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Assessment of Left Ventricular Function in Prediabetes Using Two-Dimensional Speckle Tracking Echocardiography

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Abstract

Background: Diabetes mellitus is one of the most important cardiovascular risk factors. The onset of type 2 diabetes mellitus is gradual, with nearly 70% of the patients progressing through prediabetic state which can persist for years. Multiple studies have shown prediabetes to be associated with increased risk of heart failure. Due to prolonged exposure to high glucose levels it has been supposed that prediabetes represents a state of continuous subclinical myocardial damage, which lead to left ventricular (LV) dysfunction and ultimately to heart failure. Speckle tracking echocardiography measured global longitudinal strain can detect subclinical LV dysfunction at an early stage.

Aim of Study: Is to investigate the presence of subclinical LV dysfunction with comprehensive echocardiography including STE among asymptomatic prediabetic individuals and to study possible association between glycemic status and different indices of LV systolic and diastolic function.

Patients and Method: 90 subjects divided into 45 normotensive prediabetic subjects without known cardiovascular diseases and 45 age and sex matched normoglycemic, healthy subjectswere assessed by conventional echocardiography, TDE and 2-DSTE to measure global longitudinal strain of LV.

Results: In prediabetic subjects, prolonged DT (p<0.007), higher E/e' ratio (p<0.006), higher lateral a' peak velocity (p<0.014), lower medial and lateral e'wave velocity (p<0.001, p<0.007), lower lateral s' wave peak velocity (p<0.0001) and medial e'/a' ratio (p<0.024),lower EF% (p<0.02); however still in the normal range, and lower GLS (p<0.0001) compared with controls. Among pre-DM group, several echocardiographic measurements showed significant modest correlations with one or more of the 3 glycemic indices. There was significant correlation between all glycemic indices and GLS, with fasting plasma glucose (r=–0.42, p<0.0001), 2-h plasma glucose (r=–0.45, p<0.0001) and HbA1c (r=–0.57, p<0.0001), and E/e' ratio with fasting plasma glucose (r=–0.285, p<0.007), 2-h plasma glucose (r=–0.344, p<0.001) and HbA1c (r=–0.209, p<0.048).

Conclusion: Prediabetes is associated with subclinical LV systolic and diastolic dysfunction with an association that exists between the severity of glycemia dysregulation and the extent of LV dysfunction. STE can detect early subclinical systolic longitudinal deformation abnormality before overt-impairment of indices of LV systolic function.

Key Words: *Prediabetes – LV systolic and diastolic function – Speckle tracking echocardiography.*

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Introduction

DIABETES mellitus isone of the most important risk factors for cardiovascular morbidity and heart failure (HF) development **[1]**. Prediabetes is considered as an intermediate metabolic state in the continuum of normoglycemia to diabetes with the estimation that it affects more than 7.7% of adults worldwide **[2,3]**. Prediabetes can persist for years but nearly 70% of affected individuals will develop type 2 diabetes at the end **[4]**. Multiple studies have shown prediabetes to be associated with increased risk of heart failure **[5,6]**. Due to prolonged exposure to high glucose levels it has been supposed that prediabetes represents a state of continuous subclinical myocardial damage, which lead to left ventricular (LV) dysfunction and ultimately to heart failure **[7,8]**. Understanding the subclinical changes in LV function that occurred in prediabetes is of great benefit for the early prevention of heart failure and improved survival.

At present, LV myocardial deformation, has emerged as a potential marker to detect subclinical changes in LV function in various cardiovascular diseases. Speckle tracking echocardiography (STE) is the most commonly used modality for evaluating LV myocardial deformation with the advantage of angle and load independency **[9]**. The aim of this work is to investigate the presence of subclinical LV dysfunction with comprehensive echocardiography including STE among asymptomatic prediabetic individuals and to study possible association between glycemic status and different indices of LV systolic and diastolic function.

