Diabetes Mellitus in Young Thai Adults

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Abstract

The purposes of the present study were to 1) find the prevalence of various types of diabetes; 2) determine the prevalence of glutamate decarboxylase autoantibody (anti-GAD) and 3) identify clinical characteristics which may help in predicting insulin deficiency in young Thai adults with diabetes.

Subjects consisted of 93 adults with diabetes mellitus aged 15-40 years. In each subject, basal and post glucagon C-peptide levels were determined by radioimmunoassay. Anti-GAD was measured by radioimmunoassay and mitochondrial 3243 tRNA $^{Leu(UUR)}$ gene mutation was detected by PCR-RFLP. Data were expressed as mean ± SEM.

The mean age of subjects was 31.0 ± 0.7 years with age at diagnosis of 25.6 ± 0.9 years. Thirty nine (41.9%) were males and 54 (58.1%) were females. Pancreatic calcification was found in 7 (7.5%) of the patients while 2 (2.2%) were identified as having Wolfram syndrome. Four (4.3%) had nonketotic diabetes with affected family members in multiple generations consistent with MODY. Mitochondrial 3234 tRNA $^{Leu(UUR)}$ gene mutation was detected in only one patient. After excluding 14 subjects with pancreatic calcification, Wolfram's syndrome, MODY or mitochondrial gene mutation, 45 (57.0%) were found to be insulin-deficient and 34 (43.0%) were insulin-sufficient based on post-glucagon C-peptide levels. Using stepwise logistic regression analysis, it was found that younger age at diagnosis ($p<0.001$), smaller waist circumference($p<0.01$), previous history of DKA ($p<0.01$) was significantly associated with insulin deficiency. After excluding patients with DKA, younger age at diagnosis of diabetes ($p < 0.05$) and lower BMI ($p<0.01$) were related to insulin deficiency. Concerning the role of autoimmunity, it was found that 13 (28.3%) of insulin-deficient subjects were positive for anti-GAD while 4 (11.8%) of those who were insulin-sufficient had positive results. Of the 54 patients currently on insulin, 42 (77.8%) are insulin deficient and 14 (25.9%) have positive anti-GAD. There were 10 (18.5%) who were both insulin sufficient and negative for anti-GAD suggesting that insulin therapy may not be

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required. We concluded that about half of young Thai adults with diabetes are not insulin-deficient and treatment with insulin may be unnecessary. The prevalence of glutamate decarboxylase antibody and mitochondrial 3234 tRNA $^{Leu(UUR)}$ gene mutation is low and as yet undefined factors are accountable for insulin deficiency in a significant number of patients.

Key word : Diabetes Mellitus, Autoimmunity, Glutamate Decarboxylase Antibody, Insulin Reserve, Beta Cell Function

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Diabetes mellitus is a heterogeneous disorder in regard to etiology, pathogenesis, clinical course and treatment. Two major types as defined by the World Health Organization were type 1 and 2 or previously IDDM and NIDDM[1]. However, discrimination between type 1 and type 2 DM may be difficult, particularly in young adults[2-4]. In addition to type 1 and type 2 diabetes, other disorders causing diabetes in young adults include pancreatic disease, maturity-onset diabetes of the young (MODY), specific syndromes such as Wolfram's syndrome, Turner's syndrome and the recently described maternally inherited diabetes and deafness (MIDD) which is caused by mitochondrial gene mutations[5]. Despite the heterogeneity, diabetes mellitus in young adults is often presumed to be type 1 and treated with insulin. The introduction of insulin therapy in some patients may be unnecessary resulting in inconvenience and disruption of the patients' lifestyles. It was the purpose of the present study to 1) find the prevalence of various types of diabetes in young Thai adults, 2) identify clinical characteristics which may help predicting insulin deficiency and 3) determine the prevalence of glutamate decarboxylase autoantibody (anti-GAD) in young Thai adults with diabetes.

