

A Cross-sectional Study to Assess Beta-Cell Function in Individuals with Recently Diagnosed Young-Onset Type 2 Diabetes Mellitus and Its' Complications

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Abstract

Objective. The primary objective was to assess beta-cell function of recently-diagnosed young-onset type 2 diabetes mellitus (T2DM) individuals using basal and stimulated C-peptide levels. The secondary objective was to examine the association between C-peptide with metabolic factors and diabetes complications.

Methodology. A cross-sectional study was conducted for young-onset T2DM individuals aged 18-35 years with a disease duration of not more than 5 years. Plasma C-peptide was measured before and after intravenous glucagon injection. Demographic data, medical history and complications were obtained from medical records and clinical assessment. Continuous data were expressed as median and interquartile range (IQR). Categorical variables were described as frequency or percentage. Multivariable linear regression analysis was used to determine factors associated with C-peptide levels.

Results. 113 participants with young-onset T2DM with a median (IQR) age of 29.0 (9.5) years and 24 (36) months were included in this study. The median (IQR) basal and stimulated C-peptide was 619 (655) pmol/L and 1231 (1024) pmol/L. Adequate beta-cell function was present in 78-86% of the participants based on the basal and stimulated C-peptide levels. We found hypertension, obesity and diabetic kidney disease (DKD) to be independently associated with higher C-peptide levels. In contrast, females, smokers, those on insulin therapy and with longer duration of disease had lower C-peptide levels.

Conclusion. Most recently diagnosed young-onset T2DM have adequate beta-cell function. Elevated C-peptide levels associated with obesity, hypertension and diabetic kidney disease suggest insulin resistance as the key driving factor for complications.

Key words: type 2 diabetes mellitus, young-onset, beta-cell function, C-peptide, glucagon stimulation test

INTRODUCTION

Young-onset type 2 diabetes mellitus (T2DM) is defined as onset of T2DM before the age of 40 years in the absence of secondary causes.¹ Studies have shown an alarming increase in the prevalence of young-onset T2DM globally, more so in Asia in the last few decades.² The International Diabetes Federation (IDF) estimated an increase from 23 to 63 million young adults worldwide to have T2DM from 2000 to 2013 with the biggest increase being in Africa, Southeast Asia, and the Western Pacific region.² In an Asian

study, approximately 20% of patients with type 2 diabetes were young-onset T2DM.³ This subset of individuals has been associated with accelerated disease progression and premature complications.² There are also higher rates of insulin commencement and intensification of treatment regimen early in the disease compared to the usual onset.¹

The pathophysiology of T2DM in the young has been thought to be similar to the usual onset with an interplay of beta-cell dysfunction, insulin resistance and obesity-related mechanisms.^{2,4} There is limited data with regards

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to beta-cell function in the early stage of the disease in this population. Objective assessment of beta-cell function is important to assist us in understanding the disease mechanism and rate of progression, as well as to guide therapeutic options. This is a pilot study performed in Malaysia to assess beta-cell function of recently-diagnosed young-onset T2DM individuals using fasting and glucagon stimulated C-peptide levels. C-peptide is produced in equal amounts to insulin and is an objective marker of beta-cell function.⁵ Glucagon stimulation test (GST) is an easily performed test to assess stimulated C-peptide with good sensitivity and reproducibility in clinical practice.⁶ The secondary objective of this study was to examine the association among C-peptide levels with metabolic parameters and diabetes-related complications.

METHODOLOGY

Study design and participants

This is a cross-sectional study involving young-onset T2DM individuals seen in the diabetes clinics of two urban tertiary hospitals in Malaysia between September 2019 to December 2020. Individuals aged 18 to 35 years diagnosed with T2DM for not more than five years were recruited using the universal sampling method. This was a descriptive study, therefore no formal sample size was required. We excluded individuals with chronic kidney disease stage 2 and above (estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m²); concurrent infection or inflammatory disease, recent diabetic ketoacidosis or hyperglycaemic hyperosmolar state in the last three months, positive diabetes autoantibodies, prior clinical diagnosis of other forms of diabetes (monogenic diabetes, type 1 diabetes, latent autoimmune diabetes of adult onset and secondary diabetes), fasting capillary blood glucose less than 4.0 mmol/L or more than 13.9 mmol/L on the day of testing, and those who were pregnant.⁷ Written informed consent was obtained from all study participants prior to the commencement of the study. This study was approved by the Medical Research and Ethics Committee (MREC) of the Ministry of Health of Malaysia.

