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Endothelial Dysfunction Using Flow-mediated Dilatation Among Individuals with Pre-impaired Glucose Tolerance (Pre-IGT)

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Abstract

Objectives. Pre-impaired glucose tolerance (pre-IGT) is a prediabetes stage characterized by normoglycemia and compensatory hyperinsulinemia due to insulin resistance. Hyperinsulinemia increases cardiovascular disease (CVD) risk, especially, endothelial dysfunction (ED). However, there is paucity of studies on ED with hyperinsulinemia alone, particularly in individuals with pre-IGT. This study aimed to determine the presence of ED using brachial artery flow-mediated dilatation (FMD) among adult participants with pre-IGT and its correlation with insulin levels and other related clinical parameters.

Methodology. This is a cross-sectional analytical study. We screened adult patients with risk factors for developing diabetes (first-degree relative with type 2 diabetes mellitus, obesity, history of gestational diabetes and polycystic ovary syndrome). Brachial artery FMD was performed among participants with pre-IGT and findings were correlated with CVD risk factors using Pearson's correlation and linear regression.

Results. Of the 23 pre-IGT patients, 5 (21.74%) had decreased FMD values with significant associations with serum insulin and HbA1c. It was further observed that for every 1-unit increase in second-hour serum insulin and in HbA1c, there was a decrease in FMD values by 0.38% and 0.50%, respectively. Serum insulin was elevated, while other biochemical parameters were normal. Moreover, participants with low FMD were older, with higher BMI and had higher HBA1c, total cholesterol and low-density lipoprotein (LDL) cholesterol.

Conclusion. As early as the pre-IGT stage, endothelial dysfunction using the FMD test is already present, with red flags on other CVD risk factors already developing.

Key words: endothelial dysfunction (ED), insulin resistance (IR), pre-impaired glucose tolerance (Pre-IGT), hyperinsulinemia, type 2 diabetes mellitus (T2D), cardiovascular disease (CVD) risks

INTRODUCTION

Insulin resistance (IR) is the earliest metabolic abnormality in the pathophysiology of type 2 diabetes mellitus (T2D).[1] To overcome this, the pancreatic β-cells increase insulin secretion to maintain normal blood glucose, resulting in hyperinsulinemia.[1-5] Hyperinsulinemia in IR increases levels of C-peptide, a cleavage product of pro-insulin, which has proatherogenic effects.[6] Neointimal smooth muscle cell proliferation and vascular morphological modification have been attributed to the inflammatory effects of C-peptide lodging in the vascular intima-media of T2D patients.[7,8] The circulating C-peptide levels have also been associated with subclinical myocardial injury development.[9] The resultant vascular functional alteration, known as endothelial dysfunction (ED), which is identified through arterial intima-media thickness and abnormal arterial endothelial flow-mediated dilatation (FMD), has been elaborated in long-term adverse cardiovascular disease (CVD) outcomes and the morbidity and mortality of high-risk individuals with IR and hyperinsulinemia.[10-14]

Although these vascular abnormalities were implicated in the prediabetes stage like impaired glucose tolerance (IGT), most studies consistently implied abnormal circulating blood glucose (BG) as the primary causative factor for CVD development, compounded with major CVD risk factors like hypertension, dyslipidemia and smoking.[11-14]

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Nevertheless, there is a paucity of evidence on functional vascular abnormality or endothelial dysfunction using the pre-IGT stage as a clinical model, especially in the absence of significant risk factors.

Historically, in 1975, Kraft proposed the term *diabetes mellitus in situ* to characterize patients with normal glucose but abnormal insulin tolerance.[2] In elaborating on the natural course of T2D, de Groot and Jameson labeled this stage as compensated hyperinsulinemia.[5] Cognizant that the process of compensatory hyperinsulinemia with normoglycemia precedes the stage of IGT, our group termed this prediabetes stage as *pre-impaired glucose tolerance* (pre-IGT).[15-17] This preliminary study was conducted to determine vascular functional abnormality or endothelial dysfunction (ED), using brachial artery FMD, among adult participants with pre-IGT (hyperinsulinemic and normoglycemic) and its correlation with insulin levels and other related clinical parameters.

