
OBSTETRICS

Impact of Time since Last Meal on the False Positive Result of 50 grams Glucose Challenge Test in the Pregnancy with Gestational Diabetes Mellitus Risk: A prospective cohort study

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ABSTRACT

Objectives: To study the impact of time since last meal to the rate of false positive 50 grams glucose challenge test (GCT) during pregnancy with gestational diabetes mellitus (GDM) risk.

Materials and Methods: This prospective observational study was conducted in a tertiary care from December 2019 to August 2020. The participants were the singleton who had risks of GDM. The screening test was done using 50 grams GCT and then 100 grams oral glucose tolerance test (OGTT) used for diagnosis of GDM if GCT was ≥ 140 mg/dL. The participants' information, time and type of last meal, time of 50 grams glucose intake and blood drawing, result of GCT and OGTT were recorded. The time since last meal was categorized to < 1 , < 2 and < 3 hours. Bivariate and multivariable regression analysis were applied to evaluate the effect of time since last meal to GCT.

Results: There were 426 pregnant women completed study: 30.75% had positive GCT and 19% of these were diagnosis for GDM. The time since last meal < 1 , < 2 , and < 3 hours group had 36.0, 29.8, and 25.8 % false-positive GCT compared with 20.8, 18.2, 20.3% of ≥ 1 , ≥ 2 , and ≥ 3 hours group. The adjusted risk ratio (95% confidence interval) were 1.60 (1.14-2.24), 1.53 (1.06-2.22) and 1.23 (0.75-2.04) and p value were 0.006, 0.023, and 0.397, respectively.

Conclusion: The interval between the last meal and GCT less than 2 hours significantly increased a false positive rate of the test.

Keywords: false-positive, glucose challenge test, gestational diabetes mellitus, last meal intake.

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ผลกระทบของระยะเวลาหลังอาหารมื้อสุดท้ายกับการเกิดผลบวกกลวงจากการตรวจน้ำดีกรองเบาหวานระหว่างตั้งครรภ์

ศุภารรณ ปัตตามัณฑ์, ศรีสุดา ทรงธรรมวัฒน์, เอื้อมพร สุ่มมาตย์, อังคณา หารศรี, เมธा ทรงธรรมวัฒน์

บทคัดย่อ

วัตถุประสงค์: เพื่อศึกษาผลกระทบของระยะเวลาหลังอาหารมื้อสุดท้ายก่อนตรวจน้ำดีกรองเบาหวาน 50 กรัม glucose challenge test ในสตรีตั้งครรภ์ที่มีความเสี่ยงเบาหวานต่อการเกิดผลบวกกลวง

วัสดุและวิธีการ: งานวิจัยแบบศึกษาไปข้างหน้าในสถานพยาบาลติยภูมิตั้งแต่เดือนกุมภาพันธ์ 2562 ถึง สิงหาคม 2563 โดยทำการศึกษาในสตรีตั้งครรภ์เดียวที่มีความเสี่ยงในการเกิดภาวะเบาหวานระหว่างตั้งครรภ์ที่มาฝากครรภ์และได้รับการตรวจด้วยการคัดกรองด้วยวิธี 50 g GCT หากผลตรวจน้ำดีกรองผิดปกติ ($\geq 140 \text{ mg\%}$) จะได้รับการตรวจวินิจฉัยภาวะเบาหวานด้วยวิธี 100g oral glucose tolerance test (OGTT) โดยการซักประวัติมารดาที่เข้าร่วมงานวิจัยเกี่ยวกับข้อมูลทั่วไป เวลาที่รับประทานมื้อสุดท้าย ชนิดและปริมาณของอาหารที่รับประทาน เวลาที่ได้รับการลืนน้ำตาลและเวลาที่ได้รับการเจาะเลือด โดยระยะเวลาหลังอาหารมื้อสุดท้ายจะแบ่งเป็น < 1 , < 2 และ < 3 ชั่วโมง ผลลัพธ์ของการตรวจจะถูกบันทึกและนำมาวิเคราะห์เพื่อหาผลผลกระทบและความสัมพันธ์ของระยะเวลาหลังอาหารมื้อสุดท้ายต่อระดับน้ำตาลและผลบวกกลวงโดยใช้การคำนวณทางสถิติ bivariate and multivariable regression