Study recruitment and procedures

Study participants were recruited during their routine clinical visits to the diabetes clinic. Individuals with unknown diabetes mellitus autoantibodies status were screened prior to enrolment. Those with positive diabetes autoantibodies were excluded from the study. This study involved only a single visit. Study participants were required to fast overnight for at least eight hours and to omit all insulin and oral antidiabetic agents on the morning of testing. On the day of testing, anthropometric measurements (weight, height, waist circumference) were taken using calibrated tools. Vital signs and capillary blood glucose pre-procedure were checked. GST was performed if fasting capillary glucose levels were between 4.0 to 13.9 mmol/L. Basal C-peptide and blood glucose levels were

sampled prior to administration of 1 mg of intravenous glucagon. After 6 minutes, stimulated blood glucose and C-peptide samples were collected. Study participants were monitored for adverse effects for 15 minutes post testing. All tests were conducted by a single operator. Information regarding demography, disease history, co-morbidities, complications and treatment were gathered from medical records and clinical assessment.

Measures

The primary outcome of this study, beta-cell function, was measured using C-peptide levels (fasting and stimulated). Adequate beta-cell function was defined as either a basal C-peptide level of more than 250 pmol/L or a stimulated C-peptide level of more than 600 pmol/L, or both.⁵

Independent variables examined included current age, gender, ethnicity, smoking status, family history of T2DM, age of disease onset, disease duration, fasting glucose, HbA1c, insulin therapy, waist circumference, obesity, hypertension, dyslipidaemia, macrovascular complications, microvascular complications, retinopathy, nephropathy, and neuropathy. Waist circumference was measured midway between the iliac crest and the lowermost margin of the ribs at the end of a normal respiratory expiration.⁸ Obesity was defined based on a body mass index (BMI) cut-off of ≥ 27.5 kg/m².⁹ Abdominal obesity was defined as a waist circumference of ≥ 90 cm in males or ≥ 80 cm in females.⁸ Glycated haemoglobin (HbA1c) performed in the last three months was used as a measure of glycaemic control. Hypertension was defined as the persistent elevation of systolic blood pressure of 140 mmHg or greater and/or a diastolic blood pressure of 90 mmHg or greater.¹⁰ Dyslipidemia in type 2 diabetes was diagnosed based on low-density lipoprotein (LDL) cholesterol levels of 2.60 mmol/l or greater, high-density lipoprotein (HDL) cholesterol levels of 1.02 mmol/l or less and triglyceride levels of 1.7 mmol/l or greater.¹¹

Macrovascular complication was defined as an established history of ischemic heart disease, stroke or peripheral vascular disease based on medical records. Microvascular complication was defined as the presence of one or more of the following complications: retinopathy, nephropathy or peripheral neuropathy. Retinopathy was assessed based on slit lamp examination records performed in the last 12 months by credentialed personnel at the ophthalmology clinic of respective tertiary hospitals. Participants with no recent assessment were referred for evaluation as per routine protocol. Both ophthalmology clinics used the Early Treatment for Diabetic Retinopathy Study (ETDRS) classification to diagnose and classify diabetic retinopathy. Diabetic kidney disease (DKD) was assessed based on two or more urinary spot quantification of proteinuria or urine albumin creatinine ratio (ACR) performed in the last 12 months. Microalbuminuria was defined as a urine ACR of 3-30 mg/mmol whereas overt nephropathy was defined as a urine ACR of >30 mg/mmol.¹² Subjects with chronic

kidney disease stage 2 and above (estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m²) were excluded from this study, as majority of C-peptide is metabolised by the kidney and may result in inaccurate results.⁵ Peripheral neuropathy was diagnosed based on diminished sensation to standardised monofilament examination or reduced vibration on graduated tuning fork testing. Successful weaning of initial insulin therapy was defined as the ability to discontinue insulin in those initiated on insulin within 6 months of diagnosis.

