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Islet Autoantibody and Beta Cell Secretory Status at Diagnosis in Young Bangladeshi with Phenotypically Type 2 Diabetes

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Abstract

Background. The overlapping clinical features in young-onset type 2 diabetes (T2DM) present significant diagnostic difficulties. Variable autoimmunity and beta-cell dysfunction, which are related to the phenomenon, are not sufficiently consolidated to distinguish subclasses.

Objectives. To determine the frequency of islet autoantibodies and beta-cell secretory status in phenotypically young Bangladeshi with T2DM.

Methodology. This cross-sectional study enrolled 83 patients with newly diagnosed young-onset phenotypically T2DM, aged 10 to 29 years, comprising 34 males (41%) and 48 females (59%), using non-probability purposive sampling. The demographic and clinical features of the patients were recorded. A fasting blood sample was collected for C-peptide and islet antibodies (anti-glutamic acid decarboxylase [GAD], zinc transporter 8 [ZnT8], and Islet Antigen 2 [IA-2] antibodies). C-peptide, anti-GAD Ab and IA-2 Ab were measured by chemiluminescence, while the ZnT8 Ab was measured by enzyme-linked immunosorbent assay (ELISA).

Results. An adequate beta cell secretory reserve was present in 97.6% of participants (N = 82), with a median C-peptide level of 4.3 ng/mL (IQR: 3.0-6.7). Of the 82 patients included, GAD Ab was found to be positive in 17% (n = 14), ZnT8 Ab in 2.4% (n = 2), and none were positive for IA-2 Ab or a double antibody (ZnT8 Ab + GAD Ab). The frequency of double diabetes (DD) [GAD Ab positive subjects] was 17% (14/82). Comparing the GAD Ab positive to the negative group, the former had a significantly lower homeostasis model assessment of β-cell function (HOMA-B) at 24.7 (16.3-99.1) [versus 81.9 (30-154) in the latter (p = 0.02)] and a significantly higher fasting plasma glucose (FPG) median IQR at 16 mmol/L (10-19) [compared to 9.5 (6.7-14.5) (p = 0.04) in the negative group.] The body mass index (BMI) was the only significant predictor of C-peptide (β = 0.44, p <0.001).

Conclusion. GAD Ab was the most commonly detectable antibody in this study of young-onset phenotypically T2DM patients. The concentration of GAD Ab may influence the phenotypic presentation, but it is not a predictor of C-peptide levels. Beta-cell dysfunction in this subset of patients may depend on certain yet unexplored factors.

Key words: young diabetes, phenotypic T2DM, islet autoantibodies, C-peptide

INTRODUCTION

Diabetes is one of the most prevalent non-communicable diseases and one of the fastest-growing global health emergencies of the 21st century. Common factors for the high prevalence of diabetes are related to an increased prevalence of obesity, population aging, population growth, urbanization and physical inactivity.[1] Type 2 diabetes (T2DM) is the most prevalent form of diabetes and has increased with cultural and social changes. Not only is it highly prevalent in adults, but the prevalence of T2DM has also been rising in the young. It has been observed that Asians, compared to Western populations, develop diabetes at younger ages with a risk of developing complications in early adulthood.[2] South Asians have also increased abdominal visceral fat and greater insulin resistance (IR).[3] The prevalence of young-onset DM in Bangladesh is also rising.[4] Therefore, it is necessary to know the characteristics and nature of this group of diabetic patients in Bangladesh.

