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Fault low of HbA1c value in hemoglobin E disorder
ระดับน้ำตาลสะสมต่ำลงในผู้ป่วยมีฮีโมโกลบินผิดปกติชนิดอี

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บทคัดย่อ

หลักการและเหตุผล : อายุของเม็ดเลือดแดงมีผลต่อการวัดระดับน้ำตาลสะสม การศึกษานี้มีเป้าหมายในการศึกษาความสัมพันธ์ระหว่างระดับของน้ำตาลสะสมและระดับน้ำตาลในพลาสม่าขณะอดอาหารในผู้ป่วยเบาหวานที่มีภาวะฮีโมโกลบินผิดปกติ

วิธีการศึกษา : การศึกษาแบบภาคตัดขวาง รวบรวมข้อมูลตั้งแต่ปี พ.ศ. 2552-2560 ประกอบด้วยข้อมูลทั่วไป ระดับน้ำตาลในพลาสม่าขณะอดอาหารมากกว่า 6 ชั่วโมง เปรียบเทียบกับระดับน้ำตาลสะสม โดยแบ่งกลุ่มผู้ป่วยเป็น 3 กลุ่มตาม ผลการตรวจการคัดกรองภาวะฮีโมโกลบินผิดปกติชนิดอี ประกอบด้วยกลุ่มควบคุม กลุ่มฮีโมโกลบินผิดปกติชนิดแฝงและกลุ่มฮีโมโกลบินผิดปกติชนิดอีอี ในแต่ละกลุ่มแบ่งระดับน้ำตาลสะสมเป็นกลุ่มย่อย อีก 6 กลุ่ม โดยมีสมมุติฐานว่าที่ค่าเฉลี่ยระดับน้ำตาลสะสมเท่ากันค่าเฉลี่ยของระดับน้ำตาลในพลาสม่าก็ควรจะเท่ากันด้วย หากภาวะของฮีโมโกลบินผิดปกติไม่มีผลต่อการวัดระดับน้ำตาลสะสม

ผลการศึกษา : ผู้ป่วยเบาหวานได้รับการเจาะเลือดทั้งหมด 1947 ครั้ง กลุ่มควบคุม 725 ครั้ง กลุ่มฮีโมโกลบินผิดปกติ 1,222 ครั้ง ข้อมูลทั่วไปไม่แตกต่างกัน ค่าเฉลี่ยของอายุ ระดับน้ำตาลสะสม ระดับน้ำตาลในพลาสม่า ไม่แตกต่างกัน แต่ผู้ป่วยฮีโมโกลบินผิดปกติมีภาวะซีดมากกว่า จากการแบ่งกลุ่มผู้ป่วยเป็น 6 กลุ่ม ข้อมูลผู้ป่วยส่วนใหญ่ อยู่ในกลุ่ม 6.0-6.4, 6.4-6.9 และ 7.0-7.4 จากการทดสอบด้วยอโนว่า ที่ระดับน้ำตาลสะสมเท่ากันนั้น ระดับน้ำตาลในพลาสม่ายังคงสูงกว่า โดยเฉพาะในกลุ่มฮีโมโกลบินอีอี แต่ในกลุ่มที่ระดับน้ำตาลสะสมสูงมากหรือต่ำมาก ไม่มีความแตกต่างกัน ส่วนกลุ่มฮีโมโกลบินอีชนิดแฝง มีความแตกต่าง ที่ระดับน้ำตาลสะสมเท่ากัน ระดับน้ำตาลในพลาสม่ายังคงสูงในกลุ่มระดับน้ำตาลสะสมร้อยละ 6.0-6.4 และ ร้อยละ 6.5-6.9

สรุป : การใช้ระดับน้ำตาลสะสมในการประเมินการควบคุมระดับน้ำตาลในเลือดในผู้ป่วยเบาหวานที่มีฮีโมโกลบินผิดปกติชนิดอี ควรแปลผลด้วยความระมัดระวังที่ระดับน้ำตาลสะสม ร้อยละ 6.0-7.5 นั้นระดับน้ำตาลในพลาสม่าขณะอดอาหารยังคงสูง เพราะผลต่ำลวงในการวัดระดับน้ำตาลสะสม

คำสำคัญ : เบาหวาน น้ำตาลสะสม น้ำตาลเฉลี่ย ฮีโมโกลบินผิดปกติ ทัลลัสซีเมีย

วารสารการแพทย์โรงพยาบาลศรีสะเกษ สุรินทร์ บุรีรัมย์ 2561; 33(2): 105-118

Abstract

Background : A decreasing erythrocyte life-span is associated with lower HbA1c level. This research aims to study the fault of HbA1c level in poor control of diabetes in endemic area of hemoglobinopathy.

