Low Density Lipoprotein Cholesterol and Non-high Density Lipoprotein Cholesterolin Diabetic Patients with Hemoglobin E Disorders at Surin Hospital

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ABSTRACT

Objective : To evaluate the prevalence of low density lipoprotein cholesterol (LDL) of less than100 mg/dl and non-high density lipoprotein cholesterol (non-HDL-C) of less than 130 mg/dl in diabetic patients with hemoglobin E (HbE) disorders at Surin Hospital, located in the northeastern region of Thailand.

Material and Method : The study was conducted in 3,106 diabetic patients at Surin Hospital from June, 2009 through May, 2010. Demographic and clinical data were collected. The HbE screening test and Hb typing were performed. The prevalence of HbE disorders and dyslipidemia were analyzed.

Results : The prevalence of homozygous HbE (HbEE) and HbE traits were 7.6% and 35.6% respectively. The prevalence of low density lipoprotein cholesterol (LDL) of less than 100 mg/dl and non-high density lipoprotein cholesterol (non-HDL-C) of less than 130 mg/dl in HbEE were 37.4% and 48.9%, in the HbE trait were 28.8% and 33.9%, and in the negative screening group were 28.3% and 34.2% respectively. The means of LDL and non-HDL-C were significantly lower in the HbEE than in the other groups ($p < 0.001$ and $p < 0.001$ respectively). The adjusted odds ratio with a 95% confidence interval (95% CI) of LDL less than 100 mg/dl and non-HDL-C less than 130 mg/dl in HbEE when compared with the negative screening group were 1.37 (1.01-1.85) and 1.69 (1.26-2.26) respectively. The adjusted odds ratio with 95% CI of LDL less than 100 mg/dl and non-HDL-C less than 130 mg/dl in the HbE trait when compared with the negative screening group were 1.03 (0.87-1.22) and 0.99 (0.84-1.17) respectively.

Conclusions : LDL and non-HDL-C in diabetic patients with HbEE were significantly lower than in negative screening group, whereas there were no statistical differences of LDL and non-HDL-C between diabetic patients with the HbE trait and the negative screening group.

Keywords : low density lipoprotein cholesterol, LDL, non-high density lipoprotein cholesterol, non-HDL-C, Hemoglobin E disorders, HbEE, HbE trait, Diabetes mellitus, DM, Surin Hospital

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นิพนธ์ต้นฉบับ

ความชุกของผู้มีค่าไขมันชนิดแอลดีแอลต่ำและค่าคอเลสเตอรอลที่หักค่าไขมันชนิดเอชดีแอลออกแล้วต่ำในผู้ป่วยเบาหวานที่เป็นฮีโมโกลบิน อี ในโรงพยาบาลสุรินทร์

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บทคัดย่อ

วัตถุประสงค์ : เพื่อค้นหาความชุกของผู้มีค่าไขมันชนิดแอลดีแอลต่ำและค่าคอเลสเตอรอลที่หักค่าไขมันชนิดเอชดีแอลออกแล้วต่ำในผู้ป่วยเบาหวานที่เป็นฮีโมโกลบิน อี ในโรงพยาบาลสุรินทร์

วัสดุและวิธีการ : ทำการศึกษาภาคตัดขวางในผู้ป่วยเบาหวาน จำนวน 3,106 คน ตั้งแต่เดือนมิถุนายน พ.ศ. 2552 ถึงเดือนพฤษภาคม พ.ศ. 2553 เก็บข้อมูลและสืบค้นหาความชุกของภาวะไขมันในเลือดผิดปกติและฮีโมโกลบิน อี

