RESEARCH ARTICLE

Antibodies to Glutamic Acid Decarboxylase-65 is Associated with Total Daily Dose of Insulin Requirement in Children with Type 1 Diabetes

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Abstract

BACKGROUND: Type 1 diabetes (T1D) mostly occurs due to the destruction of pancreatic beta cells due to autoimmune processes. Diagnosis of T1D can be established by examining the c-peptide levels and the markers of pancreatic autoantibodies, including glutamic acid decarboxylase 65 autoantibodies (GAD-65). However, the association between c-peptide and anti-GAD-65 toward patients' clinical manifestations needs to be further explored. Hence, the aim of current study was to identify the association of anti-GAD65 with c-peptide and clinical characteristics in children with T1D.

METHODS: Case-control study involving 47 T1D children (T1D group) and 41 healthy children (control group) younger than 18 years old was conducted. Secondary data regarding subjects' demographic characteristics and medical history were collected from subjects, and serum blood was drawn from each subject for the anti-GAD65 and c-peptide measurement. Anti-GAD65 and c-peptide levels were measured using an Enzyme-Linked Immunosorbent Assay (ELISA) methods.

RESULTS: Anti-GAD65 antibody was detected in 78.7% T1D group, while only 2.43% were detected in control group subject (p=0.0000). The c-peptide level of T1D group was 0.07±0.19 nmol/L and control group was 1.5±0.77 nmol/L (p=0.0000). The total daily dose of insulin in subjects with positive anti-GAD65 was greater than in the negative anti-GAD65 (p=0.012). The sensitivity and specificity of the anti-GAD65 were 85.4% was 66.7%, respectively.

CONCLUSION: The results of this study show that anti-GAD65 was associated with total daily dose of insulin requirement in children with T1D.

KEYWORDS: diabetes mellitus, type 1 diabetes, anti-GAD65, c-peptide

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Introduction

Diabetes can occur at any age but type 1 diabetes (T1D) is dominantly occurred in children, accounting for 86-90% of all diabetes cases.(1-3) T1D occurs due to the destruction of pancreatic beta cells due to autoimmune processes and only 2-5% are idiopathic.(1,4) Pancreatic beta cell destruction can be detected by autoantibody examination of pancreatic cells including glutamic acid decarboxylase 65 autoantibodies (GAD-65), islet cell autoantibodies (ICA), tyrosine phosphatase-like insulinoma antigen 2 (IA2), insulin autoantibodies (IAA), and β-cell-specific zinc transporter 8 autoantibodies (ZnT8).(5-8) The percentage of antibodies against T1D depends on age, duration of disease, and ethnicity.(4,9) Furthermore, autoantibody levels provide information on the degree of beta cell damage, the onset of T1D symptoms, the need for insulin, prognosis, and the risk of other autoimmune diseases. Therefore, antibody examination is important to determine the etiology of pancreatic ß cell damage.(5,10-13) Anti-GAD65 has a high predictive value in determining the presence of pancreatic ß

cell damage compared to other antibodies because GAD65 is the most stable autoantibody compared to others and can last longer.(14) It even still provides useful information up to 15 years after the diabetes diagnosis.(4)

Regarding to the destruction of pancreatic beta cell in T1D pathogenesis, in addition to the presence of diabetes-related autoantibodies, the absence of endogenous insulin secretion is often used to classify patient as having T1D. (15,16) The measurement of c-peptide is a well accepted method for the quantification of endogenous insulin because the degradation of insulin in the body is faster (halflife: 3-5 min) than that of c-peptide (halflife: of 20-30 min).(15) C-peptide is cleared in the peripheral circulation at a constant rate, whereas insulin is cleared variably making direct measurement less consistent. In insulin-treated children with diabetes, measurement of c-peptide also avoid the pitfall of cross-reaction of assay between endogenous and exogenous insulin.(15,16) Positivity of anti-GAD65 was associated with a decreased of insulin. (17) Even though both c-peptide and anti-GAD-65 have been used in establishing the diagnosis of T1D, but the association between the two markers and patients' clinical manifestations in T1D patients still needs to be further explored. Therefore, this study aimed to identify the association of anti-GAD65 with c-peptide and clinical characteristics in children with T1D.