ผลการศึกษา: สตรีตั้งครรภ์ที่เข้าร่วมงานวิจัยทั้งหมด 426 คน มีอายุเฉลี่ย 30.75 ที่ตรวจคัดกรองได้ผลผิดปกติ ในกลุ่มนี้พบว่า ร้อยละ 19 ได้รับวินิจฉัยภาวะเบาหวาน และเมื่อเปรียบเทียบความสัมพันธ์ของระยะเวลาจากอาหารมื้อสุดท้ายก่อนได้รับการตรวจน้ำดีกรองเบาหวานพบว่าที่เวลา < 1 , < 2 และ < 3 ชั่วโมง มีผลบวกกลวงจากการตรวจร้อยละ 36.0, 29.8 และ 25.8 เทียบกับที่เวลา ≥ 1 , ≥ 2 และ ≥ 3 ชั่วโมงที่มีผลบวกกลวงร้อยละ 20.8, 18.2 และ 20.3 ตามลำดับ จากการวิเคราะห์แบบ multivariable regression analysis พบร่วมนิค่า adjusted risk ratio 1.60 (1.14-2.24), 1.53 (1.06-2.22) และ 1.23 (0.75-2.04), มีค่า p value เท่ากับ 0.006, 0.023, and 0.397 ตามลำดับ

สรุป: ช่วงเวลาหลังอาหารมื้อสุดท้ายมีผลต่อตรวจคัดกรองเบาหวาน GCT พบร่วยว่าที่เวลาน้อยกว่าสองชั่วโมงเป็นต้นไป ทำให้เพิ่มผลบวกกลวงของการตรวจน้ำดีกรองเบาหวานอย่างมีนัยสำคัญทางสถิติ

คำสำคัญ: ผลบวกกลวง, การคัดกรองเบาหวาน, ภาวะเบาหวานขณะตั้งครรภ์, มื้ออาหาร

Introduction

Gestational diabetes mellitus (GDM) is a common problem that effects both maternal and fetal health during pregnancy. The effects include increased risk of fetal macrosomia, fetal anomaly, shoulder dystocia, cesarean delivery rate, fetal death and further overt DM in both the mother and the newborn^(1, 2). In Thailand, the prevalence rate of GDM is reported as between 1.5-9.3%⁽³⁻⁷⁾ compared with a prevalence rate in the United State of 9.2%⁽⁸⁾.

There is no consensus on a standard GDM diagnostic tools and this has had an effect to the variation in diagnosis criteria, screening and management by different medical centers⁽⁹⁻¹²⁾. Two-step approach for diagnosis of GDM, consists of a 1 hour 50 grams glucose challenge test (GCT) followed by a 100 grams oral glucose tolerance test (OGTT). This method is recommended by the American College of Obstricians and Gynecologists (ACOG)⁽¹⁾ and has been widely used in many medical centers in Thailand.

A false-positive GCT is definded as the patient has a positive GCT but a negative OGTT. The false-positive GCT result is inconvenient for the patients, increases cost of screening, which can cause additional unnecessary diagnostic test, treatment and increased maternal concern about their health condition⁽¹³⁾. The significance of this false-positive group is questionable, there are some studies that considered the false-positive GCT as an early form of glucose intolerance⁽¹⁴⁻¹⁶⁾ and effect to perinatal outcomes⁽¹⁷⁻²⁰⁾.

Recently, there have been some studies on the impact of meal timing and calories and their influence on glucose levels in adults⁽²¹⁻²⁴⁾. There are a few studies that investigated the effect of timing, fasting duration and calories on glucose levels following GCT screening in pregnant woman which might effect the false-positive screening GCT⁽²⁵⁻²⁸⁾. There was neither a practical cut-off point since last meal nor calories were tested for application on the GCT. Thus, the primary objective of this study was to investigate the influence of last meal timing on GDM screening result. The secondary objective was to evaluate the effect of calories of last meal on the GCT result. The aim was to create practical patient advise for at risk patients who were undergoing GCT

screening.