Methods : This cross-sectional study was conducted from 2009 to 2016. Patient's clinical information, fasting plasma glucose (FPG), and HbA1c levels were collected and divided into three groups which are control group, hemoglobin E heterozygous (HbEA) and homozygous group (HbEE). Each group was further divided into six strata according to HbA1c level. The hypothesis, at the same level of HbA1c, FPG would not higher if there is no effect in hemoglobin E disorder.

Results : As a result of 1947 test, there were 1,222 diabetic patients, in cludes hemoglobin E disorder and 725 diabetic patients with negative dichlorophenol-Indolephenol (DCIP) test. There was no significant difference regarding age, HbA1c, FPG, and serum creatinine which were found between the study and the control group. When there was a comparison between the study and the control group, anemia was more prevalent among diabetic patients with hemoglobin E homozygous ($p<0.05$). Furthers, HbA1c level was divided into six strata which were5.0-5.4, 5.5-5.9, 6.0-6.4, 6.4-6.9, 7.0-7.4, and 7.5-7.9%. The ANOVA test revealed that HbA1c levels in each stratum was not significantly different among these three

groups. FPG level was significantly different in 6.0-6.4, 6.5-6.9, and 7.0-7.4%; as well as, HbA1c stratum with FPG was higher in HbEE than the control group. In HbEA group, FPG was still higher in 6.0-6.4 and 6.5-6.9 strata.

Conclusion : Diabetic patients with hemoglobins E disorder should carefully use HbA1c level as an indicator for long-term glycemic control. At HbA1c 6.0-7.5%, FPG is higher than expected because of the fault of low HbA1c measurement.

Keywords : Diabetes mellitus, Hemoglobinopathy, Hemoglobin E disorder, Glycohemoglobin, HbA1c

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INTRODUCTION

Previous large prospective research trials in diabetic patients mellitus have demonstrated that HbA1c levels are directly related to the risk of diabetic complications.[1-3] The most important factor that determines HbA1c concentration is a long-term blood glucose level which makes HbA1c be a standard for monitoring in long-term glycemic control in diabetics.[4-6] The data from some research have shown thatintensive glucose control can lead to the increasing of Hypoglycemic which also attacks in diabetic patients.[7]

In diabetic patients, there are normal hemoglobins in which HbA1c values strongly correlate with blood glucose level. However, many studies have shown that there is the decreasing in erythrocyte life-span such as the observation of hemolytic anemias, which is associated with the lower concentration of HbA1c.[9-11] This has been suggested to be because HbA1c is correlated with the developmental stage of erythrocytes.[9-10] The concentration of minor hemoglobins in young's erythrocytes was found to be lower than older erythrocytes.[8] Therefore, HbA1c concentration has been proposed as a diagnostic parameter in anemia which is associated with short erythrocyte life-spans.[11]

More than 700 forms of hemoglobinopathy or abnormal hemoglobin variants have been reported.[12-16] Hemoglobin E disorder is the most prevalent hemoglobinopathy in Surin

province, Thailand.[16-17] Hence, diabetic patients who have concomitant hemoglobin E disorder are also frequently encountered.[17] hemoglobinopathies are routinely screened in the diabetic clinic at Surin hospital. HbA1c is a standard for monitoring long-term glycemic which controls in hemoglobin E disorder diabetics. However, alterations of HbA1c in diabetes mellitus due to the factors also affect hemoglobin levels which have not been extensively investigated. This study aims to define the fault of HbA1c level in poor control of diabetes in endemic area of hemoglobinopathy.

METHODOLOGY

This cross-sectional study was approved by the institutional reviewed board and conducted in the diabetic clinic at Surin hospital since january, 2009 to december, 2016. Among these 81 patients, there were HbEE which the blood sample was also taken for HbA1c once per year. Informed consent was obtained from all subjects. The sample size of 588 samples was calculated from the average and the variance which obtained from a previous study in 2006.[11] Number in each group was calculated to be representative of the population at 95% confidence with 1% of standard error of mean. Subjects were confirmed diabetic patients who already had been treated either with insulin, oral hypoglycemic drugs or a physician-prescribed diet. In addition, the analysis measurements from three sets of data were also used. The hypothesis at the same level of HbA1c, FPG was higher because there is a low level in hemoglobin E disorder patients.

