SUBMITTED 19 SEP 21
REVISIONS REQ. 25 NOV 21 & 19 JAN 22; REVISIONS RECD. 5 DEC 21 & 24 JAN 22
ACCEPTED 20 FEB 22
ONLINE-FIRST: MARCH 2022
DOI: https://doi.org/10.18295/squmj.3.2022.022

Establishing Trimester-Specific Hemoglobin A1c Reference Levels for Pregnant Women

A retrospective study among 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]**

Departments of [1]Endocrinology, [2]Obstetrics and 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-mails:* drpunnose@rediffmail.com; drpunnose@ststephenshospital.org

Abstract

***Objectives*:** This study aimed to define trimester-specific hemoglobin A1c (A1c) reference intervals among healthy South Asian pregnant women. ***Methods*:** In this restrospective study,1357 pregnant women without diabetes, gestational diabetes, gestational hypertension, anemia, β-thalassemia, or systemic diseases were included. They had term delivery of babies having weight appropriate for gestational age. A1c (using high performance liquid chromatography, meeting the National Glycohemoglobin Standardization Program and International Federation of Clinical Chemistry standards), hemoglobin, and RBC indices were estimated at the first antenatal visit. The A1c 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. The control group included 67 healthy non-pregnant women. Statistical tests were used to obtain the normal the normal reference values for the HbA1c . and the tests

were considered significant when p value <0.05. ***Results:*** The median HbA1c (2.5 to 97.5 percentiles) was lower among the pregnant women; 4.8 (4-5.5) % or 32 (20-39) mmol/mol than in the non pregnant women; 5.1 (4-5.7) % or 29 (20-37) mmol/mol (p <0.001). These were 4.9 (4.1-5.5) % or 30 (21-37) mmol/mol, 4.8 (4-5.3) % or 29 (20-34) mmol/mol, and 4.8 (3.9-5.6) % or 29 (19-38) mmol/mol for the T1, T2 and T3 groups, respectively; p-values:T1 vs T2=<0.001, T1 vs T3= 0.002, T2 vs T3= 0.111, T1 vs non pregnant group = <0.001. ***Conclusions:*** Compared to normal non pregnant women, the A1c was lower in normal pregnant women in South Asian population. These A1c changes were observed despite having significantly higher body max index among women in the T2 and T3 groups than in the T1 and non pregnant groups. To understand the factors determining the A1c decrease in pregnancy and to validate the findings of this study, we recommend further prospective studies among South Asian women

Keywords*:* Asian, Gestational diabetes, HbA1c , Pregnancy trimesters, Reference values.

Advances in Knowledge

- Earlier studies stressed the need to identify ethnic- and trimester-specific HbA1c reference intervals in normal pregnant women.
- Compared to the non-pregnant state, there is significant decrease in HbA1c levels in pregnancy among South Asian pregnant women; and this decrease is obvious in early pregnancy.
- Among healthy South Asian women, the suggested upper reference limits of HbA1c in first, second and third trimesters are 37,34, and 38mmol/mol, respectively

Application to patient care

- The proposed upper trimester-specific HbA1c reference values may be used as threshold values to identify women prone for gestational diabetes (GDM) and other adverse pregnancy outcomes.
- Early identification of these high risk women will open up a window of opportunity to introduce preventive strategies.
- These HbA1c reference values can guide in designing further prospective studies among South Asian pregnant women, and can develop alternate tests to OGTT for GDM diagnosis and to establish glycemic targets in pregnancies complicated by diabetes.

Introduction

Glycated hemoglobin (A1c) is widely used as a standard biomarker for glycemic control during management of diabetes mellitus in the general population,[1] but, there is uncertainty over the role of A1c for glycemic assessment during pregnancy. The accuracy of A1c estimation in pregnancy is affected by several physiological changes in pregnancy like increase in red cell production, younger red cell age distribution, and reduced red cell life span.[2] Moreover, the high prevalence of iron deficiency and the common practice of iron supplementation in pregnant women (especially in developing countries) can influence the A1c estimation in pregnancy.[3] Despite these limitations, several prestigious organizations have recommended A1c estimation in pregnancy for various reasons. The World Health Organization advocates A1c estimation at the first ante natal visit to identify women with 'Diabetes in Pregnancy'(A1c $\geq$ 6.5%, $\geq$ 48 mmol/mol).[4]