C-peptide analysis was performed in a centralised laboratory using IMMULITE-2000 C-peptide (Siemens), a solid phase, two-site chemiluminescence immunometric assay. The co-efficient of variation (CV) of this test is less than 5% based on the designated laboratory internal quality control (IQC) performance analysis. This is in keeping with target of desirable biological variation.¹³ Study participants were screened for diabetes autoantibodies including anti-islet cell antibodies (ICA), anti-glutamic acid (GAD) antibodies and anti-islet tyrosine phosphatase (IA2) antibodies using commercially available ELISA kits (Medipan GmbH, Germany). Fasting blood glucose level was determined by hexokinase method. Urinary albumin and creatinine were measured by immunoturbidimetric and enzymatic methods respectively. Lastly, HbA1c was analysed using high performance liquid chromatography (HPLC).

Statistical analysis

All statistical analysis was performed with Statistical Package for Social Science (SPSS) Version 22.0.¹⁴ Most continuous data was found to be not normally distributed, therefore expressed as median and interquartile range (IQR). Categorical variables were described as frequency or percentage. Bivariate analysis using Mann Whitney U and Kruskal Wallis test were used to examine association between categorical data. Spearman's rank correlation coefficient was used to assess relationship between continuous data. We proceeded with linear regression analysis using variables with $p < 0.25$ from bivariate analysis and clinically important outcome variables based on biological plausibility. Stepwise linear regression with forward selection was performed. The final model was tested for autocorrelation (Durbin-Watson test), multicollinearity and homoscedasticity. The significance level for all steps of analyses was $p < 0.05$.

RESULTS

A total of 151 patients were screened to be recruited in this study. Twenty-eight patients were excluded due to positive autoantibodies ($n = 20$), CKD stage 3 and above ($n = 2$), pregnancy ($n = 1$), recent diabetic ketoacidosis ($n = 2$), and hyperglycemia on day of testing ($n = 3$). Six patients declined participation and the remainder ($n = 4$) were not contactable. A final sample size of 113 participants participated in this study.

Patient characteristics (Table 1)

The median (IQR) age and disease duration of study population was 29 (9.5) years and 24 (36) months respectively. There was a female preponderance ($n = 70$, 61.9%) with the predominant ethnicity being Malay ($n = 80$, 70.8 %). More than two-thirds ($n = 84$, 74.3%) of the study population was obese. Approximately 90% of the subjects had abdominal obesity: 88.4% of the males ($n = 38$) and 91% of the females ($n = 64$). Almost all had concomitant dyslipidaemia ($n = 104$, 92.0%) while less than one-third had hypertension ($n = 34$, 30.1%). Majority had a family history of T2DM involving at least one first-degree relative ($n = 94$, 83.2%). There were 14.2% ($n = 16$) active smokers in the population studied. The average glycaemic control of the population studied was poor, with a median (IQR) HbA1c of 8.5% (4.1). More than half of the study population ($n = 66$, 58.4%) was currently on insulin therapy. It was found that 75.3% ($n = 55$) of all patients with history of insulin use were initiated on insulin therapy early (within six months from diagnosis). However, only 15.7% ($n = 8$) of those started on early insulinization were successfully weaned off. There were no documented macrovascular complications; however, microvascular complications were present in 38.1% ($n = 43$) of the cohort. DKD was the most common microvascular complication (34.5%, $n = 39$), mostly in the form of microalbuminuria. Retinopathy and peripheral neuropathy were observed in less than 10% of the cohort.

