GLYCEMIC VARIABILITY AND ITS ASSOCIATED FACTORS AMONG GESTATIONAL DIABETES MELLITUS PATIENTS IN KOTA BHARU, KELANTAN

DR. NOORMAZITA BINTI HASSAN

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF MEDICINE

(FAMILY MEDICINE)

SCHOOL OF MEDICAL SCIENCES

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ABSTRAK

Pengenalan: Variasi paras gula merupakan perubahan paras gula yang berlaku dalam tempoh tertentu disebabkan oleh perubahan fisiologi dan hormon yang terlibat dalam metabolisma gula. Di kalangan wanita mengandung yang menghidap kencing manis, kajian- kajian menunjukkan peningkatan variasi paras gula sebagai peramal yang kuat untuk mendapat kencing manis jenis 2 dan komplikasi lain kepada kedua-dua bayi dan ibu. Tujuan kajian ini adalah untuk menentukan variasi di dalam paras gula dan mengenalpasti faktor-faktor yang berkait dengan variasi paras gula di kalangan ibu mengandung yang menghidap kencing manis di Kota Bharu Kelantan.

Metodologi: Kajian ini merupakan kajian hirisan lintang paparan rekod secara retrospektif yang melibatkan 222 pesakit mengandung yang telah didiagnosis dengan kencing manis pada trimester kedua dan ketiga sama ada menerima rawatan ubat atau terapi diet di Klinik Kesihatan Bandar Kota Bharu Kelantan. Faktor-faktor sosiodemografik, perihal kehamilan dan kriteria klinikal dikenalpasti daripada buku ibu mengandung KIK/1(b)/96 yang berada di dalam simpanan klinik. 4 bacaan catatan gula diambil dari profil gula dalam darah dianalisa dari rekod yang sama dan variasi paras gula ditentukan menggunakan sisihan piawai daripada purata profil gula dalam darah untuk keseluruhan 32 bacaan gula dalam darah. Data seterusnya dianalisa menggunakan regresi linear mudah dan berganda untuk mengesan perkaitan di antara faktor-faktor sosiodemografik, perihal kehamilan dan kriteria klinikal kepada variasi paras gula. Seterusnya, regresi logistic mudah dan berganda digunakan untuk melihat perkaitan di

antara variasi paras gula dan keputusan ulangan ujian air gula yang dilakukan selepas bersalin MOGTT.

Keputusan: Seramai 222 paparan rekod pesakit terlibat dalam kajian ini. Variasi paras gula yang didapati adalah 0.71mmol/l. Faktor perihal kehamilan iaitu status pesakit mengandung yang mempunyai kencing manis di dalam rawatan mempunyai perkaitan dengan variasi paras gula. Seterusnya, variasi paras gula dibuktikan ada perkaitan dengan keputusan ulangan ujian air gula yang dilakukan selepas bersalin MOGTT dengan keputusan p= 0.002 (odds ratio [OR] = 6.0; 95% CI = 1.891,19.39).

Kesimpulan: Variasi paras gula di dalam kajian ini menunjukkan bacaan yang rendah berbanding kajian-kajian terdahulu. Walaubagaimanapun, kajian ini masih dapat merumuskan bahawa wanita mengandung yang menghidap kencing manis di dalam rawatan berisiko untuk mendapat variasi paras gula yang lebih tinggi seterusnya boleh mendatangkan komplikasi kepada kedua-dua ibu dan bayi yang dikandungkan. Malah, variasi paras gula yang tinggi ini juga akan memberi impak kepada keputusan ulangan ujian air gula MOGTT yang dilakukan 6 minggu selepas kelahiran. Oleh dengan itu, pengesanan dan pencegahan awal harus dilakukan untuk mengelakkan risiko mereka mendapat kencing manis kekal di kemudian hari.

GLYCEMIC VARIABILITY AND ITS ASSOCIATED FACTORS AMONG GESTATIONAL DIABETES MELLITUS PATIENTS IN KOTA BHARU KELANTAN

ABSTRACT

Introduction: Glycemic variability (GV) is defined as changes in glycemic control as a consequence of the physiological and circadian rhythm of hormones involved in glucose metabolism. As stated in few studies increase GV among gestational diabetes mellitus (GDM) patients will impose a risk to both mother and fetus as well as risk of developing type 2 diabetes mellitus. The aim of this study was to determine GV and its associated factors among GDM in Kota Bharu Kelantan.

Methodology: A cross-sectional study with retrospective record review was conducted among 222 patients who had been diagnosed with GDM in the second or third trimester and either on diet or medication in Klinik Kesihatan Bandar Kota Bharu Kelantan. The socio-demographic, obstetrics and clinical characteristics were obtained from the antenatal book KIK/1(b)/96 that were available in the clinic. Four points blood sugar profile (BSP) also had been analyzed and been assessed for GV using standard deviation (SD) of mean of total 32 readings of BSP. Data further was analyzed using simple and multiple linear regression to look for associated factors for glycemic variability. Furthermore, simple and multiple logistic regression was used to look for an association between GV to post-partum MOGTT.

Result: There were 222 record views involved in this study. The GV calculated was 0.71mmol/l. GDM patients which were on treatment found to have associated with GV. There was an association between GV and postpartum MOGTT result with the $p= 0.002$ (odds ratio [OR] = 6.0; 95% CI = 1.891,19.39).