Many authors recommend A1c as a screening and even as a diagnostic test for gestational diabetes mellitus (GDM).[2,5] In 2011, the California State Diabetes and Pregnancy Program 'Sweet Success' adopted a new algorithm for the diagnosis and treatment of hyperglycemia in pregnancy.[6] Accordingly, all women with A1c values of 5.7–6.4% (39–46 mmol/mol) in early pregnancy are advised to undergo GDM treatment without further confirmatory OGTT. The American Diabetes Association suggests periodic A1c estimations in pregnancy as a secondary measure of glycemic control after self-monitoring of glucose.[7] The National Institute for Health and Care Excellence in the United Kingdom proposes A1c in pregnancy as a useful guide for risk stratification and prediction of pregnancy outcomes.[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 first trimester A1c is recognized as a predictor of GDM later in pregnancy,[2,9] as well as of adverse pregnancy outcomes.[2,10] Second and third trimester A1c levels 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 the lack of strong research evidence in obstetric population. The A1c cut off points for diagnosis of 'Diabetes in pregnancy' ($\geq$ 6.5%, $\geq$ 48 mmol/mol) and GDM in the 'Sweet Success'program (5.7to 6.4%, 39-48 mmol/mol) are guided by the A1c values for diagnosis of diabetes and pre-diabetes in a non-obstetric population, respectively. However, A1c levels

in pregnancy are lower than in non obstetric population, and it shows physiological variations between trimesters.[12] There are significant racial and ethnic differences in glycation of hemoglobin for a level of glycemia.[13] Clearly, there is a need to define ethnic- and trimester-specific A1c reference levels in normal pregnant women, before it is recommended for GDM screening and diagnosis, risk stratification and for measuring metabolic control.

There is an ongoing Type 2 diabetes epidemic in the Middle East and the South Asian region including the obstetric population. India has 5.7 million women with hyperglycemia during pregnancy and ranks first in the world in this respect.[14] However, to our knowledge, A1c levels among normal pregnant South Asian women are not yet defined. Here, we identified, trimester-specific A1c levels in healthy non-diabetic South Asian pregnant women who delivered babies with an age-appropriate weight.

Methods

This retrospective study involved pregnant women who attended antenatal clinic at St.Stephen's hospital, a tertiary care hospital in Delhi, North India between January 2011 and December 2016. Our center follows a universal thalassemia screening strategy for pregnant women at the first antenatal visit. The protocol includes estimation of HbA, HbA2, and HbF through hemoglobin (Hb) electrophoresis,with concurrent estimates of A1c. All women with A1c $\geq$6.5% (48 mmol/mol) were diagnosed to have overt diabetes and those women having A1c <6.5 % (48 mmol/mol) were screened for GDM through a universal one-step 75 g OGTT between 24 and 28 gestational weeks or earlier if having high GDM risk factors. The GDM diagnosis was made by the International Association of Diabetes and Pregnancy Study Group (IADPSG) recommendations.[15] All pregnant women were on iron and folic acid supplementation.

Selection of the study population is shown in a flow diagram (Fig.1). As part of universal thalassemia screening, 9388 pregnant women had Hb electrophoresis (A1c estimation) at the first antenatal visit; all of them were evaluated for inclusion in this study. We excluded 8031 women due to unclear date of last menstrual period, delivery outside our hospital, diagnosis of diabetes and gestational diabetes, GDM risk factors, anaemia,[16] systemic diseases and delivery of babies with small- and large-for gestational age babies.[17] The remaining 1357 women in the study population were sub-categorized into three groups based on the gestational age of A1c estimation: (a) first trimester <14 weeks (T1), n=513 women; (b) second trimester-14-26 weeks (T2), n=550 women; and (c) third trimester 27-41 weeks (T3),

n=294 women. The body mass index (BMI) was calculated from the height and weight recorded at first antenatal visit. The serum thyroid stimulating hormone (TSH) was estimated at 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 of 67 non–pregnant healthy women were recruited from 750 women, who attended the pre-pregnancy counseling clinic of our hospital during the study period. The age and BMI of the control group was comparable to those of whole and T1 pregnancy groups respectively. (Table 1&2) All had FPG < 5.5 mmol/l (< 100 mg/dl) or random plasma glucose < 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 had no systemic disease. The A1c levels of the study and the control groups were compared. The reference intervals of A1c levels in each trimester were estimated and were compared for any differences. This research protocol was approved by St.Stephen's hospital ethics committee (No.SSHEC/R0136) with a waiver for patient consent form.