Glucagon stimulation test results (Table 2)

The C-peptide levels had a median (IQR) basal value of 619 (655) pmol/L and 1231 (1024) pmol/L with stimulation. There was a strong positive correlation between fasting and stimulated C-peptide levels, $r_s = 0.92$, $p < 0.001$ (data not shown in Table 2). Majority of the study participants had adequate beta-cell function based on the basal and stimulated C-peptide levels at 86% and 78% respectively. Within the subgroup of participants on insulin as current therapy, there was still a high proportion registering adequate beta-cell function at 77% and 70% respectively based on the basal and stimulated C-peptide levels. Transient and self-limiting nausea was reported in 8.8% ($n = 10$) participants immediately after the procedure.

Factors associated with basal and stimulated C-peptide levels

Table 3 shows the bivariate analysis between basal and stimulated C-peptide levels with baseline clinical characteristics, metabolic parameters and diabetes-related complications. Both basal and stimulated C-peptide were significantly associated with gender, disease duration, age of disease onset, HbA1c, insulin therapy, obesity, waist circumference and hypertension ($p < 0.05$). In addition, stimulated C-peptide was also significantly associated with current age, smoking and dyslipidaemia ($p < 0.05$). There was no significant association between basal and stimulated C-peptide with diabetes related complications.

We proceeded with linear regression analysis to determine factors independently associated C-peptide levels (Tables 4 and 5). We included 11 variables with $p < 0.25$ from the bivariable analysis and clinically important outcome variables studied into the preliminary main effect model (current age, gender, smoking, age of disease onset, disease duration, HbA1c, obesity, insulin therapy, dyslipidaemia, hypertension and DKD). We found hypertension, obesity and DKD to be independently associated with higher C-peptide levels. In contrast, females, smokers, those on insulin therapy and those with longer disease duration had lower C-peptide levels.

DISCUSSION

We found our young-onset T2DM population to be predominantly female (62%). Subjects also had a strong family history of diabetes. Almost three-quarters of them were obese by BMI criteria. More than 90% had abdominal obesity associated with dyslipidaemia while almost one-third had hypertension. This high prevalence of metabolic syndrome with multiple cardiovascular risk factors despite a young age and short disease duration is similar to the clinical characteristics reported in literature.^{1,3,15} The median HbA1c of 8.5% (69 mmol/mol) in our study cohort indicated a relatively poorer glycaemic control compared to the median HbA1c of 7.9% (63 mmol/mol) for the general diabetic population in the country.¹⁶ Our findings concur

Table 1. Patient demography, metabolic parameters, disease history and complications

	Variables	Median (IQR) ^a	n (%)
Demography	Age (years)	29.0 (9.5)	
	Gender	Male	43 (38.1)
		Female	70 (61.9)
	Ethnicity	Malay	80 (70.8)
		Chinese	15 (13.3)
Indian		18 (15.9)	
Metabolic parameters	Obesity	BMI <27.5 kg/m ²	29 (25.7)
		BMI ≥27.5 kg/m ²	84 (74.3)
	Abdominal Obesity	Male (WC ≥90 cm)	38 (88.4)
		Female (WC ≥80 cm)	64 (91.4)
	Hypertension		34 (30.1)
Dyslipidaemia		104 (92.0)	
Medical history	Diabetes duration (months)	24 (36)	
	HbA1c (%)	8.5 (4.1)	
	Family history of diabetes		94 (83.2)
	Smoking		16 (14.2)
Diabetes treatment history	Current insulin therapy		66 (58.4)
	Time to insulin initiation	≤6 months from diagnosis	55 (75.3)
		>6 months from diagnosis	18 (24.7)
Successful weaning of initial insulin ^b		8 (15.7)	
Complications	Microvascular		43 (38.1)
	Retinopathy		8 (7.1)
		Non-proliferative ^c	4 (50.0)
		Proliferative ^c	4 (50.0)
	Diabetic kidney disease		39 (34.5)
		Microalbuminuria ^d	29 (74.4)
		Overt nephropathy ^d	10 (25.6)
Peripheral neuropathy		9 (8.0)	

IQR, interquartile range; BMI, body mass index; WC, waist circumference.