ผลการศึกษา : พบค่าความชุกของโรคโลหิตจางธาลัสซีเมียชนิดอี ร้อยละ 7.6 และฮีโมโกลบิน อี แฝง ร้อยละ 35.6 พบค่าความชุกของผู้มีค่าไขมันชนิดแอลดีแอลน้อยกว่า 100 มิลลิกรัมต่อเดซิลิตร และผู้มีค่าคอเลสเตอรอลที่หักค่าไขมันชนิดเอชดีแอลออกแล้วน้อยกว่า 130 มิลลิกรัมต่อเดซิลิตร ในโรคโลหิตจางธาลัสซีเมียชนิดอี ร้อยละ 37.4 และร้อยละ 48.9 ในฮีโมโกลบิน อี แฝง ร้อยละ 28.8 และร้อยละ 33.9 ในกลุ่มที่การตรวจคัดกรองให้ผลลบร้อยละ 28.3 และร้อยละ 34.2 ตามลำดับ พบค่าเฉลี่ยของไขมันชนิดแอลดีแอลและค่าเฉลี่ยของคอเลสเตอรอลที่หักค่าไขมันชนิดเอชดีแอลออกแล้วต่ำกว่าอย่างมีนัยสำคัญทางสถิติในกลุ่มโรคโลหิตจางธาลัสซีเมียชนิดอี ($p < 0.001$ และ $p < 0.001$) ค่า Odds ratio เมื่อปรับค่าแล้ว และค่าความเชื่อมั่น 95% ของไขมันชนิดแอลดีแอลน้อยกว่า 100 มิลลิกรัมต่อเดซิลิตร และคอเลสเตอรอลที่หักค่าไขมันชนิดเอชดีแอลออกแล้วน้อยกว่า 130 มิลลิกรัมต่อเดซิลิตร ในโรคโลหิตจางธาลัสซีเมียชนิดอีเมื่อเปรียบเทียบกับกลุ่มที่การตรวจคัดกรองให้ผลลบคือ 1.37 (1.01-1.85) และ 1.69 (1.26-2.26) ตามลำดับ ค่า Odds ratio เมื่อปรับค่าแล้ว และค่าความเชื่อมั่น 95% ของไขมันชนิดแอลดีแอลน้อยกว่า 100 มิลลิกรัมต่อเดซิลิตร และคอเลสเตอรอลที่หักค่าไขมันชนิดเอชดีแอลออกแล้วน้อยกว่า 130 มิลลิกรัมต่อเดซิลิตรในฮีโมโกลบิน อี แฝง เมื่อเปรียบเทียบกับกลุ่มที่การตรวจคัดกรองให้ผลลบคือ 1.03 (0.87-1.22) และ 0.99 (0.84-1.17) ตามลำดับ

สรุป : พบไขมันชนิดแอลดีแอลและคอเลสเตอรอลที่หักค่าไขมันชนิดเอชดีแอลออกแล้วในผู้ป่วยเบาหวานที่เป็นโรคโลหิตจางธาลัสซีเมียชนิดอี ต่ำกว่ากลุ่มที่การตรวจคัดกรองให้ผลลบอย่างมีนัยสำคัญทางสถิติ แต่ไม่พบความแตกต่างทางสถิติดังกล่าวระหว่างฮีโมโกลบิน อี แฝงกับกลุ่มที่การตรวจคัดกรองให้ผลลบ

Introduction

The Third Report of the National Cholesterol Education Program Adult Treatment Panel (NCEP ATPIII) categorized diabetes mellitus (DM) in the group with coronary risk as high as patients with established coronary artery disease (CAD), and their low density lipoprotein cholesterol levels (LDL) should be lowered to less than 100 mg/dl.[1] Lipid lowering is one of the important strategies for reducing cardiovascular disease (CVD) events in diabetic patients.[2] Recent studies suggest that a more aggressive target for lipid lowering may have added benefits.[3,4] The Thailand diabetes registry project showed a high prevalence of dyslipidemia in adult Thai type 2 diabetes who attended diabetes clinics in university and tertiary-care hospitals.[5] The study also suggested that most of them would have to be treated with lipid lowering agents. The Hemoglobin E (HbE) disorder is one of the world's most common and important mutations.[6,7] Both the HbE trait and homozygous HbE (HbEE) are mild disorders. HbE occurs in high frequency at the junction of Thailand, Laos, and Cambodia.[8] A recent study crudely showed that cholesterol levels and LDL levels were significantly lower in diabetic patients with HbEE than in the negative screening group.[9]

The author evaluated the lipid levels of diabetic patients with HbE in a large scale group of cases at Surin Hospital, a tertiary-care hospital which is located in the northeastern region of Thailand at the boundary of Cambodia.