Our 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 EDTA anticoagulated blood using Beckman coulter LH 750/780 analyzer using VCS technology. We used the standard protocol for the OGTT: ingestion of 75 g 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. The glucose estimation was done by hexokinase method on a Beckman AU680/480 analyzer. Two levels of plasma glucose controls (from Bio Rad) were run daily; Level 1 - 4.53 mmol/l (81.50 mg/dl) and Level 2-15.57 mmol/l (280.2 mg/dl). The monthly coefficient of variation (CV) % calculated for the Level -1 and Level -2 controls were 1.7 % and 1.4% respectively. The blood for A1c estimation was a non fasting sample collected in EDTA vial. Estimation was done within two hours of sampling by the Ion exchange High Performance Liquid Chromatography method with a Bio-Rad D10TM machine (Bio-Rad laboratories, Hercules CA). The estimation was traceable to the reference methods of both the National Glycohemoglobin Standardization Program (NGSP) 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 A1c 5.45%, 37 mmol/mol) and high control (mean 9.95%, 86 mmol/mol) respectively. Our laboratory

participated in an External Quality Assurance Scheme (EQAS) for both glucose and A1c. The Z Score for glucose was 0.60 and 0.65 for A1c.

All study groups had the minimum of 40 subjects mandated by IFCC for identification of reference intervals.[18] The data analysis was performed using SPSS version 16 (SPSS Inc.,Chicago, USA) and R-software version 4.0.2. Continuous variables were presented with mean and standard deviation. An unpaired student's t- test was used to compare the mean between pregnant and non-pregnant women. The homogeneity of variances was checked using Leven's test. One-way analysis of variance followed by post-hoc Tukey's test were applied to compare the mean among groups T1,T2&T3. Five normality statistical tests were used to get the normal reference value of HbA1c namely: Anderson-Darling, Cramer-von Mises, Kolmogorov-Smirnov, Shapiro-Francia, and Pearson chi-square. The mean $\pm$ two standard deviations were reported as reference values when the normality condition was fulfilled or else a non-parametric method and median with percentiles (2.5^{th} and 97.5^{th}) were reported as the normal range. The 95% confidence intervals of these percentiles were determined with bootstrapping with 10000 replications using the *boot* package of r-software. The Mann-Whitney U-test was applied to compare the distribution of A1c between pregnant and non-pregnant women and between the trimesters and a p-value < 0.008 was considered significant as per Bonferroni correction (0.05/number of comparisons). A p-value < 0.05 was considered as significant for other statistical tests.

Results

Table 1 shows reference intervals for A1c for the control group, study population, and T1, T2, and T3 trimester groups expressed as median and percentiles. All five statistical tests to assess normality of A1c values showed violation of normality. The median A1c value of 4.8 % (29mmol/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) in the control group (p <0.001 for both). The A1c 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), T1 and T3 (p=0.001) and no difference between T2 and T3 (p= 0.111).

Fig. 2 presents the upper normal A1c level for the control,T1,T2 and T3 groups:5.7%(39 mmol/mol),5.5% (37mmol/mol),5.3% (34mmol/mol),and 5.6% (38 mmol/mol), respectively.Table 2 shows the clinical and laboratory parameters of the whole, trimester-specific pregnancy groups, and the control group. The women in all trimesters were age-

matched, and the gestational age at delivery and the birth weight were comparable (p-values > 0.05 for all parameters). The Hb and RBC count were lower, and the MCV, MCH, and MCHC were higher in pregnant women than in the control group; there was no difference of HCT and RDW between these groups. Compared to the T1 group, there was a decrease in Hb and RBC count and an increase in MCV, MCH and MCHC in T2 group; the RDW and HCT remained static between groups. There was a significant rise of hemoglobin and RDW in T3 versus T2; HCT, MCV, and MCHC were constant.

Discussion

The A1c is lower during pregnancy than when not pregnant in South Asian women.The A1c reference values for the first, second, and third trimesters were 4.1- 5.5% (21- 37 mmol/mol), 4-5.3% (20-34 mmol/mol), and 3.9-5.6% (19-38 mmol/mol), respectively. Earlier studies revealed some racial differences in A1c reference intervals: (a) Caucasian women in Italy, 3.5-5.7% (15 - 39mmol/mol), 3.3-5.6 %(14-38 mmol/l), and 4.3-5.6 %(23-38 mmol/l) in 15-24, 25-27, and 28-36 gestational weeks, respectively;[19] (b) Mexican women, T1 4.5-5.6% (26-38mmol/mol), T24.4-5.5% (26-37 mmol/mol), and T34.4-5.6% (25-38 mmol/mol);[20] (c) Japanese women, T1 4.7-5.7% (28-39 mmol/mol), T2 4.4-5.4% (25-36mmol/mol), T3 4.6-5.8% (27-40mmol/mol).[21] Compared to these studies, the upper A1c reference values of our South Asian cohort were marginally lower. The stringent selection criteria (exclusion of women having, GDM diagnosed by 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 hemoglobin might have contributed to this modest A1c difference.