PATIENTS AND METHOD

Subjects

Subjects consisted of 93 adults with diabetes mellitus, aged 15-40 years, recruited from the diabetic-outpatient clinics of Ramathibodi Hospital, Mahidol University and Theptarin General Hospital, Thailand during 1996-1997. Subjects were diagnosed as having diabetes mellitus according to WHO criteria[1]. Criteria for MODY included age at onset less than 25 years, diabetes presented in at least 3 generations and non-insulin requiring for more than 2 years. The study protocol was approved by the ethical clearance committee on human rights related to research involving human subjects of the Faculty of Medicine, Ramathibodi Hospital, Mahidol University. Informed consent was obtained from each patient before investigation.

Assessments

Abdominal radiograph was obtained in every patient to detect pancreatic calcification. Serum C-peptide level was measured by radioimmunoassay (Incstar, Stillwater, MN) after an overnight fast and at 6 minutes after an intravenous injection of 1 mg of glucagon. Serum was stored at -20°C for less than 6 months before the assay. The cutoff levels for basal and post-glucagon C-peptide were obtained from studying 22 young adults with diabetes mellitus. Based on clinical criteria, 10 of the subjects were classified as insulin-deficient and 12 subjects were insulin-sufficient. The clinical criteria for insulin deficiency included history of DKA, duration of diabetes of more than 2 years and insulin dependency. Clinical criteria for insulin sufficiency included the absence of DKA, absence of marked ketonuria during follow-up, good glycemic control while on oral agents and duration of diabetes of less than 5 years. The cutpoints with 100 per cent sensitivity and 90 per cent specificity for poor insulin

reserve were 0.36 nmol/L and 0.52 nmol/Lfor basal C-peptide and stimulated C-peptide, respectively. Subjects who had stimulated C-peptide above 0.52 nmol/L were classified as insulin-sufficient.

Anti-GAD was determined by radioimmunoassay (CIS Bio International, France). The control group consisted of 38 healthy non-diabetic subjects recruited by flyer or advertisement. The cutoff point for positive anti-GAD corresponding to the mean value of controls + 2 standard deviations was 0.8 U/ml.

Mitochondria 3243 $tRNA^{leu(UUR)}$ mutation was detected by PCR-RFLP. DNA was extracted from peripheral leukocytes by phenol/chloroform extraction. Mitochondrial DNA flanking the A to G transition at position 3243 was amplified by polymerase chain reaction with the following primers: forward, 5'-AGGACAAGAAGAAATAAGGCC-3'; and reverse, 5'-CACGTTGGGGCCTTTGCGTA-3' as previously described(6). The final PCR product was then digested with Apa I restriction endonuclease. The digested material was resolved on 0.8 per cent agarose gel with ethidium bromide staining.

Statistical Analysis

Baseline clinical characteristics were compared by Student's *t* test and Chi square test as appropriate. Clinical predictors for insulin deficiency were derived from stepwise multiple logistic regression analyses. Data were expressed as mean ± SEM.

RESULTS

Specific disorders associated with diabetes were presented in 14 (15.1%) subjects which included 7 (7.5%) with pancreatic calcification, 2 (2.2%) with Wolfram's syndrome, 1 (1.1%) with mitochondrial 3243 $tRNA^{Leu(UUR)}$ mutation and 4 (4.3%) with clinical features consistent with MODY. Based on stimulated C-peptide levels, in the other 79 subjects without specific disorders 45 (57.0%) were found to be insulin-deficient (C-peptide, 0.18 ± 0.001 nmol/L) and 34 (43.0%) were insulin-sufficient based on stimulated C-peptide levels (C-peptide, 1.62 ± 0.15 nmol/L). Clinical characteristics of the two groups are shown in Table 1. Insulin-deficient subjects were significantly younger, had lower BMI, smaller waist circumference (WC), smaller waist hip ratio (WHR). Using stepwise logistic regression, the clinical predictors of poor insulin reserve were younger age at diagnosis ($p < 0.001$), smaller WC ($p < 0.01$) and history of DKA ($p < 0.01$) as shown in Table 2A. All 25 patients with a history of DKA were insulin-deficient except 3 subjects. Two of these 3 subjects were obese (BMI 30.0 and 30.1 kg/m^2). All 3 patients were negative for anti-GAD. After excluding subjects with a history of DKA, the clinical predictors for insulin deficiency in the remaining 55 subjects were younger age at diagnosis ($p < 0.05$) and lower BMI ($p < 0.01$) as demonstrated in Table 2B.