Material and Method

The study was carried out in the diabetes clinic at Surin Hospital by a simple random sampling method between June 2009 and May 2010. The present study was approved by the Ethics Committee of Surin Hospital. The committee classified the proposal as an R-to-R (routine to research study). Participants were diabetic patients followed in the clinic for more than two months.

The processes of the diabetic clinic were designed and conducted by a pilot project of participatory action research (PAR) which have been described in detail elsewhere.[10] At each visit, the patients were treated by physicians and a multidisciplinary team based on the American Diabetes Association (ADA) standard recommendations which consist of position statements that represent the official ADA opinion as denoted by formal review and approval.[11,12]

The data were recorded on the day that the laboratory examinations were performed. The author included: demographic data, pertinent parts of physical examinations, laboratory examinations, specific medications, and diabetic complications verified by physician's reports. The history of diabetes was obtained through an interview. Height and weight were measured by trained health professional via standard procedures, after which body mass index (BMI) was calculated as the weight in kilograms divided by the square of height in meters. Blood pressure (BP) was obtained with an automated blood pressure machine (Omron HEM-907XL) after resting at least 15

minutes, using the appropriate cuff size and measured after being in a seated position with both feet on the floor for at least three minutes. BP measurements were repeated twice at three minutes apart and the average values were used in the analyses. The goal of BP in diabetic patients was determined as less than130/80 mmHg.[11,12] The patients who had a diagnosis of hypertension (HTN) by the physicians or had a BP more than the goal were classified as HTN.

The fasting plasma glucose (FPG), Hemoglobin A1c (HbA1c), lipid profile, complete blood count including hemoglobin concentration (Hb), BUN, and creatinine were collected on the same day after the patients had been regularly treated in the clinic for more than two months.

The combination of dichlorophenol-indolephenol (DCIP) test and low mean corpuscular volume (MCV) level were used as a HbE screening test.[13] Hemoglobin typing was performed in cases of a positive HbE screening test by the Hb Gold analyzer (Drew Scientific ltd., England) using low pressure liquid chromatography (LPLC). The interpretation of HbE from Hb Gold chromatogram was based on hematologic data in various HbE syndromes.[14] HbA1c was measured by turbidimetric inhibition immunoassay and the reagent was Tina-Quant Hemoglobin A1c II Cobas. The lipid profile consisted of a total cholesterol level (CHOL), triglyceride level (TG), LDL and high density lipoprotein cholesterol level (HDL). The CHOL and TG were measured by enzymatic colorimetric assay; the reagents were Cholesterol CHOD-PAP Cobas and Triglyceride GPO-PAP Cobas respectively. HDL and LDL were measured by homogenous enzymatic colorimetric assay; the reagents were HDL-C plus 3rd generation Cobas and LDL-C plus 2nd generation Cobas respectively. Both the HbA1c and lipid profile were analyzed by a Roche/Hitachi 917 automatic analyzer. Non-HDL cholesterol level (non-HDL-C) was calculated as CHOL minus HDL. The DCIP test was KKU-DCIP Clear reagent.[15] FPG, CBC, BUN and creatinine were determined by the central laboratory of Surin Hospital using standard methods with quality control.

The patients were classified into three groups: negative screening, HbE trait, and HbEE. The patients with dyslipidemia were defined as all patients who were taking lipid lowering agents and patients whose lipid levels were over the target of the ADA.[11,12] The cut-off point of anemia for each sex was classified by WHO standards.[16]

Statistical analysis

The demographic and clinical data are presented as numbers and percentages for categorical variables; as means and standard deviations (SD) for continuous variables. The Pearson Chi-square test was used to compare the differences between the categorical variables. Two-tailed tests were used to determine the statistical significance at a p-value of less than 0.05. Normality of distribution for each group was checked using Kolmogorov-Smirnov test. Differences in mean values of continuous variables were compared using Kruskal-Wallis test.

To compare the lipid levels between HbE disorders and the negative screening group, the correlation was calculated to define each variable that influenced the lipid levels. Confounding factors were adjusted by applied multiple logistic regression analysis, and the variables of p-value less than 0.25 were also entered into the models. The multicollinearity was clarified and unnecessary variables were excluded from the models by the backward elimination method. Descriptive statistics were analyzed using SPSS for Windows version 11.0 (SPSS Inc, Chicago, IL, U.S.). The STATA version 6.0 (STATA Corporation, TX, U.S.) was used to analyze binary logistic regression and adjusted odds ratio (ORs) with a 95% confidence interval (CI).

