Original Article
The diagnosis value of blood glucose combined glycosylated hemoglobin in gestational diabetes

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Abstract: Gestational diabetes mellitus is a common complication in gestation period, and single diagnostic test of blood glucose always results in misdiagnosis. Previous studies showed that glycosylated hemoglobin was a promising biomarker for screening, diagnosis and treatment of diabetes. Combined measurements can improve the sensitivity and specificity of diagnosis. Thus, our study aimed to explore the diagnostic value of combined measurements of blood glucose and glycosylated hemoglobin in gestational diabetes mellitus. 278 samples of pregnancy were enrolled. Duration of pregnancy was from 24 weeks to 28 weeks. 52 cases of pregnancy were confirmed gestational diabetes mellitus by 75 g oral glucose tolerance test (OGTT) and enrolled in experimental group. 123 samples of normal pregnancy were collected as control group. Samples of fasting venous blood were collected for examination the levels of blood glucose and glycosylated hemoglobin (HbA1c). Receiver operating characteristics (ROC) curve was established to analyze the diagnostic value of combined measurements. Compared with control group, experimental group had significantly higher levels of HbA1c and 75 g OGTT. HbA1c was positively correlated with blood glucose in pregnancy with gestational diabetes mellitus. Area under the curve of HbA1c ROC was 0.854 (P<0.01). When HbA1c was at cutoff value (5.43%), Youden index reached to the highest value (0.596), while the sensitivity and specificity were 0.832 and 0.764, respectively. Combined measurements of blood glucose and HbA1c reduced the sensitivity of diagnosis, while improved specificity and increased Youden index for the diagnosis of gestational diabetes mellitus. Combined measurements of blood glucose and glycosylated hemoglobin have promising diagnostic value in the diagnosis of gestational diabetes mellitus.

Keywords: Blood glucose, glycosylated hemoglobin, HbA1c, gestational diabetes mellitus

Introduction

Gestational diabetes mellitus is a common complication in gestation period with broad definitions, including impaired glucose tolerance at the diagnosis of gestation, pre-pregnancy diabetes and diagnosis of diabetes during pregnancy [1]. Incidence of gestational diabetes mellitus is gradually increased with the development of living standard and changes in life style. In addition, impaired blood glucose could cause poor prognosis for both pregnant women and fetus [2, 3]. Moreover, gestational diabetes mellitus was associated with postnatal adverse events [4]. Thus, blood glucose control is of great clinical significance for both pregnant women and fetus.

However, there is no unified standard to diagnose gestational diabetes mellitus. Early diagnosis of gestational diabetes mellitus was emphasized by many organizations, including international association of diabetic pregnancy study group (IADPSG), American Diabetes Association (ADA) and World Health Organization (WHO). However, these organizations did not reach an agreement on the threshold of detection methods [5-7]. 75 g OGTT is the current gold standard for gestational diabetes mellitus, but protocols of 75 g OGTT require multiple blood collections, which are tedious with high costs [8]. International diabetes institute has showed that glycosylated hemoglobin is an important diagnosis index with great diagnostic value in common diabetes. Glycosylated hemoglobin is stable and less affected by diets; however, it is unclear whether glycosylated hemoglobin is applicable as combined measurements for the diagnosis of gestational diabetes mellitus. Therefore, our study aimed to explore

Table 1. Analysis of basic information between experimental group and control group

Test index	Control (N=123)	Experimental (N=52)	P
Age (year)	25.3±5.2	26.1±26.1	0.33
Gestational age (week)	25.2±2.1	25.8±1.8	0.07
BMI	24.04±3.24	23.21±3.21	0.14
Caesarean section (Y/N)	49/74	21/31	0.95
Gravidity (1/2)	102/21	44/8	0.78

the diagnostic value of glycosylated hemoglobin in gestational diabetes mellitus, and the feasibility of combined measurements.

Methods

Research objects

This prospective cohort study enrolled 278 pregnant women at East Hospital, Tongji University School of Medicine from June 2015 to May 2016. 52 cases were confirmed gestational diabetes mellitus till February 2016. Diagnostic criteria were referred to the criteria of gestational diabetes mellitus released by Chinese Medical Association in 2015: All patients received 75 g OGTT. Three phenomena were confirmed as gestational diabetes mellitus, including fasting blood glucose ≥5.1 mmol/L, 1 hour blood glucose ≥10 mmol/L and 2 hour blood glucose ≥8.5 mmol/L. 123 normal pregnant women were collected as control group. Questionnaire was made to gather essential information of pregnancy, including age, gestational age, weight, height, family history, lifestyle and antenatal care. Body mass index = weight/height^2 (kg/m^2). Exclusion criteria were as follows: progestation diabetes, family history of diabetes, hypertension, major mental illness and severe organic diseases. All enrolled patients signed consent forms, and this study was under supervision and approval of East Hospital, Tongji University School of Medicine ethics committee.