Conclusion: The GV in this study was low compared to previous study done. This study shows that if patient is known to have GDM and is receiving treatment, they may have high GV during their current pregnancy which further may lead to complications for both mother and fetus. In addition, high GV during the current pregnancy would predispose GDM patients to abnormal MOGTT results six weeks after delivery. Therefore, early recognition and intervention are necessary to avoid the risk of developing type 2 diabetes mellitus in the future.

CHAPTER 1

INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as carbohydrate intolerance with the onset or first recognition during pregnancy period [1]. According to Clinical Practice Guideline (CPG) of Management of Diabetes in Pregnancy, GDM is diagnosed when 75 g oral glucose tolerance test (OGTT) showed a venous fasting plasma level of ≥5.1mmol/L after an overnight fast and at least 7.8mmol/L at two hours. GDM is significant risk factor for the development of type 2 diabetes mellitus, as stated by [2] which 35-60% of women with history of GDM will develop type 2 diabetes mellitus within ten years. Gestational diabetes mellitus imposes a risk to both mother and fetus including higher risks of spontaneous miscarriage, preterm labor, and cesarean section [3]. Among the infant of GDM mothers, they are at higher risk of macrosomia, low Apgar score, need for intensive care unit admission, hypoglycemia, congenital malformation and respiratory distress syndrome (RDS) [4]. The prevalence of GDM is highest among Asians and especially among Indian Asians [5]. In Malaysia, the prevalence of GDM in the general population is 11.6%, according to National Obstetric Registry, which showed higher in Indians compared to other ethnic groups [6].

Meanwhile, glycemic variability (GV) is defined as changes in glycemic control as a consequence of the physiological and circadian rhythm of hormones involved in glucose metabolism[7]. It refers to swings in blood glucose levels, throughout the day, including hypoglycemic periods and postprandial increases, as well as blood glucose fluctuations that occur at the same time on different days [8]. It is one of the possible factors in the etiology of complication of diabetes as few studies showed that increased glycemic variability in diabetes

mellitus plays an important role in the development of microvascular and macrovascular complications [9]. Therefore, it is very important to calculate the glucose variability among GDM mothers as it will give a huge association of complications to both mother and child [10]. Currently, several specific methods have been developed to evaluate within day blood glucose variation. Among these measures are the mean amplitude of glucose excursions (MAGEs), mean of daily differences (MODDs), meal related glycaemic excursions [mean indices of meal excursions (MIMEs)], standard deviation of blood glucose (SDBG), the M-value [which is a quantitative measure of the deviation of several blood glucose values in a specified time period from an arbitrarily selected point (e.g. 5.0 mmol/l)] and the risk of severe hypoglycaemia [measured by the low blood glucose index (LBGI)] [11].

Few methods that have been used to characterize glycemic variability and one from it is continuous glucose monitoring (CGM) as well as conventional method via self-monitoring blood glucose (SMBG) [12]. In practice, each of these tools requires different methods for their use; while some are easy to apply, others are more complex and not practical for clinical use. Today, there is no consensus regarding preferred measures of glycaemic variability; each has its own advantages and disadvantages [13]. In this study, we are using the conventional method using SMBG which portrays as blood sugar profile (BSP) as it is widely used in Malaysia and is done practically either by patients itself or in clinic by trained personnel using glucometer. BSP provides information regarding blood sugar value at different times, thereby enabling identification of pattern and tailoring of the treatment regimens to the individual requirement [14].

Factors associated with GV in GDM patients are multifactorial. Each region shows in differences in sociodemographic characteristics; thus, the associated factors vary worldwide. Since little is known regarding factors affecting glycemic variability in GDM patient in local setting, identification of it may help clinicians especially in primary setting to devise strategies

and intervention that are relevant to local practice. In this study, factors include age, employment status, ethnic group and education level under sociodemographic data had been discussed. One study in Canada proved that GV has association with GDM older than 35 years old[15] and another study in Iran and Bahrain identified women's age as one of the risk factors for GV in GDM[16]. In addition, having low education level also been proved to have association with GV among GDM [17].Factors under obstetrics and clinical characteristics include parity, family history of diabetes mellitus, gestational age at diagnosis of GDM, gestational weight gain, previous history of GDM and booking BMI also been discussed in this study. A family history of diabetes in the first-degree relatives is one of the major risk factors for development of GDM and among GDM, study by *Martina et al* showed correlation between GV among GDM mother with family history of DM[18]. Glycemic variability also correlated with pre-pregnancy BMI. Higher BMI before pregnancy and excessive maternal weight gain correlated positively with GV among GDM mother[18]. Women with a history of GDM appear to have nearly 10-fold higher risk of developing type 2 DM[19]and proved to have association with GV during the pregnancy period[20]. Glycemic variability was shown to have association with post-partum MOGTT, study done in China shows there is significant association between GV to post-partum MOGTT[21].

References

1. Jovanovic L, Pettitt DJ. Gestational diabetes mellitus. JAMA. 2001;286(20):2516-8.

2. Metzger BE, Buchanan TA, Coustan DR, de Leiva A, Dunger DB, Hadden DR, et al. Summary and recommendations of the Fifth International Workshop-Conference on Gestational Diabetes Mellitus. Diabetes Care. 2007;30 Suppl 2(Supplement 2):S251-60.

3. Green JR, Pawson IG, Schumacher LB, Perry J, Kretchmer N. Glucose tolerance in pregnancy: Ethnic variation and influence of body habitus. American Journal of Obstetrics and Gynecology. 1990;163(1, Part 1):86-92.