For the laboratory measurements, a blood sample was taken in the morning after an overnight fast and there was a test for fasting plasma glucose, completed blood count, blood urea nitrogen, creatinine, dichlorophenol-Indolephenol (DCIP) and HbA1c. Subjects were classified into one of three groups; included, negative DCIP (N), hemoglobin E trait(HbEA) and homozygous hemoglobin(HbEE). When DCIP test was positive, hemoglobin typing was further done by Hb gold analyser (Drew Scientific Ltd.,England) by using low-pressure liquid chromatography (LPCL). Control group just have negative for DCIP but it did not mean normal blood exam. HbA1c level was compared between groups. Base on the characteristric of FPG, HbA1c, completed blood count, hematocrit and creatinine, they were collected from the first visit of year 2009. The DCIP test was KKU-DCIP-Clear reagent.[18]

HbA1c was measured by using the turbidimetric inhibition immunoassay (TINIA) for hemolyzed whole blood

(Cobas®, Roche Diagnostics, USA). Testing blood sugar levels compared among thesethree groups of diabetic patients when the cumulative sugar levels were equal. While the hypothesis at the cumulative average sugar level HbA1c was equal, the average level of blood sugar was not different. By the way, testing of variance within and between groups was done by dividing HbA1c level into six strata which were 5-5.4, 5.5-5.9, 6.0-6.4, 6.5-6.9, 7.0-7.4, and 7.5-7.9%. Subjects with HbA1c level which exceed 7.9 or less than 5.0 subjects in third stage chronic kidney disease or worse and subjects with no hemoglobin typing test results were excluded from the study. The sample size in each strata, 147 was calculated with 2% of standard error of mean.

Statistical analysis was carried out by using minitab18 software. Descriptive parameters are presented as means with standard deviations. The ANOVA test was used to compare based on the characteristics. The ANOVA with F-test was used to compare the mean values among the various strata of HbA1c levels and FPG. A p-value < 0.05 was considered statistically significant. If one-way ANOVA yielded significant results, turkey method and 95% confidence was further conducted between groups.

RESULTS

There were 1947 blood tests consisting of 725 in control group 613 in HbEA group and 609 in HbEE group. A total of 627 diabetic patients treated at the Surin hospital diabetic clinic were studied during the eight-year period from January 2009 to December 2016. Among these, 81 patients were HbEE, 193 were HbEA and 353 patients were in the control group. There were 3 patients whose hemoglobin typing test result was not known. There were no significant differences in regard to age, FPG and creatinine among the groups. The hematocrit was significantly lower in HbEE group. Table 1 describes patient characteristics and results of laboratory blood tests. A total of 258 blood tests (13.3%) were excluded from analysis due to HbA1c level being less than 5.0% or greater than 7.9 among which 109 were in the DCIP negative group, 57 in the HbEA group, and 92 in the HbEE group. The HbA1c concentration was ranged from 3.5% to 14.4% with distribution (Figure 1). A total of 86.8% of blood test was included. Mean of HbA1c was 7.2% and 1.5 standard deviation. There was few data in HbA1c level at 8.0-8.7 to evaluation.

Table 1. Characteristics of the patients at baseline.

Variable	DCIP negative (n=353)	HbEA (n=193)	Homozygous HbE (n=81)	p-value*
Age(years; mean,sd)	60(10.9)	58(10.6)	60(10.4)	0.1156
Sex(Male: Female)	0.43	0.46	0.38	
Glycohemoglobin(%; mean,sd)	7.7(1.8)	7.6(1.8)	6.97(1.3)	0.0938
FPG(mg/dl; mean,sd)	150(85.7)	151(50.1)	146(45.9)	0.5660
Hematocrit(%; mean,sd)	39(5.2)	38(4.4)	32(4.1)	<0.001
Serum creatinine(mg/dl; mean,sd)	1.1(0.4)	1.0(0.4)	1.0(0.3)	0.8157

FPG= Fasing plasma glucose; p-value<0.001 significant

In addition, a table 2 describes the FPG and HbA1c values in each HbA1c stratum. When there is an analysis of the different strata of HbA1c levels, all of HbA1c levels in all strata were not significantly different among groups. Figure 2, 3, 4 presented the mean of FPG level which different in the 6.0-6.4, 6.5-6.9 and 7.0-7.4% of HbA1c stratum. In this stratum, FPG was higher in HbEE than in the control group, but there was no significant difference in FPG values between the HbEA and HbEE, except in the 6.5-6.9% of HbA1c stratum. Figure 5, 6, 7 presented an additionally, the variance of HbA1c in every stratum was different from the variance of FPG. In the HbE disorder group significant differences were found in the variance of FPG as compared to the control group.