As shown in Table 3, 13 (28.8%) of the insulin-deficient subjects were positive for anti-GAD while 4 (11.8%) of those who were insulin-sufficient were positive for anti-GAD. Three subjects in the insulin-deficient group were not on insulin. Two of these subjects were negative while one was positive for anti-GAD. The subject with positive anti-GAD subsequently developed failure to oral agents. Ten subjects who had adequate C-peptide and were negative for anti-GAD were treated with insulin. Insulin was introduced in 2 subjects because of a history of DKA. These two subjects were shown

Table 1. Comparison of clinical characteristics of subjects according to insulin reserve.

	Insulin deficient (n = 45)	Insulin sufficient (n = 34)	p-value
Sex (male:female)	18:27	13:21	NS
Age (year)	28.3 ± 1.1	33.5 ± 1.0	< 0.01
Age at diagnosis (year)	21.8 ± 1.2	30.8 ± 1.1	< 0.001
BMI (kg/m^2)	21.3 ± 0.6	25.6 ± 0.8	< 0.001
Waist (cm)	73.0 ± 1.5	86.2 ± 2.0	< 0.001
Hip (cm)	90.5 ± 1.3	98.2 ± 1.6	< 0.001
Waist-hip ratio	0.81± 0.01	0.88 ± 0.01	< 0.001
Number (%) with history of DKA	22 (48.9%)	3 (8.8%)	< 0.001

Table 2A. Factors associated with insulin deficiency from stepwise logistic regression analysis.

Clinical characteristics	Odds Ratio	95% CI
Age at diagnosis (year)	0.83	0.74 - 0.93
Waist circumference (cm)	0.90	0.90 - 0.96
History of DKA	18.0	2.9 - 112.2

Table 2B. Factors associated with insulin deficiency from stepwise logistic regression analysis in subjects without history of DKA.

Clinical characteristics	Odds Ratio	95% CI
Age at diagnosis (year)	0.77	0.66 - 0.90
BMI	0.75	0.59 - 0.94

Table 3. The number of subjects categorized according to insulin reserve and anti-GAD result. Numbers in parentheses represent the number of subjects who were on insulin.

	Insulin deficient	Insulin sufficient
Negative anti-GAD	32 (30)	30 (10)
Positive anti-GAD	13 (12)	4 (2)
Total	45 (42)	34 (12)

to have adequate insulin reserve (stimulated C-peptide = 1.48 and 0.66 nmol/L) after 4 months and 5 years of DKA. One of the two patients with positive anti-GAD in the adequate insulin reserve group subsequently developed drug failure and insulin was started.

DISCUSSION

The findings in the present study demonstrated the heterogeneity of DM in young Thai adults. Specific disorders associated with diabetes were present in 15.1 per cent of subjects. It is of note that the two subjects with pancreatic calcification never had abdominal pain or symptoms of exocrine dysfunction suggesting that routine abdominal X-ray is still a useful investigation for young adults with diabetes mellitus particularly in this region of the world. MIDD was infrequent in the present study. There was only one patient with mitochondrial 3243 tRNA Leu(UUR) mutation detected by PCR-RFLP. MIDD is uncommon and the prevalence of MIDD varies among populations depending on the subgroups of diabetic subjects screened[7-9]. Although MIDD is more prevalent in younger patients with diabetes,[10] it was rare in our population according to the results of the present study. However, the prevalence of other mitochondrial mutations related to diabetes[11-13] remains to be determined.

Autoimmunity is one of the etiologic factors for diabetes particularly in the younger age group. The autoantibodies associated with diabetes include anti-GAD, islet cell antibodies (ICA), insulin autoantibodies (IAA) and tyrosine phosphatase antibody (IA2). The frequency of ICA, IAA but not anti-GAD decreased in patients aged more than 20 years[14]. In our study, the prevalence of anti-GAD in subjects with poor insulin reserve was 28.3 per cent which was low compared to that in Caucasians[15]. Nevertheless, the prevalence was consistent with previous reports in Thais[16] and other Asian populations[17-19]. The low prevalence of anti-GAD may be due to the difference in genetic and environmental factors among populations. Besides autoimmunity, other factors can also be associated with insulin secretory defects in diabetes. For example, reduced insulin secretion is a feature of MIDD[20] and MODY[21]. However, mitochondrial 3243 tRNALeu(UUR) mutation was detected in only one subject and only 4 subjects had clinical features consistent with MODY. Hence, other undefined factors may be accountable for insulin deficiency in a sizable number of patients. Determination of other beta cell-related antibodies and mutational study of genes associated with MODY can be helpful in more precise classification of diabetes in young adults according to pathophysiological basis.