^a not normally distributed data expressed as median (IQR)

^b within insulin initiation at ≤6 months from diagnosis subgroup

^c within retinopathy subgroup

^d within diabetic kidney disease subgroup

Table 2. Glucagon stimulation test results

	Variables	Median (IQR) / n (%)
Glucose (mmol/L), median (IQR)	Fasting glucose	7.5 (3.3)
	Stimulated glucose	8.3 (3.7)
C-peptide (pmol/L), median (IQR)	Basal C-peptide	619.0 (655.0)
	Glucagon stimulated C-peptide	1231.0 (1024.0)
Beta cell function, n (%)	Adequate basal beta cell function ^a	97.0 (85.8)
	Adequate stimulated beta cell function ^b	88.0 (77.9)
	For participants on insulin as current therapy:	
	i. Adequate basal beta cell function ^a	51.0 (77.3)
ii. Adequate stimulated beta cell function ^b	46.0 (69.7)	

Results expressed in median (IQR) for not normally distributed variables and percentage for categorical variables.

^a defined as C-peptide >250 pmol/L, ^b defined as C-peptide >600 pmol/L.⁹

with several studies done in the Asian region showing poorer glycaemic control in young-onset T2DM compared to the usual onset T2DM.^{3,17}

The high percentage of insulin use within six months of diagnosis coupled with a low rate of subsequent

weaning of insulin therapy in this study indicate a delayed diagnosis with high prevalence of severe hyperglycaemia at presentation requiring insulin, followed by difficulties in restoring euglycemia despite intensive use of insulin. This is consistent with the high percentage of undiagnosed diabetes among the young age group (18 to 39 years) which is at 77%

Table 3. Bivariate analysis between c-peptide and clinical features, metabolic parameters and diabetes complications

Variables	Basal C-peptide			Stimulated C-peptide		
	Median (IQR) ^a	r_s^b	p	Median (IQR) ^a	r_s^b	p
Current age		0.164	0.083		0.259	0.006
Gender	Male	857.0 (709.0)	0.002	1705.0 (1268.0)	0.001	
	Female	541.0 (500.0)		1021.0 (980.3)		
Ethnicity	Malay	635.5 (658.5)	0.499	1247.5 (1008.3)	0.354	
	Chinese	559.0 (469.0)		751.0 (1264.0)		
	Indian	639.0 (536.5)		1276.0 (902.0)		
Smoking	Yes	852.0 (625.5)	0.177	1673.5 (851.75)	0.034	
	No	583.0 (637.0)		1086.0 (1071.5)		
Family history	Yes	625.5 (618.5)	0.693	1213.0 (952.0)	0.785	
	No	543.0 (964.0)		1241.0 (1612.0)		
Age of disease onset			0.219		0.301	0.001
Disease duration			0.238		0.227	0.015
Fasting glucose			0.069		0.074	0.434
HbA1c			0.286		0.319	0.001
Insulin therapy	Yes	460.0 (382.0)	<0.001	917.0 (884.8)	<0.001	
	No	910.0 (560.0)		1629.0 (954.0)		
Obesity^c	Yes	766.5 (604.8)	<0.001	1493.0 (1108.8)	<0.001	
	No	348.0 (322.5)		761.0 (553.0)		
Waist circumference			0.417		0.404	<0.001
Dyslipidaemia	Yes	635.5 (681.5)	0.160	1259.5 (1036.0)	0.034	
	No	450.0 (239.5)		970.0 (715.0)		
Hypertension	Yes	897.0 (870.3)	0.001	1622.0 (1085.5)	0.001	
	No	546.0 (485.0)		1039.0 (1083.0)		
Microvascular	Yes	672.0 (688.0)	0.707	1347.0 (1434.0)	0.896	
	No	594.5 (578.5)		1095.0 (975.3)		
Retinopathy	Yes	708.0 (1004.8)	0.392	1251.0 (1056.8)	0.771	
	No	606.0 (645.0)		1231.0 (1038.0)		
Nephropathy	Yes	722.0 (685.0)	0.474	1413.0 (1373.0)	0.411	
	No	581.0 (644.8)		1089.0 (1015.3)		
Neuropathy	Yes	424.0 (710.0)	0.321	1089.0 (1332.0)	0.284	
	No	625.5 (641.5)		1236.0 (1004.8)		