Table 2. Means and standard deviations of the glycohemoglobin and fasting plasma glucose in each group.

Glycohemoglobin	FPG in DCIP negative (n=725)	FPG in HbEA (n=613)	FPG in HbEE (n=609)	p-value*
5.0-5.4(%; mean,sd)	124.2(22.83)	118.9(30.84)	102.0(24.69)	0.1499
5.5-5.9(%; mean,sd)	120.1(22.39)	123.6(29.31)	109.4(39.00)	0.0795
6.0-6.4%l; mean,sd)	121.0(18.45)	128.8(26.08)	131.7(27.04)	<0.05**
6.5-6.9(%l; mean,sd)	134.6(41.09)	150.8(42.89)	130.9(32.24)	<0.05**
7.0-7.4(%l; mean,sd)	138.9(31.01)	159.5(42.49)	151.2(27.27)	<0.05**
7.5-7.9(%l; mean,sd)	150.8(42.68)	165.8(43.42)	152.3(43.56)	0.1133

FPG= Fasting plasma glucose, *p-value<0.05 significant, **significant

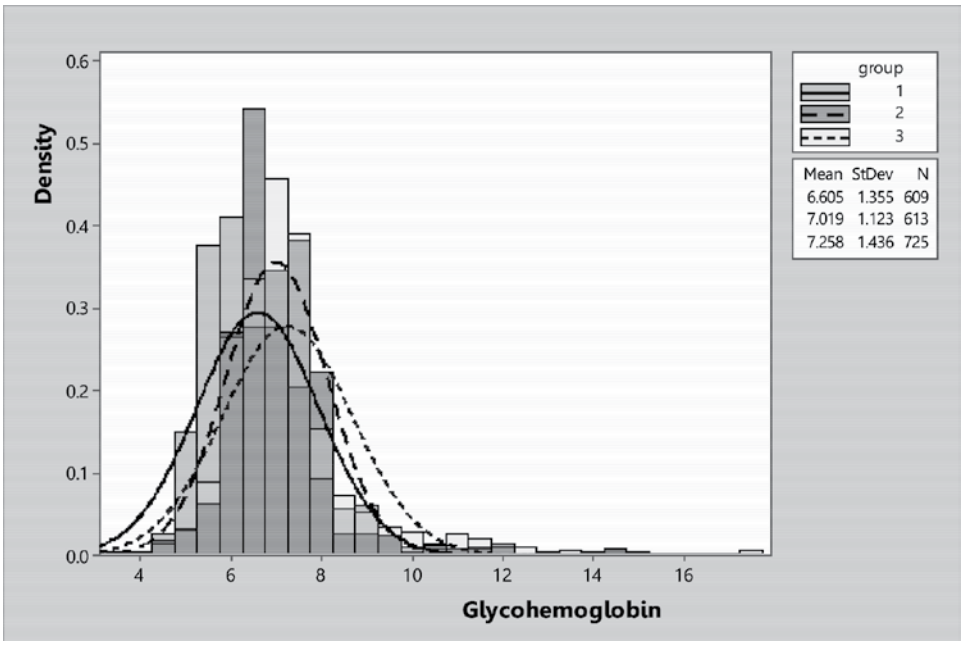

Figure 1-the variation of glycohemoglobin compared 3 groups; ----1 HbEE group, -- -- 2 HbEA group, - - -3 negative DCIP group.

