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Research Article

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Consequential of Hemoglobin E Disorder on Renal Complication of Diabetic Patients in Surin Hospital, Thailand

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Abstract

Background: *Decreased erythrocyte life-span is associated with artificial low hemoglobinA1c (HbA1c) in hemoglobin E diabetes. This research aims to study the renal complication in hemoglobin E disorder diabetic patients in Surin hospital.*

Methods: *This case control cohort study was conducted from 2009 to 2018.Patient's clinical information, fasting plasma glucose (FPG), and HbA1c levels were collected and divided into two groups, homozygous group (HbEE) and negative dichlorophenol-indolephenolgroup (control group). Subjects were confirmed diabetics who already had been treated either with insulin, oral hypoglycemic drugs or a physician-prescribed diet. Target of diabetic control follow standard treatment, not try to intensive control. The endpoint was that of new diabetes nephropathy, defined as the development of macroalbuminuria (urine albumin > 300 microgram/milligram) and rate of decline of estimated glomerular filtration rate (eGFR) per year. The hypothesis was that the cumulative average duration of disease was equal; the renal complication between two groups was not different.*

Results: *From 2009 to 2018, 132 diabetic patients with hemoglobin E disorder and 493 diabetic patients with negative DCIP were included in the study. There were no significant differences in regard to age, duration of disease, systolic blood pressure (SBP), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) among the groups. The hematocrit (Hct.), diastolic blood pressure (DBP), hemoglobin A1c (HbA1c), cholesterol (CHO), triglyceride (TG), serum creatinine (Cr) was significantly lower in HbEE group. The eGFR and FPG was significant high in HbEE group. When compared with controls, risk difference of macroalbuminuria was 4.2% lower prevalent in diabetic patients with hemoglobin E homozygous (P=0.008). The rate of decline in eGFR were significant much slower in HbEE group 0.64 ml/min/year and 0.71 ml/min/year in control group (P<0.001), after adjusted confounding factors.*

Conclusions: *With long term cohort study, hemoglobin E disorder diabetic patients have less effect on renal complication when the mean fasting plasma glucose level was higher and lower HbA1c than negative DCIP group. Diabetic patients with hemoglobin E disorder should be monitored using HbA1c level as an indicator for long-term glycemic control.*

Keywords: Diabetes Mellitus, Hemoglobinopathy, Hemoglobin E Disorder, Diabetic Nephropathy, Surin Hospital

Background

Previous large prospective research trials in patients with diabetes mellitus have demonstrated that intensive glucose lowering appears to prevent the development and progression of abnormal levels of albuminuria, suggesting the possibility of renoprotection [1-3]. The most important factor that determines hemoglobin A1C (HbA1c) concentration is long-term blood glucose level witch makes HbA1c the standard for monitoring long-term glycemic control in diabetics [4-6]. The data from some researchers have shown that too intensive glucose control can lead to increased hypoglycemic attacks in diabetic patients [7].

In patients with diabetes having normal hemoglobin, HbA1c values strongly correlate with blood glucose level. However, many studies have shown that decreased erythrocyte life-span such as observed in hemolytic anemia, is associated with lower concentration of HbA1c [8-14]. This has been suggested to be because HbA1c is correlated with the developmental stage of erythrocytes. The concentration of minor hemoglobins in young erythrocytes was found to be lower than that in the older erythrocytes. Therefore, HbA1c concentration has been proposed as a diagnostic parameter in anemia associated with short erythrocyte life-spans [15-16]. There were some reported cases for low HbA1c levels in a poorly controlled diabetic [17-20].

More than 700 forms of hemoglobinopathy or abnormal hemoglobin variants have been reported [21-26]. Hemoglobin E disorder is the most prevalent hemoglobinopathy in Surin, Thailand. Therefore,

patients with diabetes who have concomitant hemoglobin E disorder are also frequently encountered [27]. Hemoglobinopathies is routinely screened for in the diabetes clinic at Surin hospital. However, fasting plasma glucose (FPG) and HbA1c are not the only factors that need monitoring, a holistic approach in tailoring care for each patient to prevent complications in the long term should always be kept a priority. This study aimed to investigate the renal complication in hemoglobin E disorder diabetic patients.