Methods

Study Design and Subjects Recruitment

In this case control study, 47 children aged 0-18 years that were diagnosed with diabetes mellitus based on International Society Paediatric and Adolescent Diabetes 2022 consensus1 were recruited. Type of diabetes based on clinical characteristics, continuous usage of insulin from the time of diagnosis and c-peptide level cut-off of <0.6 mmol/L was considered as T1D.(18) Diabetic children with emergency conditions were excluded as subjects. Meanwhile, for the control group, 41 healthy children with same age range but did not have diabetes or autoimmune diseases was recruited. The research was conducted at the Paediatric Endocrinology Outpatient Clinic, Dr. Hasan Sadikin General Hospital from May 1, 2022 to August 31, 2022. The protocol of study was approved by the Research Ethics Committee of Dr. Hasan Sadikin General Hospital Bandung Indonesia (No. LB.02.01/X.2.2.1/15321/2022) and informed consent was waived by the Research Ethics Committee.

Data Collection for Subjects' Characteristic

We interviewed the subjects to collect data on their demographic characteristics, age, gender, history of parents' diabetes or autoimmune disease, diabetic ketoacidosis history, age at diagnosis and duration of diabetes, total daily dose of insulin, c-peptide level at newly diagnosed diabetes and HbA1c levels. Later, each subject underwent an anthropometric body weight and height measurement for the body mass index calculation.

Measurement of Anti-GAD65

Five mL of blood was drawn from the vein of the forearm of each subject was moved into a gel tube and then moved into a centrifuge to obtain serum for the measurement of anti-GAD65 levels using the Human GAD 1 Enzyme-linked Immunosorbent Assay (ELISA) kit Demeditec GDE/96 (RSR Limited, Cardiff, United Kingdom). This ELISA method employed indirect ELISA principle, limit of analytical sensitivity 0.57 u/mL and the standard measuring interval was 5 to 2,000 µ/mL. The cut-off value for the kit was 5.0 µ/mL and the coefficient of variation for the kit using ELISA was <15%. Measurement result was categorized as positive if anti-GAD level was higher than 5.0 µ/mL, and categorized as negative if anti-GAD level was less than 5.0 µ/mL.

Measurement of C-peptide

The obtained centrifuged serum was also used for the measurement of c-peptide levels using Rat C-Peptide ELISA Kit (Cat. No EA0006Ra; Bioassay Technology Laboratory, Shanghai, China). The manufacturer of ELISA kit used competitive ELISA as the method, with the limit of detection was 0.044 ng/mL and standard curve range was 0.1-40 ng/mL. Low c-peptide level was described if the c-peptide levels were <0.6 ng/mL, while above was considered as normal c-peptide level. All sample processing were conducted at Clinical Pathology Departement Laboratory of Dr. Hasan Sadikin General Hospital.

Statistical Analysis

The SPSS program version 18 for Windows (SPSS Inc. Chicago, II, USA) was used to statistically analyze the collected data. Kolmogorov-Smirnov test was used for the normality test, followed by Chi-square test or Fisher test to compare the differences of categorical data between the two groups. Meanwhile, Mann-Whitney U test was used to determine the statistical significance of differences in continuous data between two groups. The Pearson correlation test was applied for normally distributed data

and the Spearman test for categorical data not normally distributed. The $p<0.05$ was considered statistically significant.