METHODS

Research design, study participants, and sample size

We conducted a cross-sectional, preliminary, small-scale study among adult patients at risk of developing T2D [first-degree relative with T2D, history of gestational DM (GDM), polycystic ovary syndrome (PCOS), overweight or obese and acanthosis nigricans].[18] Sixty-seven individuals aged 18 to 40 years old were purposively recruited at the Ambulatory Care Services of the University of Santo Tomas Hospital (Manila, Philippines). The World Health Organization Asian Body Mass Index (BMI) classification was used to categorize BMI (overweight: BMI = 23.00 to 24.90 kg/m^2, obese: BMI ≥25 kg/m^2).[19] We excluded patients diagnosed with T1D or T2D, IGT, impaired fasting glucose, hypertension (blood pressure >140/90 mm Hg), smoking history (either previous or current), dyslipidemia, coronary artery disease, cerebrovascular disease, chronic heart failure, liver disease and steroid intake for over a month in the past 3 months.[20]

Sample size (*priori*) computation was performed using the formula recommended by Viechtbaurer et al., for pilot or small-scale studies.[21] From the study of Skaug et al., the prevalence of endothelial dysfunction among low-risk healthy patients was 16.00%.[22] Using the recommended formula, a prevalence of 16.00%, and a significance level of 0.05 or 95% confidence interval, the computed sample size was 18 participants.

Written informed consent was obtained from all participants. This study was conducted under the Declaration of Helsinki and was approved by the University of Santo Tomas Hospital Research Ethics Committee (REC) (Reference No. REC-2021-05-070-TF). The manuscript is in line with the International Committee of Medical Journal Editors (ICMJE) Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals.

Anthropometric and biochemical data

We measured BMI, systolic BP [normal value (NV) < 120 mm Hg] and diastolic BP (NV <80 mm Hg). All participants underwent the following biochemical tests with their corresponding NV: fasting BG (< 100 mg/dL); post-75-g oral glucose tolerance test (OGTT) 2-hour BG (<140 mg/dL); 2-hour serum insulin (<30 uIU/mL);[23,24] HbA1c (< 5.7%), lipid profile [total cholesterol (<200 mg/dL), triglycerides (<150 mg/dL), low-density lipoprotein (LDL) cholesterol (<130 mg/dL), high-density lipoprotein (HDL) cholesterol (>40 mg/dL)][25]; creatinine (0.5 to 0.95 mg/dL); and alanine transaminase (ALT) (10 to 35 U/L). Those with normal fasting and 2nd-hour BG but with an elevated 2nd-hour insulin level were classified as pre-IGT.[15-17] The pre-IGT participants with generally normal biochemical results were included in the study (N = 23).

Brachial artery flow-mediated dilatation (FMD)

Included participants with pre-IGT subsequently underwent brachial artery FMD test. The following were done since several variables such as food, medication, temperature and sympathetic stimulation can affect FMD: (a) The participants were asked to fast for 8 to 12 hours before the procedure; (b) The procedure was performed at the University of Santo Tomas Hospital Heart Station, which is a quiet and temperature-controlled room; (c) The participants were asked not to exercise before the procedure; and (d) They were asked to avoid ingesting caffeine, high-fat foods and vitamin C for at least 4 to 6 hours before the procedure.[26]

A single technician did the procedure using ultrasonography (Philips CX50 model) with a 3-12 MHz linear probe (Philips L12-3). The measurements were obtained on the non-dominant arm while the participant was supine, allowing 20 minutes of rest before the procedure. Hyperemia was induced by inflation of a pneumatic cuff (12.5 cm wide) at 230–250 mm Hg for 5 minutes on the most proximal portion of the upper arm. The arterial diameter measurement was repeated 45–60 seconds after sudden cuff deflation. We utilized the average of the 3 measurements for the analysis. We calculated FMD as the percentage increase in diameter from the baseline to the maximum value after the cuff deflation. Interpretation of FMD results was performed by a vascular specialist who was blinded from the participants' characteristics. A positive result for endothelial dysfunction (ED+) was present if FMD values were less than 10% of the baseline diameter.[26] According to Kuvin et al., this cut-off score has a sensitivity of 91% and a negative predictive value of 95%. However, specificity was at 54% with a positive predictive value of 39%.[27]

Statistical analysis

Statistical analyses were conducted using STATA Statistical Software, Version 13, College Station, TX: StataCorp LP. A *p*-value of 0.05 was considered statistically significant.