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The common underlying mechanism of all forms of DM is the dysfunction or destruction of pancreatic beta cells. Factors involved in this process include genetic predisposition, epigenetic processes, insulin resistance, autoimmunity, concurrent illnesses, inflammation and environmental factors. Understanding the beta cell status can help define subtypes of diabetes and guide their treatment.[5] Islet beta cell autoantibodies against glutamic acid decarboxylase (GAD), zinc transporter 8 (ZnT8) and Islet antigen 2 (IA-2) are implicated in the pathogenesis of autoimmune beta-cell destruction. Historically, T2DM has been considered primarily a metabolic disease of older individuals without involvement of the immune system. Over the years, investigations into the pathophysiology of T2DM have identified the presence of islet-specific T cells and islet autoimmunity in T2DM.[6] Using islet autoantibodies as a biomarker for islet autoimmunity in type 2 diabetes mellitus (T2DM), the prevalence of islet autoimmunity has been estimated to be between 5% and 30%.[7] One study showed that the frequency of autoimmunity was lower in T2DM compared to T1DM; however, there were still 8.1%, 30.3% and 34.8% of T2DM children and adolescents testing positive for anti-glutamic acid decarboxylase (GAD), Zn transporter 8 (ZnT8) and Islet Antigen 2 (IA-2) antibody, respectively.[8] Recently, the phenotypes of T1DM and T2DM have become less distinctive, which is why the World Health Organization (WHO) has revised the classification of diabetes and has recognized hybrid forms of DM, including slowly evolving immune-mediated DM and ketosis-prone diabetes as separate subtypes of DM. The American Diabetes Association (ADA) (2011) defined youth with type 2 diabetes as having evidence of islet cell autoimmunity with autoantibodies targeting beta cells typical of type 1 diabetes as "double diabetes" (DD). The term "double diabetes" (DD) refers to cases where a patient exhibits characteristics that are a combination of T1DM and T2DM.[9] Common symptoms of DD include obesity, insulin resistance, a positive family history and the presence of autoantibodies, specifically GAD56, IA-2 and insulin antibodies. DD can be a significant event among young-onset (11–19 years old) diabetic patients, because of weight gain and insulin resistance.[10] Identifying DD in children and adolescents is crucial as it affects the diagnostic method and choice of treatment.

The serum C-peptide level reflects insulin secretion from pancreatic islet cells and has been suggested as a valid parameter in classifying diabetes. Patients with DD need early treatment with insulin as they develop early beta cell failure and are more prone to develop ketosis. Nearly 65% of the DD group may require early insulin treatment.[11] In our country, there is no available data on the prevalence of DD and the percentage of positive islet antibodies in young T2DM patients. Hence, the objective of our study was to measure islet autoantibodies (GAD, ZnT8, and IA2) and to assess beta-cell secretory status through fasting C-peptide and HOMA-B in young-onset phenotypically T2DM individuals. Additionally, we also wanted to compare the clinical characteristics of participants with or without GAD Ab positivity and to correlate clinical and biochemical variables with GAD Ab, ZnT8 Ab levels and HOMA-B. Lastly, the study aimed to evaluate the ability of glycemic values to predict GAD antibody positivity using the Receiver Operating Characteristic (ROC) curve analysis.

METHODOLOGY

Study subjects and design

This cross-sectional study was conducted in the Department of Endocrinology at Bangabandhu Sheikh Mujib Medical University (BSMMU, Dhaka, Bangladesh). Eighty-three newly diagnosed (within 1 month of diagnosis) young patients with phenotypical T2DM, diagnosed according to the American Diabetes Association (ADA) criteria (age range: 10–29 years), were enrolled through non-probability purposive sampling. Phenotypical T2DM characteristics include: overweight/obesity, central obesity (abdominal obesity), features of insulin resistance (like acanthosis nigricans, obesity (central/ generalized), skin tags, double chin, lipodystrophy and, in females, features of androgen excess (androgenic alopecia, hirsutism, oligomenorrhea), positive family history and absence of ketosis at presentation.[12] Patients with gestational diabetes, chronic liver disease, chronic kidney disease, other endocrinopathies and current use of drugs interfering with endogenous insulin and C-peptide concentration were excluded from this study.

Sample size

Assuming a 21.2% prevalence of the most frequently detected islet autoantibody in young-onset T2DM (SEARCH for Diabetes in Youth Study Group, 2007), the required sample size was calculated with a 95% confidence level and a 10% margin of error using the following formula:

$$n = \frac{Z^2 pq}{d^2}$$

n = The desired sample size that would help to measure the different indicators.
z = The standard normal deviation, usually set at 1.96 at 5% level, which corresponds to a 95% confidence level.
p = The assumed target proportion.
q = 1-p
d = The degree of accuracy level considered as 10%. The degree of accuracy d, which is assumed, is 0.1

After calculation, the sample size was 65, but we recruited 83 young phenotypical T2DM patients.