Methods

Subject

This cases control cohort study was approved by the institutional review board and conducted in the diabetes clinic at Surin Hospital from January 2009 to December 2018. Informed consent was obtained from all subjects. The sample size was calculated from the average and variance obtained from a previous study in 2006. The number in each group was calculated to be representative of the population at 95% confidence. Subjects were confirmed diabetics who already had been treated either with insulin, oral hypoglycemic drugs or a physician-prescribed diet. Target of diabetic control follow standard treatment, not try to intensive control. For analysis measurements from 2 data sets were used. Exclusion criteria included hemoglobin H disease and hemoglobin E heterozygote.

Measurement

For the laboratory measurements, a blood sample was taken in the morning after an overnight fast and tested for fasting plasma glucose, lipid profile, complete blood count, blood urea nitrogen, creatinine, and dichlorophenol-indolephenol (DCIP) and HbA1c. Subjects were classified into one of 2 groups: negative DCIP (control group) and homozygous hemoglobin (HbEE). When DCIP test was positive, hemoglobin typing was further done by Hb Gold analyzer (Drew Scientific Ltd., England) using low-pressure liquid chromatography (LPCL). Serum creatinine levels were measured annually throughout the course of the study. The estimated glomerular filtration rate (eGFR) was estimated with the use of the Chronic Kidney Disease Epidemiology Collaboration formula. Baseline characteristrics, FPG, HbA1c, lipid profile, complete blood count including hematocrit, creatinine, systolic blood pressure and diastolic blood pressure were collected from the first visit of year 2009, retrospective to year at diagnosis and once time per year until 2018 or loss follow up or dead.HbA1c was measured using the turbidimetric inhibition immunoassay (TINIA) for hemolyzed whole blood (Cobas®, Roche Diagnostics, USA). Testing urine albumin compared with 2 groups of diabetic patients. The endpoint was that of new diabetes nephropathy, defined as the development of macroalbuminuria (urine albumin > 300 microgram/milligram) and rate of decline of eGFR per year. The hypothesis was that the cumulative average duration of disease was equal, the renal complication between two groups was not different.

Statistical analysis

Statistical analysis was carried out using STATA14. Descriptive parameters are presented as means with standard deviations or percent. The general data were compared between the negative DCIP group and HbEE group by Fisher's exact tests or unpair t-test. The risk difference regression analysis was used to compare risk of macroalbuminuria. Gaussian regression analysis was performed to identify rate of decline of eGFR per year between two groups. Two-sided P<0.05 was considered significant.

Results

A total of 635 diabetic patients treated at the diabetes clinic at Surin hospital were studied from January 2009 to December 2018. Among these, 132 patients were HbEE and 493 patients were in the control group. A total of, 2,557 blood tests were done with 1,468 in control group and 1,089 in hemoglobin E disorder group.

Demographic characteristics

(Table 1) is the list of the demographic characteristics of the patients. There were no significant differences in regard to age, duration of disease, systolic blood pressure (SBP), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) among the groups. The hematocrit (Hct.), diastolic blood pressure (DBP), hemoglobin A1c (HbA1c), cholesterol (CHO), triglyceride (TG), serum creatinine (Cr) was significantly lower in HbEE group. The eGFR and FPG was significant high in HbEE group. Table 1 describes patient characteristics and results of laboratory blood tests. The relation of FPG and duration of disease are given in (Figure 1). HbEE group have higher level of FPG and lower in HbA1c during follow-up, show in (Figure 2).

Table 1: Baseline characteristics by control and HbEE group

Variable	Neg. DCIP	% or SD	Hb EE	% or SD	P-Value
N	1468		1085		
Age	61.9	11.0	62.3	10.9	0.378
Sex M/F	493/945	34.30%	285/800	26.30%	<0.001
Duration	7.0	6.6	7.3	5.7	0.206
SBP	129.0	16.3	128.3	15.6	0.286
DBP	75.5	10.9	74.3	10.6	0.005
FPG	150.2	55.0	156.2	60.2	0.011
HbA1c	7.9	2.1	7.1	1.7	<0.001
Hct.	37.6	4.8	32.3	4.2	<0.001
Hb	12.7	6.3	11.0	6.0	<0.001
CHO	191.5	46.5	184.6	39.8	<0.001
TG	165.3	102.6	152.7	100.6	0.003
HDL	48.0	12.5	48.5	12.5	0.300
LDL	117.0	47.2	113.4	46.9	0.067
Cr	1.08	0.4	0.98	0.4	<0.001
eGFR	64.2	24.6	69.7	25.0	<0.001
DN	317.0	21.6%	189.0	17.4%	0.009