Results

Complete demographic and clinical data were available for 3,106 of the 3,128 patients in the diabetic clinic at Surin Hospital and 888 patients (28.6%) were male. The median of age and duration of DM were 62.0 and 6.8 years respectively. Most of patients (92.1%) located in the districts within the responsibility of Surin Hospital, while 14.7% of patients lived in the Surin municipal area. The prevalence of HbEE and the HbE trait were 7.6% and 35.6% respectively. Males achieved the LDL level of less than 100 mg/dl and non-HDL-C level less than 130 mg/dl more than females (33.8% V.S. 27.3% with $p < 0.001$ and 38.3% V.S. 34.0% with $p = 0.024$ respectively). The prevalence of HDL greater than 40 mg/dl in males and HDL greater than 50 mg/dl in females were 69.1% and 45.6%, respectively.

In each group there was no statistical difference between sex ($p = 0.603$), age under 60 years ($p = 0.710$), BMI <23 Kg/m^2 ($p = 0.606$), diagnosis of HTN ($p = 0.139$), FPG less than 130 mg/dl ($p = 0.993$), HDL achieved targets ($p = 0.873$), and fibrate therapy ($p = 0.324$). The negative screening group significantly had more patients with a duration of DM of over five years ($p = 0.001$). Diabetes with HbEE significantly had the highest patients of HbA1c less than 6.5% ($p < 0.001$), HbA1c less than 7% ($p < 0.001$), LDL less than 100 mg/dl ($p = 0.014$), Non-HDL-C less than130 mg/dl ($p < 0.001$), TG less than 150 mg/dl ($p = 0.028$), and anemia ($p < 0.001$), whereas patients of statin therapy were significantly the lowest in the HbEE group ($p < 0.001$) (Table 1). There were no statistical differences in means of age, BMI, FPG, BUN, creatinine, HDL of each sex among the groups. The means of HbA1c, Hb, CHOL, TG, LDL, non-HDL-C and LDL/HDL were significantly lowest in the HbEE group (Table 2).

Table 1 Prevalence of HbE disorders and the variables among each group

Characteristics	All (%)	Negative screening (%)	HbE trait (%)	HbEE (%)	p-value*
Cases	3106	1765(56.8)	1106(35.6)	235(7.6)	
Male	888(28.6)	517(29.3)	307(27.8)	64(27.2)	0.603
Age over 60 years	1426(45.9)	813(46.1)	500(45.2)	113(48.1)	0.710
Duration of DM over five years	1475(47.5)	889(50.4)	480(43.4)	106(45.1)	0.001
BMI <23 kg/m^2	1423(45.8)	805(45.6)	503(45.5)	115(48.9)	0.606
Diagnosis of HTN	2280(73.4)	1317(74.6)	800(72.3)	163(69.4)	0.139
FPG <130 mg/dl	1443(46.5)	820(46.5)	513(46.4)	110(46.8)	0.993
HbA1c <6.5%	977(31.5)	475(26.9)	349(31.6)	153(65.1)	<0.001
HbA1c <7.0%	1451(46.7)	728(41.2)	539(48.7)	184(78.3)	<0.001
LDL <100 mg/dl	905(29.1)	499(28.3)	318(28.8)	88(37.4)	0.014
Non-HDL-C <130 mg/dl	1094(35.2)	604(34.2)	375(33.9)	115(48.9)	<0.001
TG <150 mg/dl	1566(50.4)	883(50.0)	545(49.3)	138(58.7)	0.028
HDL achieved targets**	1626(52.4)	931(52.7)	574(51.9)	121(51.5)	0.873
Statin therapy	1612(51.9)	936(53.0)	578(52.3)	98(41.7)	0.005
Fibrate therapy	387(12.5)	233(13.2)	125(11.3)	29(12.3)	0.324
Anemia	1577(50.8)	800(45.3)	582(52.6)	195(83.0)	<0.001