Study procedure

A detailed history and thorough examination were done of each individual. Height was measured by using a stadiometer. Weight was measured by a balance on a hard, flat surface. Waist circumference (WC) was measured to the nearest centimeter with a flexible steel tape while

the participants were in a standing position at the end of gentle expiration. Hip circumference was measured at the level of the widest portion of the buttocks. Blood pressure (BP) was measured in millimeters of mercury by a standard sphygmomanometer. The demographic and clinical features of the patients (mode of presentation, physical activity level, family history, glycemic status, treatment history, anthropometric measurements, features of insulin resistance- acanthosis nigricans, obesity [central/generalized], skin tags, double chin, lipodystrophy and in females, features of androgen excess [androgenic alopecia, hirsutism, oligomenorrhea]) were recorded in a standard pre-tested structured datasheet. Physical activity level was defined as (1) vigorous, (2) moderate or (3) light physical activity. Vigorous activity was defined as any activity that caused a significant increase in breathing or heart rate, if continued for at least 10 minutes (e.g., running, carrying heavy loads, digging or construction work). Moderate activity was defined as any activity that caused a slight increase in breathing or heart rate, provided it was continued for at least 10 minutes (such as brisk walking or carrying light loads). Light physical activity was defined as activities such as office work.[13] Body mass index interpretation was done for those less than 18 years of age according to the growth charts of the US Centers for Disease Control and Prevention: children with normal weight (BMI from the 5th to the 85th percentile), overweight (BMI from the 85th to the 95th percentile) and obese (BMI above the 95th percentile).[14] For those 18 years and older, body mass index interpretation was done according to the WHO adult obesity category for Asians. An abnormal waist circumference in individuals under 18 years of age was defined as exceeding the 90th percentile for age and sex, according to the CDC's waist circumference growth charts for age. For those 18 years old and above, an abnormal WC was at least 90 cm in males and at least 80 cm in females.[15] A fasting blood sample (10 mL) was collected from each participant for the measurement of C-peptide and islet autoantibodies. C-peptide-based homeostasis model assessment[16] was determined using the following formulae: HOMA-B = 0.27 × fasting C-peptide (FCP)/(FPG-3.5) [If FCP in ng/mL then FPG in mg/dL; if FCP in pmol/L then FPG in mmol/L]; and, HOMA-IR = 1.5 + (FPG (mg/dL) x FCP (ng/mL)) / 2800, with a cut-off of 0.997 (sensitivity = 85.4, specificity = 52.4). Estimated glucose disposal rate (eGDR) (mg kg^{-1}/min) was calculated using the following formula: eGDR = 21.158 − (0.09 x WC) − (3.407 x HT) − (0.551 x HbA1c), where WC = waist circumference (cm), HT = hypertension (yes = 1/no = 0) and HbA1c = HbA1c (%).[17]

Analytic method

The quantitative determination of C-peptide was performed using a chemiluminescent immunometric assay with a measuring range of 0.01-20 ng/mL. The C-peptide assay is a sandwich chemiluminescence immunoassay in a single assay run. The intra-assay coefficient of variation (CV) was 5%. Quantitative determination of glutamic acid decarboxylase antibody (GAD65) measured through chemiluminescent immunometric assay, with a measuring range of 1.0-280 IU/mL, with sensitivity of 73% and specificity of 96%. Intra-assay CV was 3.6%. Also, quantitative determination of tyrosine phosphatase-like protein antibody (Anti-IA-2) was measured by the chemiluminescent immunometric assay with a measuring range of 2.5-280 U/mL. Intra-assay CV was 3.37%. The measurements of C-peptide, GAD65, and anti-IA-2 were performed on the Snibe MAGLUMI 2000 Plus Chemiluminescence Immunoassay (CLIA) System (China). Serum ZnT8 Ab was estimated by an ELISA kit by ElisaRSR™ ZnT8 Ab™, RSR Ltd. (Cardiff, UK) with a 72% sensitivity and a 99% specificity. Intra-assay CV was 4.39%.

A GAD Ab level greater than or equal to 5 U/mL, a ZnT8 Ab level greater than or equal to 15 U/mL and an IA-2 Ab level greater than or equal to 7 U/mL were considered positive. A C-peptide greater than or equal to 0.9 ng/mL was considered adequate.

Statistical analysis

Quantitative data were expressed as mean ± SD or median and interquartile range (IQR), whereas qualitative data were expressed in frequency distribution and percentages. Statistical analyses were performed using IBM SPSS Statistics for Windows (Version 25.0). The association between categorical variables was analyzed using the $\chi 2$ test, and the continuous variables were analyzed using Student's *t*-test and one-way analysis of variance (ANOVA). A continuous variable with a skewed distribution was compared using the Mann–Whitney *U*-test. Correlation between the variables was done using Kendall's tau-b test. A *p*-value of less than 0.05 was considered statistically significant for all statistical tests.