Descriptive statistics included mean and standard and frequency and proportion for nominal data. Shapiro-Wilk's test was employed to determine data normality. Comparative analyses of the demographic and clinical characteristics according to ED status [without ED (ED-) vs. ED+)] were conducted using Chi-Square Test or Fisher's Exact test, if the assumption of at least 5 observations per cell is not met, for categorical variables; and Mann-Whitney U Test for ordinal and non-normally distributed, continuous data. Correlation analyses, using Pearson's Correlation, were initially utilized to determine the association of FMD with clinical parameters. Afterward, clinical parameters with significant associations were regressed with FMD values using linear regression. Crude analyses were initially performed and were adjusted to control for the confounders (age, sex, duration of being overweight or obese) using a 10% change in criterion estimate.

RESULTS

Demographics and clinical profiles

Among the 67 patients screened in this study, 17 were normal (normoglycemic and normoinsulinemic), 41 had pre-IGT (normoglycemic and hyperinsulinemic), 8 had IGT, and 1 had T2D. Of the 41 patients with pre-IGT, 23 were eligible for this study. We excluded 16 patients who had dyslipidemia (total cholesterol >200 mg/dL) and another 2 patients with elevated ALT.

As shown in Table 1, the mean age of participants with pre-IGT was 26.17 years (SD = 4.80). The majority were female (82.61%) and had a family history of T2D (73.91%). The mean BMI was 24.65 kg/m^2 (SD = 3.59). The mean OGTT BG at fasting and 2-hour post-load were both normal at 85.98 mg/dL (SD = 7.03) and 104.33 mg/dL (SD = 15.54), respectively. Likewise, the mean HbA1c was 5.25% (SD = 0.38), which was normal. The mean insulin at 2-hour was 109.90 uIU/mL (SD = 108.25). All participants were normotensive (SBP: 107.78 ± 11.95 mm Hg, range: 90-120 mm Hg; DBP: 71.48 ± 9.83 mm Hg, range: 60-80 mm Hg) and normolipidemic (total cholesterol: 170.76 mg/dL ± 17.83, range:143.24-199.61 mg/dL; triglycerides: 91.54 ± 55.13 mg/dL, range:34.52-145.14 mg/dL; LDL cholesterol: 95.03 ± 17.14 mg/dL, range: 67.57-129.73 mg/dL; HDL cholesterol: 57.36 ± 12.32 mg/dL, range: 40.00-85.71 mg/dL). Results also showed that the mean serum creatinine was 0.71 mg/dL (SD = 0.15) and the mean ALT was 24.95 U/L (SD = 32.81), which were both normal. Comparative analyses indicated that none of the demographic and clinical characteristics were significantly different between those with ED+ and ED– endothelial dysfunction (p >0.05). None had a history of GDM.

Table 1. Demographic and clinical profile of participants with pre-IGT according to endothelial dysfunction status (N = 23)