^a Mann-Whitney U test used for non-parametric binomial data and Kruskal Wallis for data with >2 categories

^b Spearman's rank correlation coefficient (rs) used for non-parametric data

^c Obesity defined as BMI ≥ 27.5 kg

Table 4. Factors independently associated with basal c-peptide levels from multivariable linear regression

Variables	Coefficient	95% Confidence Interval		p
		Lower limit	Upper limit	
Insulin therapy	-392.8	-550.1	-235.4	<0.001
Hypertension	192.0	22.6	361.5	0.027
Obesity	252.6	78.9	426.3	0.005
Female	-266.8	-434.9	-99.6	0.002
Nephropathy	186.0	27.2	344.9	0.022
Smoker	-264.7	-501.5	-28.0	0.029

Stepwise multiple linear regression was applied. The Durbin-Watson statistic, $d=1.947$ was between 1.5 and 2.5 and therefore data was not autocorrelated. Multicollinearity and homoscedasticity were checked and not found. A significant regression equation was found [F (6, 104) = 13.076, $p<0.001$], with R^2 of 0.43.

Table 5. Factors independently associated with stimulated c-peptide levels from multivariable linear regression

Variables	Coefficient	95% Confidence Interval		p
		Lower limit	Upper limit	
Insulin therapy	-393.4	-689.6	-97.3	0.010
Hypertension	533.5	201.6	865.4	0.002
Obesity	382.4	49.2	715.8	0.025
Female	-355.1	-644.1	-66.1	0.017
Duration of Disease	-7.2	-14.4	-0.2	0.044

Stepwise multiple linear regression was applied. The Durbin-Watson statistic, $d=1.907$ was between 1.5 and 2.5 and therefore data was not autocorrelated. Multicollinearity and homoscedasticity were checked and not found. A significant regression equation was found [F (5, 105) = 11.879, $p<0.001$], with R^2 of 0.3.

according to the National Health and Morbidity Survey in 2019.¹⁸ In the Treatment Options for Type 2 Diabetes in Adolescents and Youth (TODAY) cohort, only 13% were initiated on insulin at diagnosis and nearly all were weaned off insulin during the run-in period of two to six months using metformin titration and diabetes education.¹⁷ In the Asian region, Pan et al., showed that only 18% of newly-diagnosed young-onset diabetes patients used insulin during the first year of disease, despite approximately 10% of the study population having classical type 1 diabetes.¹⁷

There was a high prevalence of microvascular complication at 38% in our study cohort despite only a median disease duration of two years. There was a striking predominance of DKD among the microvascular complications, with majority having microalbuminuria. Literature shows the prevalence of microalbuminuria is higher among individuals with young onset T2DM at diagnosis and tends to occur after a shorter duration of disease.² The TODAY cohort showed a 6% prevalence of microalbuminuria at baseline which increased to 17% after four years.¹⁹ The Pima Indian youth with T2DM (aged <20 years) had a much higher prevalence of microalbuminuria at diagnosis at 22% and was projected to reach 60% before the age of 30.² Kim et al., also reported a high prevalence of microalbuminuria at 34.3%, nearly 2-fold higher in those with newly-diagnosed young-onset T2DM compared to those diagnosed after the age of 40 years.¹⁵ This indicates an increased risk of early-onset DKD with rapid progression among the young-onset T2DM individuals compared to usual onset, with possible ethnic variation.