Patients and Methods

Study population:

This was a cross-sectional study conducted in Kasr Al-Aini Hospital from September 2020 till March 2022. The overall study population consisted of 90 asymptomatic individuals who visited the out-patient clinic by self-referral or by recommendation for medical check-up. Exclusion criteria were: Age over 50 years or under 18 years; diabetes mellitus; current or past history of smoking; arterial hypertension; valvular heart disease beyond mild severity; history of coronary artery disease; atrial fibrillation; cardiac pacing; body mass index (BMI) $\geq 40 kg/m^2$, chronic obstructive pulmonary disease; or chronic kidney disease with estimated glomerular filtration rate <90ml/min [10]. Written consent was taken from all participants. Approval of the Local Ethics Board was received for the study protocol.

Participating individuals were divided into two groups: Prediabetes group (Pre-DM); this included 45 normotensive prediabetics without known cardiovascular diseases and control group that included 45 age and sex matched normoglycemic healthy individual. Diagnosis of prediabetes was made if any of the following criteria was met: (1) Impaired fasting glucose (IFG) defined as fasting plasma glucose (FPG) level of 100 to 125mg/dL (2) Impaired glucose tolerance (IGT) defined as two-hour glucose levels of 140 to 199mg per dL on the 75-g oral glucose tolerance test (3) HbA1c levels between 5.7 to 6.4% [11]. All the participating individuals had clinical evaluation at the initial visit and laboratory and echocardiography assessment at a second visit.

Echocardiographic examination:

All echocardiographic examinations were performed byone experienced cardiologist (El Deeb. H) who performed the study blinded to glycemic status. Two-dimensional grey scale harmonic high quality electrocardiographic gated images were obtained using Philips Affinity 50Cmachine equipped with 2.5 MHz transducer and measurements were obtained according to the recommendations of American Society of Echocardiography [12].

Each participant underwent echocardiographic examination including two-dimensional, M-mode, and subsequent Tissue Doppler echocardiography (TDE). The left atrial antro-posterior diameter, thickness of the interventricular septum and posterior LV wall, left ventricular end-diastolic diameter (LVEDD) and left ventricular end-systolic diameter (LVESD) were obtained from the M-mode echocardiographic tracing under the guidance of 2D imaging. LV ejection fraction (LVEF) was calculated by M- mode. The pulsed Doppler sample volume was positioned at the mitral leaflet tips and the following measurements were obtained: early diastolic peak flow velocity (E), late diastolic peak flow velocity (A), E/A ratio; E-wave deceleration time (DT), and is ovolumetric relaxation time (IVRT). Pulsed wave tissue Doppler imaging was used to obtain LV myocardial velocities in the apical four chamber view with a 5mm sample volume on the medial and lateral corner of the mitral annulus. Filters were set to minimize high-frequency signals, and Nyquist limit was adjusted to a velocity range of –15 to 20cm/s. The myocardial velocities in the septal and lateral mitral annuli in systole (s'), early diastole (e'), late diastole (a'), and e'/a' ratio were obtained. The mean of the septal and lateral e' waves were used to calculate the averaged E/e'ratio. All parameters were taken in three consecutive cardiac cycles at end expiration breath and averaged.

For the measurement of LV longitudinal strain, image loops from the apical four, two and three-chamber views were selected from the stored dataset and the automated cardiac motion Quantification (aCMQ) software was applied. The operator visually assessed the accuracy of the software in tracking the LV motion and made any small manual adjustment necessary to correct any machine misinterpretation [13]. All images were stored in cine-loop format, and data were transferred to workstation for further offline analysis using (QLAB 10). The myocardium in the three standard apical views was automatically divided into 6 segments (basal, mid and apical segments of the opposing walls in each view) and the analyzed values for all resulting 16 segments were shown in specific traces. Global longitudinal strain (GLS) was calculated as the average of all 3 views.

Statistical analysis:

Statistical analysis was performed using the SPSS 22nd edition. Data are expressed as mean ± standard deviation for continuous variables, and as number of observations and percentage for categorical variables. Significance of differences in terms of features obtained by measurements from control and patient groups were analyzed with Student's *t*-test or Mann-Whitney U-test. Pearson's Chi-square test was used for comparison of categorical variables. The association between glycemic status and echocardiographic indices was assessed using a Spearman correlation matrix. A *p*-value less than 0.05 was considered statistically significant.

Results

Among all baseline demographic, clinical and laboratory data, individuals in the pre-DM group showed significantly higher levels of all glycemic indices (p<0.0001) as well as increased triglyceride and lower HDL-cholesterol levels compared to control group (Table 1).