SD:standard deviation, M:male, F:female, SBP:systolic blood pressure
DBP:diastolic blood pressure, FPG:fasting plasma glucose
HbA1c:hemoglobin A1c, Hct.:hematocrit, Hb:hemoglobin
CHO:cholesteral, TG:triglyceride, HDL:high-density lipoprotein
LDL:low-density lipoprotein, Cr:creatinine
DN: diabetic nephropathy, eGFR:estimated glomerular filtration rate
CI:confidence interval HbEE:hemoglobin E homozygote
Neg.DCIP:negativedichlorophenol-indolephenol
P<0.05 for the comparison betaween neg. DCIP group and HbEE group with the use of the unpair t test or Fisher's exact test

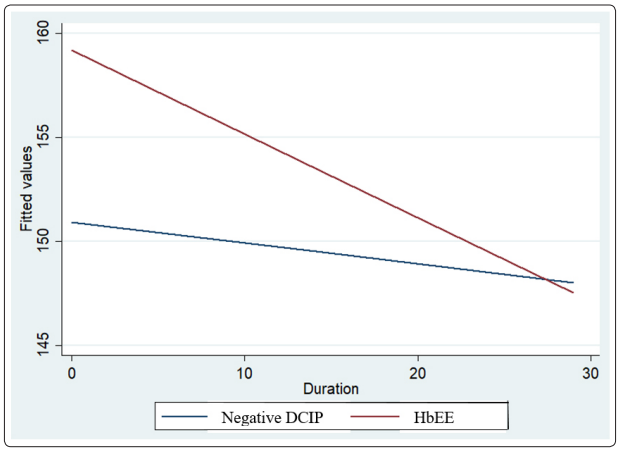

Figure 1: FPG level during follow up between negative DCIP and HbEE group, P=0.011

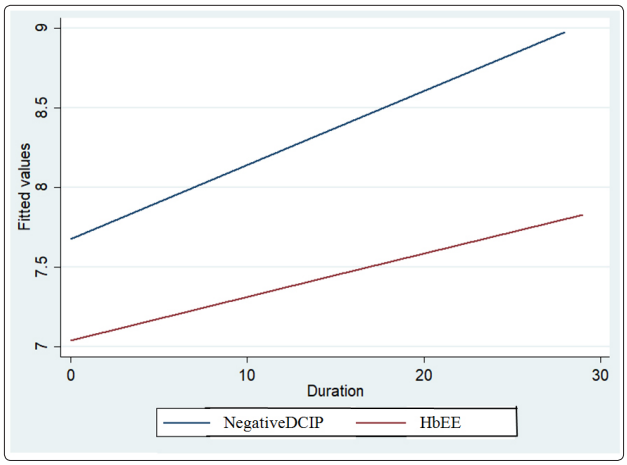

Figure 2: HbA1c level during follow up between negative DCIP and HbEE group, P<0.001

Renal complication

A univariable risk difference regression of macroalbuminuria was show in (Table 2). HbEE group had significant lower risk of macroalbuminuria than control group (risk difference 0.0417±0.03, P=0.008). (Table 3) show when compared with controls, risk difference of macroalbuminuria was 4.2% lower prevalent in diabetic patients with hemoglobin E homozygous (P=0.007), after adjustment for other confonders.