Characteristics	Endothelial dysfunction status[a]			Test statistic[b]	*p*-value (two-tailed)
	Without endothelial dysfunction or FMD ≥10% (n = 18)	With endothelial dysfunction or FMD <10% (n = 5)	Total (n = 23)		
Age (Years; x̄, SD)	25.83 (4.99)	27.40 (4.34)	26.17 (4.80)	0.48	0.502
Sex (f, %)				0.03	1.000
Male	3 (16.67%)	1 (20.00%)	4 (17.39%)		
Female	15 (83.33%)	4 (80.00%)	19 (82.61%)		
Family history of diabetes mellitus (f, %)	13 (72.22%)	4 (80.00%)	17 (73.91%)	0.12	1.000
Body mass index (kg/m^2; x̄, SD)	24.37 (3.34)	25.65 (4.69)	24.65 (3.59)	0.46	0.491
CVD risk factors (x̄, SD)					
Oral Glucose Tolerance Test (OGTT; mg/dL)					
Fasting blood glucose	85.93 (6.86)	86.17 (8.44)	85.98 (7.03)	0.88	0.914
2-hours post-OGTT	103.06 (15.90)	108.88 (14.86)	104.33 (15.54)	0.43	0.455
HbA1c (%)	5.21 (0.39)	5.38 (0.33)	5.25 (0.38)	0.33	0.352
2-hours insulin post-OGTT (uIU/mL)	92.21 (97.60)	173.58 (132.21)	109.90 (108.25)	0.30	0.325
Other CVD risk factors (x̄, SD)					
Systolic blood pressure (mm Hg)	108.50 (12.55)	105.20 (10.26)	107.78 (11.95)	0.53	0.633
Diastolic blood pressure (mm Hg)	72.22 (9.98)	68.80 (9.86)	71.48 (9.83)	0.54	0.614
Other biochemical tests (x̄, SD)					
Total cholesterol (mg/dL)	170.33 (18.65)	172.28 (16.28)	170.76 (17.82)	0.97	0.982
Triglyceride (mg/dL)	94.75 (59.74)	80.01 (36.51)	91.54 (55.13)	0.71	0.732
LDL-C (mg/dL)	93.59 (18.27)	100.23 (12.38)	95.03 (17.14)	0.31	0.331
HDL-C (mg/dL)	57.70 (12.56)	56.14 (12.73)	57.36 (12.32)	0.77	0.790
Serum Creatinine (mg/dL)	0.70 (0.12)	0.77 (0.22)	0.71 (0.15)	0.30	0.316
ALT (U/L)	24.73 (12.88)	25.72 (25.01)	24.95 (15.56)	0.46	0.491
Related CVD risk factors					
Duration of being overweight or obese (Years; x̄, SD)	5.33 (3.34)	3.60 (1.82)	4.96 (3.13)	0.21	0.232
Duration of PCOS (Months; x̄, SD)	13.13 (37.42)	0.25 (0.50)	10.42 (33.44)	0.59	0.664
History of gestational diabetes mellitus (f, %)	0 (0.00%)	0 (0.00%)	0 (0.00%)	–	–

Abbreviations and/or Symbols: x̄ = Mean, SD = Standard Deviation; f = Frequency, % = Percentage, CVD = Cardiovascular Disease, LDL = Low Density Lipoprotein, HDL = High Density Lipoprotein, ALT = Alanine Aminotransferase, PCOS = Polycystic Ovary Syndrome

[a]Note: FMD <10% denotes positive for endothelial dysfunction (ED+), while FMD >10% indicates negative for endothelial dysfunction (ED–)

[b]Note: Comparisons were conducted using Chi-Square Test or Fisher's Exact Test, for categorical variables, and Mann-Whitney U Test, for ordinal and non-normally-distributed continuous variables.

*Significant at 0.05

Table 2. Comparison of flow-mediated dilatation (FMD) among participants with pre-IGT according to endothelial dysfunction status (N = 23)

Characteristics	Endothelial dysfunction status			z-value[a]	*p*-value (two-tailed)
	Without endothelial dysfunction or FMD ≥10% (n = 18)	With endothelial dysfunction or FMD <10% (n = 5)	Total (n = 23)		
Flow-mediated dilatation score (x̄, SD)	25.25 (18.93)	6.34 (2.83)	21.14 (18.49)	3.35*	0.001

Abbreviations and/or Symbols: x̄=Mean, SD=Standard Deviation
[a]Note: Comparison was conducted using Mann-Whitney U Test.
*Significant at 0.05

As expected among pre-IGT participants, serum insulin was elevated with all other biochemical parameters within normal levels (Table 1). Although statistically not significant, comparative analyses showed that participants with low FMD (FMD <10% or ED+) were older, had higher BMI and higher fasting and 2-hour BG, HbA1c, total cholesterol and LDL cholesterol.

Endothelial dysfunction through FMD measurement

Among the 23 participants with pre-IGT who had brachial FMD test, 5 had low FMD <10% or ED+ signifying endothelial dysfunction (with a prevalence of 21.74% (95% CI: 7.46% to 43.70%). The FMD values of pre-IGT participants with FMD <10% (ED+) and FMD ≥ 10% (ED–) were 6.34% (SD: 2.83) and 25.25% (SD: 18.93), respectively (Table 2). Comparative analysis using the Mann-Whitney U Test showed that the FMD score of ED+ participants was significantly lower ($z = 3.35$, $p = 0.001$) than ED– participants.