There were significant differences in several echocardiographic indices of diastolic function in-between the 2 study groups (Tables 2,3). Individuals in the Pre- DM group showed prolonged E-DT (p<0.007), elevated E/e' ratio (p<0.006), lower medial and lateral e'wave peak velocities (p<0.0001, p<0.007 respectively), higher lateral a' wave peak velocity (p<0.014) and lower medial e'/a' ratio (p<0.024).

As regard the LV systolic function, compared to control group individuals in the pre-DM group showed significantly lower GLS and lower medial and lateral s' wave peak velocities that reached statistical significance only for the lateral annular velocity. Although the lower LVEF in the pre-DM group compared to the control group, it is noteworthy that all participants in the study had LVEF above 55%.

Among pre-DM group, several echocardiographic measurements showed significant modest correlations with one or more of the 3 glycemic indices (Table 4). Importantly, there was significant correlation between all glycemic indices and GLS, lateral e' velocity, and E/e' ratio.

Table (1): Demographics, clinical and laboratory data.

Variable	Pre-DM group (n=45)	Control group (n=45)	p< value
Age, year	38.5±8.5	39.4±10.4	0.79
Male	18 (40%)	21 (46.7)	0.50
BMI, kg/m^2	32.3±4.5	32.3±2.9	0.98
Systolic blood pressure, mmHg	110.6±23.3	110.2±23	0.89
Diastolic blood pressure, mmHg	73.8±7.9	73.3±6	0.62
Urea, mg/dl	25±7.4	25.6±6	0.53
Creatinine, mg/dl	0.7±0.2	0.8±0.1	0.18
eGFR, ml/min	144.2±36.1	153.6±31.4	0.10
Total cholesterol, mg/dl	183.6±32.2	176.1±20.7	0.12
Triglycerides,mg/dl	142.3±36.4	125.8±26.8	0.016
LDL-C, mg/dl	119.7±32.7	111.9±15.7	0.05
HDL-C, mg/dl	39.9±6.9	41.5±4.4	0.021
Fasting blood glucose, mg/dl	108±9.6	91.3±5.2	0.0001
2h- postprandial glucose, mg/dl	144.1±13	121.1±11.7	0.0001
HbA1c, %	5.9±0.4	5.3±0.2	0.0001

BMI : Body mass index.
eGFR : Estimated glomerular filtration rate.
LDL-C : Low density lipoprotein-cholesterol.
HDL-C : High density lipoprotein- cholesterol.
HbA1 c : Glycated hemoglobin.

Table (2): Conventional echocardiography measurements.

Variable	Pre-DM group (n=45)	Control group (n=45)	p< value
Left atrial diameter (cm)	3.5±0.4	3.4±0.3	0.15
LV end-diastolic diameter (cm)	4.9±0.5	5.0±0.3	0.98
LV end-systolic diameter (cm)	3.1±0.3	3.1±0.3	0.78
Interventricular septum thickness (cm)	0.82±0.10	0.90±0.10	0.62
Posterior wall thickness (cm)	0.81±0.11	0.82±0.11	0.13
LV Ejection Fraction %	64.1±5.3	67.1±3.9	0.021
Mitral inflow by PW Doppler:			
E/A ratio	1.2±0.2	1.3±0.2	0.15
E-DT (ms)	174.1±88.1	137.8±23.6	0.007
IVRT (ms)	86.8±13	90.4±9.7	0.062

LV : Left ventricle. IVRT: Isovolumetric relaxation time.
PW : Pulsed wave.

Table (3): Tissue Doppler imaging & Speckle tracking echocardiographic measurements.

Variable	Pre-DM group (n=45)	Control group (n=45)	p-value
Septal s'(cm/s)	8±1.3	8.4±1	0.06
Septal e'(cm/s)	10.2±2.3	11.6±3	0.001
Septal a'(cm/s)	8.8±2	8.8±2.2	0.71
Lateral s'(cm/s)	10.3±2.7	12±2.3	0.0001
Lateral e'(cm/s)	14.7±3.4	16.7±3.4	0.007
Lateral a' (cm/s)	10±3	8.4±2	0.014
Septal e'/a'	1.2±0.4	1.5±0.5	0.024
Lateral e'/a'	1.6±0.5	2.1±0.9	0.08
Average E/e'	6.9±1.6	6.1±1	0.006
GLS %	19.6±2.1	22.4±1.3	0.0001

GLS: Global Longitudinal Strain.

