30, 29 and 29 mmol/mol), respectively, with significant differences between T1 and T2 (P <0.001) and T1 and T3 (P = 0.002), but no difference between T2 and T3 (P = 0.111) [Table 2].

When comparing the control group to the women in all trimesters, the gestational ages at delivery and the birth weights were comparable (P >0.05 for all parameters) [Table 3]. The Hb and RBC counts were lower and the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) values were higher in pregnant women compared to the control group; there was no difference in the haematocrit (HCT) and RBC distribution width (RDW) values between these groups. Compared to the T1 group, there was a decrease in the Hb and RBC counts and an increase in the MCV, MCH and MCHC values in the T2 group; the RDW and HCT values were similar in the two groups. The Hb and RDW values in T3 were significantly higher compared to the T2 group; the HCT, MCV and MCHC values were similar [Table 3].

A comparison of the HbA1c reference intervals estimated by parametric method and non parametric method is shown in Table 4. The upper reference values of HbA1c in all groups identified by parametric method (95% confidence interval of mean) and non parametric methods (97.5th percentile) were comparable.

The upper normal HBA1c level for the control, T1, T2 and T3 groups were 5.7% (39 mmol/mol), 5.5% (37 mmol/mol), 5.3% (34 mmol/mol) and 5.6% (38 mmol/mol), respectively [Figure 2].

Discussion

This study found that the HbA1c level in pregnant women was lower than in non-pregnant women in South Asia. The HbA1c reference values for the first, second and third trimesters were 4.1–5.5% (21–37 mmol/mol), 4.0–5.3% (20–34 mmol/mol) and 3.9–5.6% (19–38 mmol/mol), respectively. Earlier studies revealed some racial differences in HbA1c reference intervals; these values were 3.8–5.5% (19–37 mmol/mol), 4.0–5.5% (20–37 mmol/l) and 4.4–5.5% (25–37 mmol/l) in 15–24 (T1 group), 25–27 (T2 group) and 28–36 gestational weeks (T3 group), respectively, for Caucasian women in Italy.[19] For Mexican women, the intervals were 4.5–5.6% (26–38mmol/mol), 4.4–5.5% (26–37 mmol/mol) and 4.4–5.6% (25–38 mmol/mol) in the T1, T2 and T3 groups, respectively.[20] The intervals for Japanese women were 4.7–5.7% (28–39 mmol/mol), 4.4–5.4% (25–36 mmol/mol) and 4.6–5.8% (27–40 mmol/mol) in the T1, T2 and T3 groups, respectively.[21] Compared to these studies, the upper HbA1c reference values of the South Asian cohort in this study were marginally lower. The stringent selection criteria (exclusion of women with GDM diagnosed using the most liberal IADPSG criteria and those with several GDM risk factors and large or small for gestational age babies), as well as the racial differences in the glycation of haemoglobin, might have contributed to this modest HbA1c difference.

Compared to the first trimester, a significant decrease in the HbA1c level was noted in the second trimester, but this remained constant in the third trimester [Table 1]. The differences in HbA1c levels between trimesters varied markedly between studies. In most populations, there was a decrease in the HbA1c level from the first to the second trimester and this decrease was often followed by a significant HbA1c rise in the third trimester (biphasic response).[19,20–24] The rise in the HbA1c level in the third trimester was not seen in some studies but a decrease was reported in one study.[12,25,26] In a Japanese study, Hashimoto *et al*. reported that the HbA1c rise in late pregnancy was mainly due to iron deficiencies in the third trimester.[27] Significant racial differences in trimester-related HbA1c variations were reported in a multi-ethnic population in the United Kingdom by Hartland *et al*.; both Caucasians and Asians had a lower HbA1c level in the second trimester compared to the first trimester, but the HbA1c rise in the third trimester was observed only in Caucasian women and not in Asians, as in this study.[22]

The metabolic changes leading to the significant decline in the HbA1c levels in mid-pregnancy were

Table 4: Comparison of haemoglobin A1c reference intervals estimated by parametric method (after normalisation by Box-Cox transformation) and non-parametric method (N = 1,424)

Group	HbA1c reference values in %	
	Parametric method, mean (95% CI)*	Non-parametric method, median (2.5th to 97.5th percentile)†
Non-pregnant women (n = 67)	5.11 (4.13-5.76)	5.10 (4.00-5.70)
All pregnant women (n = 1,357)	4.80 (4.00-5.50)	4.80 (4.00-5.50)
T1 (n = 513)	4.87 (4.10-5.51)	4.90 (4.10-5.50)
T2 (n = 550)	4.75 (3.94-5.33)	4.80 (4.00-5.30)
T3 (n = 294)	4.78 (4.00-5.55)	4.80 (3.90-5.60)

HbA1c = haemoglobin A1c; CI = confidence interval; T1 = first trimester (0–13 weeks); T2 = second trimester (14–26 weeks); T3 = third trimester (27–41 weeks).

