

To determine the reference intervals of A1C in healthy pregnant women from urban area of Karachi, Pakistan

Tayyaba Ashiq, Erum Khalid, Iram Manzoor, Sana Mubarik, Muhammad Arif Memon

Hamdard Medical and Dental College, Karachi Pakistan

ABSTRACT

Objective: To determine the reference interval of A1C in healthy pregnant women from urban areas of Karachi, Pakistan.

Material and Methods: This cross-sectional study was conducted at Hamdard university hospital, Taj medical complex, from 1st July to 31st December 2024. The study was a cross-sectional study involving healthy pregnant women (18-45 years) with normal 75g oral glucose tolerance test (OGTT). Women with diabetes, hypertension, hemoglobin <10 g/dl, comorbidities, and infections were excluded. On the basis of informed consent, demographic and clinical data were obtained. A1C was measured in venous samples by Turbidimetric inhibition immunoassay (Roche® c-311) with close calibration and exclusion of compromised samples to assure accuracy.

Results: Mean HbA1c was highest in the second trimester ($4.14 \pm 0.79\%$) with a slight rise in the third ($4.33 \pm 0.78\%$), reflecting expected physiological trends. Reference ranges were 2.7–5.7% (second trimester) and 2.8–5.5% (third), showing minor variation from global data. These findings highlight gestational variation in HbA1c and support the use of locally defined trimester-specific ranges for accurate interpretation in South Asian populations.

Conclusions: Trimester wise HbA1c reference values in healthy pregnant women are more effective to measure glycaemic control compared with generic non-pregnant reference ranges. An HbA1c of >5.6% can suggest a higher risk of gestational diabetes mellitus and should be followed by additional testing which is the gold standard oral glucose tolerance test (OGTT).

Keywords: Anemia, Gestational diabetes, Iron-deficiency, Pregnant women

This article can be cited as: Ashiq T, Khalid E, Mansoor I, Mubarik S, Memon MA. To determine the reference intervals of A1C in healthy pregnant women from urban area of Karachi, Pakistan. Pak J Pathol. 2026; 37(1): 25-29. DOI: <https://doi.org/10.55629/pakipathol.v37i1.961>

INTRODUCTION

Gestational diabetes mellitus (GDM) is a major issue worldwide with the numbers of cases of hyperglycemia in pregnancy showing that 23.0 million (19.7%) cases of live births occurred in 2024 (International Diabetes Federation, 2024) [1]. GDM occurs when the increased placental hormones cause resistance to insulin to feed the fetus, and with poor compensation by the β -cell, the mother experiences hyperglycemia to the fetus [2]. Even the slight hyperglycemia predisposes maternal-fetal risks and demands closer monitoring and perhaps further interventions in the course of pregnancy and childbirth [3].

The 75-g oral glucose tolerance test (OGTT) is currently the gold standard test for the diagnosis of GDM with lower thresholds than that used in non-pregnant women [4]. To monitor continuously, postprandial glucose and fasting plasma glucose are

frequently used. Fasting samples and frequent blood draws are however burdensome to pregnant women thus resulting in low compliance [5]. Hemoglobin A1C (HbA1c) is a retrospective measure of cumulative exposure to plasma glucose over the past few weeks, which is the duration of the life of red blood cells in the circulation. It is also convenient to patients because it does not necessitate fasting and it provides better pre-analytical stability than glucose-based tests [6]. HbA1c has been well known to be used in the long-term glycemic control in non-pregnant adults, but its use in pregnancy remains less definite. Since the second trimester, physiological alterations like decreased red blood cell life and the overwhelming prevalence of anemia could decrease HbA1c levels and conceal the actual level of maternal glycemia [7]. Consequently, the use of generic A1C cut-offs during pregnancy is inadvisable and trimester-specific reference ranges are a more accurate way of determining glycemic status [8].

Moreover, ethnic diversity is present in A1C patterns, and South Asians might not conform to patterns observed in other populations [9]. During pregnancy, hyperglycemia can be better detected using trimester-specific HbA1c reference intervals [10].