Results

General Characteristic of Subjects

Table 1 showed that 23.4% in T1D group had a history of DM parents compared to only 7.3% of the control group ($p=0.041$). There was an autoimmune history in 4.3% of the T1D group and 2.4% of the control ($p=0.641$), however, the difference was not significant. In parents' educational status, it was found that most fathers had medium and high education levels (44.7% and 40.4%), and almost similar to mothers' education levels (53.2% of medium and 31.9% of high educational levels). Most of the T1D group had good nutritional status (68.1%) and normal height (72.3%), although it still found 21.3% with obesity risk, 4.3% obesity and 6.4% with malnutrition (Table 2).

Clinical Characteristic of Subjects

Table 3 showed that 41 of 47 T1D subjects had low c-peptide and 6 subjects still had normal c-peptide. There was a significant difference in the c-peptide level between T1D group and control as 0.07±0.19 nmol/L and 1.5±0.77 nmol/L respectively ($p=0.0000$). In total, 37 out of 47 T1D subjects were positive for anti-GAD65 (78.7%) while only one control subject was positive for anti-GAD65 ($p=0.0000$). In this study, sensitivity of the anti-GAD65 test in determining T1D was 78.7% and specificity was 97.5%, meanwhile sensitivity of c-peptide was 87.2% and specificity was 95.1%. There was no significant association between the prevalence of anti-GAD65 with c-peptide levels, age at diagnosis, duration of diabetes, gender, DKA at diagnosis, and HbA1c levels (Table 4). All T1D subjects received insulin therapy with a basal-bolus regimen, the daily dose requirement of all subjects was 1.07 units/kg body weight/day. The total daily dose of insulin in subjects with positive anti-GAD65 (1.12±0.34 units per kg body weight/day) was significantly greater than in the negative anti-GAD65 0.87±0.17 units per kg body weight/day ($p=0.0128$).

Discussion

Our data showed that 21.3% of subjects with T1D had a risk of obesity and 4.3% were obese. Overweight and obesity were already common among T1D adolescents, and a recent study found that T1D youth are more likely to be overweight than their counterparts without the condition.(19) Obesity is a cause of oxidative stress and insulin resistance (20), and several case studies have reported overweight and obese youth with T1D develop type 2 diabetes (T2D) or 'double diabetes' (19). All the T1D received insulin therapy to maintain normoglycemia. The main function of insulin as an anabolic hormone that play role in inducing lipogenesis, inhibiting protein catabolism, and resulting lipid accumulation.(21,22) Intensity of insulin therapy, type of insulin and physical inactivity in T1D increase the risk of obesity.(21)

In this study, the prevalence of anti-GAD65 autoantibodies was 78.7% in T1D Indonesian children in concordance with the high prevalence of anti-GAD65 in T1D children reported in Western countries, 73.2% in the US and 86% in Europe.(8) Also, these results are in line

Table 1. Characteristics of study subject.

Characteristics	T1D (n=47)	Control (n=41)	*p*- value
Gender, n (%)			
Male	28 (59.6%)	17 (41.5%)	0.920
Female	19 (40.4%)	24 (58.5%)	
Age (year), mean±SD	12.73±3.90	14.51±1.82	0.124
BMI for Age, mean±SD	-0.84±1.5	-0.39 ± 1.47	0.148
DM History, n (%)			
Yes	11 (23.4%)	3 (7.3%)	0.041*
No	36 (76.6%)	38 (92.7%)	
Autoimmune History, n (%)			
Yes	2 (4.3%)	1 (2.4%)	0.641
No	45 (95.7%)	40 (97.6%)	

BMI: body mass index; DM: diabetes mellitus. *significant if $p<0.05$.

Table 2. Clinical-related characteristics of T1D subjects.