4. Gonzalez-Quintero VH, Istwan NB, Rhea DJ, Rodriguez LI, Cotter A, Carter J, et al. The impact of glycemic control on neonatal outcome in singleton pregnancies complicated by gestational diabetes. Diabetes Care. 2007;30(3):467-70.

5. Chu SY, Abe K, Hall LR, Kim SY, Njoroge T, Qin C. Gestational diabetes mellitus: all Asians are not alike. Prev Med. 2009;49(2-3):265-8.

6. Yong HY, Mohd Shariff Z, Rejali Z, Mohd Yusof BN, Yasmin F, Palaniveloo L. Seremban Cohort Study (SECOST): a prospective study of determinants and pregnancy outcomes of maternal glycaemia in Malaysia. BMJ Open. 2018;8(1):e018321.

7. Frontoni S, Di Bartolo P, Avogaro A, Bosi E, Paolisso G, Ceriello A. Glucose variability: An emerging target for the treatment of diabetes mellitus. Diabetes Res Clin Pract. 2013;102(2):86-95.

8. Suh S, Kim JH. Glycemic Variability: How Do We Measure It and Why Is It Important? Diabetes Metab J. 2015;39(4):273-82.

9. Smith-Palmer J, Brandle M, Trevisan R, Orsini Federici M, Liabat S, Valentine W. Assessment of the association between glycemic variability and diabetes-related complications in type 1 and type 2 diabetes. Diabetes Res Clin Pract. 2014;105(3):273-84.

10. Panyakat WS, Phatihattakorn C, Sriwijitkamol A, Sunsaneevithayakul P, Phaophan A, Phichitkanka A. Correlation Between Third Trimester Glycemic Variability in Non-Insulin-Dependent Gestational Diabetes Mellitus and Adverse Pregnancy and Fetal Outcomes. J Diabetes Sci Technol. 2018;12(3):622-9.

11. Nalysnyk L, Hernandez-Medina M, Krishnarajah G. Glycaemic variability and complications in patients with diabetes mellitus: evidence from a systematic review of the literature. Diabetes Obes Metab. 2010;12(4):288-98.

12. Yu F, Lv L, Liang Z, Wang Y, Wen J, Lin X, et al. Continuous glucose monitoring effects on maternal glycemic control and pregnancy outcomes in patients with gestational diabetes mellitus: a prospective cohort study. J Clin Endocrinol Metab. 2014;99(12):4674-82.

13. Guerci B. [Asymptomatic glycemic instability: how to measure it and which clinical applications?]. Diabetes Metab. 2003;29(2 Pt 1):179-88.

14. Dalfra MG, Sartore G, Di Cianni G, Mello G, Lencioni C, Ottanelli S, et al. Glucose variability in diabetic pregnancy. Diabetes Technol Ther. 2011;13(8):853-9.

15. Carolan M, Davey MA, Biro MA, Kealy M. Maternal age, ethnicity and gestational diabetes mellitus. Midwifery. 2012;28(6):778-83.

16. Bakiner O, Bozkirli E, Ozsahin K, Sariturk C, Ertorer E. Risk Factors That can Predict Antenatal Insulin Need in Gestational Diabetes. J Clin Med Res. 2013;5(5):381-8.

17. Ogonowski J, Miazgowski T. The prevalence of 6 weeks postpartum abnormal glucose tolerance in Caucasian women with gestational diabetes. Diabetes Res Clin Pract. 2009;84(3):239-44.

CHAPTER 2

OBJECTIVE

General objective

To determine the glycemic variability and its associated factors among GDM patients in Klinik Kesihatan Bandar, Kota Bharu Kelantan

Specific objectives

1. To determine the glycemic variability among GDM patients in Klinik Kesihatan Bandar, Kota Bharu Kelantan.
2. To determine the associated factors of glycemic variability among GDM patients in Klinik Kesihatan Bandar, Kota Bharu Kelantan.
3. To determine the association between postpartum MOGTT and glycemic variability among GDM patients in Klinik Kesihatan Bandar, Kota Bharu Kelantan.

Operative Definition

1. Gestational diabetes mellitus is diagnosed when 75g oral glucose test (OGTT) showed a venous fasting plasma level of ≥5.1mmol/L after an overnight fast and at least 7.8mmol/L at two hours according to CPG of Management of Diabetes in Pregnancy.

2. Glycemic variability is defined as changes in glycemic control because of the physiological and circadian rhythm of hormone involved in glucose metabolism. In this study, it is calculated using formula of the standard deviation (SD) of the mean of 32 readings of blood sugar profile.

3. Blood sugar profile (BSP) consist of three times pre meal glucose level (prebreakfast, prelunch and predinner) and one prebed glucose level measured using glucometer machine. It is measured in clinic setting every two or four weeks depending either on diet control or medication. In this study, 4 points BSP is measured according to CPG which are prebreakfast, 2hours post breakfast, 2 hours post lunch and 2hours post dinner.

4. Postpartum MOGTT result is a measurement of MOGTT taken after 6 weeks post-delivery.

CHAPTER 3

MANUSCRIPT

TITLE PAGE:

GLYCEMIC VARIABILITY AND ITS ASSOCIATED FACTORS AMONG GESTATIONAL DIABETES MELLITUS PATIENTS IN KOTA BHARU KELANTAN: A RETROSPECTIVE RECORD VIEW

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Ethics approval
This study was approved by the Universiti Sains Malaysia Human Research Ethics Committee (USM/JEPeM/20060332) and by MREC NMRR ID: NMRR-20-2983-55121.

Conflicts of interest
The authors declare no conflicts of interest which might compromise the professional judgment in conducting or reporting this study.