Compared to first trimester, a significant decrease in A1c level was noted in the second trimester, but this remained constant in the third trimester [Table 1]. The differences in A1c levels between trimesters varied markedly between studies. In most populations, there was a decrease in A1c level from the first to second trimester[20-24] and this decrease was often followed by a significant A1c rise in the third trimester (biphasic response).[19,20-24]The A1c rise in the third trimester was not seen in some studies,[25,26] but a decrease was reported in one study.[12] In a Japanese study, Hashimoto et al reported that the A1c rise in late pregnancy is mainly due to iron deficiencies in the third trimester.[27] Significant racial differences in trimester-related A1c variations were reported in a multiethnic population in the United Kingdom by Hartland et al; both Caucasians and Asians had a lower A1c in the second

trimester than in the first trimester, but theA1c rise in the third trimester was observed only in Causasian women (not in Asians, as in our study).[22]

The metabolic changes leading to the significant decline in A1c levels in mid-pregnancy was apparent in a longitudinal study by Mills et al.[28] This study demonstrated a significant drop in plasma glucose values between 6 and 10 weeks of gestation which was followed by a decrease in A1c levels in second trimester. The authors speculated that the maternal metabolic and hormonal factors alter the plasma glucose concentration early in pregnancy, independently of foetal glucose utilisation. Another proposed mechanism for lowering plasma glucose in 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 in pregnancy like high erythrocyte turnover and hemodilution. Subsequent compensatory mechanisms like maternal plasma reduction and increased atrial natriuretic peptide, can again raise Hb in the third trimester. [29] The high prevalence of iron deficiency anemia and the common practice of universal iron supplementation in pregnancy especially in developing countries, can modify A1c levels.[3] We excluded women with anemia and thalassemia and the changes in Hb, MCV, MCH, MCHC,and RBC over trimesters is attributable to the physiological changes in pregnancy and to iron supplementation.[30] (Table 2)

The proposed upper reference HbA1c levels in early pregnancy in this study, can be clinically relevant in the early identification of women prone for GDM and adverse pregnancy outcomes. This approach can open up a window of opportunity for early initiation of GDM preventive strategies. Strikingly, the suggested upper reference values (5.5 % and 5.3 % in first and second trimesters respectively) are lower than the generally recommended threshold A1c value of 5.7 % (39 mmol/mol) for diagnosis of 'pre-diabetes in pregnancy'.[2] In an earlier study, we observed that the first trimester A1c $\geq$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 A1c estimation between 24 and 28 weeks of gestation for GDM diagnosis in 607 Asian Indian pregnant women.[7] In that study, the A1c of 5.25% (34mmol/mol) was a reliable cut off value for identification of GDM women when IADPSG criteria was applied for GDM diagnosis. The A1c threshold values identified for GDM diagnosis in first and second trimesters in these studies agree well with the corresponding upper reference values of our study. Further, Maine et al also assessed the relationship of A1c level in the first trimester with adverse pregnancy outcomes among a cohort of multiethnic

pregnant women residing in Spain.[17]

The risk for eclampsia, LGA and macrosomia increased at A1c threshold values of 5.3 %, 5.4 % and 5.7 % (34,36 and 39 mmol/mol) respectively for the South Asian pregnant women in this cohort. These cut off values are near (though not exact) to the first trimester A1c upper reference value of 5.5% (37mmol/mol) in our study. The studies above suggest that the risks for GDM and other adverse pregnancy events start in A1c levels lower than the 'prediabetic' level of 5.7 % (39 mmols/mol).We recommend further prospective studies to validate the proposed trimester specific A1c reference levels for prediction and identification of various adverse events among South Asian pregnant women.

Our study has several limitations. The A1c reference values of this study are derived from a cross-sectional analysis of different women who attended our antenatal clinic over three trimesters. A longitudinal study on the sequential changes of HbA1c levels of a cohort of same women over different trimesters would have been ideal. The impact of this limitation is alleviated significantly in this study: Age, gravidity, family history of DM, history of GDM and abortion, gestational age at delivery, birthweight, Hb, HbA2 and HbF of women in different trimesters and the BMI between control and T1 groups were comparable (Table 3). The BMI rise in T2 and T3 groups are due to physiological gestational weight gain.The lack of data on iron, folate and B12 status of women in different trimesters is a limitation, but the RBC indices of these women do not suggest any major deficiencies of these factors. The strengths of this study include the large study population, with identification and exclusion of GDM by universal OGTT based screening as per IADPSG guidelines. All women with GDM risk factors,anaemia and thalassemia (the common hemoglobinopathy of the region) were excluded in this study. Being a single center hospital based study, the blood samples were sampled and processed under optimal conditions in one laboratory.

