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1,5 Anhydroglucitol in gestational diabetes mellitus

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ABSTRACT

Objective: 1,5 Anhydroglucitol (1,5 AG) is reported to be a more sensitive marker of glucose variability and short-term glycemic control (1–2 weeks) in patients with type1 and type 2 diabetes. However, the role of 1,5 AG in gestational diabetes mellitus (GDM) is not clear. We estimated the serum levels of 1,5 AG in pregnant women with and without GDM.

Methods: We recruited 220 pregnant women, 145 without and 75 with GDM visiting antenatal clinics in Tamil Nadu in South India. Oral glucose tolerance tests (OGTTs) were carried out using 82.5 g oral glucose (equivalent to 75 g of anhydrous glucose) and GDM was diagnosed based on the International Association of Diabetes and Pregnancy Study Group criteria. Serum 1,5 AG levels were measured using an enzymatic, colorimetric assay kit (Glycomark®, New York, NY). Receiver operating characteristic (ROC) curves were used to identify 1,5 AG cut-off points to identify GDM.

Results: The mean levels of the 1,5 AG were significantly lower in women with GDM (11.8 ± 5.7 μg/mL, $p < 0.001$) compared to women without GDM (16.2 ± 6.2 μg/mL). In multiple logistic regression analysis, 1.5 AG showed a significant association with GDM (odds ratio [OR]: 0.876, 95% confidence interval [CI]: 0.812–0.944, $p < 0.001$) after adjusting for potential confounders. 1,5 AG had a C statistic of 0.693 compared to Fructosamine (0.671) and HbA1c (0.581) for identifying GDM. A 1,5 AG cut-off of 13.21 μg/mL had a C statistic of 0.6936 (95% CI: 0.6107–0.7583, $p < 0.001$), sensitivity of 67.6%, and specificity of 65.3% to identify GDM.

Conclusion: 1,5AG levels are lower in pregnant women with GDM compared to individuals without GDM.

1. Introduction

The prevalence of gestational diabetes mellitus (GDM), one of the most common metabolic disorders during pregnancy, is increasing worldwide possibly due to advancing maternal age and increasing obesity rates.[1] Women with GDM are also more prone to develop type 2 diabetes mellitus (T2DM) in the future.[2] Women with GDM need to maintain good glycemic control to reduce the incidence of maternal and fetal complications.[3,4] Some studies have demonstrated that abnormal fetal growth depends not only on chronic hyperglycemia but also on glucose variability.[5] Markers of glycemic control such as glycated hemoglobin (HbA1c) and Fructosamine (FA) are routinely used as chronic glycemic control over 2–3 months and 2–3 weeks respectively. However, they do not provide information about glycemic variability.[6]

1,5 Anhydroglucitol (1,5 AG) is one of the major polyols in human fluids and is structurally similar to glucose.[7] It is absorbed mainly from ingested food and is distributed to all organs and tissues.[8] Under normal glycemic conditions, levels of 1,5 AG is constantly maintained through renal filtration followed by reabsorption in the proximal tubules.[9] As it competes with glucose for its reabsorption in the renal tubules, glycosuria will lead to reduction in the serum 1,5 AG level.[10] Hence, when blood glucose levels exceed the threshold of glucosuria, reabsorption of 1,5 AG is competitively inhibited and excreted in urine.[11] This mechanism hence allows it to be utilized as a marker of glycemic control and

Abbreviations: 1,5 AG, 1,5 Anhydroglucitol; GDM, gestational diabetes mellitus; OGTT, Oral glucose tolerance tests; ROC, Receiver operating characteristic.