Examination for blood samples

5 ml fasting peripheral blood was collected from all enrolled patients followed by centrifuging and separating the serum for examination the level of fasting blood glucose. 1 hour blood glucose and 2 hour blood were examined after 75 g OGTT with the same method. Roche modu-

lar analysis chemical system (Roche, USA) was used to examine blood glucose. Hemoglobin analyzer (Tosoh, USA) was used to examine the level of HbA1c.

Statistical analysis

SPSS 16.0 software was used for data processing. Measurement data with normal distribution were expressed as mean ± standard deviation (SD). Student t test was performed for comparison of the data with normal distribution and equal variance. Rank sum test was performed for analysis of data which were not falling into normal distribution. χ^2 test was performed for enumeration data and inspection level α=0.05 receiver operating characteristic (ROC) curve was established to evaluate diagnostic value of HbA1c. P<0.05 was considered as statistical significance.

Results

Analysis of basic information

No significant differences were observed on the basic information from experimental group and control group (P<0.05), including age, gestational age, BMI and history of caesarean section. This suggested equilibrium and comparability between two groups (**Table 1**).

Increased levels of blood glucose and HbA1c in gestational diabetes mellitus

75 g OGTT and HbA1c analysis showed that, compared with normal pregnancy, pregnancy with gestational diabetes mellitus had significantly higher levels of fasting blood glucose, one hour plasma glucose and two hour plasma glucose (**Table 2**, P<0.001) with 25.4%, 39.6% and 59.6% increased, respectively. The level of HbA1c was higher in gestational diabetes mellitus (**Table 2**, P<0.001).

As correlation analysis showed in **Table 3**, HbA1c was associated with the levels of blood glucose at each stage (P<0.05).

Glycosylated hemoglobin has promising diagnostic value for gestational diabetes mellitus

ROC curve is an important tool to assess the diagnostic value of clinical index. Analysis showed that the area under the curve of HbA1c

Table 2. Analysis of blood glucose and HbA1c

Test index	Control (N=123)	Experimental (N=52)	P
Hemoglobin (g/dL)	10.30±0.54	10.43±0.25	0.09
Fasting blood glucose (mmol/L)	4.32±0.45	5.42±0.43	P<0.001
1 h blood glucose (mmol/L)	7.56±1.76	10.56±2.30	P<0.001
2 h blood glucose (mmol/L)	5.67±1.32	9.05±1.90	P<0.001
HbA1c (%)	4.69±1.03	5.76±1.23	P<0.001

Table 3. Correlation analysis between HbA1c and blood glucose at each stage of 75 g OGTT

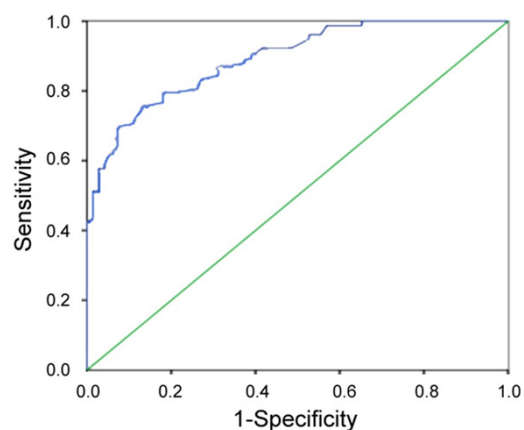| Test index	Fasting blood glucose	1 h blood glucose	2 h blood glucose
r	0.41	0.245	0.35
P	0.013	0.021	0.032

Figure 1. ROC curve for the diagnostic value of glycosylated hemoglobin.

ROC was 0.854 (**Figure 1**, 95% CI 0.813-0.901, P<0.01), suggesting HbA1c could be used as a predictor of gestational diabetes mellitus. When HbA1c was at the value of 5.43%, Youden index reached to the highest value (0.596). Thus, 5.43% was selected as the diagnostic cutoff value of HbA1c.