**Lower and upper values of 95% confidence interval are the lower and upper reference values, respectively. †2.5th and 97.5th percentiles are the lower and upper reference values, respectively.*

apparent in a longitudinal study by Mills *et al*.[28] The study demonstrated a significant drop in plasma glucose values between 6 and 10 weeks of gestation, which was followed by a decrease in the HbA1c levels in the second trimester. The authors speculated that maternal metabolic and hormonal factors alter the plasma glucose concentration early in pregnancy, independently of fetal glucose utilisation. Another proposed mechanism for the decrease in the plasma glucose level in the late first trimester is the decrease in progesterone secretion during the luteoplacental shift.[28] The HbA1c reduction in the second trimester is further exacerbated by the physiological changes during pregnancy, such as high erythrocyte turnover and haemodilution. Subsequent compensatory mechanisms, such as maternal plasma reduction and increased atrial natriuretic peptide, can again increase the HbA1c level in the third trimester.[29] The high prevalence of iron deficiency anaemia and the common practice of iron supplementation during pregnancy, especially in developing countries, can also modify HbA1c levels.[3] This study excluded women with anaemia and thalassaemia, and the changes in the Hb, MCV, MCH, MCHC and RBC levels over trimesters were attributable to the physiological changes in pregnancy and iron supplementation.[30]

The proposed upper reference HbA1c levels in early pregnancy in the current study can be clinically relevant in the early identification of women prone to GDM and adverse pregnancy outcomes. This

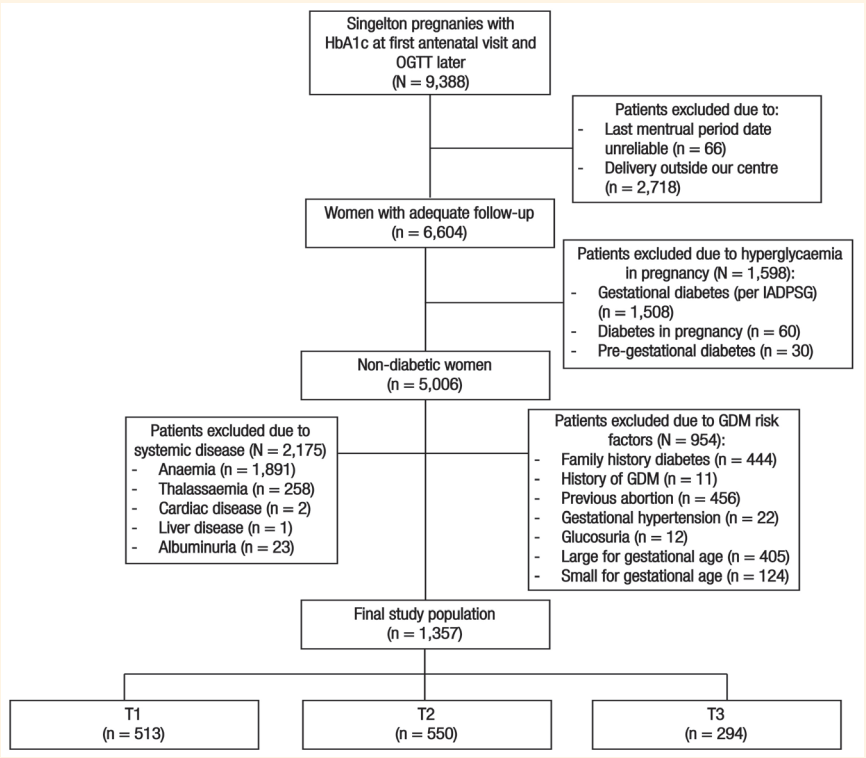

Figure 1: Flowchart showing the study population selection process.

HbA1c = haemoglobin A1c; OGTT = oral glucose tolerance test; IADPSG = International Association of Diabetes and Pregnancy Study Group; GDM = gestational diabetes mellitus (having fasting plasma glucose [PG] between 5.1–6.9 mmol/L, 1-hour PG >10 mmol/L, 2-hour PG between 8.5–11.1 mmol/L in OGTT); T1 = first trimester (0–13 weeks); T2 = second trimester (14–26 weeks); T3 = third trimester (27–41 weeks).

