Original article

Early screening biomarker HbA1c and Hematocrit for gestational diabetes mellitus

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ABSTRACT

Gestational diabetes mellitus (GDM) is a metabolic disturbance in pregnancy, traditionally defined as glucose intolerance with onset or first recognition during pregnancy with the outcome of perinatal and offspring complications. The common diagnostic criteria of oral glucose tolerance test in the second or third trimester of pregnant women help to detect the later complications of GDM. Early detection is lacking and highly essential to prevent GDM. We aim to assess the role of glycated hemoglobin and hematocrit for the early screening of GDM.

We recruited 403 study participants, collected demographic profiles and medical histories. The levels of glucose, hemoglobin (Hb), glycated hemoglobin (HbA1c), hematocrit (PCV), and thyroid-stimulating hormone (TSH) were measured in the serum samples of study participants. The ROC curve was plotted, cut-off, an area under the curve, specificity, and sensitivity of glycated hemoglobin and hematocrit was calculated using statistical software.

In our study, we observed there is a significant difference in the levels of Hb, PCV, and HbA1c between non-GDM and GDM patients. We found the prevalence of GDM 20.88% in our study population. An HbA1c cut of at 5.25% and PCV 34.5%, the sensitivity and specificity of HbA1c as 64.4% and 36.1%, the sensitivity and specificity of PCV as 58.6% and 43.1% respectively. The combined HbA1c & PCV screening results had shown the sensitivity of 36.8% and specificity of 85.4% using the ROC curve.

In conclusion, the cut-off values, specificity, and sensitivity of HbA1c and Haematocrit and its combined calculation in the first trimester of pregnant women will be more specific and sensitive early screening tools for GDM.

1. Introduction

Gestational Diabetes Mellitus (GDM) is a complication in pregnancy associated with adverse outcomes in the maternal and fetal state with the increased risk of type 2 diabetes, macrosomia, obesity, cardiovascular diseases, morbidity, and mortality of fetus. The outcomes of perinatal and long-term offspring have a strong association with glycemic control in pregnancy. Some of the birth complications like birth injury, cesarean mode of delivery and large size fetus, fetal hypoxia, fetal hyperinsulinemia, hypoglycemia, polycythemia were well recognized high-risk complications in offsprings of GDM women. The long-

term childhood complications include excess adiposity, neuro-developmental impairment, glucose intolerance, disorders of glucose metabolism, type 2 diabetes mellitus, obesity, memory deficiency, psychological defects, developmental effects, and increased risk of autism has been documented in offsprings of GDM mothers.¹

The criteria for screening tests for gestational diabetes mellitus were first suggested by Carpenter Coustan in 1982.² National Diabetes Data Group (NDDG) in 1979 well explained the classification and diagnosis of diabetes mellitus and other categories of glucose intolerance.³ The diagnostic criteria were recommended by the international association of diabetes and pregnancy study groups (IADPSG) and adopted by the

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world health organization (WHO). IADPSG criteria-based hyperglycemia and adverse pregnancy outcome (HAPO) studies found that the prevalence of GDM in the south Indian population is 17.8%.^{4–7} The standard value for fasting plasma glucose <140 mg/dL and oral glucose tolerance test values 140–199 mg/dL was recommended by DIPSI criteria for gestational diabetes.⁸

Current treatments for GDM include dietary lifestyle interventions,^{9,10} medical nutrition therapy with meal planning,¹¹ moderate physical activities for 30 min per day, and first-line pharmacological therapy with insulin^{12,13} or oral agents like glyburide¹⁴ or metformin.¹⁵ Frequent monitoring of glucose levels, scheduled dietary and medications, physical exercise will be highly helpful to eradicate the complications of gestational diabetes mellitus.

The oral glucose tolerance test (OGTT) is the common diagnostic screening method for pregnant women performed at 24–28 weeks of gestation who may have risk factors of GDM. As OGTT is quite a time-consuming, costly, low reproducibility, inconvenient confirmation of GDM, an alternative early screening strategy should be identified for the diagnosis and prevention of GDM risk in pregnant women.¹⁶

The glycated hemoglobin (HbA1c) is an irreversible non-enzymatic plasma protein glucose binding product, especially in hemoglobin. The mean level of plasma glucose on the erythrocyte is correlated with glycosylation degree. Measurement of this HbA1c will serve as an indicator of glucose control in 3–4 weeks of gestation may be used for early detection of women at risk of GDM. Very few prior studies examined and suggested HbA1c levels measured in the first trimester will be helpful for the prediction of GDM.^{16–19}

Studies had shown that hematocrit (HCT or PCV) is associated with insulin sensitivity. There is a strong relationship between insulin resistance and high values of hematocrit in pregnancy. The high levels of hematocrit measured in the first trimester in perinatal care were found to be an independent predictor for GDM.^{20,21} Based on this background our primary aim is to examine the differences in levels of HbA1c and hematocrit between non-GDM and GDM, potential identification of cutoff values, specificity and sensitivity of HbA1c and PCV for GDM screening in the first trimester as well as to evaluate the association of HbA1c and hematocrit PCV in early prediction of GDM.