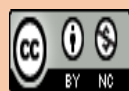
Correspondence: Dr. Tayyaba Ashiq, Assistant Professor, Department of Pathology, Hamdard Medical and Dental College, Karachi Pakistan

Email: tayyaba.a@hotmail.com

Receiving Date: 22 Oct 2025

Revision Date: 09 Jan 2026

Acceptance Date: 17 Mar 2026



Copyright © 2025. Tayyaba Ashiq, et al. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License, which permits unrestricted use, distribution & reproduction in any medium provided that original work is cited properly.

Thus, this study will identify the reference limits and cutoffs of A1C in healthy pregnant women in an urban area of Karachi, Pakistan. The development of locally specific ranges will enhance the accuracy of glycemic control during pregnancy and eliminate the reliance on the inconvenient OGTT and offer a more patient-friendly method of antenatal care.

MATERIAL AND METHODS

This cross-sectional study was conducted at Hamdard University Hospital, Taj Medical Complex, from 1st July to 31st December 2024, over a period of 6 months, following approval by the institutional ethical review (Ethical committee approval number: HCM&D 686/2024). After a thorough literature search, the sample size was calculated to be 285 using the WHO calculator, keeping the margin of error at 5.25% a confidence level at 95%, power of test 80% and reference population 28% [11]. As IFCC recommends the sample size of 120 for each partition, for a reference value study for non parametric data. Thus, using non-probability consecutive sampling technique, enrolled 122 cases of second trimester & 163 cases of third trimester in this study. Informed written consent was taken from the participants. Current study focused on 2nd and 3rd trimester only as in 1st trimester the hemodynamic and metabolic changes are not prominent.

The study included all healthy pregnant women who had fasting plasma glucose levels less than 92mg/dL and who were pregnant with a single intrauterine pregnancy confirmed by ultrasound. Included cases were followed till delivery to record their baby birth weight (outcome).

Pregnant women with multiple pregnancies (twins or higher order pregnancies), known type 1 or type 2 diabetes mellitus, prior gestational diabetes mellitus, hypertension or preeclampsia, major comorbidities including liver or kidney disease, autoimmune or inflammatory diseases including rheumatoid arthritis and systemic lupus erythematosus, anemia with hemoglobin less than 10 g/dL, recent bleeding or blood transfusion, and those with active infections including hepatitis B or C, and lastly mothers of babies with birth weight of 4kgs or more were also excluded from the study.

Demographic data, medical and obstetric history, and physical examination were noted at enrollment. Gestational age was estimated by last menstrual period or first-trimester ultrasound. To avoid diabetes mellitus and gestational diabetes, all participants underwent an oral glucose tolerance test (OGTT) of 75 g after fasting overnight (8 to 12 hours). The venous

blood was taken at fasting, one hour and two hours following administration of glucose and findings were interpreted. Sysmex XP was used to measure hemoglobin levels. Hepatitis B, hepatitis C and HIV infection screening were confirmed in the medical records or carried out in case of the absence of documentation.

To perform A1C analysis, 3 mL of venous blood were taken into a K-EDTA vacuum tube. Tubes were carefully inverted to mix and placed in 2-8°C until processed. All the samples were analyzed at the limits recommended by the manufacturer. Samples that presented clotting, hemolysis, or that were insufficient in volume were discarded. Turbidimetric inhibitory immunoassay (TINIA) A1C measurement was performed on the Roche® c-311 auto-analyzer as per the manufacturer instructions. Calibration was done at lot change, and as per the kit protocol. Any samples identified by the analyzer as having the risk of interference were not analyzed.

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 22:00 and MS Excel 2016 software. Normality of quantitative variables data was checked by using Kolmogorov-Smirnov test. Age, birth weight, HbA1c and Hemoglobin were non-normally distributed and represented by median (IQR). Qualitative variable (Trimester) was presented as frequency and percentages. HbA1c data was arranged in ascending order. Outliers were removed using Dixon reed method. The values at 2.5th and 97th were taken as lower and upper reference values respectively. The 90% confidence limits were determined manually. The Mann Whitney U test was applied to check the statistical different among the study groups. The p value ≤ 0.05 was considered as significant.