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in contrast to HbA1c and FA whose levels are higher in uncontrolled diabetes, where as levels of 1,5 AG are lower.[7,12] There are some reports that 1,5 AG levels predicted T2DM better than HbA1c.[13]

Clinical studies have also reported 1,5 AG as a short-term postprandial marker for hyperglycemia.[14] We recently showed that circulatory levels of 1,5 AG significantly reduced as glucose intolerance increases with NGT individuals having the highest values followed by IGT and T2DM and also reported on the usefullness of 1,5 AG as an additional tool to assess short-term glycemic control, compared to serum FA and HbA1c in Asian Indians.[15]

Earlier studies have demonstrated that changes in plasma levels of 1,5AG during pregnancy may reflect a mild alteration of carbohydrate metabolism and that 1,5 AG could act as an adjunct marker for HbA1c in pregnant women with diabetes.[16,17] Boritza et al. reported that 1,5 AG could be used as a reliable marker for moderate glycemic control during early pregnancy.[18] In this context, it is useful to study the association of 1,5 AG with GDM among Asian Indians who have higher prevalence of GDM, an earlier age of onset and lower body mass index.[19] Asian Indian women with GDM are also known to be more susceptible to T2DM, and the conversion to T2DM occurs earlier in South Asians compared to other ethnic groups.[20] The aim of this study was to determine the association of 1,5 AG in Asian Indian pregnant women with and without GDM.

2. Materials and methods

The study subjects for this case-control study were recruited from antenatal clinics in rural and urban centers in Tamil Nadu, Southern India. Consecutive pregnant women with gestational age <28 weeks visiting the antenatal clinics were included in the study. Inclusion criteria were as follows: pregnant women who consented for testing, aged ≥18 years of age, gestational age <28 weeks and singleton pregnancy. The study groups comprised 75 women with GDM and 145 women without GDM. Clinical information including obstetric history, family history of diabetes as well as current and past medications was collected using a structured questionnaire. The oral glucose tolerance test (OGTT) was done using 82.5 g oral glucose (equivalent to 75 g of anhydrous glucose) which was dissolved in 300 mL of water and given to the pregnant women who consumed it within 5 min. Further venous samples were drawn at fasting, 1 h and 2 h on oral glucose tolerance test and measurement of 1,5 AG were simultaneously performed in 7% (n = 15), 82% (n = 180), and 11% (n = 25) of pregnant women in the first, second and third trimester of their pregnancy, respectively. In addition HbA1c and FA was measured in the fasting state.

We calculated a sample size of 98 which had a power of >95% to detect a difference of 4.4 μg/mL between groups for 1,5 AG, with a standard deviation of 0.5 and an alpha error of 0.05. GDM was diagnosed using the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria if 1 or more values equaled or exceeded the following thresholds: fasting plasma glucose of 5.1 mmol/L (92 mg/dL), 1-h plasma glucose level of 10.0 mmol/L (180 mg/dL) or a 2-h plasma glucose level of 8.5 mmol/L (153 mg/dL).[21] We included women with singleton pregnancy and excluded those with pre-existing diabetes mellitus, hyper or hypothyroidism, or renal disease. A complete medical history was obtained, including any past or family history of diabetes, as well as current medications. Institutional Ethics Committee approval was obtained before the start of the study and written informed consent was obtained from the study participants.

2.1. Biochemical test

Plasma glucose (hexokinase method) was measured using Beckman Coulter AU2700 (Fullerton, CA, USA). HbA1c was measured by high-performance liquid chromatography using the Variant II Turbo (Bio-Rad, Hercules, CA, USA). The intra and inter-assay coefficients of variation for the biochemical assays ranged between 3.1% and 7.6%. FA (NBT/kinetic) was also measured using Beckman Coulter AU2700 (Fullerton, CA, USA). The assay has a sensitivity of 10 μmol/L, and intra- and inter-assay coefficients of variation ranged between 0.9% and 2.9%. All measurements were performed in our laboratory, which is certified by the College of American Pathologists (CAP) and the National Accreditation Board for Testing and Calibration Laboratories (NABL).

2.2. 1,5 Anhydroglucitol measurements

Serum 1,5 AG levels were assessed using an enzymatic, colorimetric assay kit (Glycomark®, New York, NY)[22] using the Beckman Coulter AU2700 (Fullerton, CA, USA). The 1,5 AG assay had, a sensitivity of 1.5 μg/mL, linearity <50 μg/mL, and coefficients of variation ranged between 2.4% and 4.9%.