Potential confounders of the assessment of beta cell secretory status and islet autoantibodies, such as chronic liver disease, chronic kidney disease, other endocrinopathies and the use of drugs that interfere with insulin secretion, were excluded from the study. An appropriate multivariable regression model was used to adjust for covariates associated with GAD antibody positivity.

Ethical considerations

The project commenced after approval of the Departmental Technical Committee and the Institutional Review Board (IRB). Voluntary, informed written consent was obtained from each subject and/or their legal guardian after a thorough explanation of the procedure and the purpose of the study. Each participant enjoyed the right to participate, refuse, or even withdraw from the study at any point in time. Proper medical services and advice were provided to all subjects, regardless of their enrollment status. Information about the patients was kept confidential. Proper counseling was done before the collection of blood samples. Adequate safety measures were taken at every step of sample collection.

RESULTS

The present study enrolled 83 young subjects with newly diagnosed phenotypic T2DM to assess their islet auto-antibodies and beta cell secretory status. Subsequently, one patient was diagnosed with fibrocalculus pancreatic diabetes and excluded from the study. No one fulfilled the criteria for T1DM based on auto-antibody positivity and C-peptide level, so we analyzed the data from 82 participants.

Table 1. Demographic and clinical characteristics of study participants (N=82)

Variables	n (%)	Median (IQR)
Age (years)		26.5 (22- 28.5)
Gender		
Male	34 (41%)	
Female	48 (59%)	
Family history†		
Present	49 (60%)	
Absent	33 (40%)	
Acanthosis Nigricans		
Present	55 (67%)	
Absent	27 (33%)	
WC (cm)		
Normal	27 (33%)	
High	55 (67%)	
Physical activity level		
Light	60 (73%)	
Moderate	21 (25.5%)	
Vigorous	1 (1.5%)	
Symptoms of hyperglycemia††		
Present	57 (69%)	
Absent	25 (31%)	
WHR		
Normal	7 (8.5%)	
High	75 (91.5%)	
BMI (kg/m²) (mean ± SD)		25.8 ± 5.2
Normal	23 (28%)	
Overweight/Obese	59 (72%)	

Mean ± SD for normally distributed data and median (IQR) for skewed data.

† Family history of DM in 10 relatives

†† Polyurea, polydipsia, weight loss, tiredness.

DM: diabetes mellitus of young; HC: hip circumference; BP: blood pressure; BMI: body mass index, WC: waist circumference; Obese, M>90, F>80; WHR: waist-hip ratio; Abdominal obesity, M>0.88, F>0.81

Clinical characteristics of the study subjects are presented in Table 1. The participants' ages varied from 10 to 29 years (median 26.5 [IQR 22-28.5]). Median glycemic values of the participants were: fasting plasma glucose of 10.4 mmol/L (IQR: 6.9-15.8), 2-hour plasma glucose after 75 g test of 18.0 mmol/L (IQR:12.9-23.7) and HbA1c of 8.5 % (IQR: 6.8-10.8). There was an adequate beta cell secretory reserve, with a median C-peptide of 4.3 ng/mL (IQR: 3.0-6.7), in the majority of participants (97.6%).

GAD Ab was found in 17% (14 out of 82) of patients, whereas ZnT8 Ab was found in 2.5% (2 out of 82). None of the participants were positive for IA-2 Ab or double antibody (ZnT8 Ab + GAD Ab). Subjects who were positive for GAD Ab were referred to as DD. There was a trend of a higher frequency of GAD Ab positivity in those under 18 years old (25% [3/12] vs. 15.7% [11/70], $p = 0.279$).

The clinico-biochemical characteristics between the GAD Ab-positive and negative groups are shown in Table 2. Beta cell secretory capacity assessed by HOMA-B was significantly lower [24.7 (16.3-99.1) vs. 81.9 (30- 154), $p = 0.02$] and fasting plasma glucose was significantly higher [16 (10-19) vs. 9.5 (6.7-14.5), $p = 0.04$] in the GAD Ab positive than those of the negative groups. The estimated glucose disposal rate (eGDR) was also significantly lower [6.49 (4.84-7.33) vs. 8.00 (6.35-9.11), $p = 0.013$] in the GAD Ab-positive groups.