C-peptide level was chosen as the measure of beta-cell function because it is a reliable indicator of insulin secretion. It has a longer half-life and is less affected by first-pass hepatic metabolism when compared to insulin. It also can be used to assess endogenous insulin production including patients on insulin treatment.^{5,6} The median C-peptide in our cohort was 619 pmol/L (basal) and 1231 pmol/L (glucagon stimulated). Based on the recommended thresholds of 250 pmol/L (basal) and 600 pmol/L (stimulated) C-peptide levels, majority (78-86%) of our patients have adequate beta-cell function.⁵ These results are comparable to other cohorts reported in the region.^{15,17}

Kim et al., reported a median basal C-peptide of 788 pmol/L and 2-hour postprandial C-peptide of 2023 pmol/L in their newly-diagnosed young-onset T2DM.¹⁵ Pan et al., reported a mean basal C-peptide of 700 pmol/L with an almost identical percentage (80-83%) of adequate beta-cell function which was assessed using basal and glucagon stimulation C-peptide testing in their newly diagnosed young-onset diabetes individuals consisting mainly of T2DM.¹⁷ This is in contrast to some other studies that reported significant beta cell dysfunction in newly-diagnosed young-onset T2DM.^{20,21} Even within the subgroup of insulin users, there was a high proportion of at least 70% having adequate beta-cell function. Pan et al., showed a lower percentage, with approximately 50% of their young newly-diagnosed DM on insulin with adequate basal and stimulated beta-cell

function using similar C-peptide cut off levels.¹⁷ However, their study population included those with T1DM.¹⁷

We postulated that the high fasting and stimulated C-peptide level shown in our study may be a surrogate marker of insulin resistance in this population.^{6,22} Therefore, we proceeded with post-hoc analysis to calculate modified HOMA-IR levels to assess insulin resistance in this cohort using basal C-peptide levels.²³ We found a mean \pm standard deviation (SD) modified HOMA-IR of 3.48 ± 1.36 . The modified HOMA-IR values showed a strong positive correlation with basal C-peptide levels, $r_s = 0.90$, $p < 0.001$. Based on previous studies done in the Asian region, a HOMA-IR value of more than 2.5 has been used to define insulin resistance.^{24,25} Using this cut off, 78% of our study population were found to be insulin resistant. This further supports our hypothesis that our recently-diagnosed young-onset T2DM population was predominantly insulin resistant as opposed to having inadequate beta-cell function.

Linear regression analysis in this study revealed females to have lower C-peptide levels than males. This may indicate a possible difference in beta-cell function between genders among the young-onset T2DM. Despite the known preponderance of females in the young-onset T2DM, there has been no conclusive evidence to suggest difference in beta-cell function between genders to date.²

Obesity and hypertension were independently associated with higher basal and stimulated C-peptide levels. This is presumably because an elevated C-peptide is known to be a surrogate marker of insulin resistance in individuals with the metabolic syndrome phenotype.⁶

As expected, patients on insulin therapy were found to have significantly lower basal and stimulated C-peptide levels. There has been concern in literature about suppression of endogenous insulin and C-peptide secretion in those on exogenous insulin therapy when beta-cell function is assessed. However, it has been proven that acute normalization of glucose concentration or hypoglycaemia is responsible for suppression of C-peptide levels during testing instead of the direct effect of the exogenous insulin.^{6,26}

Gjessing et al., has shown that contrary to the hypothesis of beta-cell function attenuation due to glucotoxicity, fasting hyperglycaemia in fact potentiates stimulated C-peptide levels during mixed-meal tolerance testing or glucagon stimulation testing in T2DM.²⁷ In this study, we ensured that the blood glucose of study participants was between 4.0 to 13.9 mmol/L at the time of testing to minimize the possible effect of extreme glucose concentrations on C-peptide values.⁷

We also found active smokers to have lower basal C-peptide levels than non-smokers. Although there are epidemiological studies confirming smoking as a risk factor for the development of T2DM, the effect of smoking on beta-cell function and insulin resistance is not conclusive.²⁸⁻³¹

DKD was found to be independently associated with elevated basal C-peptide levels in this study. In our study cohort, there was a high prevalence of DKD early in the disease despite majority of them having adequate beta-cell function. Older studies done in the usual-onset T2DM with longer disease duration showed an opposite relationship with lower C-peptide levels associated with DKD.^{7,22}