Conclusion

The trimester specific hemoglobin A1c levels are not yet defined for healthy South Asian pregnant women. This study evaluated the upper reference limits for first, second and third trimesters as 37, 34, and 38 mmol/mol, respectively. These trimester-specific A1c values can be of clinical relevance for prediction and diagnosis of GDM and for risk stratification of other adverse events among South Asian pregnant women. Further prospective studies to

validate the proposed A1c reference intervals are recommended.

Conflicts of interest

The authors declare that they have no conflict of interest

Funding

The present study is an unfunded project.

Acknowledgements

We gratefully acknowledge the immense help received from Sheeba Samuel and Sapna Robinson (diabetes nurses) as well as Aashish Kumar, Nikita Tyali, and Deepali Garg (diabetes educators) at the Dept of Endocrinology, St. Stephen's hospital for the collection of data for this study

Author contributions

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

References

1. American Diabetes Association. Glycemic Targets: Standards of Medical Care in Diabetes—2021 Diabetes Care 2021; 44 (Supplement 1): S73-S84. https://doi.org/10.2337/dc21-S006
2. Hughes RCE, Rowan J, Florkowski CM. Is there a role for HbA1c in pregnancy? Curr Diab Rep 2016; 6: 5-14. DOI 10.1007/s11892-015-0698-y.
3. FOGSI General Clinical Practice Recommendations. Management of Iron deficiency anemia in pregnancy available at The Federation of Obstetric & Gynecological Societies of India. From: www.fogsi.org/wp-content/uploads/2017/07/gcpr-recommendation-ida.pdf. Accessed; Nov 2021.
4. World Health Organization. Diagnostic Criteria and Classification of Hyperglycaemia First Detected in Pregnancy 2013.

From:http://apps.who.int/iris/bitstream/10665/85975/1/WHO_NMH_MND_13.2_eng.pdf. Accessed; Nov 2021.

5. Rajput R, Yogesh Y, Rajput M, Nanda S. Utility of HbA1c for diagnosis of gestational diabetes mellitus. Diabetes Res Clin Pract. 2012; 98:104-7.
6. California State Diabetes and Pregnancy Program sweet Success. From: http://perinatology.com/Reference/CDAPP%20SS%20Guidelines%202012.pdf. Accessed; Nov 2021.
7. American Diabetes Association. Management of Diabetes in Pregnancy: Standards of Medical Care in Diabetes—2021 Diabetes Care 2021; 44 (Supplement 1): S200-S210. https://doi.org/10.2337/dc21-S014
8. NICE guideline. National Institute for Health and Care Excellence (2015), Diabetes in pregnancy: management Diabetes in pregnancy: management from preconception to the postnatal from preconception to the postnatal period. From: https://www.nice.org.uk/guidance/ng3. Accessed; Nov 2021.
9. Punnose J, Malhotra RK, Sukhija K, Mathew A, Sharma A, Choudhary N. Glycated haemoglobin in the first trimester: A predictor of gestational diabetes mellitus in pregnant Asian Indian women. Diabetes Res Clin Pract. 2020 ;159: Article107953 . doi:10.1016/j.diabres.2019.107953.
10. Hughes RC, Moore MP, Gullam JE, Joanna EG, Muhammed K, Rowan J. An early pregnancy HbA1c >5.9% (41 mmol/mol) is optimal for detecting diabetes and identifies women at increased risk of adverse pregnancy outcomes. Diabetes Care. 2014; 37(11):2953–9.
11. Mañé L, Flores-Le Roux JA, Gómez N, Chillarón JJ, Llauradó G, Gortazar L, et al. Association of first-trimester HbA1c levels with adverse pregnancy outcomes in different ethnic groups Diabetes Res Clin Pract. 2019;150:202-210. doi: 10.1016/j.diabres.2019.03.017.Epub 2019 Mar 15.
12. Nielsen LR, Ekbom P, Damm P, Glumer C, Frandsen MM, Jensen DM, et al. HbA1clevels are significantly lower in early and late pregnancy. Diabetes Care 2004; 5:1200–1. doi: 10.2337/diacare.27.5.1200
13. Venkataraman K, Kao SL, Thai AC, Salim A, Lee JJM, Heng D et al. Ethnicity modifies the relation between fasting plasma glucose and HbA1c in Indians, Malays and Chinese. Diabet Med. 2012; 29 (7):911-917.