Abstract

Introduction: Gestational diabetes mellitus (GDM) is associated with many adverse obstetric outcomes and neonatal outcomes. Studies have shown that glycemic variability (GV) can cause a series of adverse reactions, and good control of GV can reduce the incidence of adverse pregnancy outcomes. The aim for this study was to determine GV and its associated factors among GDM in Kota Bharu Kelantan.

Methods: A cross-sectional study with retrospective record review was conducted among 222 GDM patients in Klinik Kesihatan Bandar Kota Bharu on. The socio-demographic, obstetrics and clinical characteristics were obtained from the clinic's antenatal book KIK/1(b)/96 through randomized sampling method. GV was determined using standard deviation of mean blood sugar profile (BSP). Data was analyzed using simple and multiple linear regression to look for associated factors and using simple and multiple logistic regression to look for association between glycemic variability to post-partum MOGTT.

Result: The glycemic variability of this study was 0.71mmol/l. It was calculated using formula standard deviation (SD) of mean of 32 readings of BSP. Patients with GDM on treatment was significantly associated with glycemic variability (Adj. RC= 5.1; 95% CI=2.86,9.23; *p<0.001*). There is a significant association between glycemic variability and post-partum MOGTT p= 0.002 (odds ratio [OR] = 6.0; 95% CI = 1.891,19.39).

Conclusion: GV in this study is low. Patients on treatment is significantly associated with GV. There is a significant association between GV and post-partum MOGTT.

Keywords: gestational diabetes mellitus, glycemic variability, post-partum MOGTT

Introduction

Gestational diabetes mellitus (GDM) is defined as carbohydrate intolerance with the first onset or first recognition during pregnancy period[1]. According to Clinical Practice Guideline (CPG) of Management of Diabetes Mellitus in Pregnancy 2017, GDM is diagnosed when 75g oral glucose test (OGTT) showed a venous fasting plasma level of ≥5.1 mmol/L after an overnight fast and at least 7.8mmol/L at two hours. In Malaysia, the prevalence of GDM in general population is 11.6% according to National Obstetrics Registry which showed higher in Indian population compared to other ethnic groups. Besides, GDM is significant risk factors for development of type 2 diabetes mellitus, as stated by *Metzger BE et* al which 35%-60% of women with history of GDM will develop type 2 diabetes mellitus within 10 years[2]. GDM generally imposes a risk to both mother and fetus including higher risks of spontaneous miscarriage, preterm labour and caesarean section [22]. Among the infants of GDM mothers, they are at higher risk of macrosomia, low Apgar score, need for intensive care admission, hypoglycemia, congenital malformation and respiratory distress syndrome. Therefore, it is very important to control GDM which is mainly done through monitoring the blood sugar profile (BSP).

BSP is a translation from self-blood glucose monitoring (SMBG) that is widely used among diabetic patients and it is done practically either by patients themselves or in the clinic by the

trained personnel using a glucometer [23]. In GDM, BSP is a 'snapshot' of blood glucose fluctuations throughout the day, and it is measured either by 4-points BSP or 7-points BSP.

According to Clinical Practice Guideline (CPG) of Management of Diabetes in Pregnancy 2017, 7-points BSP include measurement of blood glucose at pre-breakfast, post breakfast, pre-lunch, post lunch, pre-dinner, post dinner and pre-bed. While the 4-points BSP measures the blood glucose at pre-breakfast, pre-lunch, pre-dinner and pre-bed. The timing for BSP is flexible pertaining to the patient's meal schedule as long as it contains all three main meals and post prandial can be done either at one hour or two hours post prandial. Based on the CPG, the blood glucose targets should be as the following which is fasting or preprandial: ≤5.3 mmol/L or 1 hour postprandial: ≤7.8 mmol/L or 2 hours postprandial: ≤6.7mmol/L. The frequency of SMBG should be individualized based on mode of treatment and glycemic control.

Meanwhile, glycemic variability (GV) is defined as changes in glycemic control as a consequence of the physiological and circadian rhythm of hormones involved in glucose metabolism. It refers to swings in blood glucose levels, throughout the day including hypoglycemic periods and postprandial increases, as well as blood glucose fluctuations that occur at the same time on different days [8]. Glycemic variability is currently defined by a large and increasing number of metrics, which represent either short-term (within-day and between-day variability) or long-term glycemic variability, which is usually based on serial measurements of HbA1c or other measures of glycaemia over a longer period of time[24].Currently, several specific methods have been developed to evaluate within day blood glucose variation. Among these measures are the mean amplitude of glucose excursions (MAGEs), mean of daily differences (MODDs), meal related glycemic excursions [mean indices of meal excursions (MIMEs)], standard deviation of blood glucose (SDBG), the M-value [which is a quantitative measure of the deviation of several blood glucose values in a specified time period from an arbitrarily selected point (e.g. 5.0 mmol/l)] and the risk of severe hypoglycaemia [measured by the low blood glucose index (LBGI)] [11]. In diabetic management, GV plays as an emerging target as in addition to hbA1c and fasting plasma glucose (FPG). GV is a component of dysglycemia that may contribute independently to the pathogenesis of chronic diabetes complications. Studies investigating the relationship between GV, and diabetic macrovascular complications showed that elevated GV independently predicted the risk of one-year major adverse cardiac events in patients after acute myocardial infarction, whereas HbA1c did not[25].