RESULTS

Out of the total 285 pregnant women, 122 (42.8%) were in their second trimester and 163 (57.2%) were in the third trimester. The median (IQR) birth weight was 2.80 (3.00 – 2.50) kg, ranging from 2.00 to 3.80 kg. Median age of study population was 25.80 (27.30 – 24.00) years in the second trimester and 26.00 (27.20 – 24.70) years in the third trimester. However, median of hemoglobin level of second and third trimester was 11.00 (12.00 – 10.00) (g/dl) and 12.00 (13.00 – 11.00) (g/dl) respectively. Furthermore, mean of HbA1C in second trimester was 4.15 (4.80 – 3.60) % and third trimester was 4.50 (4.90 – 3.70) % respectively. The HbA1c levels, hemoglobin levels, and age of the study subjects in both trimesters were shown in Table-I.

The median HbA1c values were a little higher in the third trimester [4.50] compared to the second trimester [4.15]. However, there was found statistically significant difference as $p = 0.030$. The lower limit of second trimester was 2.7 with 90% confidence interval (2.4-2.8) and the upper limit was 5.7 with 90% confidence intervals (5.2 – 5.8). Similar, the lower limit

of third trimester was 2.8 with 90% confidence interval (2.8-2.9) and the upper limit was 5.5 with 90% confidence intervals (5.2 – 5.8). However, the reference intervals for Glycosylated haemoglobin (HbA1c) were in second and third trimester were 2.7 – 5.7 and 2.8 – 5.5 respectively shown in Table-II.

Table-I: Demographic and clinical Parameters of Study Participants (n=285)

Parameters	Second Trimester Median (IQR) (n=122)	Third Trimester Median (IQR) (n=163)
Age (years)	25.80 (27.30 – 24.00)	26.00 (27.20 – 24.70)
HbA1c (%)	4.15 (4.80 – 3.60)	4.50 (4.90 – 3.70)
Hemoglobin(g/dl)	11.00 (12.00 – 10.00)	12.00 (13.00 – 11.00)

Table-II: HbA1c Values in second third trimester (n=285)

Trimester	Lower Limit (90% CI)	Upper Limit (90% CI)	Reference Range	Median (IQR)	p-value
Second	2.7 (2.4-2.8)	5.7 (5.2-5.8)	2.7 – 5.7	4.15 (4.80 – 3.60)	0.030
Third	2.8 (2.8-2.9)	5.5(5.3-5.8)	2.8 – 5.5	4.50 (4.90 – 3.70)	

Table III: Comparison of Trimester-specific HbA1c Reference Intervals with Literature

Study / Population	1st Trimester (%)	2nd Trimester (%)	3rd Trimester (%)
Present Study (Karachi, Pakistan)	– (not studied)	2.7 – 5.7	2.8 – 5.5
Khan <i>et al.</i> , 2023 (Quetta, Pakistan)	3.8 – 5.2	4.1 – 5.4	4.2 – 5.7
O'Connor <i>et al.</i> , 2012 (Ireland)	4.3 – 5.4	4.4 – 5.4	4.7 – 5.7
Deng <i>et al.</i> , 2023 (China)	4.0–5.5%	3.9 – 5.3	4.1 – 5.7
Hiramatsu <i>et al.</i> , 2012 (Japan)	4.7 – 5.7	4.4 – 5.4	4.6 – 5.8
Sánchez-González <i>et al.</i> , 2018 (Mexico)	4.5 – 5.6	4.4 – 5.5	4.5 – 5.6

DISCUSSION

The current research determined trimester-specific reference intervals (RIs) of HbA1c in healthy pregnant women in Karachi. The values of mean HbA1c were low in absolute terms, and a small increase was noted between the second and the third trimester (4.14% to 4.33%). Though this difference was near significance, the fact that the confidence interval overlapped indicates only a small physiological difference between gestation. The derived RIs were 2.7-5.7% in the second trimester and 2.8-5.5% in the third trimester with a narrow and clinically significant range. It was found that the use of generic, non-pregnant cut-offs of HbA1c may potentially mischaracterize glycemic status during pregnancy. The mean values in this cohort are low and this highlights why trimester specific interpretation is important. This finding is consistent with the literature that has noted a mid-pregnancy nadir in HbA1c with a slight increase to term. In an Irish study, RIs were found to be 4.354.4% (T1), 4.457.4% (T2), and 4.757.9% (T3), with a definite reduction during the early to mid-gestation stage and an increase during late pregnancy [12]. Other large cohort study in China have also reported upper limits in the range of 5.3-5.7% and show a steady pattern of lower levels in the second trimester and higher levels in the third trimester [13], Hiramatsu *et al.*, 2012 reported the reduction of HbA1c in the second