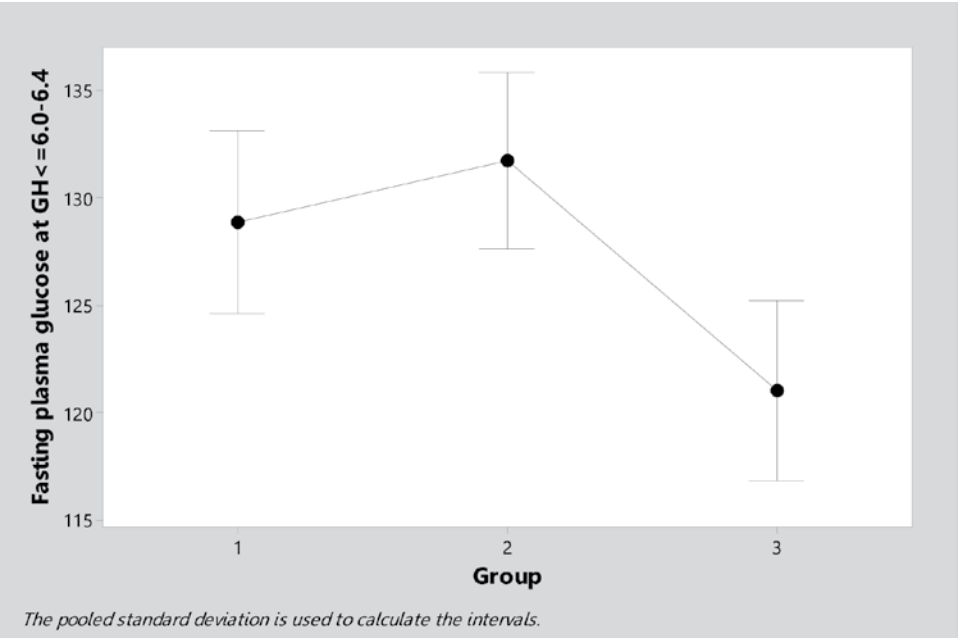

Figure 2- interval plot of 95% confidence of mean fasting plasma glucose at GH >= 6.0-6.4% compared 3 groups; 1 HbEE group, 2 HbEA group, 3 negative DCIP group.

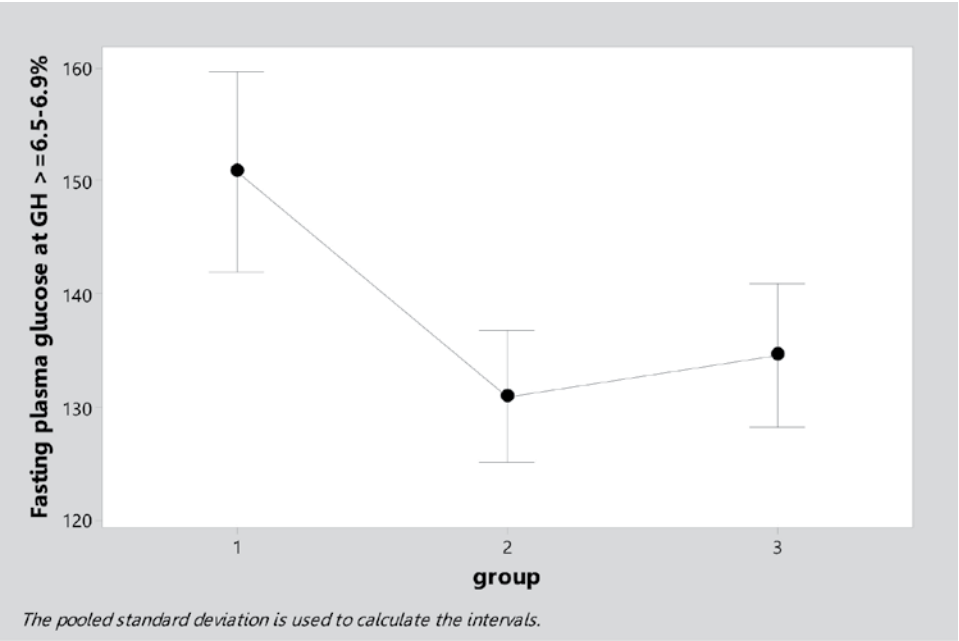

Figure 3- interval plot of 95% confidence of mean fasting plasma glucose at GH >=6.5-6.9% compared 3 groups; 1 HbEE group, 2 HbEA group, 3 negative DCIP group.