2.3. Statistical analysis

Student's *t*-test or one-way ANOVA (with Tukey's HSD) as appropriate were used to compare groups for continuous variables and the Chi-square test or Fisher's exact test as appropriate was used to compare proportions. Pearson's correlation analysis was carried out to determine the correlation between 1,5 AG, Fasting, 1 h plasma glucose (1HrPG) and 2 h plasma glucose (2HrPG). Multiple logistic regression analysis was performed using GDM as the dependent variable and 1,5 AG as the independent variable after adjusting for age, HbA1c, and family history of diabetes. Receiver operating characteristic (ROC) curves were plotted for 1,5 AG and sensitivity and specificity for identifying GDM were calculated for various 1,5 AG cut-off points. The C statistic was estimated, and by interpolation from the area under the curve, the point closest to the upper-left corner, which maximized sensitivity and specificity, was selected as the optimal cut-off point; this identified the highest number of subjects with or without GDM.[23] All analyses were done using the Windows-based SPSS statistical package (version 22.0, SPSS Inc., Chicago, IL) and $p < 0.05$ was considered statistically significant.

3. Results

The clinical and biochemical features of the study participants are shown in Table 1. Age ($p < 0.001$), FA ($p < 0.001$), fasting blood glucose ($p < 0.001$), 1HrPG ($p < 0.001$), and 2HrPG ($p < 0.001$) were higher in individuals with GDM. However HbA1c levels were not statistically different between the groups.

Fig. 1 shows that the mean levels of the 1,5 AG were significantly lower in GDM women (11.8 ± 5.7 μg/mL,) compared to women without GDM (16.2 ± 6.2 μg/mL ($p < 0.001$).

Pearson correlation analysis showed that 1HrPG ($r = -0.175$, $p < 0.01$) and 2HrPG ($r = -0.174$, $p < 0.01$) were significantly correlated with 1,5 AG. However there was no correlation between 1,5 AG and fasting plasma glucose ($r = -0.108$, $p = 0.110$).

Multiple logistic regression analysis was performed using GDM as the dependent variable and 1,5AG as the independent variable

Table 1
Clinical and biochemical characteristics of the pregnant women.

Variable	Without GDM (n = 145)	With GDM (n = 75)	*p* value
Age (years)	24.9 ± 2.6	27.4 ± 4.8	<**0.001**
Mean period of gestation (weeks)	21 ± 4.7	23.3 ± 5.6	<**0.001**
Family history of diabetes n (%)	21(14.5%)	38(50.7)	<**0.001**
HbA1c (%)	5.0 ± 0.4	5.1 ± 0.7	0.179
Fructosamine (μmol/L)	154.9 ± 46	182.8 ± 51	<**0.001**
Fasting plasma glucose (mg/dL)	80 ± 7	87 ± 12	<**0.001**
1 h plasma glucose (mg/dL)	121 ± 25	164 ± 46	<**0.001**
2 h plasma glucose (mg/dL)	99 ± 16	146 ± 42	<**0.001**

Abbreviations: GDM - gestational diabetes mellitus, HbA1c - glycated hemoglobin. Bold values indicates $p < 0.001$ is statistically significant.
Data are reported as mean ± SD unless indicated otherwise.