Materials and Methods

This prospective cohort study was conducted at the antenatal care (ANC) clinic, Udonthani Hospital, Udonthani, Thailand, from December 2019 to August 2020. The study protocol was approved by Human Research Ethical Committee of Udonthani Hospital (No.62/2562). All participants were counselled and gave their consent before participating in this study.

The inclusion criteria were all singleton pregnant women, who had risk for GDM according to the hospital's protocol including: maternal age \geq 35 years at expected date of delivery, pre-pregnancy weight \geq 70 kg or BMI $>$ 30 kg/m², family member with DM, previous GDM, previous fetal macrosomia ($>$ 4,000 g), previous unknown cause of intrauterine fetal death, previous child with shoulder dystocia, history of impaired blood glucose and glucosuria $>$ 2⁺ at least 2 times. This inclusion criteria were the Udonthani Hospital's protocol which applied from Royal Thai College of Obstetricians and Gynecologists and Srinagarind Hospital GDM guideline practice^(29, 30). The exclusion criteria were (1) known case of overt DM, (2) pregnant woman who had positive GCT, but did not receive 100 g OGTT confirmation, (3) unwilling to participate with this study. The screening test was conducted at first time ANC and repeat at 24-28 gestational age if negative result for first time screening.

All participants were asked for their: characteristics, risk of GDM factors as inclusion criteria protocols, time, and type of last meal intake. The time of glucose ingestion and blood drawing for glucose test were collected by nurses who were counselled the method to collect data at the ANC clinic. The time, since last meal until glucose intake, was divided into two groups: group 1 (less than 2 hours) and group 2 (equal to or more than 2 hours)^(25, 26). The calories were evaluated by calories table of Thai public health department and type of meal was divided into light ($<$ 300 calories) and heavy meal (\geq 300 calories)⁽³¹⁾. Blood glucose at 1 hour after 50 grams glucose intake was measured.

Two step approach was applied to test in pregnant woman who had risks for GDM were performed a 50 grams GCT without starvation and venous plasma

glucose was measured at 1 hour after ingestion. All The positive GCT test was the blood glucose at 1 hour after 50 grams glucose intake ≥ 140 mg/dl. The confirmatory test by 100 grams OGTT was done within one week. The positive OGTT test was defined as at least 2 values of blood glucose levels at fasting, 1, 2, and 3 hours after 100 grams glucose intake were $\geq 105, 190, 165, 145$ mg/dl, respectively according to the National Diabetes Data Group (NDDG) criteria⁽¹⁾. The false positive GCT test was defined as the positive result of GCT (GCT ≥ 140 mg/dl) with negative result of 100 grams OGTT by NDDG criteria. The blood glucose was tested by hexokinase technique (Achitech 46000 machine, Abbott Laboratories Company).

Statistical analysis

The sample size was calculated by Stata statistical program using formula for Chi-squared test comparing two independent proportions. The proportion of false positive GCT in time since last meal equal to or more than 2 hours group (control) and less than 2 hours group from pilot study in our center were 0.15 and 0.30. The 0.01 significance level was used. The calculated sample size was 180 per group. The estimated prevalence of potential risk for GDM in our center was 30%. Therefore, the estimated time for collection of cases at ANC clinic was nine months, all cases, which compatible with the inclusion criteria between the study period, were collected.

The participants' characteristics were presented

in term of number, percentage, mean and standard deviation. The comparison of factors between two groups was calculated by Pearson's chi square, Fisher exact or student t test depending on the characteristic of variables. The crude and adjusted risk ratio with 95% confidence interval were calculated by bivariable and multivariable regression analysis. The p value < 0.2 was used for selecting variable to multivariable analysis and p value < 0.05 was used for statistically significance. All analyses were performed using Stata Release 13 statistical software (Stata Corp, College Station, TX).