* Pearson chi-square

** HDL>40 mg/dl in male or >50 mg/dl in female

HbE = hemoglobin E; HbEE = homozygous HbE; BMI = body mass index; HTN = hypertension; FPG = fasting plasma glucose; HbA1c = hemoglobin A1c; LDL = low density lipoprotein; non-HDL-C = non-high density lipoprotein cholesterol; TG = triglyceride; HDL = high density lipoprotein

Table 2 Means and standard deviations of the variables of each group

Characteristics	All	Negative screening	HbE trait	HbEE	p value*
	Mean(SD)	Mean(SD)	Mean(SD)	Mean(SD)	
Age (year)	59.2(10.7)	59.1(10.8)	59.0(10.5)	60.0(10.4)	0.58
BMI (kg/m^2)	23.7(4.09)	23.8(4.07)	23.7(4.18)	23.3(3.86)	0.234
FPG (mg/dl)	141.3(45.1)	140.4(45.0)	142.8(48.3)	141.3(44.2)	0.487
HbA1c (%)	7.48(1.81)	7.65(1.84)	7.43(1.78)	6.44(1.41)	<0.001
Hb (g/dl)	12.1(1.73)	12.3(1.75)	11.9(1.63)	10.8(1.49)	<0.001
CHOL (mg/dl)	199.6(43.5)	201.3(43.8)	200.6(44.0)	182.2(35.5)	<0.001
TG (mg/dl)	175.3(110.3)	176.1(114.8)	177.7(106.2)	158.8(92.1)	0.008
LDL (mg/dl)	112.5(37.5)	123.5(37.5)	123.7(38.2)	109.3(30.7)	<0.001
Non-HDL-C (mg/dl)	149.9(43.4)	151.4(43.8)	150.9(43.6)	133.5(35.3)	<0.001
HDL in male (mg/dl)	47.5(12.6)	47.7(12.5)	47.4(13.1)	45.3(10.8)	0.227
HDL in female(mg/dl)	50.6(12.5)	50.7(12.7)	50.6(11.8)	50.1(13.9)	0.749
LDL/HDL	2.61(1.0)	2.63(1.01)	2.62(0.98)	2.39(0.9)	0.002

* Kruskal-Wallis test

HbE = hemoglobin E; HbEE = homozygous HbE; BMI = body mass index; FPG = fasting plasma glucose; HbA1c = hemoglobin A1c; Hb = hemoglobin; CHOL = cholesterol; TG = triglyceride; LDL = low density lipoprotein; non-HDL-C = non-high density lipoprotein cholesterol; HDL = high density lipoprotein

When compared with the negative screening group, diabetic patients with HbEE significantly had more LDL of less than100 mg/dl (p = 0.004), non-HDL-C less than130 mg/dl (p <0.001). After a multiple logistic regression analysis was performed to adjust for sex, age over 60 years, living in the municipal area, diagnosis of HTN, duration of diabetes, BMI, anemia, FPG less than130 mg/dl, statin therapy, and fibrate therapy respectively; the adjusted odds ratio with 95% CI of LDL less than100 mg/dl and non-HDL-C less than130 mg/dl in HbEE when compared with the negative screening group were 1.37 (1.01-1.85) and 1.69 (1.26-2.26) respectively (Table 3).

Table 3 Adjusted odds ratios of HbE compared with negative screening group

	Negative screening		HbE trait		HbEE	
	n (%)	ORs	n (%)	ORs (95% CI)	n (%)	ORs (95% CI)
LDL <100 mg/dl	499(28.3)	1	318(28.8)	1.03(0.87-1.22)	88(37.4)	1.37(1.01-1.85)
Non-HDL-C <130 mg/dl	604(34.2)	1	375(33.9)	0.99(0.84-1.17)	115(48.9)	1.69(1.26-2.26)
TG <150 mg/dl	883(50)	1	545(49.3)	0.99(0.85-1.16)	138(58.7)	1.34(1.00-1.80)
HDL achieved targets*	931(52.7)	1	574(51.9)	1.03(0.88-1.21)	121(51.5)	1.14(0.85-1.53)

* HDL >40 mg/dl in male and >50 mg/dl in female

HbE = hemoglobin E; HbEE = homozygous HbE; ORs = Odds ratios; 95% CI = 95% confidence interval; LDL = low density lipoprotein; non-HDL-C = non-high density lipoprotein cholesterol; TG = triglyceride; HDL = high density lipoprotein

Whereas the adjusted odds ratio with 95% CI of LDL less than100 mg/dl and non-HDL-C less than 130 mg/dl in the HbE trait when compared with the negative screening group were 1.03 (0.87-1.22) and 0.99 (0.84-1.17) respectively.