trimester, which indicates the population disparity in glycemic patterns during pregnancy [14]. Similarly, in a comparison of pregnant ($n = 445$) and non-pregnant women ($n = 384$), a study found the upper reference limits to be consistently lower in the case of pregnant women. Their trimester-specific ranges were 3.8-5.5% at 15-24 weeks, 4.0-5.5% at 25-27 weeks, 4.4-5.5% at 28-36 weeks, and thus, it was confirmed that the physiological condition of pregnancy is linked with low HbA1c levels compared to non-pregnant populations [15]. All these studies together support the finding of this study, that reference intervals at gestational age are needed to prevent misclassification of maternal glycemia. However, other studies have also reported different patterns to current findings. Gonzales *et al.*, 2018 performed a cross-sectional study on disease-free pregnant Mexican women and reported upper reference limits of HbA1c as 5.6% in the first trimester and 5.5% in the second trimester that were greater compared to the values in our study [16]. An earlier Pakistani study had recorded upper reference ranges of 5.2, 5.4 and 5.7 percent in the first, second and third trimesters respectively and showed an increasing trend in HbA1c with increasing gestation [17]. Similar findings were also described by Versantvoort *et al.*, 2013 where the first to third trimester level of HbA1c was significantly elevated [18]. All these findings point towards the development of gestational age-specific

cut-offs and strengthen the clinical utility of the pregnancy-adjusted HbA1c intervals in assessing maternal glycemia. These disparities indicate that the difference, which is observed among cohorts, may be explained by population-specific, ethnic, and methodological factors.

These findings suggest that an increase of HbA1c levels beyond the locally determined values (5.7% in the second trimester and 5.5% in the third trimester) can be considered abnormal and can be used as diagnostic criteria of gestational diabetes mellitus (GDM). It is in line with previous meta-analyses and cohort studies, which revealed that HbA1c levels of 5.5-5.9% are a good diagnostic measure of GDM [19, 20].

A number of studies have shown that the HbA1c levels of South Asian populations are higher than those of White Europeans with the same glycemic level suggesting that international reference ranges cannot be directly applied to this population. Current study RIs of the Karachi-specific variety overcome this limitation by offering population-specific standards and thus reducing the chances of misclassification by ethnic variability.²¹ New Zealand study also found that Asians had higher HbA1C compared to Europeans despite the fact that the glucose levels had been adjusted in cross-sectional study [22].

The HbA1c reference ranges (2.7-5.7% in the second trimester and 2.8-5.5% in the third trimester) were below the non-pregnancy-specific ranges by a consistent margin and this confirms that pregnancy needs its own cutoffs. Current studies values were close to both Chinese and Japanese studies (upper limits 5.3-5.6%) [13, 14]. Mexican cohort upper limits were nearly the same (5.5%) [16], with a recent Pakistani study in Quetta indicating a little higher third trimester (5.7%) [17]. These variations of 0.2-2.4% indicate that there is both population-specific variation and the need to come up with locally derived reference intervals. The physiological changes of pregnancy like shortened red cell lifespan, hemodilution, and anemia lead to lower HbA1c cutoffs than in non-pregnant adults.

Present study analysis reveals that HbA1c among Karachi women decreases in the middle of pregnancy with a small increase thereafter, though the upper reference limits are a bit different to other populations. This demonstrates the need to use locally-derived trimester-specific RIs to avoid misclassification and maximize accuracy in South Asian women.