Table 2: Effects of HbEE compared with control group on macroalbuminuria by univariable analysis

DN	Risk Diff.	95% CI		P-value
HbEE	-0.0417	-0.07	-0.01	0.008
constant	0.22	0.19	0.24	<0.001

HbEE:hemoglobin E homozygote
DN: diabetic nephropathy
CI:confidence interval

P<0.05 for the comparison betaween neg. DCIP group and HbEE group
with the use of risk difference regression analysis

Table 3: Effects of HbEE compared with control group on macroalbuminuria by multivariable analysis

DN	Risk Diff.	95% CI		P-value
HbEE	-0.0420	-0.07	-0.01	0.007
Age	0.00	0.00	0.01	<0.001
SEX	0.00	-0.03	0.04	0.827
SBP	0.00	0.00	0.00	<0.001
DBP	0.00	0.00	0.00	0.001
Duration	-0.01	-0.01	-0.01	<0.001

HbEE:hemoglobin E homozygote
DN: diabetic nephropathy
CI:confidence interval
SBP:systolic blood pressure
DBP:diastolic blood pressure
P<0.05 for the comparison betaween neg. DCIP group and HbEE group
with the use of risk difference regression analysis

During the investigation period, the mean rate of decline in eGFR was 0.63±0.16 ml/min/year. The HbEE group had a significantly lower rate of decline in eGFR as compared with the control group (0.56±0.21 vs. 0.68±0.23 ml/min/year, respectively, P<0.001), by univariable analysis. The rate of decline in eGFR were significant much slower in HbEE group 0.64 ml/min/year and 0.71 ml/min/year in control group (P<0.001), after adjusted confounding factors, SBP, DBP, CHO, TG, HDL and LDL, show in (Table 4 & Table5). The rate of decline in eGFR faster in macroalbuminuria group (1.37± 0.32 ml/min/year vs. 0.57±0.17 ml/min/year, P<0.001), show in (Table 6).

Table 4: Effects of HbEE compared with control group on eGFR by univariable analysis

eGFR	Coef.	95% CI		P-value
Neg. DCIP	-0.68	-0.85	-0.50	<0.001
HbEE	-0.56	-0.76	-0.35	<0.001

HbEE:hemoglobin E homozygote
CI:confidence interval
eGFR:estimated glomerular filtration rate
Neg.DCIP:negativedichlorophenol-indolephenol
P<0.05 for the comparison betaween neg. DCIP group and HbEE group
with the use of Gaussian regression analysis

Table 5: Effects of HbEE compared with control group on eGFR by multivariable analysis

eGFR	Coef.	95% CI		P-value
Neg. DCIP	-0.71	-0.89	-0.54	<0.001
HbEE	-0.64	-0.87	-0.41	<0.001
SBP	-0.36	-0.43	-0.30	<0.001
DBP	0.41	0.31	0.51	<0.001
CHO	0.02	-0.01	0.05	0.249
HDL	0.04	-0.04	0.13	0.338
LDL	0.01	-0.01	0.04	0.302
TG	-0.03	-0.05	-0.02	<0.001

SBP:systolic blood pressure
DBP:diastolic blood pressure, FPG:fasting plasma glucose
CHO:cholesteral, TG:triglyceride, HDL:high-density lipoprotein
LDL:low-density lipoprotein, Cr:creatinine
eGFR:estimated glomerular filtration rate
CI:confidence interval
Neg.DCIP:negativedichlorophenol-indolephenol
P<0.05 for the comparison betaween neg. DCIP group and HbEE group
with the use of Gaussian regression analysis

Table 6: Effects of macroalbuminuria compared with no albuminuria on eGFR by multivariable analysis

eGFR	Coef.	95% CI		P-value
no proteinuria	-0.57	-0.74	-0.40	<0.001
DN	-1.37	-1.65	-1.09	<0.001
SBP	-0.34	-0.41	-0.27	<0.001
DBP	0.38	0.28	0.48	<0.001
CHO	0.01	-0.02	0.05	0.409
HDL	0.04	-0.04	0.13	0.321
LDL	0.01	-0.01	0.04	0.295
TG	-0.03	-0.04	-0.02	<0.001

SBP:systolic blood pressure
DBP:diastolic blood pressure, FPG:fasting plasma glucose
CHO:cholesteral, TG:triglyceride, HDL:high-density lipoprotein
LDL:low-density lipoprotein, Cr:creatinine
eGFR:estimated glomerular filtration rate
CI:confidence interval
Neg.DCIP:negativedichlorophenol-indolephenol
P<0.05 for the comparison betaween neg. DCIP group and HbEE group
with the use of Gaussian regression analysis