Few methods that have been used to characterize GV variability and one from it is continuous glucose monitoring (CGM) as well as conventional method via self-monitoring blood glucose (SMBG) [12]. In practice, each of these tools requires different methods for their use; while some are easy to apply, others are more complex and not practical for clinical use. Today, there is no consensus regarding preferred measures of glycemic variability; each has its own advantages and disadvantages [13]. In this study, the conventional method using SMBG which portrays as blood sugar profile (BSP) has been used as it is widely used in Malaysia and is done practically either by patients itself or in clinic by trained personnel using glucometer. BSP provides information regarding blood sugar value at different times, thereby enabling identification of pattern and tailoring of the treatment regimens to the individual requirement [14].

It is very important to calculate GV in GDM because a higher variability can predict the outcome and complications for both mother and child. There are many factors associated with glycemic

variability in GDM patients. Each region shows difference in sociodemographic characteristics and clinical backgrounds; thus, the associated factors vary worldwide[26]. Sociodemographic factors and obstetric factors have shown significant association with glycemic variability in many studies[14, 18]. In local setting, identification of it may help clinicians especially in primary care setting to devise strategies and intervention that are relevant to clinical practice. Therefore, this study mainly to determine the glycemic variability and its associated factors among gestational diabetes mellitus patients.

Methods

This is a cross-sectional study with retrospective record review conducted in Klinik Kesihatan Bandar Kota Bharu (KKBKB) Kelantan from 1 January 2020 to 15 January 2021. The obstetric data record, blood sugar profile and post-partum MOGTT result had been retrieved and reviewed from the clinic's antenatal card (Rekod Kesihatan Ibu KIK/ 1(b)/96) through randomized sampling method. It involved GDM patients who attended KKBKB. The inclusion criteria include those who has diagnosed with gestational diabetes mellitus at second trimester or third trimester and either on diet control or medication. Patients who established diagnosis of diabetes mellitus or having other comorbidities such as human immunodeficiency virus (HIV), blood disorder or autoimmune disease were excluded from this study. The incomplete or missing BSP data from the antenatal book also was excluded from this study. The sample size of 222 calculated using single mean formula.

Study tools

The clinical research tool used in this study consisted of a case report form, generated from the Rekod Kesihatan Ibu KIK/ 1(b)/96. The case report form consisted of two sections: Section 1 included socio-demographic information and patients' clinical data (age, ethnicity, parity, level of education, employment status, family history of GDM, previous history of GDM, pre pregnancy BMI, type of GDM, gestational age at the diagnosis of GDM, total weight gain and post-partum MOGTT result). While section 2 included patient's BSP throughout pregnancy period. The BSP result is monitored in 4-points BSP; pre-breakfast, pre-lunch, pre-dinner and pre-bed started from 14weeks of gestation until delivery. For each sample, there were total of 8 readings of BSP retrieved from the record. Out of 222 samples, only 48 GDM patients or 22% did BSP monitoring by themselves at home while the rest of 174 GDM patients or 78% did BSP in clinic setting. The GV is calculated based on standard deviation (SD) of the mean of their 32 readings of BSP.

Data analysis

The formula of SD of mean of 32 BSP readings is used to determine GV. Simple and multiple linear regression is used to look for associated factors. Confirmatory analysis multiple logistic regression (MLR) is used to look for association between GV to post-partum MOGTT.

The research design was approved by the Human Ethical Committee of the School of Medical Science, Universiti Sains Malaysia USM/JEPem/20060332.

Results

i) Glycemic variability of GDM patient
The GV calculated was 0.71 (SD 0.26) mmol/l. it was calculated using formula SD of mean of 32 reading of BSP readings retrieved from the antenatal record GDM patients.

ii) Sociodemographic and clinical characteristics of GDM patient
Of the reviewed antenatal records, 222 (100%) were completed in proforma and hence included in the analysis. The characteristics of pregnant women in this study are presented in Table 1. The majority of participants were Malay (n=200, 90.1%), were aged below 35 years (n=138, 62.2%), were unemployed (n=124, 55.9%) and had a low education level (n=135, 60.8%). The mean of age of participants is 33 years old. Most were multiparous (n=130, 58.6%) and been overweight or obese before pregnant (n=198, 88.3%). With regards to the obstetrics factors, majority of participants had previous history of GDM (n=196, 88.3%) and has background family history of diabetes mellitus (n=165, 74.3%). Most GDM patients were on diet control (n=137, 61.7%) compared to those on treatment either oral medication or insulin. Mean gestational age at diagnosis of GDM was 22 weeks of gestation and total weight gain until the diagnosis of GDM was 6 kg from the booking weight. All participants did MOGTT at 6-weeks post-partum and 119 participants or 53.6% were had abnormal MOGTT results.

Table 1. Sociodemographic, obstetrics and clinical characteristic (n = 222)

Variable	Mean (SD)	n (%)
Age (years)		
Less than 35		138 (62.2)
35 and more		84 (37.8)
Ethnicity		
Malay		200 (90.1)
Non-Malay		22 (9.9)
Parity		
<2		47 (21.1)
2-5		130 (58.6)
>5		45 (20.3)
Education level		
Low (primary school)		135 (60.8)
High (secondary school above)		87 (39.2)
Employment status		
Yes		98 (44.1)
No		124 (55.9)
GDM status		
Diet control		137 (61.7)
Treatment (oral medications/insulin)		85 (38.3)