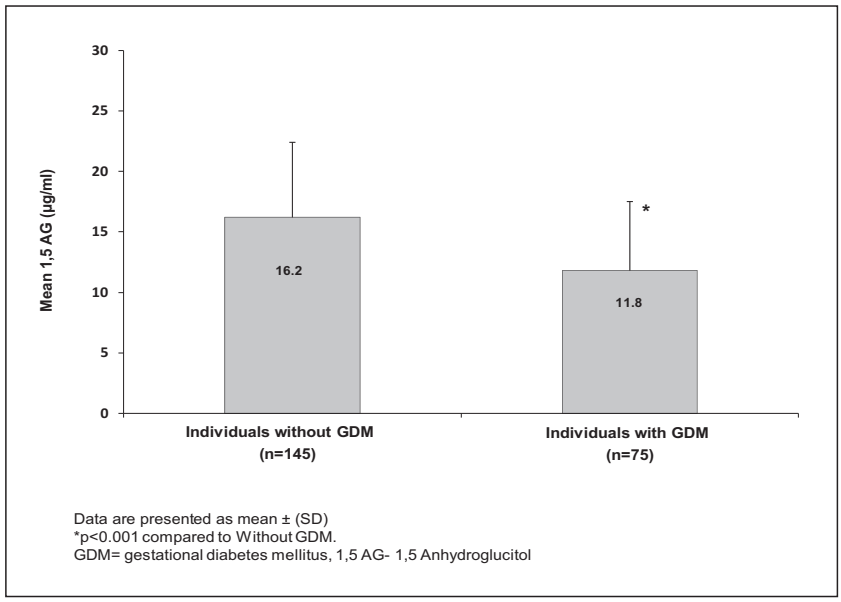


Fig. 1. Mean levels of 1,5 AG in individuals with and without GDM.

(Table 2). 1,5 AG showed a significant association with GDM (odds ratio [OR]: 0.880, 95% confidence interval [CI]: 0.834–0.929, $p < 0.001$). This association remained statistically significant even after adjusting for age, family history of diabetes, BMI and gestational age (OR: 0.876, 95% CI: 0.812–0.944, $p < 0.001$).

ROC curves were constructed to derive the cut-off point for 1,5AG levels with the best sensitivity and specificity to identify women with GDM. A 1,5 AG cut-off point of 13.21 μg/mL had a C statistic of 0.6936 (95% CI: 0.6107–0.7583, $p < 0.001$), a sensitivity of 67.6% and specificity of 65.3% for identifying GDM. The C statistic improved to 0.7801 after adjusting for age and family history of diabetes (Fig. 2). The ROC curves with various glycemic parameters 1,5 AG, FA and HbA1c to detect GDM is shown in Supplementary Fig. 1 a. Of the three, 1,5 AG had the highest AUC for GDM (AUC = 0.693) followed by FA (AUC = 0.671) and HbA1c (AUC = 0.581). On analyzing combinations, it was found that the combination of fasting plasma glucose and 1,5 AG predicted GDM better (AUC = 0.752) compared to traditional glycemic markers as shown in Supplementary Fig. 1 b.

If we use 1,5AG cut-off less than13.21 μg/mL, we found that 65.3% of GDM compared to 32.4% of individuals without GDM had values below the cut point (Fig. 3).

We also stratified GDM individuals according to their HbA1c levels <6% and found that 65% of those with HbA1c <6% had 1,5 AG of <13.2 μg/mL (Supplementary Fig. 2).