Associations of FMD with clinical parameters

A significant, negative correlation of FMD score, albeit weak, was found with the 2-hour serum insulin ($r = –0.37$, $p = 0.048$) and HbA1c ($r = –0.48$ $p = 0.020$) (Table 3). Although insignificant, negative correlations with FMD were obtained with the following cardiometabolic parameters: fasting and 2-hour BG post-OGTT and duration of being overweight or obese.

Table 3. Correlation analyses using Pearson's R of the associations of flow-mediated dilatation (FMD) with different variables among patients with pre-IGT (N = 23)

Clinical parameters	FMD	
	r-value	*p*-value (two-tailed)
2-hour insulin post-OGTT (uIU/mL)	-0.37	0.048
HbA1c (%)	-0.48*	0.020
Fasting blood glucose (mg/dL)	-0.21	0.348
2-hour blood glucose post-OGTT (mg/dL)	-0.22	0.307
Body mass index (BMI; kg/m^2)	0.01	0.985
Duration of being overweight or obese (years)	-0.06	0.799
Status of polycystic ovary syndrome (PCOS)	0.20	0.423
Duration of PCOS (months)	0.13	0.595
Systolic blood pressure (mm Hg)	0.11	0.621
Diastolic blood pressure (mm Hg)	0.03	0.885

*Significant at 0.05

Results of the crude and adjusted linear regression analyses (Table 4) indicated that, after controlling for significant confounders (age, sex and duration of being overweight or obese), both 2-hour serum insulin ($\beta = –0.38$, $p = 0.050$) and HbA1c ($\beta = –0.50$, $p = 0.031$) had negative and significant associations with FMD. In particular, every 1-unit increase in 2-hour serum insulin leads to a 0.38% decrease in FMD values (1 unit of insulin is equivalent to 1 uIU/mL). Similarly, every 1-unit increase in HbA1c values denotes a 0.50% decrease in FMD values (1 unit of HbA1c is equivalent to 1%).

DISCUSSION

OGTT as a measure for IR and hyperinsulinemia

With OGTT, Stumvoll and colleagues demonstrated a high correlation between glucose metabolic clearance rate (MCR), insulin sensitivity index (ISI) and the first- and second-phase insulin release.[28] The group had shown that BMI, insulin (120 min) and glucose (90 min) were highly correlated with MCR ($r = 0.80$, $p < 0.00005$) and ISI ($r = 0.79$, $p < 0.00005$). Interestingly, the parameters predicted by the equations correlated better with the measured parameters than homeostasis model assessment (HOMA-IR) for secretion and resistance, the delta 30-min insulin/delta 30-min glucose ratio for secretion and insulin (120 min) for insulin resistance taken from the OGTT.

In the study by Crofts et al., over half of the 4,185 participants with normal glucose tolerance showed hyperinsulinemia after 75-g OGTT. In their report, however, fasting insulin had limited value in diagnosing hyperinsulinemia.[23] In their follow-up study, a 2-hour insulin level post-OGTT >30 uU/mL had the highest sensitivity of 98% in predicting

Table 4. Crude and adjusted analyses using linear regression of the association of the insulin at 2-hours post-prandial and HbA1c with flow-mediated dilatation of the participants with pre-IGT (N = 23)

Risk factors	Flow-mediated dilatation (FMD)					
	Crude or unadjusted analyses			Adjusted analyses		
	Crude β coefficient	Standard error (SE)	*p*-value (two-tailed)	Crude β coefficient	Standard error (SE)	*p*-value (two-tailed)
Insulin 2 hours	–0.37*	0.03	0.048	–0.38*	0.04	0.050
HbA1c	–0.48*	9.42	0.020	–0.50*	10.46	0.031

[a]The beta coefficients were adjusted for the confounding effects of Age, Sex, and Duration of Body Mass Index.
*Significant at 0.05