2. Materials and methods

2.1. Study design

Our study was designed as a cross-sectional study. The Institutional Ethics Committee approved this study. Study participants attending the department of obstetrics and gynecology, SRM Medical College Hospital and Research Centre, Tamilnadu, India were included after getting informed consent from the participating women.

2.2. Participants criteria

Pregnant women with and without gestational diabetes mellitus, between the age group of 18–35 years and, singleton pregnancy between 8 and 28 weeks of gestation were included in the study. Patients above 35 years, multiple pregnancies, with a history of type 2 diabetes, hypertension, hyperemesis, hematological disorders, organ dysfunction, thyroid disease, and in-vitro fertilization-embryo transfer were excluded from the study. GDM was diagnosed using International Association of Diabetes and Pregnancy Study Groups criteria (IADPSG): one or more of fasting plasma glucose level of ≥ 92 mg/dL, 1-h plasma glucose level of ≥ 180 mg/dL, and 2-h plasma glucose level of ≥ 153 mg/dL following a 75-g oral glucose tolerance test. Pregnant women with normal glucose tolerance and no other comorbidities are taken as control subjects.

2.3. Demographic profile

Study participants' profiles including age, height, weight, singleton

or multiple pregnancies, period of gestation, family history and history of diabetes, other complications, were collected after receiving consent. Body mass index (BMI) was calculated based on the height and weight of the participants.

2.4. Laboratory investigations

Blood samples were collected from the study participants in a sterile vacutainer. Hb, hematocrit PCV (packed cell volume), HbA1c was assessed in the blood samples. Serum samples were separated by centrifugation of blood at 3000 rpm for 10 min and utilized for analysis of biochemical parameters using an automated clinical chemistry autoanalyzer (Beckman Coulter, California, USA) and hormones are analyzed in VITROS® ECIQ Immunodiagnostic System.

2.5. Statistical analysis

The statistical analysis was done using SPSS software version 21. Students-test was done for the comparison of the parameters between non-GDM and GDM. A receiver operating characteristic (ROC) curve was plotted, sensitivity, specificity, and area under the curve was calculated to identify the cut-off values of HbA1c and hematocrit PCV. P-value <0.05 was considered to be statistically significant.

3. Results and discussion

3.1. Study population

Based on the inclusion and exclusion criteria of the participants, 403 subjects were included in the study. Among 403 subjects, 318 were non-GDM participants and 85 were GDM participants. The average age group of non-GDM subjects is 26.44 ± 2.34 years and GDM subjects are 27.0 ± 2.87 years. The mean $\pm$ SD values of BMI of non-GDM subjects were 26.54 ± 3.45 kg/m² and GDM subjects were 30.10 ± 2.33 kg/m² (Table 1).

3.2. Clinical parameters

Hemoglobin values of non-GDM subjects were 11.09 ± 0.07 g/dL and GDM subjects were 11.47 ± 0.14 g/dL Student t-test revealed a statistically significant difference in Hb values between GDM and non-GDM subjects ($P = 0.020$). The hematocrit PCV percent of non-GDM was $33.85 \pm 0.28\%$ and GDM $34.85 \pm 0.37\%$ which was statistically significant ($P = 0.034$). HbA1c values for non-GDM were 5.18 ± 0.04 and GDM were 5.62 ± 0.09 with the p-value of 0.0001. The thyroid levels of non-GDM and GDM was found to be 1.97 ± 0.08 mU/L and 1.86 ± 0.13 mU/L respectively (Table 1). We did not find any statistically significant difference in age, BMI, and thyroid levels between non-GDM and GDM participants in the study.

Table 1

Comparison of clinical characters of females with and without GDM.

Parameters	Non-GDM (n = 318)	GDM (n = 85)	T test	P value
Age (years)	26.44 ± 2.34	27.0 ± 2.87	0.567	0.568
Body Mass Index (kg/m ²)	26.54 ± 3.45	30.10 ± 2.33	0.477	0.634
Hemoglobin (gm/dl)	11.09 ± 0.07	11.47 ± 0.14	−2.345	0.020*
Hematocrit (%) PCV	33.85 ± 0.28	34.85 ± 0.37	−1.2135	0.034*
HbA1c(%)	5.18 ± 0.04	5.62 ± 0.09	−4.849	0.0001*
TSH (mU/L)	1.97 ± 0.08	1.86 ± 0.13	0.708	0.479

*P < 0.05 is statistically significant. P value is calculated by student t-test. TSH- Thyroid stimulating hormone, PCV-packed cell volume, HbA1c-Glycated hemoglobin.