A number of studies have evaluated insulin reserve by assessing C-peptide levels in both type 1 and type 2 diabetes. The findings as well as the cutoff point for differentiating insulin reserve varied and were dependent on study design, clinical criteria for the types of diabetes, age and duration of the disease. Madsbad and others[22-24] found that C-peptide value of 0.59 nmol/L, 6 min after an IV injection of 1 mg glucagon, had a high accuracy for predicting insulin dependency regardless of the type of diabetes. The cutoff value may not be suitable in other populations since it may be confounded

by the variation among C-peptide assays used. Nevertheless, the cutoff stimulated C-peptide level of 0.52 nmol/L in our study was close to the previously suggested value of 0.59 nmol/L even although it was independently determined. In patients with borderline C-peptide value, clinical data may assist in the decision for the choice of treatment. The history of DKA, younger age at onset and lower BMI were associated with insulin deficiency in the present study. This is in keeping with previous reports which found that practically all diabetic persons before age 20 belonged to the IDDM group[2,3] except in some racial populations such as Pima Indian[25] and Nauruan[26] which have a high incidence of young obese NIDDM. Furthermore, per cent desirable body weight has been reported to be helpful in the classification of newly diagnosed diabetic patients[27]. It is of note that almost half of the young adults with diabetes in our study were insulin sufficient. Initiating insulin treatment in such patients may induce inconvenience and disrupt their lifestyles. Direct assessment of insulin reserve or estimating the risk of insulin deficiency from clinical factors may be helpful in avoiding unnecessary insulin administration.

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เบาหวานในผู้ป่วยไทยที่เป็นเบาหวานตั้งแต่อายุน้อย

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ได้ทำการศึกษาชนิดของเบาหวานและการทำงานของ pancreatic (cells ในผู้ป่วยเบาหวานไทยอายุ 15–40 ปี จำนวน 93 ราย พบว่าร้อยละ 14 ของผู้ป่วยมีสาเหตุจำเพาะของโรคเบาหวานได้แก่ pancreatic calcification, Wolfram syndrome, mitochondrial 3234 tRNA Leu(UUR) gene mutation และ MODY ในผู้ป่วยที่ไม่มีสาเหตุจำเพาะพบว่ามีภาวะพร่องอินสุลินร้อยละ 57 และร้อยละ 43 ไม่พบว่ามีภาวะพร่องอินสุลิน ลักษณะทางคลินิกที่มีความสัมพันธ์กับภาวะพร่องอินสุลินได้แก่ อายุเมื่อเริ่มเป็นเบาหวานน้อย ($p < 0.001$), เส้นรอบเอวน้อย ($p < 0.01$) และประวัติของการเกิด diabetic ketoacidosis ($p < 0.01$) ในผู้ป่วยที่มีภาวะพร่องอินสุลินร้อยละ 28.3 ให้ผลบวกต่อการตรวจ glutamate decarboxylase antibody ผู้ป่วยที่ไม่พร่องอินสุลินและตรวจไม่พบ glutamate decarboxylase antibody ได้รับการรักษาด้วยยาฉีดอินสุลินร้อยละ 18.5 การศึกษานี้ชี้ให้เห็นถึงความหลากหลายของชนิดของโรคเบาหวานในผู้ป่วยที่เริ่มเป็นเมื่ออายุน้อย และประมาณครึ่งหนึ่งของผู้ป่วยเบาหวานเหล่านี้ไม่มีภาวะพร่องอินสุลิน glutamate decarboxylase antibody และ mitochondrial 3234 tRNA Leu(UUR) gene mutation พบได้น้อย เชื่อว่ายังมีปัจจัยอื่นที่เป็นสาเหตุของภาวะพร่องอินสุลินที่ต้องการการศึกษาเพิ่มเติม

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