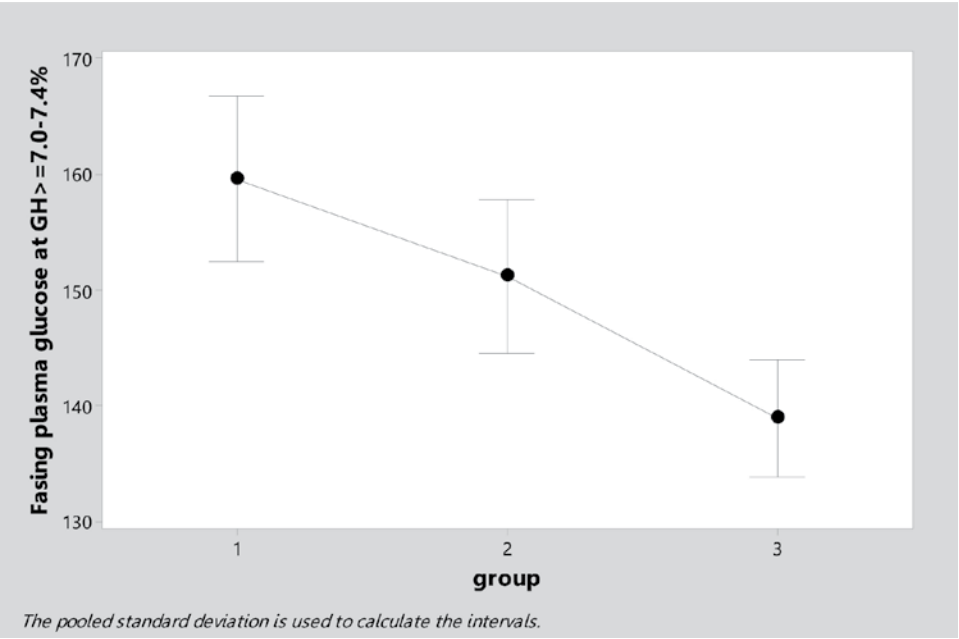

Figure 4- interval plot of 95% confidence of mean fasting plasma glucose at GH >=7.0-7.4% compared 3 groups; 1 HbEE group, 2 HbEA group, 3 negative DCIP group.

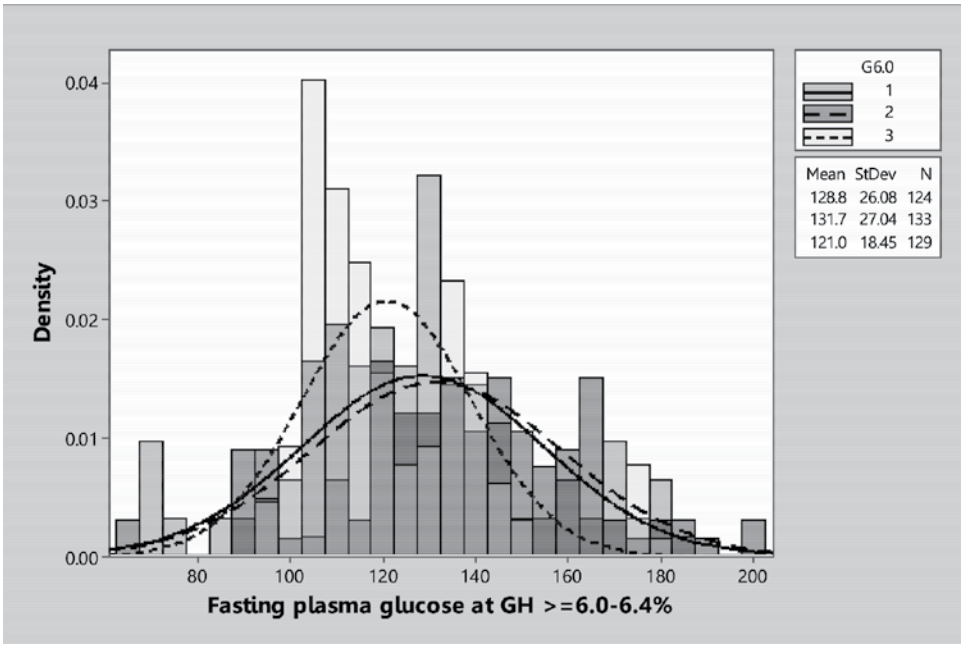

Figure 5-the variation of fasting plasma glucose at GH >=6.0-6.4% compared 3 groups; ----1 HbEE group, -- -- 2 HbEA group, - - -3 negative DCIP group.