Family history of DM		
Yes		165 (74.3)
No		57 (25.7)
Previous history of GDM		
Yes		196 (88.3)
No		26 (11.7)
Pre-pregnancy BMI (kg/m^2)	28.16 (4.80)	
Overweight/obese		198 (89.2)
Not overweight/obese		24 (10.8)
Gestational age at diagnosis (week)	22.09 (5.54)	
Total weight gain until diagnosis (kg)	6.00 (14.20)[a]	
Postpartum MOGTT 6 weeks		
Normal		103 (46.4)
Abnormal		119 (53.6)

a: Median (IQR)

iii) Factor associated with GV among GDM patient

Table 2 shows the association the sociodemographic factors, clinical and obstetrics factors to GV in GDM patients. Using simple linear regression analysis, 11 independent variables including age, ethnicity, education level, employment status, GDM status, family history of DM, previous history of GDM, pre pregnancy BMI, gestational age at diagnosis, total weight gain and post-partum MOGTT involved in the analysis. Multiple linear regression showed that only the variable of GDM status had significant association with GV with *p<0.001* (Adj. RC= 5.1; 95% CI=2.86,9.23; *p<0.001*) The model explains 14% of variation of the glycemic variability in the study sample (R^2: 0.14).

Table 2 Associated factors for GV among GDM using simple and multiple linear regression analyses (n=222)

Variable	Simple Linear Regression				Multiple Linear Regression			
	Crude RC	(95% CI)	χ^2 Stat. (df)[a]	p value[a]	Adjusted RC	(95% CI)	χ^2 Stat. (df)[a]	*p value*[a]
Sociodemographic Factors								
Age (years)								
35 and more	1.13	(0.510,	0.194 (1)	0.659				
Less than 35	1.00	1.532)						
Ethnicity								
Malay	0.817	0.337-1.980	0.199 (1)	0.654				
Non-Malay	1.000							
Education level								
Low	1.027	0.594-1.775	0.009 (1)	0.925				
High	1.000							
Employment status								
No	1.093	0.637-1.874	0.104 (1)	0.748				
Yes	1.000							
Obstetric And Clinical Characteristics								
GDM status								
Treatment	5.144	2.864-9.236	32.331 (1)	<0.001	5.144	2.864-9.236	32.331 (1)	**<0.001**
Diet control	1.000				1.000			
Family history of DM								
Yes	0.940							
No	1.000	0.510-1.731	0.039 (1)	0.843				

Previous history of GDM				
Yes	0.788			
No	1.000			
		0.346-1.792	0.321 (1)	0.570
Pre-pregnancy BMI (kg/m^2)	1.041	0.984-1.101	1.970 (1)	0.162
Gestational age at diagnosis (week)	0.954	0.908-1.002	3.565 (1)	0.061
Total weight gain until diagnosis (kg)	0.942	0.846-1.050	1.174 (1)	0.282

[c] Crude regression coefficient
[d] Confidence Interval
[e] *t statistic*
[f] Adjusted regression coefficient
MLR: Forward and backward method applied. R^2=0.14. The model reasonably fits well; Model assumption are met. There is no interaction between independent variables, and no multicollinearity problem. 2 variables of pre pregnancy BMI and gestational age of diagnosis cannot fit into MLR since R^2 0.14 which is very small.

In table 3, simple and multiple logistic regression analysis was used to check the association between GV and post-partum MOGTT. Multiple logistic regression showed post-partum MOGTT has significant association to GV, p= 0.002 (odds ratio [OR] = 6.0; 95% CI = 1.891,19.39) where other confounders were controlled including pre-pregnancy BMI, family history of DM and previous history of GDM.

Table 3: The association between postpartum MOGTT and glycemic variability using multiple logistic confirmatory analyses.

Variable	Simple Logistic Regression				Multiple Logistic Regression					
	Crude OR	(95% CI)	χ^2 Stat. (df)[a]	*p value*	B	S.E	Wald	Exp (B)	(95% CI)	p value
Glycemic variability	6.15	1.931, 19.622	10.777	0.002	1.801	0.594	9.198	6.056	1.891-19.394	**0.002**

Discussion

Sociodemographic and clinical characteristics of GDM patient

The majority of participants were Malay (n=200, 90.1%), were aged below 35 years (n=138, 62.2%), were unemployed (n=124, 55.9%) and had a low education level (n=135, 60.8%). The mean of age of participants is 33 years old. Most were multiparous (n=130, 58.6%) and been overweight or obese before pregnant (n=198, 88.3%). With regards to the obstetrics factors, majority of participants had previous history of GDM (n=196, 88.3%) and has background family history of diabetes mellitus (n=165, 74.3%). Most GDM patients were on diet control (n=137, 61.7%) compared to those on treatment either oral medication or insulin. Mean gestational age at diagnosis of GDM was 22 weeks of gestation and total weight gain until the diagnosis of GDM was 6 kg from the booking weight. All participants did MOGTT at 6-weeks post-partum and 119 participants or 53.6% were had abnormal MOGTT results.