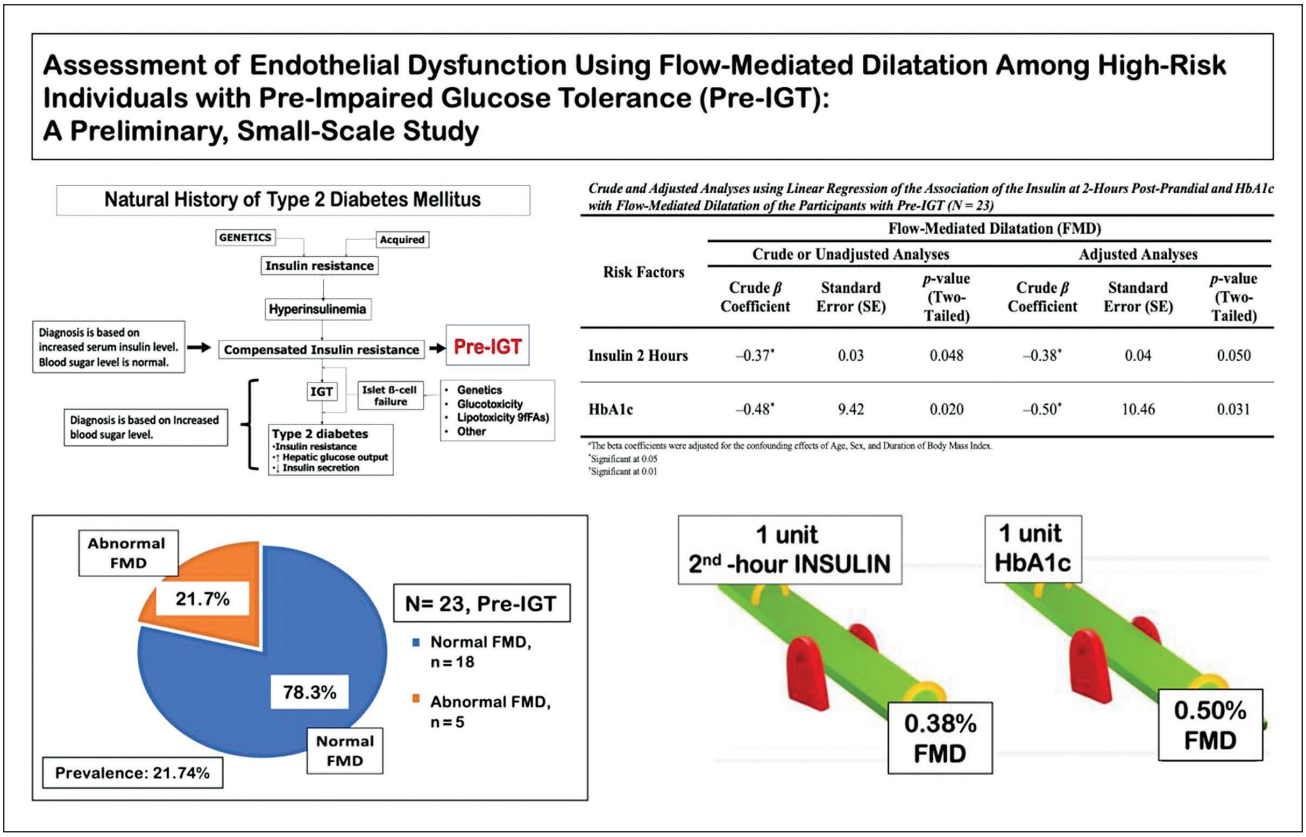

Figure 1. Graphical Abstract.

or screening patients with a hyperinsulinemic pattern, followed by a value >45 uU/mL (sensitivity = 85.00%).[24] Our group[15-17] has been utilizing the 2-hour plasma insulin level >30 mU/mL to identify high-risk individuals with pre-IGT.

Pre-IGT, hyperinsulinemia, and endothelial dysfunction

Pre-IGT as a potential model for CVD outcomes of hyperinsulinemic patients

The influence of various cardiometabolic factors (IGT, T2D, obesity, dyslipidemia and smoking) in ED development makes it challenging to quantify hyperinsulinemia's isolated contribution in assessing CVD development among high-risk individuals with IR. Although the development of vascular functional abnormalities like ED has been extensively elaborated in the prediabetes stage, the abnormal circulating BG levels consistently remained the significant contributory factor of contention in addition to other CVD risk factors like hypertension, dyslipidemia and smoking.[10-13] An example is in the report by Sciacqua et al., where the 1-hour BG post-OGTT of at least 155 mg/dL among the hypertensive participants was the major determinant of all indices of vascular stiffness.[29]