4. Discussion

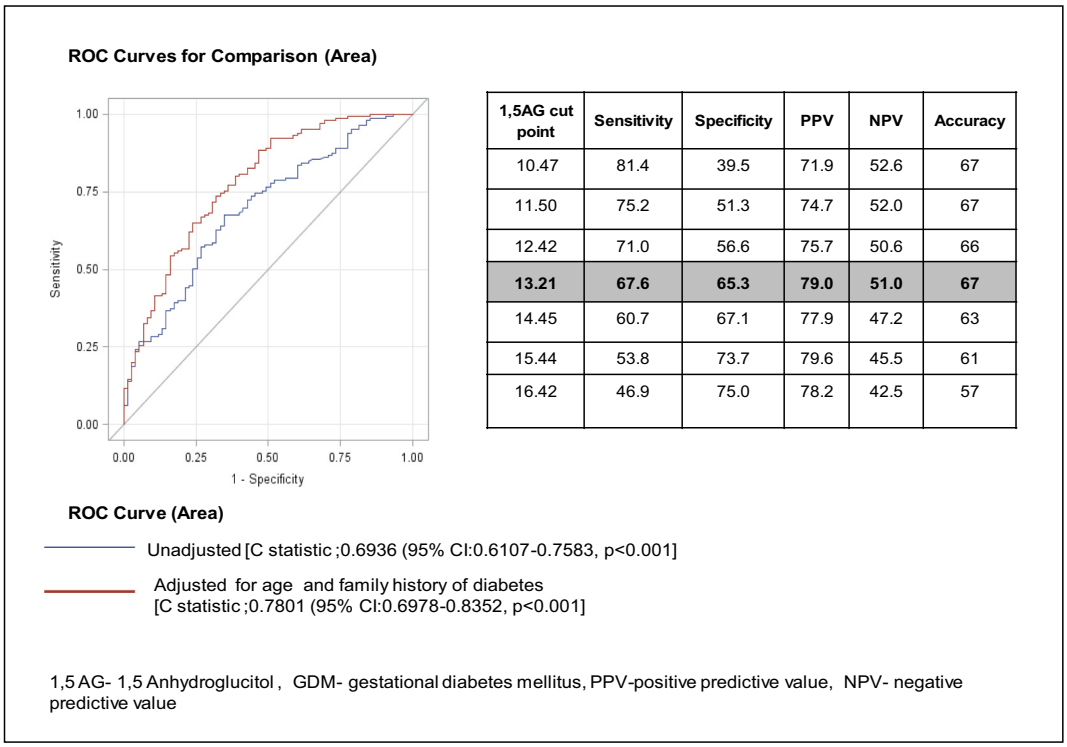
This study shows the following findings. First, the mean 1,5 AG levels were significantly lower in subjects with GDM. Second, 1,5 AG showed a significant correlation with 1HrPG and 2HrPG. Third, 1,5 AG was significantly associated with GDM after adjusting for age, family history of diabetes, BMI and gestational age. Fourth, among the three individual glycemic parameters, we observed that the 1,5 AG had the best C statistic compared to FA and HbA1c. Additionally, a 1,5 AG cut off-point of 13.21 μg/mL could be used to identify 65% of GDM.

There is considerable controversy regarding the ideal screening and diagnosis test for GDM.[24] Furthermore, the current management of diabetes typically consists of daily pre- and post-prandial blood glucose monitoring. Glycated hemoglobin levels are currently not recommended for diagnosis or monitoring of GDM due to its lack of sensitivity. Moreover since HbA1c is a measurement of 2 to 3 months glycemic control and GDM typically set in the second trimester i.e. 24–28 weeks of pregnancy utility of HbA1c is rather limited. Due to the technical nature of assay, there is a mixed evidence on the utility of fructosamine for screening or diagnosing GDM.[25,26,27] 1,5 AG reflects hyperglycemia and glucose variability, even in patients with well or moderately controlled diabetes.[28,29] Earlier studies have shown that 1,5 AG is more sensitive than HbA1C and FA in response to short-term glycemic therapy[30,31] and its levels were reduced in patients with T2DM.[15] A

Table 2
Multiple logistic regression analysis using GDM as the dependent variable and 1,5 AG as independent variable.

Variable	OR	95% CI	*p* value
Unadjusted 1,5 AG	0.880	0.834–0.929	<**0.001**
Model 1: Adjusted for age	0.887	0.838–0.938	<**0.001**
Model 2: Model 1 plus family history of diabetes	0.898	0.847–0.952	<**0.001**
Model 3: Model 2 plus BMI	0.875	0.812–0.944	<**0.001**
Model 4: Model 3 plus gestational age	0.876	0.812–0.944	<**0.001**

Abbreviations: 1,5 AG - 1,5 Anhydroglucitol, BMI - Body Mass Index, OR - odds ratio, CI - confidence interval



1,5AG cut point	Sensitivity	Specificity	PPV	NPV	Accuracy
10.47	81.4	39.5	71.9	52.6	67
11.50	75.2	51.3	74.7	52.0	67
12.42	71.0	56.6	75.7	50.6	66
13.21	**67.6**	**65.3**	**79.0**	**51.0**	**67**
14.45	60.7	67.1	77.9	47.2	63
15.44	53.8	73.7	79.6	45.5	61
16.42	46.9	75.0	78.2	42.5	57