A recent large data-driven cluster analysis by Ahlqvist et al., in Scandinavia identified five novel subgroups in their T2DM population. These five subgroups were classified as severe autoimmune diabetes (SAID), severe insulin-deficient diabetes (SIDD), severe insulin-resistant diabetes (SIRD), mild obesity-related diabetes (MOD) and mild age-related diabetes (MARD).³² The SIRD subgroup characterized by obesity and insulin resistance had a higher risk of DKD independent of their glycaemic control compared to the other subgroups.³² Zou et al., confirmed these findings in the Chinese and United States populations.³³ However, Anjana et al., identified an additional subgroup, Combined Insulin Resistant and Deficient Diabetes (CIRDD), unique to the Asian Indian population. This subgroup was younger in onset, had combined insulin deficiency and resistance with poor glycaemic control and increased hazards of kidney disease and retinopathy.³⁴ A similar analysis in our young-onset T2DM population will be beneficial to understand the subgroups within and their characteristics, including risk of complications.

Limitations

This study had a few limitations. Although this was a descriptive study not requiring a formal sample size, the modest sample size may have underscored the power in detecting the association between C-peptide with metabolic parameters and diabetes-related complications. Therefore, less emphasis can be placed on significance testing of results in this study. The cross-sectional study design was not able to establish causal relationship between measured variables and outcomes.

Our study population was comprised of patients attending diabetes clinics in two urban tertiary hospitals and was not fully representative of the general population of young-onset T2DM in the country. Despite attempts to exclude those with monogenic diabetes based on prior clinical diagnosis, the lack of genetic testing is a limiting factor.

Lastly, the slit lamp examination not being done by designated ophthalmologists may have lead to operator bias in the results of retinopathy screening. However, it was verified that the standard operating procedure of both of the ophthalmology clinics required their personnel to be credentialed and privileged to perform the test and used the Early Treatment for Diabetic Retinopathy Study (ETDRS) classification to diagnose and classify diabetic retinopathy.

This is the first study assessing beta-cell function in recently diagnosed, exclusively young-onset T2DM in the country

and one of the few in Asia. Various measures to minimize the effects of confounding factors on C-peptide measurement were undertaken including stringent eligibility criteria, exclusion of extreme blood glucose levels and omission of exogenous insulin on the day of testing to improve the accuracy of C-peptide testing.

CONCLUSION

Majority of the participants who have young-onset diabetes have adequate basal and stimulated pancreatic beta-cell function. There was a high rate of metabolic syndrome in this study cohort associated with early-onset DKD. The glycaemic control was poor despite high rates of insulin use. Elevated C-peptide levels associated with obesity, hypertension and DKD suggest that insulin resistance rather than beta-cell dysfunction is the key driving factor of complications. Our study paves the direction for future prospective studies involving a larger cohort of individuals with direct measurement of insulin sensitivity to confirm our findings. Treatment strategies in this population should be tailored, focusing on early diagnosis before development of severe hyperglycaemia with a combination of intensive lifestyle modification and pharmacotherapy to address obesity, insulin resistance and avoiding overtreatment with insulin. These individuals should be screened for microvascular complications especially DKD upon diagnosis and monitored closely thereafter to prevent premature morbidity and mortality.

Statement of Authorship

The authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement

SN: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Resources, Data Curation, Writing - original draft preparation, Visualization, Project administration, Funding acquisition; **SR:** Conceptualization, Methodology, Validation, Investigation, Resources, Data Curation, Writing - review and editing, Visualization; **MBLB:** Conceptualization, Methodology, Validation, Investigation, Resources, Data Curation, Writing - review and editing, Visualization; **NSCR:** Conceptualization, Methodology, Validation, Investigation, Resources, Visualization; **ST:** Conceptualization, Methodology, Validation, Investigation, Resources, Visualization; **NA:** Conceptualization, Methodology, Validation, Investigation, Resources, Visualization; **YMC:** Conceptualization, Methodology, Validation, Investigation, Resources, Visualization; **SHF:** Conceptualization, Methodology, Validation, Investigation, Resources, Data Curation, Writing - review and editing, Supervision, Project administration, Funding acquisition.

Author Disclosure

The authors declared no conflict of interest.

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