Glycemic variabililty

Studies about glycemic variability (GV) are gaining more attention among the researchers. In the recent few years majority of the studies were done using the continuous glucose monitoring (CGM) [8]. There are few methods to calculate GV, for example by using self-monitoring blood glucose (SMBG) [27] and the more convenient way was by using blood sugar profile (BSP) especially in the community where many patients cannot afford to have a glucometer at home. However, thus far, no study had yet used the BSP as a tool to calculate GV among the GDM patients. This study used secondary data which were available in the BSP chart of patient, even patient did comply to the 4 points BSP, it is considered wide range for proper variability. The GV value of this studied population was 0.66 (IQR 0.29) mmol/l which is smaller than previous findings, where calculated GV among the population were 2.9-3.0mmol/L [28]. Study by *Umpierrez et al* showed that GV calculated using CGM was 2.9mmol/L. By using CGM, it measures interstitial fluid every 10 seconds and an average blood glucose value every five minutes 24 hours a day which give accuracy as compared to BSP[28]. By using BSP, data collection time was not controlled and open to bias based on mealtime and diet taken as well as the type of activities they participate prior to the BSP blood collection. All these factors may contribute to different result of BSP [29]. Furthermore, using BSP may have inaccurate self-reported which heavily rely on the patient's compliance. Study by *Boris et al* also showed the GV value of 0.61mmol/L, similar to this study and used BSP to calculate GV[30]. BSP still one of the mainstays of modern diabetes management. It is widely used and easy to be practiced by patients. There is a need for more frequent glucose monitoring, improve patient's compliance hence increase in the accuracy in reported data.

Associated factors for GV

Status of GDM on treatment was found to be significant in this study which has association with GV. This finding is consistent with *Czupryniak L et al* which reported that GDM patients on treatment especially insulin have high GV and mainly in third trimester[31]. Another study in Japan showed that GDM on treatment have higher GV as the fluctuation of glucose is variable and wider as compared to GDM on diet control [32]. It was contributed to positively to treatment as clinical practitioner and patient both more aware on hypoglycemic and hyperglycemia episodes, so as to moderate the strategies of their treatment by altering dosage and frequency[27].

Other associated factors that proved to have significant association with GV in other study include age older than 35 years old, previous history of GDM, previous family history of DM

and booking BMI. The factor of family history of DM is strongly associated to risk of type 2 diabetes itself [33], thus lifestyle changes, diabetes screening and awareness need to be emphasized. In one cohort study done in Japan [34] found there was an association among those with strong family history of diabetes in first degree relative to the glycemic variability. Thus, it is very crucial to maintain a constant level of glucose in non-diabetic people to prevent complication later. GV also correlated with pre-pregnancy BMI. Higher BMI before pregnancy correlated positively with GV among GDM mother[18]. Women with a history of GDM appear to have nearly 10-fold higher risk of developing type 2 DM[19]and proved to have association with GV during the pregnancy period[20].One study in Canada proved that GV has association with GDM mother older than 35 years old[15] and another study in Iran and Bahrain identified women's age as one of the risk factors for GV in GDM[16]. The difference from those studies include they were using CGM as a tool to calculate GV as well as large population size compared to this study.

Nevertheless, the other associated factors for GV (parity, ethnic group, education level, employment status, gestational age of diagnosis of GDM and total weight gain) were statistically not significant in this study. Study by *Panyakat et al* proved no correlation was found between glycemic variability and gestational weight gain with optimal weight gain in pregnancy is 12kg[10]

Association between GV to postpartum MOGTT

There was significant association between post-partum MOGTT and GV. Study by *Kramer et al* [35]suggested that increased GV strongly associated with abnormal post-partum MOGTT result as an early reflection for pre-diabetes status of patient. Another randomized controlled trial study showed that GV together with high HbA1c of >33mmol/l (5.2%) strongly affect postpartum MOGTT result [36]. However, the reliability of HbA1c in pregnancy is questionable due to confounding factors such as dilutional anaemia and shortened erythrocyte lifespan [37].It is also shown that high GV has been proposed to be an additional risk factor for long term complication including developing of type 2 diabetes or other future cardiovascular events [8].

Limitation of study

Data was collected through retrospective record review with 4 points BSP retrieved from the record as 4 points BSP is practically done in primary care setting in Malaysia. However, available published article with 7 points SMBG reflects more interday glucose fluctuation and give more accurate GV.[38]

Conclusion

Glycemic variability is 0.71mmol/l in this study. GDM patients on treatment is significant associated factor for glycemic variability. There is a significant association between GV and post-partum MOGTT.

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Ethical approval: The research was approved by the Human Ethical Committee of the School of Medical Science, Universiti Sains Malaysia USM/JEPem/20060332.

Conflicts of interest

The authors declare no conflicts of interest.

How does this paper make a difference in general practice?

- This paper provides the glycemic variability among GDM patients and its associated factors in Kota Bharu Kelantan.
- The two significant risk factors found in the study (GDM status and post-partum MOGTT) warrant education and early diet intervention for women with such risks.

REFERENCES

1. Jovanovic L, Pettitt DJ. Gestational diabetes mellitus. *JAMA.* 2001;286(20):2516-8.

2. Metzger BE, Buchanan TA, Coustan DR, et al. Summary and recommendations of the Fifth International Workshop-Conference on Gestational Diabetes Mellitus. *Diabetes Care.* 2007;30 Suppl 2(Supplement 2):S251-60.

3. Green JR, Pawson IG, Schumacher LB, Perry J, Kretchmer N. Glucose tolerance in pregnancy: ethnic variation and influence of body habitus. *Am J Obstet Gynecol.* 1990;163(1 Pt 1):86-92.

4. Magon N, Seshiah V. Gestational diabetes mellitus: Non-insulin management. *Indian journal of endocrinology and metabolism*. 2011;15(4):284.

5. Suh S, Kim JH. Glycemic Variability: How Do We Measure It and Why Is It Important? *Diabetes Metab J.* 2015;39(4):273-82.

6. Ceriello A, Monnier L, Owens D. Glycaemic variability in diabetes: clinical and therapeutic implications. *Lancet Diabetes Endocrinol*. 2019;7(3):221-30.