Results

There were 443 participants who meet the inclusion criteria for GDM screening from December 2019 to August 2020. There were 17 participants who were excluded: 2 were overt DM, 2 were unwilling to participate in this study and 13 were unobtainable to confirm diagnostic test (Fig. 1). The total number of participants was 426 which were divided into 2 group by timing from last meal to ingesting 50 grams glucose. These two groups were: group 1 (last meal intake time less than 2 hours) and group 2 (last meal intake equal to or more than 2 hours). The number of participants were 245 and 181 respectively. Mean age \pm standard deviation (SD) of all participants was 27.5 ± 6.6 years and the mean BMI \pm SD was 24.5 ± 5.8 . Mean gestational age was 21.4 weeks and other data are presented in Table 1. There were no significant different of epidemiological characteristics in both groups.

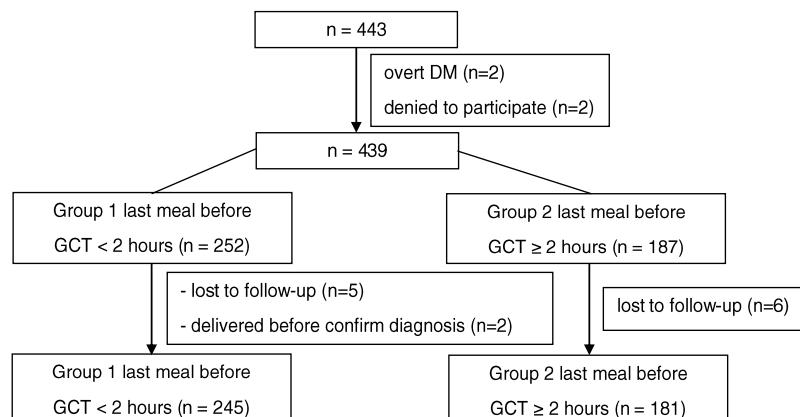


Fig. 1. Flow diagram of identification process for this study.

Table 1. Comparison of epidemiological characteristics between group 1 (time since last meal less than 2 hours) and group 2 (time since last meal equal to or more than 2 hours).

Characteristics	Total (n = 426)	Group 1 (n = 245)	Group 2 (n = 181)	p value
Age (years)	27.5 ± 6.6	27.8 ± 6.8	27.1 ± 6.3	0.31
Primigravida, n (%)	158 (37.1%)	87 (35.5%)	68 (37.6%)	0.676
Gestational age (weeks)	21.4 ± 9.7	21.6 ± 9.5	21.2 ± 9.9	0.694
Weight (kgs)	61.8 ± 14.6	61.9 ± 14.1	61.6 ± 15.3	0.813
BMI (kgs/m ²)	24.5 ± 5.8	24.5 ± 5.5	24.5 ± 6.1	0.741
Category (n, %)				0.408
< 18.5	43 (10.1%)	20 (8.1%)	23 (12.7%)	
18.5 to < 25	221 (51.8%)	131 (53.5%)	90 (49.7%)	
25 to < 30	100 (23.5%)	59 (24.1%)	41 (22.6%)	
30 to < 40	56 (13.2%)	33 (13.5%)	23 (12.7%)	
≥ 40	6 (1.4%)	2 (0.8%)	4 (2.2%)	
Underlying disease				0.31
Hypertension	8 (1.9%)	2 (0.8%)	6 (3.3%)	
Hyperthyroid	4 (0.9%)	3 (1.2%)	1 (0.6%)	
SLE	3 (0.7%)	1 (0.4%)	2 (1.1%)	
Occupation				0.723
Housewife	212 (49.7%)	126 (51.4%)	86 (47.5%)	
Employee	130 (30.5%)	70 (28.5%)	60 (33.2%)	
Merchant	44 (10.3%)	26 (10.7%)	18 (9.9%)	
Government worker	37 (8.9%)	22 (8.9%)	15 (8.3%)	
Other	3 (0.7%)	1 (0.4%)	2 (1.1%)	
Relatives				0.6
First degree relatives	105 (24.6%)	65 (26.5%)	40 (22.1%)	
Second degree relatives	159 (37.3%)	96 (39.1%)	63 (34.8%)	
Previous pregnancy				0.597
Fetal macrosomia	5 (1.2%)	1 (0.4%)	4 (2.2%)	
Fetal anomalies	5 (1.2%)	3 (1.2%)	2 (1.1%)	
Previous GDM	4 (0.9%)	2 (0.8%)	2 (1.1%)	
Preeclampsia	3 (0.7%)	2 (0.8%)	1 (0.6%)	

Data are presented as mean ± standard deviation unless specified otherwise.