**Overt diabetes first diagnosed in pregnancy (HbA1c >48 mmol/mol or fasting plasma glucose >7 mmol/L or 2-h PG >11.1 mmol/L).*

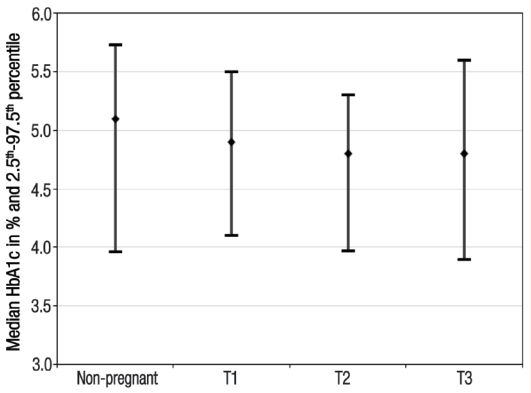

Figure 2: Median and percentile for haemoglobin A1c for non-pregnant women and pregnant women in different trimesters.

approach can provide an opportunity for the early initiation of GDM preventive strategies. Strikingly, the suggested upper reference values (5.5% and 5.3% in the first and second trimesters, respectively) are lower than the generally recommended threshold HbA1c value of 5.7% (39 mmol/mol) for the diagnosis of 'prediabetes during pregnancy'.[2] In an earlier study, the first-trimester HbA1c level, which was >5.5% (37 mmol/mol), was a strong predictor (adjusted odds ratio = 2.6; P <0.001) of GDM later in pregnancy.[12] Similarly, Rajput *et al*. studied the utility of HbA1c estimation between 24 and 28 weeks of gestation for GDM diagnosis in 607 pregnant Indian women.[5] In their study, the HbA1c level was 5.25% (34 mmol/mol), which was a reliable cut-off value for the identification of women with GDM when the IADPSG criteria were applied. The upper reference values in the present study corroborate with the HbA1c threshold values identified for GDM diagnosis in the first and second trimesters in several previous studies.[5,12] Further, Maine *et al*. assessed the relationship between the HbA1c level in the first trimester and adverse pregnancy outcomes among a cohort of multi-ethnic pregnant women in Spain.[17] The risk for eclampsia, LGA and macrosomia increased at HbA1c threshold values of 5.3%, 5.4% and 5.7% (34, 36 and 39 mmol/mol), respectively, for the pregnant South Asian women in this cohort. These cut-off values are close to the first trimester HbA1c upper reference value of 5.5% (37 mmol/mol) in this study. Previous studies suggest that the risks for GDM and other adverse pregnancy events start at HbA1c levels lower than the 'prediabetic' level of 5.7% (39 mmol/mol).[9,11] The authors recommend further prospective studies to validate the proposed trimester-specific HbA1c reference levels for the prediction and identification of various risks and possible adverse outcomes among pregnant South Asian women.

This study had several limitations. The HbA1c reference values were derived from a cross-sectional analysis of different women who visited the antenatal clinic over three trimesters. A longitudinal study on the sequential changes in HbA1c levels of a cohort of the same women over different trimesters would have been ideal. However, the impact of this limitation was significantly alleviated in this study: age, gravidity, family history of diabetes mellitus, history of GDM and abortion, gestational age at delivery, birth weight, the Hb, HbA2 and HbF levels of women in different trimesters as well as the BMI between the control and T1 groups were comparable. The BMI increase in the T2 and T3 groups was due to physiological gestational weight gain. Another limitation was the lack of data on the iron, folate and B12 status of women in different trimesters, but the RBC indices of these women did not suggest any major deficiencies of these factors. The strengths of this study included the large study population and the identification and exclusion of GDM by universal OGTT-based screening as per IADPSG guidelines. All women with GDM risk factors, anaemia and thalassaemia (the common haemoglobinopathy of the region) were excluded from this study. Furthermore, being a single-centre hospital-based study, the blood samples were sampled and processed under optimal conditions in one laboratory.

Conclusion

The trimester-specific haemoglobin HbA1c levels are not yet defined for healthy, pregnant South Asian women. This study evaluated the upper reference limits for the first, second and third trimesters as 37, 34 and 38 mmol/mol, respectively. These trimester-specific HbA1c values can be of clinical relevance for the prediction and diagnosis of GDM and the risk stratification of other adverse events among pregnant South Asian women. Further prospective studies to validate the proposed HbA1c reference intervals are recommended.

AUTHORS' CONTRIBUTION

JP conceptualised the idea of and prepared the manuscript. RM carried out statistical analysis and contributed substantially to the discussion and preparation of the manuscript. KS and RMR contributed to the discussion and provided constructive criticism regarding the manuscript. AS, PV and NC assisted in clinical data collection and analysis and contributed to manuscript preparation and discussion. RJ assisted in the analysis of laboratory data and contributed to the manuscript. All authors approved the final version of the manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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