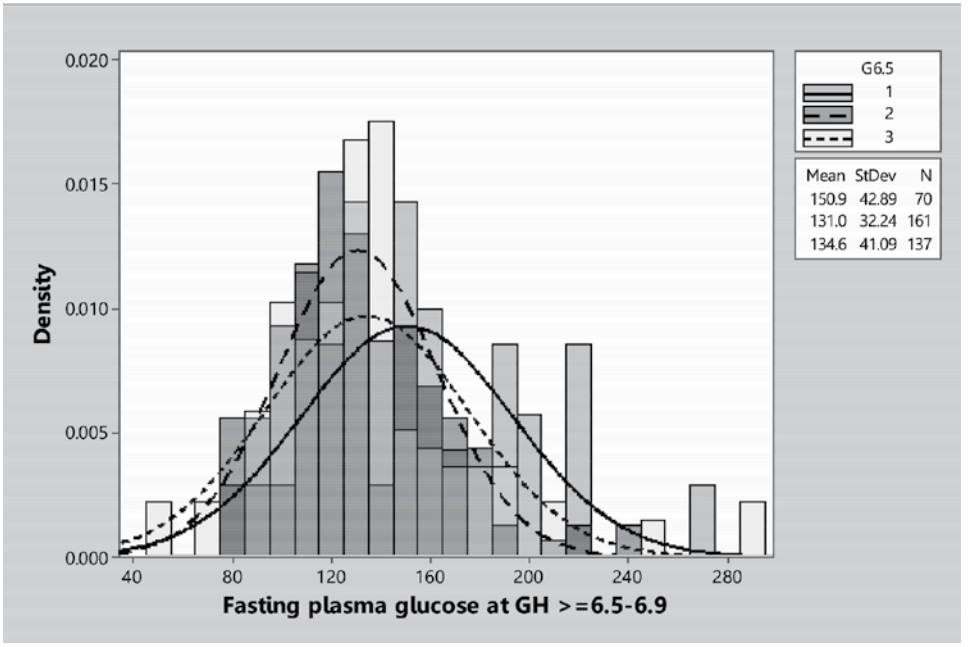

Figure 6-the variable of fasting plasma glucose at GH >=6.5-6.9% compared 3 groups; ----1 HbEE group, -- -- 2 HbEA group, - - - 3 negative DCIP group.

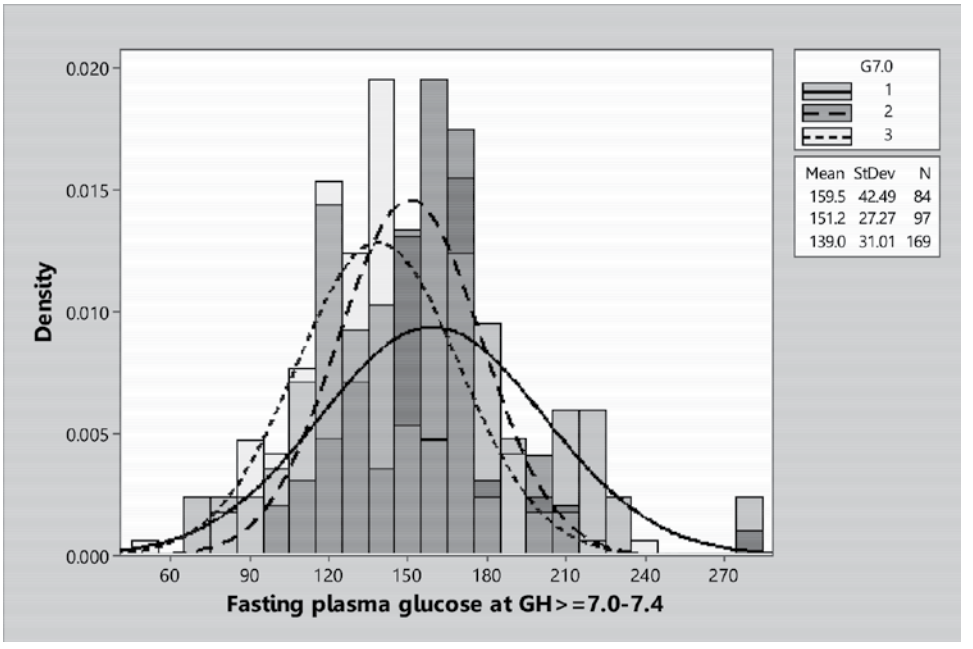

Figure 7- the variation of fasting plasma glucose at GH >=7.0-7.4% compared 3 groups; ----1 HbEE group, -- -- 2 HbEA group, - - - 3 negative DCIP group.