LIMITATION

The population used in the study was relatively small and single center which might have contributed to variation in nutritional status since women of different socioeconomic statuses were included in the study. Since malnutrition rates are high in Karachi, anemia could not be completely ruled out and might have affected the levels of HbA1c even after a screening. Moreover, hematologic indices like iron status were not put under control and the study did not examine the direct correlation between the HbA1c levels and pregnancy outcomes thus limiting the scope of clinical interpretation and generalizability.

CONCLUSION

This study established trimester-specific HbA1c reference ranges among healthy pregnant women in Karachi and established lower values than non-pregnant cut-offs and a second-trimester peak, thereby confirming the need to develop locally based standards to improve glycemic testing and maternal-fetal care. The A1c values above the determined cutoffs in the respective trimester should be taken as GDM and managed accordingly.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHORS CONTRIBUTION

Tayyaba Ashiq: Substantial contributions to study design, acquisition of data, manuscript drafting, final approval, accountable for all aspects of publication.

Erum Khalid: Substantial contribution to acquisition of data, final approval, accountable for all aspects of publication.

Iram Mansoor: Substantial contribution to acquisition of data, interpretation of data, final approval, final approval, accountable for all aspects of publication.

Sana Mubarik: Substantial contribution to acquisition of data, critical review, final approval, accountable for all aspects of publication.

Muhammad Arif Memon: Overall supervision, final approval, accountable for all aspects of publication.

REFERENCES

1. Yuen L, Hannah W, Hare M, Simmons D. Hyperglycaemia in pregnancy: the role of ethnicity and geography in risk and outcomes. *Diabetologia*. 2025; 68 (11); 2340-61
DOI: <https://doi.org/10.1007/s00125-025-06510-7>
2. Olmos-Ortiz A, Flores-Espinosa P, Díaz L, Velázquez P, Ramírez-Isarraraz C, Zaga-