Combined measurements of blood glucose and glycosylated hemoglobin improved diagnosis of gestational diabetes mellitus

The diagnostic efficacy was assessed at the diagnostic cutoff value of HbA1c, and analysis showed that the sensitivity and specificity were 0.832 and 0.764, respectively. Sensitivity of the combined measurements was significantly higher than blood glucose single diagnosis, the same was observed in specificity and Youden index (**Table 4**).

Discussion

Incidence of gestational diabetes mellitus has gradually increased in recent years. Clinical trials showed that morbidity of gestational diabetes mellitus was nearly 5.0% in China, while up to 20% in developed countries [9]. Moreover, untreated gestational diabetes mellitus results in many complications with a poor prognosis.

As current golden standard for the diagnosis of gestational diabetes mellitus, OGTT's efficacy is largely limited by complicated protocols, which requires fasting before examination, twice collections of blood samples and long duration for examination [10]. Thus, a simple and reliable examination was required to improve the diagnosis of gestational diabetes mellitus. American diabetes association recommends glycosylated hemoglobin as a diagnosis biomarker for gestational diabetes mellitus, which does not need fasting and has promising diagnostic efficacy. Previous studies proved that the level of glycosylated hemoglobin was positively associated with the concentration of blood glucose [11], and had a promising sensitivity and specificity [12].

Our study demonstrated that pregnancy with gestational diabetes mellitus had significantly higher levels of blood glucose, including fasting blood glucose, 1 h blood glucose and 2 h blood glucose. What's more, compared with normal pregnancy, the level of glycosylated hemoglobin indeed was increased in pregnancy with gestational diabetes mellitus, consistent with previous study with a meta-analysis [13]. Furthermore, our study showed that glycosylated hemoglobin was associated with multiple index of blood glucose and not influenced by diet situations, suggesting glycosylated hemoglobin was a stable biomarker for the diagnosis of gestational diabetes mellitus. A Korean retrospective study showed that glycosylated hemoglobin was associated with blood glucose at different stages of pregnancy [14]. We determined to set up a cutoff value of HbA1c at 5.43%. Compared with OGTT examination in

Table 4. Analysis of diagnostic efficacy for two strategies

Test index	Sensitivity	Sensitivity	Positive predictive value	Positive predictive value	Youden index
Fasted blood glucose	0.821	0.716	0.632	0.856	0.612
HbA1c	0.832	0.764	0.593	0.901	0.596
HbA1c+ fasting blood glucose	0.725	0.903	0.672	0.912	0.628

current clinical scenario, HbA1c (cutoff value, 5.43%) had a false positive rate of 41.7% and positive predictive value of 58.3%. Accordingly, we suggested that glycosylated hemoglobin could not totally replace OGTT at the diagnosis of gestational diabetes mellitus. Previous studies also indicated that, although glycosylated hemoglobin level was remarkably increased in gestational diabetes mellitus, it was not a reliable diagnostic index for gestational diabetes mellitus due to outrageous false positive rate and false negative rate [15-17]. The possible mechanism is that the life span of erythrocytes was influenced by glycosylated hemoglobin, while antioxidant inhibited glycosylation [18].

Amylidi et al. examined the level of glycosylated hemoglobin during the first trimester and found glycosylated hemoglobin level was increased in gestational diabetes mellitus. Moreover, HbA1c (≥6%) had a promising diagnostic efficacy for gestational diabetes mellitus [19]. Our study further confirmed the diagnostic efficacy of HbA1c, verified by analysis of ROC curve. However, blood glucose is also an important diagnostic index for gestational diabetes mellitus [20]. In addition, combined measurements might improve the accuracy of diagnosis [21]. We firstly explored the diagnostic value of combined measurements of blood glucose and glycosylated hemoglobin in gestational diabetes mellitus, and found that combined measurements had higher sensitivity and specificity, suggesting diagnostic value of combined measurements was superior to single diagnostic test of blood glucose.

Our study has some limitations. 1) It needs further large cohort clinical studies to confirm the diagnostic value of combined measurements of blood glucose and glycosylated hemoglobin for gestational diabetes mellitus. 2) Territoriality was a potential confounding due to the relatively small sample size. 3) The diagnostic value of combined measurements should be tested at multiple periods of pregnancy.

In conclusion, glycosylated hemoglobin is an important diagnostic index for gestational diabetes mellitus. Combined measurements of blood glucose and glycosylated hemoglobin have promising diagnostic value for the diagnosis of gestational diabetes mellitus.

Disclosure of conflict of interest

None.

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References

[1] American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2012; 35 Suppl 1: S64-S71.