There was a significant negative correlation between age (r = -0.18, $p = 0.02$) and HOMA-B (r = -0.17, $p = 0.02$) with GAD

Table 2. Clinical and biochemical characteristics in GAD Ab positive and negative participants (N=82)

Characteristics	Group		*P*
	GAD Ab Positive (n=14)	GAD Ab Negative (n=68)	
Age (years, median; IQR)	23 (18.7-28.2)	27 (22.2-28.7)	0.20
Sex; n (%)			
Male	7 (50 %)	27 (39.7%)	0.47
Female	7 (50 %)	41 (60.2%)	
BMI (kg/m², mean ± SD)	24.4 ± 6.7	25.9 ± 4.8	0.73
WHR (cm, median; IQR)	0.91 (0.88-0.97)	0.90 (0.88-0.95)	0.59
Acanthosis nigricans; n (%)	7 (50%)	48 (70%)	0.13
Family history of DM; n (%)	8 (57%)	41 (60%)	0.87
Symptoms of hyperglycemia; n (%)	11 (78.5%)	46 (67.5%)	0.41
FPG (mmol/L, median; IQR)	16 (10-19)	9.5 (6.7-14.5)	**0.04**
2h PG (mmol/L, median; IQR)	22.5 (13.9-27.9)	16.6 (12.8-22.9)	0.10
HbA1c (median; IQR)	10.6 (6.8-12.5)	8.4 (6.8-10.2)	0.16
HOMA-B (median; IQR)	24.7 (16.3-99.1)	81.9 (30- 154)	**0.02**
HOMA-IR (median; IQR)	1.81 (1.71-1.89)	1.80 (1.60-1.94)	0.97
eGDR (mg/kg/min, median; IQR)	6.49 (4.84-7.33)	8.00 (6.35-9.11)	**0.013**

Significance level was measured by $\chi 2$, Fisher's Exact test, and Mann-Whitney U test as applicable.

Within parentheses are percentages over the column total if not otherwise mentioned. HOMA-IR: homeostatic model assessment for insulin resistance; HOMA-B: homeostasis model assessment of β-cell function; WHR: waist-hip ratio; GAD Ab: glutamic acid decarboxylase antibody; BMI: body mass index; FPG: fasting plasma glucose; 2-hr PG: 2 hours after 75 g plasma glucose; HbA1c: glycated hemoglobin; eGDR: estimated glucose disposal rate.

Ab levels, and a positive correlation between HbA1c (r = 0.14, p = 0.04) and GAD Ab levels. However, there was no significant correlation between ZnT8 Ab level and any of the other variables (Table 3).

There was a significant positive correlation between BMI (r = 0.50, p <0.001) and symptoms of hyperglycemia (r = 0.26, p = 0.004) with HOMA-B and a negative correlation between HOMA-B and positive GAD Ab (r = -0.17, p = 0.02). There was no correlation between HOMA-B and ZnT8 Ab positivity (Table 4).

Multiple logistic regression (Table 5) was used to assess the ability of control measures (age, positive family history, BMI, Acanthosis nigricans, HOMA-B) to predict levels of GAD Ab positivity. Still, there were no statistically significant predictors of GAD Ab positivity.

Table 3. Correlation of GAD Ab and ZnT8 Ab level with clinical and biochemical characteristics

Determinants of 'r'	GAD Ab		ZnT8 Ab	
	r	*p*	r	*P*
Age (years)	-0.180	**0.02**	0.13	0.08
BMI (kg/m²)	-0.090	0.24	-0.01	0.81
HOMA-B	-0.178	**0.02**	0.04	0.57
HOMA-IR	-0.025	0.74	0.04	0.57
FPG (mmol/L)	0.122	0.11	-0.03	0.63
2h-PG (mmol/L)	0.143	0.06	-0.07	0.35
HbA1c (%)	0.148	**0.04**	-0.05	0.49

Correlation between variables was done by Kendall's tau-b.

r: correlation coefficient; HOMA-IR: homeostatic model assessment for insulin resistance; HOMA-B: homeostasis model assessment of β-cell function; FPG: fasting plasma glucose; 2h PG: 2 hours after 75 plasma glucose; HbA1c: glycated hemoglobin, BMI: body mass index; GAD Ab: glutamic acid decarboxylase antibody; ZnT8 Ab: zinc transporter 8 antibody.

Table 4. Correlation of HOMA-B with clinical characteristics and autoantibodies

Determinants of 'r'	r	*p*
HOMA-B vs BMI	0.50	**<0.001**
HOMA-B vs GAD Ab	-0.17	**0.02**
HOMA-B vs ZnT8 Ab	0.04	0.57
HOMA-B vs family history	0.15	0.09
HOMA-B vs. symptoms of hyperglycemia	0.26	**0.004**

Correlation between variables was done by Kendall's tau-b.

r: correlation coefficient; HOMA-B: homeostasis model assessment of β-cell function; BMI: body mass index, GAD Ab: glutamic acid decarboxylase antibody; ZnT8 Ab: zinc transporter-8 antibody.