- Clavellina V. Immunoendocrine dysregulation during gestational diabetes mellitus: The central role of the placenta. *Int J Mol Sci.* 2021; 22(15): 8087. DOI: <https://doi.org/10.3390/ijms22158087>
3. Abbas S, Khattak M, Hameed S, Saeed S, Shabir R. HbA1c as an early diagnostic marker in gestational diabetes mellitus. *Professional Med J.* 2024; 31(05): 750-6. DOI: <https://doi.org/10.29309/TPMJ/2024.31.05.7993>
4. Beunen K, Neys A, Van Crombrugge P, Moyson C, Verhaeghe J, Vandeginste S, *et al.* Fasting plasma glucose level to guide the need for an OGTT to screen for gestational diabetes mellitus. *Acta Diabetol.* 2022; 59(3): 381-94. DOI: <https://doi.org/10.1007/s00592-021-01812-9>
5. Bogdanet D, O'Shea P, Lyons C, Shafat A, Dunne F. The oral glucose tolerance test—is it time for a change? —a literature review with an emphasis on pregnancy. *J Clin Med.* 2020; 9(11): 3451. DOI: <https://doi.org/10.3390/jcm9113451>
6. Silke O, Yim IS, Shaikh K, Lalani S, Ghani F, Wanyonyi S, *et al.* Is HbA1c associated with birth weight? A multivariable analysis of Pakistani pregnant women. *J Obstet Gynaecol Res.* 2023; 49(8): 2048-55. DOI: <https://doi.org/10.1111/jog.15706>
7. Obeagu GU, Altraide BO, Obeagu EI. Iron deficiency anemia in pregnancy and related complications with specific insight in Rivers State, Nigeria: A narrative review. *Ann Med Surg.* 2025; 87(6): 3435-44. DOI: <https://doi.org/10.1097/ms9.0000000000003224>
8. Punnose J, Malhotra RK, Sukhija K, Rijhwani RM, Sharma A, Choudhary N, *et al.* Establishing trimester-specific haemoglobin A1c reference intervals in pregnant women: a retrospective study of healthy south Asian women with normal pregnancy outcomes. *Sultan Qaboos Univ Med J.* 2023; 23(1): 81-9. DOI: <https://doi.org/10.18295/sgumj.3.2022.022>
9. Cavagnoli G, Pimentel AL, Freitas PA, Gross JL, Camargo JL. Effect of ethnicity on HbA1c levels in individuals without diabetes: Systematic review and meta-analysis. *PloS One.* 2017; 12(2): e0171315. DOI: <https://doi.org/10.1371/journal.pone.0171315>
10. Chigusa Y, Ikeda Y, Yamamura A, Mogami H, Nishimura A, Morita S, *et al.* Role of random blood glucose and HbA1c levels in optimizing glucose tolerance screening in early pregnancy: A retrospective cohort study. *Obstet Gynecol Sci.* 2025; 68(4): 273-82. DOI: <https://doi.org/10.5468/ogs.25052>
11. Nisa KU, Taj A, Dahri B, Sajida S, Hashmi H, Wahid N. The frequency of gestational diabetes mellitus & its responsible factors among pregnant women presenting at a tertiary care hospital, Karachi. *Int J Health Sci.* 2023; 7(S1): 386-94. DOI: <https://doi.org/10.53730/ijhs.v7nS1.14211>
12. O'Connor C, O'Shea PM, Owens LA, Carmody L, Avalos G, Nestor L, *et al.* Trimester-specific reference intervals for haemoglobin A1c (HbA1c) in pregnancy. *Clin Chem Lab Med.* 2012; 50(5): 905-9. DOI: <https://doi.org/10.1515/cclm.2011.397>
13. Deng Y, Cheng D, Liao G, Tan X, Yang J. Trimester-specific reference intervals for hemoglobin A1c in non-diabetic pregnancy in a Chinese population. *BMC Pregnancy Childbirth.* 2023; 23(1): 671. DOI: <https://doi.org/10.1186/s12884-023-05980-0>
14. 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. DOI: <https://doi.org/10.1507/endocrj.K10E-410>
15. Mosca A, Paleari R, Dalfra MG, Di Cianni G, Cuccuru I, Pellegrini G, *et al.* Reference intervals for hemoglobin A1c in pregnant women: Data from an Italian multicenter study. *Clin Chem.* 2006; 52(6): 1138-43. DOI: <https://doi.org/10.1373/clinchem.2005.064899>
16. 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 Childbirth.* 2018; 18(1): 424. DOI: <https://doi.org/10.1186/s12884-018-2057-x>
17. Khan A, Asif N, Ullah R, Khan TA, Khan A, Malik SS. Establishing reference intervals for HBA1C in all three trimesters of pregnancy; A Cross-sectional study on healthy pregnant women of Quetta, Baluchistan. *Pak Armed Forces Med J.* 2023; 73(1): 84. DOI: <https://doi.org/10.51253/pafmj.v73i1.4814>
18. Versantvoort AR, Van Roosmalen J, Radder JK. Course of HbA1c in non-diabetic pregnancy related to birth weight. *Neth J Med.* 2013; 71(1): 22-5. Available from: <https://www.nijmonline.nl/getpdf.php?id=1258>
19. Lowe Jr WL, Scholtens DM, Kuang A, Linder B, Lawrence JM, Leberthal Y, *et al.* Hyperglycemia and adverse pregnancy outcome follow-up study (HAPO FUS): maternal gestational diabetes mellitus and childhood glucose metabolism. *Diabetes Care.* 2019; 42(3): 372-80. DOI: <https://doi.org/10.2337/dc18-1646>
20. Renz PB, Cavagnoli G, Weinert LS, Silveiro SP, Camargo JL. HbA1c test as a tool in the diagnosis of gestational diabetes mellitus. *PloS One.* 2015; 10(8): e0135989. DOI: <https://doi.org/10.1371/journal.pone.0135989>
21. Mostafa SA, Davies MJ, Webb DR, Srinivasan BT, Gray LJ, Khunti K. Independent effect of ethnicity on glycemia in South Asians and white Europeans. *Diabetes Care.* 2012; 35(8): 1746-8. DOI: <https://doi.org/10.2337/dc11-2079>
22. Metcalf P, Kyle C, Kenealy TW, Sundborn G, Jackson RT. Ethnic differences in HbA1c in adults in New Zealand: A cross-sectional study. *Integr Obesity Diabetes.* 2018; 4(2): 1-7. DOI: <https://doi.org/10.15761/IOD.1000205>