Table (4): Correlations between glycemic indices and Echocardiographic measurements.

Variable	Fasting blood glucose, mg/dl	2-hour post-prandial blood glucose, mg/dl	HbA1 C, %
Septal s'(cm/s):			
r=	–0.109	–0.027	–0.204
p-value	0.308	0.802	0.053
Septal e'(cm/s):			
r	–0.304	–0.213	–0.231
p	0.004	0.044	0.029
Septal a'(cm/s):			
r	0.147	0.261	–0.068
p	0.166	0.013	0.525
Lateral s'(cm/s):			
r	–0.289	–0.238	–.0311
p	0.006	0.024	0.003
Lateral e'(cm/s):			
r	–0.389	–0.347	–0.251
p	0.0001	0.001	0.017
Lateral a' (cm/s):			
r	0.0475	0.433	–0.001
p	0.0001	0.0001	0.991
Average E/e':			
r	0.285	0.344	0.209
p	0.007	0.001	0.048
E/A:			
r	–0.299	–0.326	–0.06
p	0.012	0.006	0.623
E-DT (ms):			
r	0.134	0.091	0.338
p	0.207	0.395	0.001
IVRT (ms):			
r	–0.082	–0.12	–0.10
p	0.441	0.258	0.35
GLS %:			
r	–0.425	–0.453	–0.576
p	0.0001	0.0001	0.0001

DT : Deceleration time.
IVRT : Isovolumetric relaxation time.
GLS : Global Longitudinal Strain.

Discussion

The main findings of the current study are: (1) Prediabetes is associated with subclinical LV systolic and diastolic dysfunction and (2) An association exists between the severity of glycemia dysregulation and the extent of LV dysfunction.

LV dysfunction in prediabetic individuals has been shown in several prior studies **[14,15,16]**. However several studies suffered from some limitations that preclude firm conclusions; these limitations include (1) The recruitment of heterogenous diabetic and prediabetic populations **[15,16,17]**, (2) Not all studies studied systolic and diastolic LV function **[17,18,19]**, and (3) The presence of several confounding variables (e.g, older age, hypertension, smoking) that could impact the association between prediabetes status and LV function **[15,16,20]**. Our study avoided such limitations by recruiting only individuals with prediabetes and by having several exclusion criteria to avoid the possible impacts of confounding variables. The current study studied both LV systolic and diastolic functions and it utilized STE to characterize subclinical LV function. STE has the advantage of more objective quantification, being angle independent and being less affected than tissue Doppler imaging by overall heart motion **[21,22]**.

Left ventricular diastolic dysfunction in prediabetes:

Our study showed abnormalities in several indices of diastolic function as estimated by analysis of mitral inflow using conventional pulsed and tissue doppler modalities: Prolonged DT; lower medial and lateral e' velocity, higher lateral a' velocities; lower e'/a' ratio; and elevated E/e' ratio.

Similar findings were reported by other researches. In a study by Mil widsky et al., **[18]**, middle aged adults with impaired fasting glucose were 43% more likely to have LV diastolic dysfunctioncompared with euglycemic individuals after adjusting for other factors. In another study by Capaldo et al., **[19]**, both impaired fasting glucose and impaired glucose tolerance were related to lower ratios of peak velocities of early to late transmitral flow. Akcay el al., **[14]** compared diastolic function using TDI in normotensive prediabetic individuals versus controls. Individuals with prediabetes showed significantly lower septal e' wave and e'/a' ratio and higher E/e' ratio, myocardial is ovolumetric relaxation time, myocardial is ovolumetric contraction time and myocardial performance index. In another study by Zhou et al., **[15]**, global longitudinal peak diastolic strain rate was significantly lower in prediabetic individuals when compared to healthy controls.

Left ventricular systolic dysfunction in prediabetes:

Our study showed evidence of subclinical impairment of LV systolic function among prediabetic individuals. Importantly, LVGLS was significantly reduced among prediabetic individuals compared to healthy control. This was detected despite the fact all participants had normal LVEF.