Discussion

Surin Province is located in the northeast of Thailand, near the Thai-Cambodian border. In this region, Thalassemia and Hemoglobin E disorder is more prevalent than in other areas of the world. Patients with diabetes, therefore, are often found to have concomitant hemoglobin E disorder with an estimate of approximately 30-50% of all diabetes patients, which adds a layer of complexity in caring for these patients [23, 26, 27]. The American Diabetes Association (ADA) recommends HbA1c as the standard laboratory assessment of long-term glycemic control and efficacy of treatment of diabetic patients [4-6]. HbA1c better correlates with complications than dose FPG, However, a factor that affects HbA1c level is the lifespan of the red blood cells. In patients with hemoglobinopathies where, the lifespan of red blood cell is shorter than normal, HbA1c may also be lower than usual. For this reason, in ADA guidelines self mornitoring blood glucose is recommended for use to monitor diabetic patients with abnormal hemoglobin [4].

For this reason, HbA1c should also be used for monitoring of glycemic control in diabetic patients with hemoglobinopathies to ensure minimizing long-term diabetic complications while avoiding hypoglycemic attacks, but its use need to take into account the confounding effect of shortened red blood cells lifespan. HbA1c is the result of an irreversible non-enzymatic glycation of the beta chain of hemoglobin A. It is normally present in circulating red cells because of the glycosylation reaction between hemoglobin and circulating glucose. In the pesence of excessive plasma glucose, the hemoglobin beta-chain becomes increasingly glycosylated, making the HbA1c a useful index of long-term glycemic control. This is data are in accordance with the findings that the concentration of minor hemoglobins in young erythrocytes was found to be lower than in the older erythrocytes, if FPG is lower than 170mg%. HbA1c concentrations in diabetic patients with HbEE group were found to be significantly lower than the control group. When the 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 because normal values for HbA1c are based on individuals with a normal hematological profile. For patients in whom 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 combined with HbA1c monitoring. However, FPG and HbA1c are not the only factors that need monitoring; a holistic approach in tailoring care for each patient to prevent complications in the long term should always be kept a priority.

In this study, all physicians were follow the ADA recommended glycemic goals for non-pregnant individuals are based on data of HbA1c. The listed blood glucose goals are levels that appear to correlate with achievement of HbA1c of <7%. During follow up, HbEE group have higher level of FPG and lower in HbA1c. If the lower HbA1c was the artificial low, the patients in HbEE group may have more complication. Because the physician were not try to intensive control when FPG were still high with low HbA1c.

Diabetic nephropathy is a major cause of renal failure. Proteinuria contributes to the progression loss of renal function, while other factors are still debate. In this study show when compared with controls, risk difference of macroalbuminuria was 4.2% lower prevalent in diabetic patients with hemoglobin E homozygous (P=0.008), by univariable and multivariable risk difference regression analysis. The decline in glomerular filtration rate is highly variable, ranging from 2-20 ml/min/year, with a median of 12 ml/min/year [28]. In this data, show very slow rate of decline in eGFR 0.64 ml/min/year (0.14-0.87, 95% confidence interval). Lower than lower range from previous study. The natural history of non-diabetic Caucasians is median decline of GFR 0.4 ml/min/year [29]. Data from this studies evaluated the rate of decline in eGFR were significant slower in HbEE group, but higher 1.6 time and 1.7 time in control group, if compared with normal Caucasians population.

Several limitations are worth mentioning in this study. First, patients with hemoglobin E heterozygote were excluded. Second, missed data in antihypertensive drugs that may be effect in renal complication. Last, many clinical endpoints was not completed evaluated, all caused mortality, macro-vascular complication and other micro-vascular complication. Therefore, FPG is likely not appropriate for monitoring in HbEE patients and instead, HbA1c should be used in these patients population.

Conclusion

Since HbA1c levels are presently the best indicator of long term glycemic control. With long term cohort study, hemoglobin E disorder diabetic patients have less effect on renal complication when

the mean fasting plasma glucose level was higher and lower HbA1c than negative DCIP group. Diabetic patients with hemoglobin E disorder should be monitored using HbA1c level as an indicator for long-term glycemic control.

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