BMI: Body Mass Index, SLE: Systemic Lupus Erythematosus, GDM: Gestational Diabetes Mellitus

Fig. 2 and Table 2 demonstrate the primary outcome of this study. The mean of 50 g GCT of all participants was 127.4 mg/dl and 131 participants (30.8%) were GCT positive when used the cut-off point at 140 mg/dl (41.3% when used the cut-off at 130 mg/dl). There was 19.0% form this group had

positive for diagnostic test by using NDDG criteria (26.7% using Carpenter's criteria), meanwhile prevalence of GDM in at risk group from this study was 5.8% (8.2% using Carpenter's criteria).

The positive OGTT (GDM) prevalence was higher in group 2 (time since last meal equal to or more than

2 hours). The GCT false positive was 106 participants from the total GCT screening (24.9%) which was higher in group 1 (time since last meal less than 2 hours) with

statistical significance. The power of study calculated by number of participants and proportion of false positive cases was 0.83 with the alpha error at 0.05.

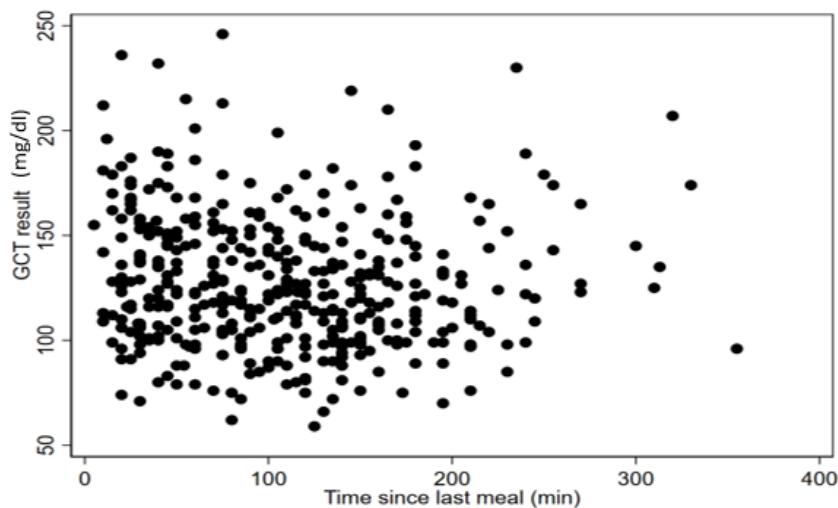


Fig. 2. Distribution of time since last meal (minutes) and 50 grams glucose challenge test (GCT) result (mg/dl).

Table 2. Result of GCT and OGTT between group 1 (time since last meal less than 2 hours) and group 2 (time since last meal equal to or more than 2 hours).

Results	Total (n = 426)	Group 1 (n = 245)	Group 2 (n = 181)	p value
50 grams GCT, mean \pm SD (95%CI)	127.4 \pm 31.7 mg/dl (124.4-130.4)	130.2 \pm 31.9 mg/dl (126.1-134.2)	123.6 \pm 31.1 mg/dl (119.1-128.2)	0.033
Positive GCT, n (%)	131 (30.8%)	85 (34.7%)	46 (25.4%)	0.040
Positive OGTT (GDM), n (%)	25/131 (19.1%)	12/85 (14.1%)	13/46 (28.3%)	0.049
False positive GCT/total GCT	106/426 (24.9%)	73/245 (29.8%)	33/181 (18.2%)	0.006

GCT: glucose challenge test, OGTT: oral glucose tolerance test, SD: standard deviation, CI: confidence interval, GDM: Gestational Diabetes Mellitus