In our present study, we noted that hyperinsulinemia may also be implicated in the development of endothelial dysfunction, and this notion is aligned with the assertions of previous literature in the early identification of populations at risk of DM for prompt diagnosis and clinical management.[30-32] Cabrera de Leon et al., demonstrated in the general population, who were followed up for 3.5 years, an increased risk of myocardial infarction and coronary artery disease by 2.8 times and 2.4 times, respectively, with increased C-peptide levels.[33] Notably, Layton and colleagues have demonstrated that in over 17,000 individuals followed up for 15 to 25 years, those with a genetic predisposition to develop T2D already showed significant differences in the clinical and biochemical markers at an early asymptomatic stage versus those with no genetic risk.[34] Corollary to these reports, albeit statistically insignificant, we noted that patients with asymptomatic ED+ had higher age, BMI and elevated biochemical profiles such as fasting and 2-hour BG, HbA1c, total cholesterol and LDL cholesterol values.

Albeit a preliminary and small-scale study using FMD as a measure of ED and after controlling for significant confounders (age, sex and duration of being overweight or obese), we noted a negative, moderate association between 2-hour serum insulin and HbA1c. This is the first study to report the potential association of ED with hyperinsulinemia in the absence of other major CVD risk factors, and these findings are consistent with the idea that early identification of high-risk individuals with IR may be beneficial for clinical management. This concept has also been advanced in several other studies.[30-32]

Recently, the assessment of endothelial function has become a valuable technique in the study of atherosclerosis.[35] Several studies have shown that the endothelium-dependent

vasomotor function in the brachial and coronary arteries predicts long-term cardiovascular risk. These studies, however, only included a subset of high-risk individuals and the predictive significance in low-risk population is not well documented.[36] Thus, it is imperative to compare ED in the normal population and patients with pre-IGT in the future.

Limitations and recommendations

Albeit the presented findings, this study has certain limitations. The small-scale nature of the study warrants a more significant number of participants for more conclusive, precise results and better generalization. Although our study identified the prevalence of ED and its associations with insulin and HbA1c among high-risk adult patients with no other CVD risk factors, the reported statistics may be overestimated even after sufficient statistical control due to the small sample size. Moreover, the biological plausibility of the presented associations may need to be clarified due to the acquired sample size, further supporting the need for large-scale studies. Certain clinical parameters, such as the 1-hour insulin level post-OGTT, were not measured.

Nonetheless, our findings have shown that ED can potentially occur in the hyperinsulinemic, normoglycemic stage of T2D and thus may support the pre-IGT model in CVD outcome development. This preliminary knowledge provides impetus not only for further research endeavors but also for clinicians to intensify the identification of this subpopulation through sufficient history taking and assessment. Since the implicated contributory factors for such occurrence include CVD risk factors and their duration, thus leading to IR, it is imperative to accentuate the extraction of this information during history taking and assessment. To add to this, the current findings may serve as a stepping stone for future endeavors, especially those with consistent findings, in paving the way for a call to action for robust early diagnosis and management of IR, especially among the members of the population who are not overtly at risk or have no or have unknown CVD risk factors.

CONCLUSION

Pre-IGT is a prediabetes stage characterized by compensatory hyperinsulinemia due to insulin resistance with still normal or absent major cardiometabolic risk factors like hyperglycemia, hypertension and dyslipidemia. In our study, ED was observed in 21.74% of patients. In the absence of other CVD risk factors and at the early stage of T2D, hyperinsulinemia and high HbA1c appear to be associated with endothelial dysfunction.

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

CRediT Author Statement
JAS: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration, Funding acquisition; **ALM:** Conceptualization, Methodology, Validation, Investigation, Resources, Data Curation, Writing – review and editing, Project administration; **RJGS:** Conceptualization, Methodology, Validation, Resources, Data Curation, Writing – review and editing, Project administration; **FP:** Conceptualization, Methodology, Validation, Resources, Data Curation, Writing – review and editing, Project administration; **JRM:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Project administration; **LMA:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Data Curation, Writing – original draft preparation, Writing – review and editing, Visualization, Supervision, Project administration, Funding acquisition

Author Disclosure
The authors declared no conflict of interest.

Data Availability Statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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