Table 5. Multiple logistic regression for positive GAD Ab

Variables	OR	*P*	95% CI	
			Lower	Upper
Age (each year increase)	0.89	0.08	0.79	1.01
Family history of diabetes	1.20	0.78	0.31	4.6
BMI (kg/m²)	1.04	0.58	0.90	1.20
Acanthosis nigricans	0.21	0.07	0.44	1.05
HOMA-B	0.99	0.33	0.98	1.00

R^2 8.5-14.2%

OR: Exp(B); p <0.05 is significant; 95% CI: 95% confidence interval

HOMA-B: homeostasis model assessment of β-cell function; BMI: body mass index; GAD Ab: glutamic acid decarboxylase antibody.

Multiple regressions were used to assess the ability of control measures (age, positive family history, BMI, Acanthosis nigricans, waist-hip ratio, physical activity, GAD Ab, and ZnT8 Ab) to predict levels of C-peptide. BMI was a statistically significant predictor (B = 0.44, p <0.001). Every 1 kg/m^2 increase in BMI will increase 0.44 units of C-peptide.

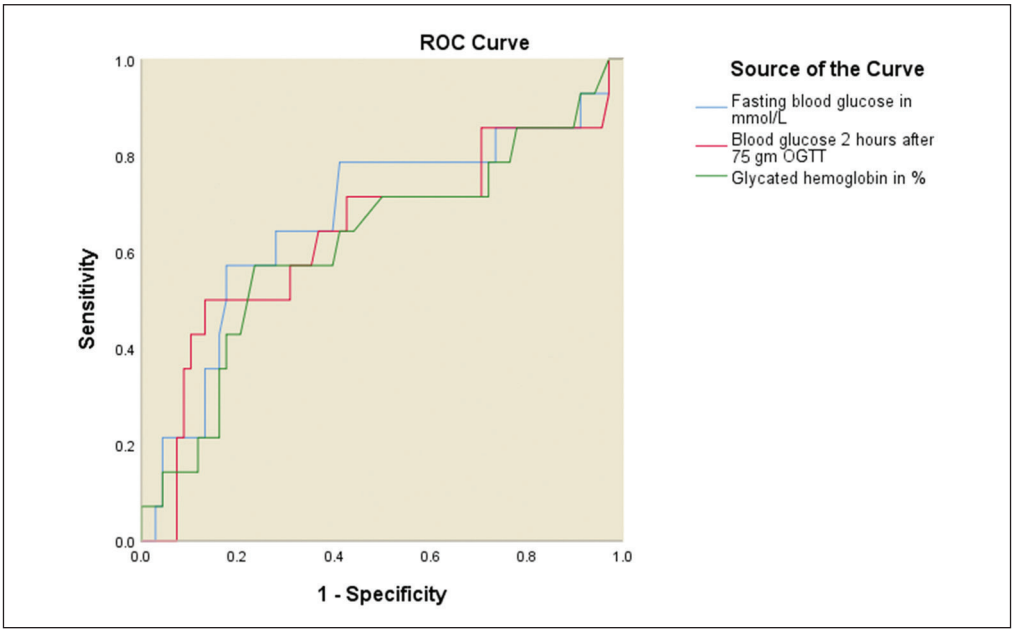
The ROC curve (Figure 1) analysis of glycemic values as a marker of GAD Ab positivity showed that the FPG value at a threshold of 11.3 mmol/L predicts the GAD Ab positivity with a sensitivity of 68% and specificity of 63%. Plasma glucose value 2 hours after a 75-gram glucose drink (2h-PG) at a threshold of 20.25 mmol/L predicts the GAD Ab positivity with a sensitivity of 56% and specificity of 72%. HbA1c at a threshold of 8.6% predicts the GAD Ab positivity with a sensitivity of 63% and specificity of 63%.

DISCUSSION

The classification of diabetes is crucial for developing an effective management plan for the condition. But considerable diagnostic hurdles exist in classifying young diabetic subjects due to overlapping clinical features, variable autoimmunity, and varying degrees of beta-cell dysfunction. Diabetes is often classified based on the presence of islet autoantibodies and C-peptide level. T2DM is the most polygenic and the most challenging to define because genetic, epigenetic, environmental variables, islet autoimmunity and islet-specific T cell response all play a role in its development. This ambiguity in diagnosis may contribute to a delay in initiating appropriate treatment, which leads to subsequent poor glycemic control.