1,5 AG- 1,5 Anhydroglucitol , GDM- gestational diabetes mellitus, PPV-positive predictive value, NPV- negative predictive value

Fig. 2. ROC curves of 1,5 AG among individuals with GDM and without GDM for the unadjusted and adjusted models.

similar trend was also observed even among subjects with GDM in this study, as women in GDM had significantly lower levels of 1,5 AG compared to pregnant women without GDM. We also observe a significant association between 1,5 AG and GDM even after adjusting for age and family history of diabetes. This observation is supported by Dworacka et al.[16] who demonstrated a significant correlation between 1,5 AG and GDM. Prospective studies are needed to understand the association of 1,5 AG with GDM and its prognostic significance.

Earlier studies have reported that 1,5 AG was significantly associated with maximum glucose concentration among pregnant women.[32] We found that 1,5 AG showed inverse correlation with 1HrPG glucose on OGTT and this is an important observation as elevated 1HrPG glucose during OGTT provides a better tool to identify subjects with beta-cell dysfunction compared with HbA1c and these individuals are more prone to developing T2DM.[33,34] Additionally, it has been demonstrated that the combination of fasting and 1HrPG glucose gives higher predictability for large-for-gestational-age newborns of mothers with GDM.[35]

Earlier studies have reported that 1,5 AG was more sensitive and specific than HbA1c in predicting postprandial hyperglycemia.[28] We observed that there is no significant difference in HbA1c levels among pregnant women with and without GDM. However, 65% of GDM with lower levels of 1,5 AG (<13.21 μg/mL) had clinically acceptable HbA1c and this suggests that over half of them may have glycemic variability. This suggests the clinical importance of measuring 1,5 AG in studying glycemic variability. The strength of this study is that it is the first study which derive a cut-point for 1,5 AG in Asian Indians with GDM. Second, 1,5 AG correlates with 1HrPG and 2HrPG values and could be used as surrogate marker for expensive glucose profiling to assess glycemic variability. Longitudinal studies with serial measurements of 1,5-AG need to be done at different stages of pregnancy to further clarify the role of 1,5-AG as a marker of GDM. One of the limitations of this study is that of being a cross-sectional study and we could not assess the cause and effect relationship. Second, the sample size was relatively small.

Finally we did not perform continuous glucose monitoring or self monitoring of glucose to correlate them with 1,5 AG levels.

In summary, our data suggest that 1,5 AG levels are lower in Asian Indian pregnant women with GDM compared to individuals without GDM. More studies are needed to see if 1,5 AG could be used to predict GDM.

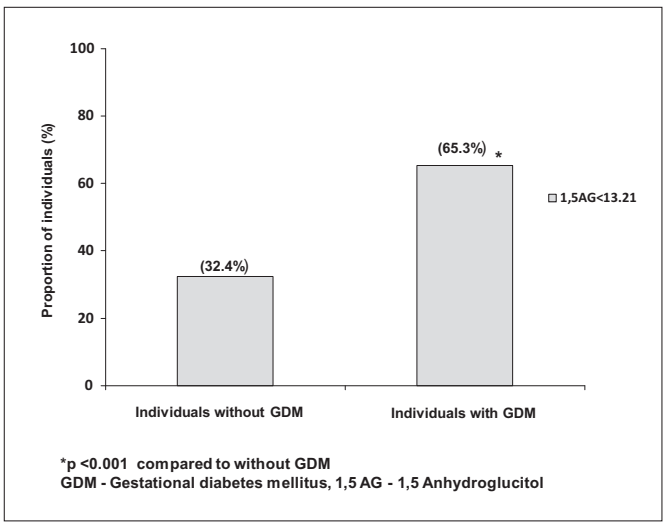


Fig. 3. Proportion of individuals with and without GDM based on the 1,5 AG cut-off value of 13.21 μg/mL.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jdiacomp.2018.11.010.

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