The comparison of false positive GCT to various time since last meal and calories were presented in Table 3. The time since last meal of less than 1 and 2 hours had an effect to a false positive GCT with a risk ratio (RR) 1.72 (1.42-2.86) and 1.63 (1.13-2.35), respectively while 3 hours since last meal had no significant difference for false positive GCT with RR 1.27 (0.77-2.09). The mean calories of last meal intake were 389.8 kcal and the amount of last

meal calories had no significant affect to the false positive GCT ($p = 0.64$). The blood collection time distribution is shown in Fig. 3. The only associated factor with the false positive GCT, which had a p value of less than 0.2, was the period of test (morning or afternoon). The multivariable analysis was done and there was no significant effect with the false positive GCT rate when it was adjusted with the time since last meal (Table 3).

Table 3. Comparison of false-positive result of 50 grams GCT between various time since last meal and calories of meal.

Group and type of meal	Total GCT (n)	False positive GCT (%)	Risk ratio (95%CI)	Adjusted risk ratio (95%CI)	p value
Time since last meal					
< 1 hour	114	41 (36.0%)	1.72 (1.42-2.86)	1.60 (1.14-2.24) ^a	0.006
≥ 1 hour	312	65 (20.8%)			
< 2 hours	245	73 (29.8%)	1.63 (1.13-2.35)	1.53 (1.06-2.22) ^a	0.023
≥ 2 hours	181	33 (18.2%)			
< 3 hours	357	92 (25.8%)	1.27 (0.77-2.09)	1.23 (0.75-2.04) ^a	0.397
≥ 3 hours	69	14 (20.3%)			
Calories of last meal					
Mean ± SD	389.8 ± 188.6	380.3 ± 188.9	1.00 (0.99-1.00)	NA	0.546
Light meal	150	37 (24.7%)	1.01 (0.71-1.43)	NA	0.939
Heavy meal	276	69 (25%)			
Light meal within 2 hours	80	24 (30%)	0.98 (0.66-1.49)	NA	0.961
Heavy meal within 2 hours	165	49 (29.7%)			
Time of GCT (blood collecting time)					
8.00 am to 12.00 pm	296	64 (21.6%)	1.49 (1.07-2.07)	1.36 (0.98-1.91) ^b	0.07
12.01 pm to 16.00 pm	130	42 (32.3%)			

^a adjusted with time of GCT

^b adjusted with time since last meal ≥ 2 hours

GCT: glucose challenge test, CI: confidence interval, SD: standard deviation

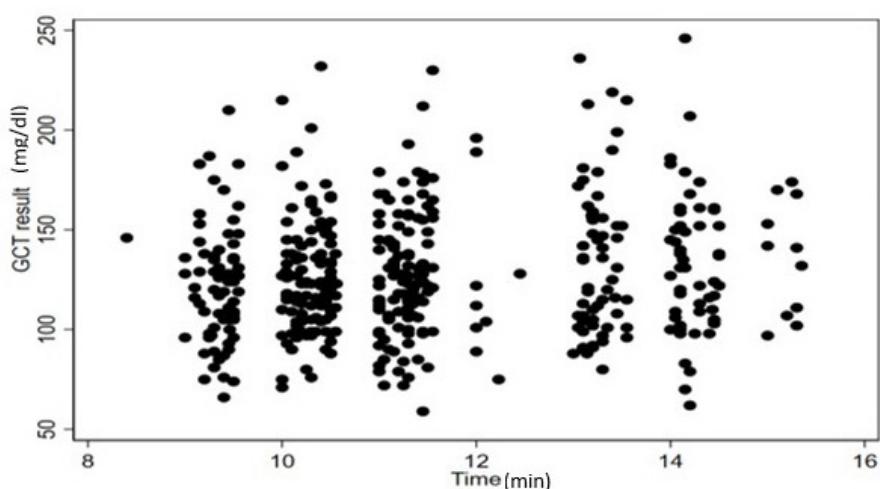


Fig. 3. Distribution between time of blood collection and result of 50 grams glucose challenge test (mg/dl).