Discussion

Despite well-publicized treatment guidelines, elevated cholesterol levels remain high in diabetic patients. The prevalence of dyslipidemia in adult Thai type 2 diabetes who attended diabetes clinics in university and tertiary-care hospitals in recent years was very high.[6] Presently, the prevalence of LDL less than 100 mg/dl and non-HDL-C less than130 mg/dl were around one third and predominated in males. This rate of achieving an LDL target was not much better than the previous report of non-pharmacological therapy for lowering LDL levels in diabetic patients at Surin Hospital.[17] Although statins are highly effective for decreasing LDL levels in patients with diabetic dyslipidemia, failure to reach LDL targets remains common.[18]

In North America, the failure to achieve goals for dyslipidemia can be initiated from many causes, including a lack of follow up and improper titration of the starting statin dose, but perhaps the most important reason is poor adherence to treatment.[18,19]. The titration of the statin dose may be a major cause of the failure at Surin Hospital because the budget from the government supported health plan was limited on the frequency of blood examinations and types of statins.

The mean of HbA1c was statistically lowest and achieving HbA1c targets were statistically highest in HbEE, the interference of HbE on HbA1c measurement may confound the results.[20,21] The author recently reported that HbA1c was significantly lower in diabetic patients with HbEE at Surin Hospital.[8] The effect of HbE on the immunoassays used for HbA1c measurement may cause bias so the variables of HbA1c were not entered into the model of the regression analysis. HbE disorders especially HbEE cause anemia as showed in the tables.

HbE is the most common hemoglobinopathy in Southeast Asia with most of the data of these genetic abnormalities coming from Thailand.[8,15,22] However there are few studies about the effects of HbE on diabetes.[9,20,21] The present study shows a large portion of diabetic patients with HbE, the available patients are two times greater than the recent study that evaluated HbE screening tests.[13] This study clearly confirmed the earlier finding of more patients with LDL <100 mg/dl in diabetes with HbEE than in negative screening group[9] and also found a greater odds ratio of non-HDL-C less than 130 mg/dl in diabetes with HbEE. Abnormal lipid composition and organization can be found in some subpopulations of RBC in hemoglobiniopathies,[23] and the reduction of cholesteryl linoleate is a severity index of atherogenesis risk in beta-thalassemia/ Hb E.[24] Even though LDL and non-HDL-C are predictors of CVD risk in patients with diabetes,[25] the benefit of these finding in HbEE were not clarified.

Conclusion

Hemoglobin E disorders are highly prevalent in diabetic patients at Surin Hospital. LDL and non-HDL-C in diabetic patients with HbEE were significantly lower than in negative screening group, whereas there were no statistical differences of LDL and non-HDL-C between diabetic patients with HbE trait and the negative screening group. The benefit of these findings in diabetic patients with HbEE regarding cardiovascular disease risk should be further evaluated.