14. Guariguata L, Linnenkamp U, Beagley J, Whiting DR, Cho NH. Global estimates of the prevalence of hyperglycemia in pregnancy. Diabetes Res Clin Pract 2014;103:176–185.
15. Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, Damm P ,et al. International Association of Diabetes and Pregnancy Study Group. Consensus.International Association of Diabetes and Pregnancy Study Groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care 2010; 33:676–682.
16. World Health Organization, Vitamin and Mineral Nutrition Information System, WHO, Geneva, 2011 (WHO/NMH/NHD/MNM/11.1). From: https://www.who.int/vmnis/indicators/haemoglobin/en/ Accessed; Sept 2020.
17. National Neonatology Forum. National Neonatal-Perinatal Database (2002-03). From: www.newbornwhocc.org/pdf/nnpd_report_2002-03.PDF. Accessed; Nov 2021
18. Solberg HE. The IFCC recommendation on estimation of reference intervals. The Ref Val program. Clin Chem Lab Med.2004; 42 (7):710–4.
19. Mosca A, Paleari R, Dalfrà MG, Di Cianni G, Cuccuru I, Pellegrini G, et al. Reference intervals for hemoglobin Alc in pregnant women: data from an Italian multicenter study. Clin Chem. 2006; 52(6):1138–43.
20. Sánchez- González CM, Castillo-Mora A, Alvarado-Maldonado IN, Ortega-González C, Martínez Cruz N, Arce –Sánchez L et al . Reference intervals for hemoglobin A1c (HbA1c) in healthy Mexican pregnant women: a cross-sectional study. BMC Pregnancy and Childbirth. 2018; 18:424-429. doi: 10.1186/s12884-018-2057-x
21. Hiramatsu Y, Shimizu I, Omori Y, Nakabayashi M. Determination of reference intervals of glycated albumin and hemoglobin A1c in healthy pregnant Japanese women and analysis of their time courses and influencing factors during pregnancy. Endocr J. 2012;59 (2):145–51.
22. Hartland AJ, Smith JM, Clark PMS, Webber J, Chowdhury T, Dunne F. Establishing trimester- and ethnic group-related reference ranges for fructosamine and HbA1c in non-diabetic pregnant women. Ann Clin Biochem 1999; 36:235–7.
23. Phelps RL, Honig GR, Green D, Metzger BE, Frederiksen MC, Freinkel N. Biphasic changes in haemoglobin A1c concentrations during normal human pregnancy. Am J Obstet Gynecol 1983;147: 651-653.

24. Worth R, Potter JM, Drury J, Fraser RB, Cullen DR. Glycosylated haemoglobin in normal pregnancy: a longitudinal study with two independent methods. Diabetologia 1985; 28:76.
25. O'Kane MJ, Lynch PLM, Moles KW, Magee SE. Determination of a diabetes control and complications trial-aligned Hb A1c reference range in pregnancy. Clin Chim Acta 2001; 311:157–9.
26. Parentoni LS, de Faria EC, Bartelega MJLF, Moda VMS, Facin ACC, Castilho LN. Glycated haemoglobin reference limits obtained by high performance liquid chromatography in adults and pregnant women. Clin Chim Acta 1998; 274:105–9.
27. Hashimoto K , Noguchi S, Morimoto Y, Hamada S, Wasada K, Imai S, et al. A1C but not serum glycated albumin is elevated in late pregnancy owing to iron deficiency Diabetes Care 2008; 31(10):1945-8. doi: 10.2337/dc08-0352. Epub 2008 Jul 3.
28. Mills JL, Jovanovic L, Knopp R, Aarons J, Conley M, Park E , Lee YJ, et al : Physiological reduction in fasting plasma glucose concentration in the first trimester of normal pregnancy: The Diabetes in Early Pregnancy Study. Metabolism 1998; 47(9):1140–1144. doi: 10.1016/s0026-0495(98)90290-6
29. Lurie S, Mamet Y. Red blood cell survival and kinetics during pregnancy. Eur J Obstet Gynecol Reprod Biol.2000; 93:185–192.
30. Milman N, Byg KE, Agger AO. Hemoglobin and erythrocyte indices during normal pregnancy and postpartum in 206 women with and without iron supplementation. Acta Obstet Gynecol Scand. 2000; 79(2):89-98. doi: 10.1034/j.1600-0412.2000.079002089.x.PMID:10696955.

Table 1: The median and percentiles of HbA1c in non pregnant women, whole study population, and in first (T1), second (T2) and third (T3) trimester groups.

Study Group	**N**		**HbA1c %**				
		Median (0.95 CI)	**Percentile (0.95 CI)[a]**		**Range (min-max)**	**P value**	**Type of distribution**
			2.5th (0.95 CI)	**97.5th (0.95CI)**			
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[b]	Non-Gaussian(5)[f]
Pregnant (whole group)	1357	4.8 (4.8-4.8)	4.0 (3.9-4.1)	5.5(5.4-5.5)	3.2-5.9	<0.001[b]	Non-Gaussian(5)

T1 0-13weeks	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[c]	Non-Gaussian(5)
T2 14-26 weeks	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[d]	Non-Gaussian(5)
T3 27-41 weeks	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[d] 0.111[e]	Non-Gaussian(5)

n= number of women, [a] CI: Confidence Intervals , [b] P value for comparison of non-pregnant with pregnant groups, [c] P value for comparison with non pregnant group , [d] P value for comparison with T1 group , [e] P value for comparison of T2 and T3 groups, [f] Values in parentheses indicate number of tests for goodness of fit with $p<0.05$

Table 2: Comparison of clinical and Laboratory parameters in mean ± standard deviation. (A) Whole study population versus control group (B) Between First (T1), Second (T2) and Third (T3) trimesters.