DISCUSSION

Surin Province is located in the northeast of Thailand, near the Thai-Cambodian border. In this region, thalassemia and hemoglobin E disorder are more prevalent than other areas.[14-15] Therefore, the diabetic patients are often found to have concomitant hemoglobin E disorder with an estimate of approximately 30-50% of all diabetic patients, which also add a level of complexity in caring for these patients.[14,16-17] The American Diabetes Association (ADA) recommends HbA1c as the standard laboratory assessment of long-term glycemic control and efficient treatment of diabetic patients.[4-6] The HbA1c better correlates with complications than FPG. However, a factor that affects HbA1c level is a lifespan of the red blood cells.[9] In patients with hemoglobinopathies, the lifespan of red blood cell is shorter than normal which HbA1c may also be lower than usual.[9-11] For this reason, in American guidelines, FPG is recommended for using to monitor diabetic patients with abnormal hemoglobin.[4] However, a study has shown that monitoring diabetic patients by using FPG may lead to aggressive blood sugar lowering interventions which causes the increasing of death rate.[7]

For this reason, HbA1c should also be used for monitoring of glycemic control in diabetic patients with hemoglobinopathies to ensure the minimizing long-term diabetic complications while avoid hypoglycemic attacks. Nevertheless, it needs to take into the account of the confounding effect of shortened red blood cell lifespan.[9] HbA1c is the result of an irreversible non-enzymatic glycation of the beta chain of hemoglobins A. It is normally presented in circulating red cells because of the glycosylation reaction between hemoglobins and circulating glucose.[8] In the presence of excessive plasma glucose, the hemoglobins beta-chain increases glycosylated which makes the HbA1c be a useful index of long-term glycemic control.[8] This data are consistent with the findings that the concentration of minor hemoglobins in young erythrocytes was found to be higher than in the older erythrocytes, if HbA1c is higher than 7.4. HbA1c concentrations in diabetic patients with hemoglobin E disorder was found to be not significantly higher than the control group with similar very low glycemic control level. Most of data (71.4%) generally in 6.0-7.4%, there was a powerful sample size to evaluated

and proved that there was had false low HbA1c. Several limitations are worth mentioning in this study. First, most patients with thalassemia HbEE also have low hemoglobins. Besides, hemoglobins level is also correlated with HbA1c level. Moreover, there is a high prevalence of patients with thalassemia HbE who have less or no symptoms. If FPG level is not statistically significantly high; hence, these patients can be monitored in the same criteria which is used in patients who don't have red blood cells problems. This would allow coverage of most patients and the patients who need to be carefully monitored will be reduced to 10% of the population of the diabetic patients. The recommendations of glycemic goals for non-pregnant individuals are based on data of HbA1c. The goals of blood glucose are at the levels which appear to correlate with achievement of HbA1c less than 7%. [4-6] However, in some clinical situations, laboratory assessment using HbA1c may provide unreliable information. When the glycohemoglobin such as HbA1c result is inconsistent with a patient's clinical situation, conditions that affect red blood cell lifespan and hemoglobinopathies must be considered as possible causes. It is because normal values for HbA1c are based on individuals who have normal hematological profiles. For patients that HbA1c and measured blood glucose appear discrepant, clinicians should consider the possibilities of hemoglobinopathy or altered red cell turnover, and the options of more frequent and/or different timing of self-monitoring of blood glucose which combined with HbA1c monitoring.[4]

This study found that at the same level of HbA1c among these three groups of the patients such as negative DCIP group, thalassemia trait group, and thalassemia HbE homozygote group, the average FPG which tested by ANOVA was not significantly different. The variances between the groups were not different from the variances within the sample group. Test of variances between each groupsshown that the patients with HbE disorder had a greater variability of blood sugar. Thus, FPG is likely not appropriated for monitoring these patients; on the other hands, HbA1c should be used in these patients. Nonetheless, FPG and HbA1c are not the only factors that used in monitoring but a holistic approach in tailoring care for each patient to prevent complications in the long term should always be kept in a priority.

CONCLUSION

At the HbA1c stratum of 6.0-6.4, 6.5-6.9 and 7.0-7.5%, FPG is higher than expected because of the fault low of HbA1c measurement. Self monitoring in blood sugar should be performed.[4] Diabetic patients with hemoglobins E disorder should carefully use HbA1c level as an indicator for long-term glycemic control. Diabetic patients with unexpected by low HbA1c value should be identified hemoglobins variant.

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