Discussion

Gestational diabetes is the most common endocrine problem in pregnancy which has an impact as to pregnancy outcomes. There is still not a satisfactory detection method for this disease⁽¹⁻¹⁰⁾. At present, there is no consensus regarding diagnostic tools between the one and two step methods, although the one step approach has greater proportion of women diagnosed with GDM but outcome of feto-maternal complications such as fetal macrosomia, cesarean section rate, birth trauma, etc. still no significant in both one and two step diagnostic methods⁽⁹⁻¹²⁾. A two-step approach method was applied in various countries for GDM diagnosis with different cut-off times for the 3 hour OGTT by NDDG and Carpenter and Coustan. The Carpenter and Coustan threshold could include a greater amount of pregnant women with GDM implied to a greater detection of feto-maternal complications^(32, 33) but absence of clear comparative trials. Due to unclear benefit between Carpenter and NDDG cut-off, so our hospital decided to use NDDG cut-off in clinical practice. The two steps approach method could be applied to non-fasting pregnant woman that is more facilitative to use in various countries including Thailand^(1, 34).

This study showed: first, GDM prevalence was 5.8% which was compatible with the previous studies which reported prevalence between 1.5-9.3%⁽³⁻⁷⁾. Second, false positive GCT prevalence was 24.9% which was similar to a previous report of between 8.8%-37.4%^(19, 20, 35). The prevalence increased if the Carpenter's criteria was used for diagnosis. The wide range for GDM prevalence and false positive GCT prevalence from previous studies were also due to the different diagnostic methods and GCT cut-off points, GDM detection and diagnosis^(9, 10, 34, 36, 37).

The results from this study found that the time since last meal had an impact on the blood glucose level after a 50g GCT and a false positive GCT test (positive GCT but negative 100 g OGTT) especially when a last meal within 1 hour and 2 hours with RR 1.72 (1.42-2.86) and 1.63 (1.13-2.35), respectively. The results were comparable with previous studies by

Sermer et al⁽²⁵⁾ and Cetin et al⁽²⁶⁾ who had reported the impact of time since last meal had an effect on mean plasma glucose. These former prior studies suggested the new cut-off level of 50 grams GCT if the time since last meal was < 2 hours in order to increase the results positive predictive value and specificity.

There were some previous studies about the effects of the period of the day, especially in the afternoon and at night, could decrease blood glucose GCT due to maternal metabolism and β -cell function^(27, 28). In contrast, Wong et al⁽³⁷⁾ and McElduff et al⁽³⁸⁾ reported that the afternoon GCT had higher positive rate but lower rate of GDM diagnosis due to cortisol metabolism. Data from this study found that the afternoon GCT (12.00 pm -16.00 pm) after the adjusted effect of time since last meal had no effect on the false positive GCT result. The calories of meal in this study had also less effect to glucose level of GCT which was compatible with a study that found a diet with low, medium, or high glycemic index had no effect on the GCT result⁽³⁹⁾.

The strength of this study were: first, a prospective observational cohort study which had the strength of potential relationship between exposure and outcome. Second, although the step approach to GDM diagnosis is one of the methods widely used, there are few studies about the factors for false positive in this test. This study found the factor of food and time since last meal to affect the false positive GCT result. Third, from the previous studies review this topic was the first study, which focused on the time and type of meal to false positive effect of GCT and last, the result of this study is easy to advice and apply for practical use.

There had some limitations with this study: first, recall bias, because even this study was a prospective cohort study, the information from participants about the previous meal and time intake might not be precise information. Second, the definition of light and heavy meals was applied from other countries (due to a lack of a Thai classification), thus the difference in food type might have some misclassification. Third, the risk approach screening GCT was used in this study because it is the routine practice of our center and many other centers in Thailand. A higher rate of GDM is assumed if routine GCT screening is performed.

Finally, there were lack of information of the effect of false positive GCT to the pregnancy outcome in this study. The long-term study is needed to access this effect.

Conclusion

Time since last meal had an impact to the false positive GCT result. A Time interval of more than 2 hours before the 50 grams GCT is suggested to avoid the unnecessary investigation for GDM screening.

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Potential conflicts of interest

The authors declare no conflicts of interest.

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