The present study aimed to investigate the presence of islet-specific autoantibodies (GAD Ab, ZnT8 Ab and IA-2 Ab) and beta-cell secretory status in 83 young (aged 10-29 years) phenotypic T2DM subjects. GAD Ab was positive in one of six participants, ZnT8 Ab in one of twenty participants, while none were positive for IA-2 Ab or a double antibody (ZnT8 Ab+ GAD Ab). The frequency of double diabetes (GAD Ab positive) was 17% (14/82). GAD Ab-positive participants phenotypically simulated T1DM, with a lower BMI, less acanthosis nigricans, an infrequent family history of DM, and a higher prevalence of hyperglycemic symptoms. There were no independent predictors of GAD Ab positivity. HOMA-B had a significant positive correlation with BMI and symptoms of hyperglycemia. C-peptide concentration had an inverse relationship with GAD Ab titer, and BMI was the only statistically significant predictor of C-peptide level.

In the SEARCH study (2014), 21.2% of children aged 10 to 19 years diagnosed as T2DM tested positive for GAD-65 antibodies. However, in the TODAY study (2010), 9.8% (118 of 1,206) of young people with T2DM were positive for GAD-65 and/or IA-2 antibodies. In this study, GAD Ab was positive in 17% (14/82) of the participants. The higher rate of GAD Ab identified in young T2DM subjects in the



	Values	Area under curve	Sensitivity (%)	Specificity (%)	Youden index
FPG (mmol/L)	11.30	0.69	68	63	1.43
2h-PG (mmol/L)	20.25	0.67	56	72	1.40
HbA1c (%)	8.6	0.66	63	63	1.41

FPG: Fasting plasma glucose; 2h-PG: 2 hours after plasma glucose; HbA1c: glycated hemoglobin.
ROC curve: receiver operating characteristic curve

Figure 1. ROC curve analysis of glycemic values as a marker of GAD Ab positivity (N=82).

SEARCH study may be due to a higher background of the non-Hispanic white population. Tosur et al., also found that the proportion of autoantibody positivity varies by race/ethnic background, with the highest rates in white non-Hispanics, followed by Asians.[18]

Islet autoimmunity observed in T2DM possesses unique characteristics in terms of the presence of a single auto-antibody, ethnicity, age of onset of DM and insulin treatment. The presence of a single islet cell antibody is a unique feature of the Asian cohorts, whereas double antibody positivity was primarily observed in white Europeans.[19] ZnT8 Ab and IA-2 Ab positivity was present in 60–80% of individuals with new-onset T1DM compared to <2% of controls and <3% of individuals with phenotypic T2DM. ZnT8 Ab positivity is associated with older age, inflammation and acute metabolic complications in adults with both T1DM and phenotypic T2DM.[20] Wenzlau et al., found a low prevalence of ZnT8 Ab positivity (0.43%) in adolescents with T2DM.[21] We also found that ZnT8 Ab was positive in 2.4% (2/82) of participants, and none of the participants were positive for IA-2 Ab or double antibody (ZnT8 Ab + GAD Ab). In contrast, Mishra et al., found a 13% positivity rate for IA-2 Ab among DM participants under 25 years of age, where they included all DM subjects regardless of type; following antibody testing, 51% were identified with T1DM.[21] However, because this study only enrolled phenotypical T2DM patients, the observed low titer of IA-2 Ab in our study may be attributable to this. This low prevalence of ZnT8 Ab and IA-2 Ab indicates that ZnT8 and IA-2 Ab autoantibody positivity is not the etiology of a significant number of cases of T2DM in young people in our country.

The SEARCH study, TODAY study, and others found that GAD Ab-positive T2DM participants, denoted as "DD," were less likely to present with clinical and phenotypic traits of T2DM; instead, they were more likely to demonstrate a T1DM phenotype, characterized by hyperglycemic symptoms and a low BMI, among other manifestations.[19,22] We also found that GAD Ab-positive subjects had a lower BMI, fewer cases of acanthosis nigricans, a positive family history, and a higher frequency of symptoms of hyperglycemia compared to GAD Ab-negative subjects. Most studies found these phenotypic differences to be statistically significant. Since we only recruited phenotypic T2DM patients who had a high BMI and some signs of insulin resistance at the time of enrollment, they were not too likely to exhibit the phenomenon of positive GAD autoimmunity.