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Reference

1. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation,and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001; 285: 2486-97.
2. Pyorala K, Pedersen TR, Kjekshus J, Faergeman O, Olsson AG, Thorgeirsson G. Cholesterol lowering with simvastatin improves prognosis of diabetic patients with coronary heart disease. A subgroup analysis of the Scandinavian Simvastatin Survival Study (4S). Diabetes Care 1997; 20: 614-20.
3. Collins R, Armitage J, Parish S, Sleigh P, Peto R. Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 5963 people with diabetes: a randomised placebo-controlled trial. Lancet 2003; 361: 2005-16.
4. Colhoun HM, Betteridge DJ, Durrington PN, Hitman GA, Neil HA, Livingstone SJ, et al. Primary prevention of cardiovascular disease withatorvastatin in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): multicentre randomised placebo-controlled trial. Lancet 2004; 364: 685-96.
5. Pratipanawatr T, Rawdaree P, Chetthakul T, Bunnag P, Ngarmukos C, Benjasuratwong Y, et al. Thailand diabetes registry project: Current status of dyslipidemia in Thai diabetic patients. J Med Assoc Thai 2006; 89(Suppl 1): S60-5.
6. Old JM, Olivieri NF, Thein SL. Avoidance and population control. In: Weatherall DJ, Clegg JB, Gibsons R, Higgs DR, Old JM, Olivieri NF, editors. The thalassaemia syndromes. Oxford: Blackwell Science; 2001: 597-629.
7. Weatherall DJ. Introduction to the problem of hemoglobin E-beta thalassemia. J Pediatr Hematol Oncol 2000; 22: 551.
8. Wasi P. Haemoglobinopathies including thalassaemia. Part 1: Tropical Asia. Clin Haematol 1981; 10: 707-29.
9. Srisurin W. Prevalence and effect of hemoglobin E disorders on HbA1c and lipid profile of diabetic patients at Surin Hospital. J Med Assoc Thai 2011; 94 (1): 36-42
10. Srisurin W. 12 years of participatory action research in diabetes clinic at Surin Hospital. Med J Srisaket Surin Buriram Hosp 2010; 25: 117-134.
11. American Diabetes Association. Standards of medical care in diabetes 2009. Diabetes Care 2009; 32(Suppl 1): S13-61.
12. American Diabetes Association. Standards of medical care in diabetes 2010. Diabetes Care 2010; 33(Suppl 1): S11-61.
13. Prayongratana K, Polprasert C, Raungrongmorakot K, Tatone K, Santiwatanakul S. Low cost combination of DCIP and MCV was better than that of DCIP and OF in the screening for hemoglobin E. J Med Assoc Thai 2008; 91: 1499-504.

14. Vichinsky E. Hemoglobin E syndromes. Hematology 2007; 1: 79-83.
15. Fucharoen G, Sanchaisuriya K, Sae-ung N, Dangwibul S, Fucharoen S. A simplified screening strategy for thalassaemia and haemoglobin E in rural communities in south-east Asia. Bull World Health Organ 2004; 82: 364-72.
16. World Health Organization. Methods of assessing iron status. In: Iron deficiency anaemia: assessment, prevention and control. A guide for programme managers. WHO/NHD/01.3. Geneva: WHO; 2001: 33-45.
17. Srisurin W. Non-pharmacological therapy for low-density lipid-cholesterol levels in type-2 diabetic patients at Surin Hospital. Intern Med J Thai 2002; 18: 238-44.
18. Parris ES, Lawrence DB, Mohn LA, Long BL. Adherence to statin therapy and LDL cholesterol goal attainment by patients with diabetes and dyslipidemia. Diabetes Care 2005; 28: 595–9.
19. Benner JS, Glynn RJ, Mogun H, Neumann PJ, Weinstein MC, Avorn J. Long-term persistence in use of statin therapy in elderly patients. JAMA 2002; 288: 455–61.
20. Little RR, Rohlfing CL, Hanson S, Connolly S, Higgins T, Weykamp CW, et al. Effects of hemoglobin (Hb) E and HbD traits on measurements of glycated Hb (HbA1c) by 23 methods. Clin Chem 2008; 54: 1277-82.
21. Paisooksantivatana K, Kongsomgan A, Banyatsuppasin W, Khupulsup K. Influence of hemoglobin E on measurement of hemoglobin A1c by immunoassays. Diabetes Res Clin Pract 2009; 83: e84-85.
22. Wasi P, Pootrakul S, Pootrakul P, Prawatmuang P, Winichagoon P, Fucharoen S. Thalassemia in Thailand. Ann N Y Acad Sci 1980; 344: 352-63.
23. Kuypers FA. Membrane lipid alterations in hemoglobinopathies. Hematology Am Soc Hematol Educ Program 2007; 68-73.
24. Luechapudiporn R, Morales NP, Fucharoen S, Chantharaksri U. The reduction of cholesteryl linoleate in lipoproteins: an index of clinical severity in beta-thalassemia/Hb E. Clin Chem Lab Med 2006; 44: 574-81.
25. Lu W, Resnick HE, Jablonski KA, Jones KL, Jain AK, Howard WJ, et al. Non-HDL cholesterol as a predictor of cardiovascular disease in type 2 diabetes. Diabetes Care 2003; 26: 16–23.