7. Nalysnyk L, Hernandez-Medina M, Krishnarajah G. Glycaemic variability and complications in patients with diabetes mellitus: evidence from a systematic review of the literature. *Diabetes Obes Metab.* 2010;12(4):288-98.

8. Tay J, Thompson CH, Brinkworth GD. Glycemic variability: assessing glycemia differently and the implications for dietary management of diabetes. *Annual review of nutrition*. 2015;35:389-424.

9. Yu F, Lv L, Liang Z, et al. Continuous glucose monitoring effects on maternal glycemic control and pregnancy outcomes in patients with gestational diabetes mellitus: a prospective cohort study. *J Clin Endocrinol Metab*. 2014;99(12):4674-82.

10. Guerci B. [Asymptomatic glycemic instability: how to measure it and which clinical applications?]. *Diabetes Metab.* 2003;29(2 Pt 1):179-88.

11. Dalfra MG, Sartore G, Di Cianni G, et al. Glucose variability in diabetic pregnancy. *Diabetes Technol Ther*. 2011;13(8):853-9.

12. Bergenstal RM. Glycemic variability and diabetes complications: does it matter? Simply put, there are better glycemic markers! *Diabetes care*. 2015;38(8):1615-21.

13. Gáborová M, Doničová V, Bačová I, et al. Glycaemic Variability and Risk Factors of Pregnant Women with and without Gestational Diabetes Mellitus Measured by Continuous Glucose Monitoring. *IJERPH* 2021;18(7):3402.

14. Yu Q, Aris IM, Tan KH, Li L-J. Application and Utility of Continuous Glucose Monitoring in Pregnancy: A Systematic Review. *Frontiers in Endocrinology.* 2019;10(697).

15. Umpierrez GE, B PK. Glycemic Variability: How to Measure and Its Clinical Implication for Type 2 Diabetes. *Am J Med Sci*. 2018;356(6):518-27.

16. Bolinder J, Hagström-Toft E, Ungerstedt U, Arner P. Self-monitoring of blood glucose in type I diabetic patients: comparison with continuous microdialysis measurements of glucose in subcutaneous adipose tissue during ordinary life conditions. *Diabetes Care.* 1997;20(1):64-70.

17. Kovatchev BP, Otto E, Cox D, Gonder-Frederick L, Clarke W. Evaluation of a new measure of blood glucose variability in diabetes. *Diabetes care.* 2006;29(11):2433-8.

18. Czupryniak L, Barkai L, Bolgarska S, et al. Self-monitoring of blood glucose in diabetes: from evidence to clinical reality in Central and Eastern Europe—recommendations from the international Central-Eastern European expert group. *Diabetes technology & therapeutics*. 2014;16(7):460-75.

19. Glucose Variability in Diabetic Pregnancy. *Diabetes Technology & Therapeutics*. 2011;13(8):853-9.

20. Qureshi N, Kai J. Informing patients of familial diabetes mellitus risk: how do they respond? A cross-sectional survey. *BMC Health Services Research*. 2008;8(1):1-8.

21. Kobayashi D, Noto H, Takahashi O, Shimbo T. Glycemic variability and subsequent malignancies among the population without diabetes. *Diabetes Research and Clinical Practice.* 2020;159:107987.

22. Vounzoulaki E, Khunti K, Abner SC, Tan BK, Davies MJ, Gillies CL. Progression to type 2 diabetes in women with a known history of gestational diabetes: systematic review and meta-analysis. *Bmj*. 2020;369.

23. Fan Y, Li W, Liu H, et al. Effects of obesity and a history of gestational diabetes on the risk of postpartum diabetes and hyperglycemia in Chinese women: obesity, GDM and diabetes risk. *Diabetes research and clinical practice.* 2019;156:107828.

24. Somani B, Arora M, Bhatia K, Arora D, Banerjee M. A comparative study of the different diagnostic criteria of gestational diabetes mellitus and its incidence. *Medical Journal Armed Forces India*. 2012;68(1):6-11.

25. Rajab KE, Issa AA, Hasan ZA, Rajab E, Jaradat AA. Incidence of gestational diabetes mellitus in Bahrain from 2002 to 2010. *International Journal of Gynecology & Obstetrics*. 2012;117(1):74-7.

26. Panyakat WS, Phatihattakorn C, Sriwijitkamol A, Sunsaneevithayakul P, Phaophan A, Phichitkanka A. Correlation Between Third Trimester Glycemic Variability in Non-Insulin-Dependent Gestational Diabetes Mellitus and Adverse Pregnancy and Fetal Outcomes. J *Diabetes Sci Technol.* 2018;12(3):622-9.

27. Kramer CK, Choi H, Zinman B, Retnakaran R. Glycemic variability in patients with early type 2 diabetes: the impact of improvement in β-cell function. *Diabetes Care*. 2014;37(4):1116-23.

28. Paramasivam S, Chinna K, Singh A, et al. Continuous glucose monitoring results in lower HbA1c in Malaysian women with insulin-treated gestational diabetes: a randomized controlled trial. *Diabetic Medicine*. 2018;35(8):1118-29.

29. Weykamp C, John WG, Mosca A. A review of the challenge in measuring hemoglobin A1c. *Journal of diabetes science and technology*. 2009;3(3):439-45.

30. Lachin JM, Bebu I, Bergenstal RM, et al. Association of glycemic variability in type 1 diabetes with progression of microvascular outcomes in the diabetes control and complications trial. *Diabetes care.* 2017;40(6):777-83.

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