Clinical Variable	T1D (n=47)
Father's Education, n (%)	
Low level	7 (14.9%)
Medium level	21 (44.7%)
High level	19 (40.4%)
Mother's Education, n (%)	
Low level	7 (14.9%)
Medium level	25 (53.2%)
High level	15 (31.9%)
Anthropometry, mean±SD	
Body weight (kg)	41.41±15.01
Body height (cm)	144.58±18.82
Nutritional Status (BMI for Age), n (%)	
Obesity	2 (4.3%)
Risk of overweight	10 (21.3%)
Normal	32 (68.1%)
Malnutrition	3 (6.4%)
Stature (Height for Age), n (%)	
Normal	34 (72.3%)
Short stature	11 (23.2%)
Very short stature	2 (4.3%)
DKA at diagnosis, n (%)	
Yes	24 (51.1%)
No	23 (48.9%)
TDD Insulin (U/kgBW/day), mean±SD	1.07±0.33

TDD: total daily dose.

with a study in Korea that reported that 71% of T1D patients were positive for anti-GAD65 (23) and 77.5% in Sudan (24) but in contrast to previous data that the most idiopathic types are found in Asian and African countries according to low positivity autoimmune markers.(8,14) An anti-GAD65 positivity of 53% has been reported in India (9), 44.3% in Singapore, 34.1% in Syria and 49.5% in Jordan (14). The reason that several proportion of children with diabetes have no detectable anti-GAD65 might be that there is a truly antibody-negative form of diabetes in children and adolescents where the beta cell destruction is mediated by mechanisms other than organ-specific autoimmunity.(24) Another explanation could be that phenomenon is caused by insensitive antibody assay or need others autoantibody measurement.(25) There were fewer anti-GAD65 positive participants in the control group (2.43%) in concordance with studies in Syria (1.3%) and Jordan (2.0%).(14) Anti-GAD65 antibodies have been found in a subset of non-diabetic persons who do not develop diabetes over time and do not have close relatives with autoimmune diabetes, according to a study in children. The prevalence of anti-GAD65 positive in non-diabetic individuals varies between research (0.7-4.8%), and the clinical significance of positivity is debatable.(26)

Genetic factors, particularly the HLA DQA1-DQB1 genotypes, may be the cause of the variations in anti-GAD65 prevalence among various ethnic communities with T1D.(25) A recent international prospective cohort study also discovered some non-HLA genes to be genetic contributors to the development of islet autoimmunity and T1D.(27) The total requirement dose of insulin in subjects with positive anti-GAD65 was significantly higher than in the negative anti-GAD65 (p=0.012). This suggests that anti-GAD65 levels can be used as a predictor of high insulin requirements in the initial management of T1D at the newly of diagnosis.(5,21) The sensitivity of the anti-GAD65 measurement was 78.7% and specificity was 97.5%. This demonstrates that the anti-GAD65 test is moderate for identifying the existence of damaged pancreatic beta cells and insulin deficiency.

C-peptide is the component of proinsulin which is cleaved prior to co-secretion with insulin from pancreatic beta cells, and modern techniques detect c-peptide levels that can be utilized to aid in the diagnosis of T1D.(15)

Table 3. Comparation anti-GAD65 and c-peptide levels.

Characteristic	T1D (n=47)	Control (n=41)	*p*-value
C-peptide Level, mean±SD	0.07±0.19	1.50±0.77	0.000*
C-peptide, n (%)			
C-peptide low	41 (87.2%)	2 (4.9%)	
C-peptide normal	6 (12.8%)	39 (95.1%)	
Anti-GAD65 Level, mean±SD	79.64±1.28	0.38±0.98	0.000*
Anti GAD65, n (%)			
Positive	37 (78.7%)	1 (2.4%)	
Negative	10 (21.3%)	40 (97.6%)	

Sensitivity of c-peptide: 87.2%; specificity of c-peptide: 95.1%; sensitivity of anti-GAD65: 78.7%, specificity of anti-GAD65: 97.5%.

Table 4. Correlation between anti-GAD65 with subjects' clinical characteristics.