[2] Holst S, Kjær SK, Jørgensen ME, Damm P, Jensen A. Fertility problems and risk of gestational diabetes mellitus: a nationwide cohort study. Fertil Steril 2016; 106: 427-434, e1.

[3] Xiao Q, Cui YY, Lu J, Zhang GZ, Zeng FL. Risk for gestational diabetes mellitus and adverse birth outcomes in Chinese women with polycystic ovary syndrome. Int J Endocrinol 2016; 2016: 5787104.

[4] HAPO Study Cooperative Research Group, Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, Hadden DR, McCance DR, Hod M, McIntyre HD, Oats JJ, Persson B, Rogers MS, Sacks DA. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med 2008; 358: 1991-2002.

[5] International Association of Diabetes and Pregnancy Study Groups Consensus Panel, Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, Damm P, Dyer AR, Leiva AD, Hod M, Kitzmiler JL, Lowe LP, McIntyre HD, Oats JJ, Omori Y, Schmidt MI. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care 2010; 33: 676-682.

[6] World Health Organization. Diagnostic criteria and classification of hyperglycaemia first de-

tected in pregnancy: a world health organization guideline. Diabetes Res Clin Pract 2014; 103: 341-363.

[7] Kilpatrick ES, Bloomgarden ZT, Zimmet PZ. International expert committee report on the role of the A1C assay in the diagnosis of diabetes: response to the international expert committee. Diabetes Care 2009; 32: e159; author reply e160.

[8] Mishra S, Rao CR, Shetty A. Trends in the diagnosis of gestational diabetes mellitus. Scientifica (Cairo) 2016; 2016: 5489015.

[9] Simeoni U, Barker DJ. Offspring of diabetic pregnancy: long-term outcomes. Semin Fetal Neonatal Med 2009; 14: 119-124.

[10] Rani PR, Begum J. Screening and diagnosis of gestational diabetes mellitus, where do we stand. J Clin Diagn Res 2016; 10: E1-E4.

[11] Capula C, Mazza T, Vero R, Costante G. HbA1c levels in patients with gestational diabetes mellitus: relationship with pre-pregnancy BMI and pregnancy outcome. J Endocrinol Invest 2013; 36: 1038-1045.

[12] Odsæter IH, Åsberg A, Vanky E, Carlsen SM. HbA1c as screening for gestational diabetes mellitus in women with polycystic ovary syndrome. BMC Endocr Disord 2015; 15: 38.

[13] Gong T, Wang J, Yang M, Shao Y, Liu J, Wu Q, Xu Q, Wang H, He X, Chen Y, Xu R, Wang Y. Serum homocysteine level and gestational diabetes mellitus: a meta-analysis. J Diabetes Investig 2016; 7: 622-8.

[14] Ryu AJ, Moon HJ, Na JO, Kim YJ, Kim SJ, Mo SI, Byun JR. The usefulness of the glycosylated hemoglobin level for the diagnosis of gestational diabetes mellitus in the Korean population. Diabetes Metab J 2015; 39: 507-511.

[15] Frisoli G, Naranjo L, Shehab N. Glycohemoglobins in normal and diabetic pregnancy. Am J Perinatol 1985; 2: 183-188.

[16] Artal R, Mosley GM, Dorey FJ. Glycohemoglobin as a screening test for gestational diabetes. Am J Obstet Gynecol 1984; 148: 412-414.

[17] Morris MA, Grandis AS, Litton J. Glycosylated hemoglobin: a sensitive indicator of gestational diabetes. Obstet Gynecol 1986; 68: 357-361.

[18] Rajput R, Yogesh Yadav, Rajput M, Nanda S. Utility of HbA1c for diagnosis of gestational diabetes mellitus. Diabetes Res Clin Pract 2012; 98: 104-107.

[19] Amylidi S, Mosimann B, Stettler C, Fiedler GM, Surbek D, Raio L. First-trimester glycosylated hemoglobin in women at high risk for gestational diabetes. Acta Obstet Gynecol Scand 2016; 95: 93-97.

[20] Ye M, Liu Y, Cao X, Yao F, Liu B, Li Y, Wang Z, Xiao H. The utility of HbA1c for screening gestational diabetes mellitus and its relationship with adverse pregnancy outcomes. Diabetes Res Clin Pract 2016; 114: 43-49.

[21] Sagili H, Kamalanathan S, Sahoo J, Lakshminarayanan S, Rani R, Jayalakshmi D, Kumar KT. Comparison of different criteria for diagnosis of gestational diabetes mellitus. Indian J Endocrinol Metab 2015; 19: 824-828.