Parameter	**A Whole study population**			**B Women in different Trimesters**					
	women all trimesters n=1357	**Non pregnant control group n= 67**	**P value**	**0-13 weeks n = 513 (T1)**	**14-26 weeks n = 550 (T2)**	**27-41 weeks n= 294 (T3)**	**P value T1 vs T2**	**P value T1 vs T3**	**P value T2 vs T3**
Age (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 (weeks)	--	--	--	9.53± 2.47	18.81±3.62	31.23 ± 3.02	-	--	--
Hemoglobin-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- 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 - 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- %	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- 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- %	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 (weeks)	--	--	--	38.53 ± 1.02	38.56 ± 1.02	38.53±1.00	0.921	0.999	0.925
Birth Weight- kg	--	--	--	2.89±0.29	2.87 ±0.36	2.85±0.28	0.562	0.199	0.660

For (A) Unpaired students t-test was applied to compare the mean value between the groups and for (B) One-way analysis of variance followed by post-hoc Turkey's test. GA = Gestational age, MCH = Mean corpuscular hemoglobin, MCV = Mean corpuscular volume, MCHC = Mean corpuscular hemoglobin concentration, RDW = RBC diameter width. n= number of women.

Figure 1

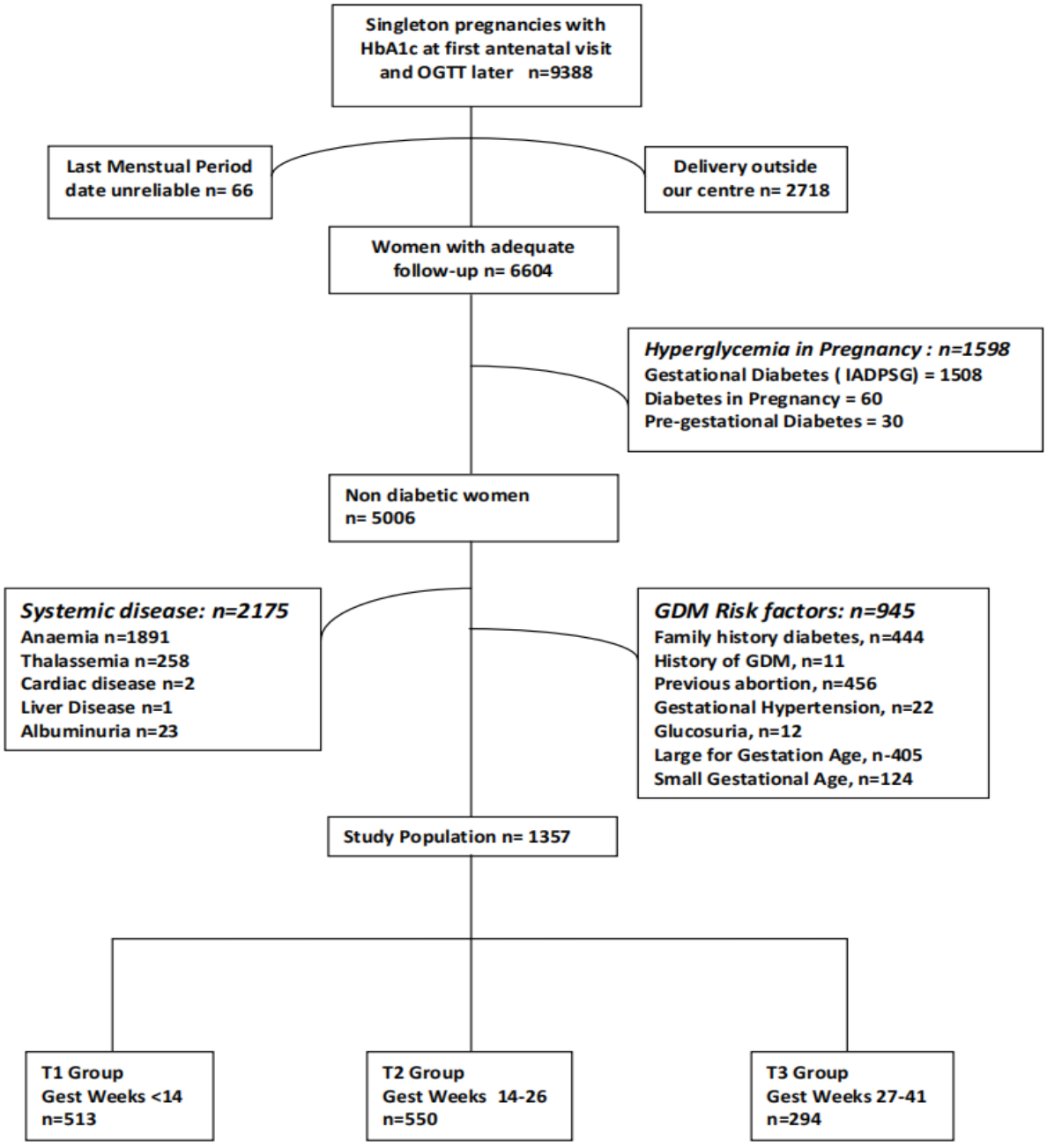

Figure 1: Flow diagram on selection of study population. OGTT= Oral Glucose Tolerance Test, GDM= Gestational Diabetes mellitus; having fasting plasma glucose (PG) between 5.1-6.9 mmols/L, 1-hour PG > 10 mmol/L , 2-hour PG between 8.5 – 11.1 mmol/L in OGTT , IADPSG = International Association of Diabetes and Pregnancy Study Group, Pre-gestational diabetes = Diabetes diagnosed before pregnancy , Diabetes in pregnancy = Overt diabetes first diagnosed in pregnancy; HbA1c > 48 mmol/mol or FPG > 7 mmol/L or 2-h PG > 11.1 mmol/L

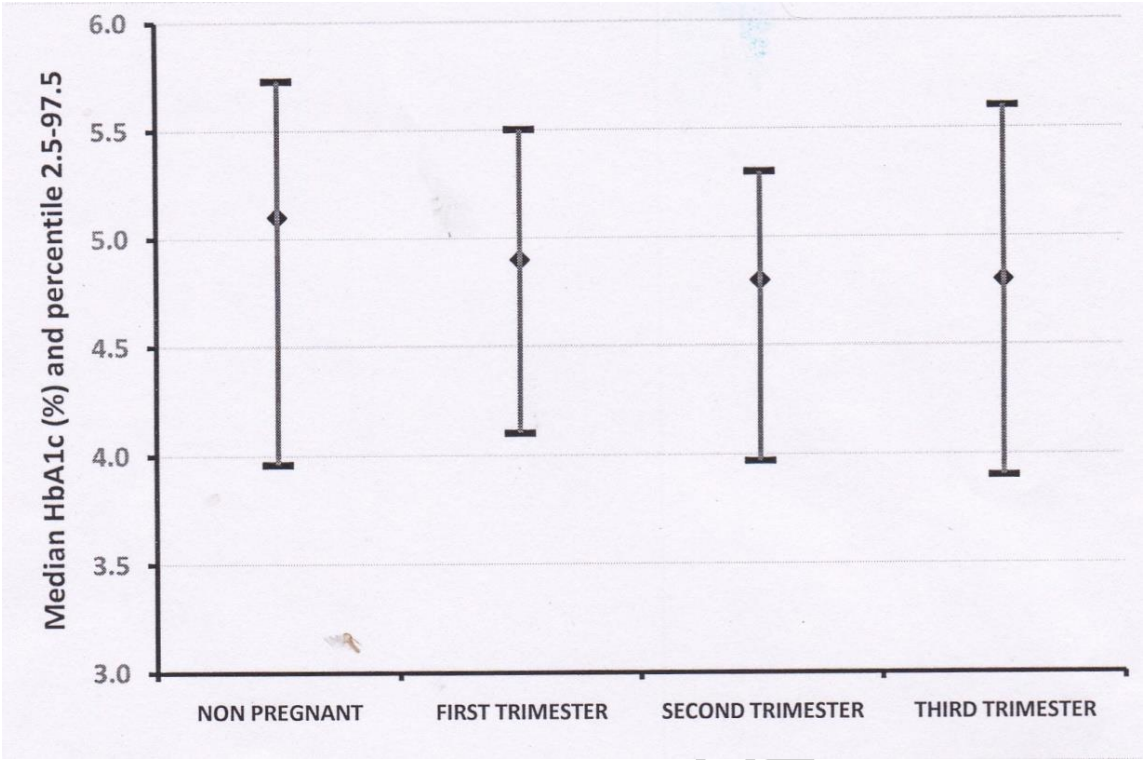

Figure 2: Median and percentile (2.5 to 97.5) for hemoglobin A1c (%) for women in non-pregnant and different trimesters.