3.3. Cut-off value, specificity, and sensitivity of HbA1c and PCV in GDM

A ROC curve was plotted to find the specificity and sensitivity of HbA1c at 12–28 weeks of gestation in GDM detection. In our study, the cut-off value of HbA1c was found to be 5.25% and PCV to be 34.5% (Fig. 1). The area under the ROC curve in HbA1c and PCV was found to be 0.715 and 0.604 respectively. HbA1c showed a sensitivity of 64.4% and specificity of 36.1%, whereas PCV showed 58.6% of sensitivity and 43.1% specificity (Table 2). Combined HbA1c and PCV showed 36.8% of sensitivity and 85.4% of specificity under the ROC plot determination.

4. Discussion

Women with GDM have a greater risk of obstetrical hitches and adversative pregnancy consequences like preeclampsia and macrosomia. Previous studies had shown the worldwide prevalence of GDM as 4.8% and 15% by CDC and prevention whereas IADPSG (2010) and Seshiah et al. found 17.8% GDM prevalence in the south Indian population.⁴ The pooled GDM prevalence in Asia was reported as 11.5% Lee KW et al. 2018.²² In our study, we observed 20.88% of prevalence of GDM.

Over the previous years, HbA1c, a marker representative of the normal plasma glucose level in the preceding 8–12 weeks, has been recommended as a diagnostic indicator for diabetes mellitus in non-pregnant women. Compared to OGTT, HbA1c has advantages as the blood sample can be drawn in a non-fasting state and there is no need for ingestion of glucose or blood sampling at different time intervals.^{23,24}

In nonpregnant women, though a level of $\geq 6.5\%$ is suggested for diagnosis of diabetes, no harmony has been reached on the HbA1c efficacy for the diagnosis of diabetes during pregnancy. Still, HbA1c levels $\geq 6.5\%$ have been used in detecting DM in early pregnancy on the basis that HbA1c levels in early pregnancy are comparable to those women who are not nonpregnant (4.5%–5.7% in early pregnancy vs. 4.7%–6.3% in non-pregnant women).^{25–27}

Many investigators have tried to use HbA1c in the management of GDM patients. A study of Indian pregnant women conducted by Balaji and colleagues on HbA1c reported higher mean HbA1c values in GDM women compared to normal pregnant women ($5.96 \pm 0.63\%$ [42 mmol/mol] vs. $5.36 \pm 0.36\%$ [35 mmol/mol]).²⁸ One more study by Rajput et al. reported significantly higher HbA1c levels in GDM women ($5.73 \pm 0.34\%$ [39 mmol/mol] vs. $5.34 \pm 0.35\%$ [35 mmol/mol], $p < 0.001$), and reported the analytical performance of HbA1c for GDM during the pregnant women in the second trimester.²⁹

We also assessed the indicative performance of HbA1c for GDM compared to the reference method, 75-g OGTT. In this study, when an HbA1c level of 5.25% was used as a cut-off value, it showed high sensitivity (64.4%), suggesting that measurement of HbA1c can be used as a screening tool for GDM, although the specificity was low (36.1%).

In addition, there were variances in the cut-off value of HbA1c between our study and previous studies. We expect that these inconsistencies are caused by different ethnicities, and the mechanism of ethnic difference of HbA1c has yet been known clearly. Uneven relation between blood glucose levels and HbA1c can be explained by the hemoglobin glycation difference.^{30,31}

Although HbA1c is not very virtuous at diagnosing GDM, it may have the potential to envisage adverse outcomes in pregnancy. HbA1c at pregnancy of 18–22 weeks, or 32–36 weeks or HbA1c along with other clinical data in large sample size may help to screen for GDM and potentially reduce the number of OGTTs. Further, HbA1c could predict preeclampsia and birth weight.

5. Conclusion

HbA1c presented high sensitivity with comparatively low specificity for the GDM diagnosis in pregnant women and is a possible prognosticator of postpartum diabetes mellitus. HbA1c may have a potential for

Fig. 1. ROC curve showing the sensitivity and specificity of HbA1c and PCV.

Table 2
Sensitivity and specificity of HbA1c and PCV from ROC curve.

Parameters	Area under the curve	Cut-off value	Sensitivity	Specificity
HbA1c	0.715	5.25	64.4%	36.1%
PCV	0.604	34.5	58.6%	43.1%

screening for GDM since it is likely to eradicate GDM in a considerable percentage of women and a less invasive substitute screening test for OGTT in GDM patients. Whether HbA1c alone or combined with other data like hematocrit can be useful in envisaging adverse outcomes in pregnancy among healthy women is unclear, and more research is needed as early management improves outcomes and decreases the forthcoming risk of diseases like DM, obesity, cardiovascular disease, and metabolic syndrome.

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Declaration of competing interest

None.

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