Tfayli et al., found that insulin sensitivity was significantly impaired in auto-antibody negative young T2DM compared with the young with islet autoimmunity and young obese controls.[23] Because the young patients with auto-immunity do not have the same level of insulin resistance as those with auto-antibody negative T2DM, the progression to diabetes must be due to a bigger component of beta cell failure than in auto-antibody negative young T2DM, presumably due to islet autoimmunity.

Hosen et al., from our study group found that C-peptide levels may not be low at diagnosis in young-onset DM subjects, which supports our findings of an adequate C-peptide level at diagnosis.[24] The GAD Ab positive T2DM group in SEARCH had a mean fasting C-peptide level of 2.83 ± 1.8 ng/mL, which was modestly higher than the TODAY study subjects (2.30 ± 1.62 ng/mL) but lower than that of the antibody-negative participants in both the TODAY study (4.13 ± 2.22 ng/mL) and SEARCH (3.71 ± 2.2 ng/mL). However, the C-peptide was substantially higher than that found in individuals with physician-diagnosed T1DM in SEARCH (fasting C-peptide 0.6 ng/mL). We found that HOMA-B and C-peptides were low, and fasting plasma glucose was significantly high in GAD Ab-positive groups. The median C-peptide concentration was slightly high in our study, as we measured C-peptide at diagnosis.

It was found that BMI (OR -0.11, p = 0.014), GAD Ab (OR 0.14, p = 0.007), C-peptide (OR -0.90, p = <0.001), and HOMA-IR (OR 0.92, p <0.001) were significant independent predictors for both fasting plasma glucose and 2-h plasma glucose. This indicates that both insulin resistance and beta-cell dysfunction contribute to glycemic status, with a prominent impairment in insulin secretion, as evidenced by a weak relationship (r = 0.23) with markers of insulin resistance (HOMA-IR) but a strong relationship (r = -0.73) with C-peptide. GAD Ab was a weak predictor, but HOMA-IR and C-peptide were strong predictors for the glycemic status of the participants. These findings are similar to other findings in Asians. Asian T2DM patients are generally non-obese, have a prominent impairment in insulin secretion and a better insulin sensitivity than non-Asians.[25] The steepest rates of glycemic deterioration were among those with young-onset T2DM, especially those diagnosed at age 20 years.[23] Aggressive glycemic deterioration in young-onset T2DM contributed to persistently increased HbA1c levels. A national Chinese cross-sectional survey of people with newly diagnosed T2DM showed that HOMA-β was similar across ages at diagnosis, but the difference in HOMA-β between people with T2DM and age-matched control individuals was much greater among people with young-onset than in people with usual-adult onset T2DM.[26]

In summary, the most commonly detectable antibody was the GAD Ab in our study of young-onset phenotypic T2DM subjects. The frequency of IA-2 Ab and ZnT8 Ab was lower, and none were positive for double-antibodies. The concentration of GAD Ab may dictate phenotypic presentation. The C-peptide level may be adequate at diagnosis, and GAD Ab may not be a reliable predictor of C-peptide level.

CONCLUSION

GAD Ab was the most commonly detectable antibody in young-onset phenotypic T2DM patients. The frequency of ZnT8 Ab and IA-2 Ab was very low to undetectable, and none were positive for double-antibodies, indicating that IA-2 Ab and ZnT8 Ab may not be characteristic of young-onset T2DM. The concentration of GAD Ab may dictate phenotypic presentation. Though GAD Ab may not be a predictor of C-peptide level, it may be concluded that beta cell dysfunction in young patients with diabetes might be dependent on other unexplored factors for classifying the early-onset T2DM.

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Statement of Authorship
All authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement
KKS: Conceptualization, Methodology, Software, Validation, Formal Analysis, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Project administration, Funding acquisition**;** **MH:** Conceptualization, Methodology, Software, Validation, Formal Analysis, Investigation, Data Curation, Writing – original draft preparation, Writing – review and editing, Supervision, Project administration; **NS:** Conceptualization, Methodology, Investigation; **SBAS:** Investigation, Resources; **HM:** Investigation, Resources; **TF:** Investigation, Resources, Writing – review and editing; **MAH:** Conceptualization, Methodology, Validation, Investigation, Data Curation, Writing – original draft preparation, Writing – review and editing, Supervision, Project administration, Funding acquisition.

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