Characteristic	Anti-GAD65 Positive (n=37)	Anti-GAD65 Negative (n=10)	Control (n= 41)	*p* -value
Age (year), mean±SD	12.99±4.11	11.77±2.97	14.51±1.82	
Age (year), n (%)				
0-12 year	12 (32.4%)	5 (50.0%)	1 (2.4%)	0.167[a]
>12 year	25 (67.6%)	5 (50.0%)	40 (97.6%)	
Age at Diagnosis, n (%)	8.38±3.38	7.29±3.81	n/a	0.370[a]
Gender, n (%)				
Male	21 (56.7%)	7 (70.0%)	17 (41.4%)	0.460[a]
Female	16 (43.3%)	3 (30.0%)	24 (58.6%)	
Diabetes Duration (year), mean±SD	4.76±2.57	4.51±2.89	n/a	
Diabetes Duration, n (%)				
0-4 year	16 (43.2%)	5 (50.0%)		0.640[a]
4-8 year	17 (45.9%)	4 (40.0%)		
>8 year	4 (10.9%)	1 (10.0%)		
C-peptide Levels, mean±SD	0.05±0.07	0.17±0.40	1.52±0.78	
C-peptide Levels, n (%)				
Low	35 (94.6%)	8 (80.0%)	2 (4.8%)	0.295[a]
Normal	2 (5.4%)	2 (20.0%)	39 (95.2%)	
HbA1C, mean±SD	9.09±1.68	10.25±2.67	n/a	0.100[b]
DKA at Diagnosis, n (%)				
Yes	21 (56.7%)	3 (30.0%)	n/a	0.140[a]
No	18 (43.3%)	7 (70%)		
TDD Insulin, mean±SD	1.12 ± 0.34	0.87±0.17	n/a	0.012[a,*]

HbA1c: hemoglobin A1C, DKA: diabetic ketoacidosis, TDD: total daily dose. [a]Tested with Spearman Test, [b]Tested with Pearson Test, *significant if $p<0.05$.

In this study, the level of c-peptide in T1D group was significantly lower than control 0.07±0.19 *vs.* 1.5±0.77 nmol/L (p=0.0000). This result in line with a study in Massachusetts and Tanzania that described that low c-peptide levels have clinical significance and helpful in defining declining in insulin secretion.(28,29) C-peptide levels in the positive anti-GAD65 group were lower than those in the negative anti-GAD65 group (0.05 nmol/L *vs.* 0.17 nmol/L) although not significantly different. This is in line with previous study that found c-peptide levels in the anti-GAD65 positive group were lower than those in the anti-GAD65 negative group.(30) In this study, 6 children were found in T1D subjects with normal c-peptide levels. This suggests that anti-GAD65 testing is more effective at onset of diagnosis, when c-peptide was less beneficial due to the honeymoon phase, during which people with T1D are temporarily still able to secrete insulin.(31)

The anti-GAD65 was recently identified as an autoantibody marker in T1D, which is detected in early diagnosis and in the longstanding phase of diabetes, increasing diagnostic sensitivity of T1D. Current study report the association of anti-GAD65 with c-peptide and clinical characteristics in Indonesian children with T1D, however only conducted in a single centre study. Further studies are required to be performed multicentre and to investigate the role of anti-GAD65 in newly diagnosed diabetes in children and understand the genetic polymorphism on anti-GAD65 autoimmunity in patients with T1D.

Conclusion

In conclusion, anti-GAD65 may be useful to ascertain the cause of pancreatic beta cell destruction and has a potential benefit for classifying types of diabetes in children. However, there was no significant association between anti-GAD65 with c-peptide levels and clinical parameters, but the positivity of anti-GAD65 was associated with total insulin daily dose requirement in children with T1D.

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Authors Contribution

F, NR, and MF were involved in planning of the study. F collected the data, performed the analysis, and wrote the manuscript; MF and NR supervised the work and aided in interpreting the result. N and EN collected the subject of the study and performed the measurements. All authors discussed the results and commented on the manuscript.

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