Lower LVGLS has been shown to be associated with future major adverse cardiovascular events including the development of heart failure. This occurs independent of LVEF, age, gender and

presence of hypertension [23]. Furthermore, reduced LV GLS is associated with increased all-cause mortality [24].

Although measurements of LVEF and LVGLS in all prediabetic individuals were within the normal range, these measurements were significantly reduced when compared to healthy control. It is possible that some of prediabetic individuals in this study were in the transition phase between normal LV function and incipient LV systolic dysfunction. The relatively younger age of prediabetic individual in our study may entail a shorter duration of glycemia dysregulation with consequently less evident impact on LV systolic function.

The impairment of LV systolic dysfunction was previously reported. Akcay el al., [14], reported lower peak velocity of septal s' wave and higher myocardial performance index values in normotensive prediabetic individuals. In another study by Zhou et al., [15], LVGLS measured by magnetic resonance imaging was significantly reduced in individuals with prediabetes and diabetes with no significant difference in global radial strain, global circumferential strain, global radial, circumferential, and longitudinal peak systolic strain rate and LVEF among the studied groups. Similar findings were found by El Hini et al., [25].

Glycemic status and LV dysfunction:

The impairment f LV diastolic and systolic functions among prediabetic individuals may be due to multiple factors:

Increased arterial stiffness with subsequent increased LV after load [26]; increased risk of underlying coronary artery disease [27], microvascular dysfunction [28], increased oxidative stress, impaired myocardial metabolism and intracellular calcium handling may also play a role in the development or progression of HF associated with prediabetes [29].

Hyperglycemia and hyperinsulinemia are expected to have a central role in these pathophysiological mechanisms including: Deposition of reactive advanced glycation end products in the myocardium with subsequent myocardial stiffness and hypertrophy [30]; apoptotic cell death and myocyte necrosis [31], chronic sympathetic nervous system activation, increased oxidative stress [32]; myocardial lipotoxicity [33]; and endothelial dysfunction [34].

Our study found significant correlations between the three glycemic indices (HbA1c, fasting blood glucose and impaired glucose tolerance) and different parameters of LV diastolic and systolic dysfunction among individuals with prediabetes. Similar findings were reported in previous studies [15,16,25] though in many studies the correlation was restricted only to one or two but not all glycemic indices [15,16].

The inverse correlation between HbA1c and LVGLS was reported in previous studies (El Hini et al., [25]. In our study, among all glycemic indices, HbA1c showed the strongest inverse correlation with LVGLS. This may be related to the fact that HbA1c has low variability in glucose measurement compared with fasting and oral glucose tolerance test [35]. Actually, HbA1C was reported to be superior to fasting blood glucose in the prediction for coronary heart disease, stroke, and total mortality [36].

The E/e' ratio is considered as a surrogate for LV filling pressure. The significant positive correlation between the 3 glycemic indices and E/e' ratio among our participants with prediabetes is a reflection of the fact that LV diastolic dysfunction is the classical and the most common detected LV functional abnormality in diabetic patients [37].

Conclusion:

Prediabetes is associated with subclinical LV systolic and diastolic dysfunction with an association that exists between the severity of glycemia dysregulation and the extent of LV dysfunction. Our findings give further support to the concept that prediabetes should be considered as a high-risk state for the development of diabetes as well as a pertinent risk factor for the development of congestive heart failure. Prediabetic individuals with normal LVEF and subclinical evidences of LV dysfunction (e.g., reduced LVGLS; elevated E/e' ratio) may benefit from closer long-term follow-up for the development of LV dysfunction, symptoms of heart failure or arrhythmia.

Limitations:

This study has some limitations including being a single center study; the relatively small number of participants; and the lack of follow-up. In addition, the presence of several exclusion criteria may limit the generalizability of the results of the study. Furthermore, coronary artery disease was excluded based on history and normal electrocardiography and echocardiography and not by performing functional stress tests or coronary angiography. The high body mass index in the participants may act as a confounding variable; however both overweight and obesity are common association.

Conflict of interest:

We declare having no financial or personal relationships with other people or organizations that could inappropriately influence (bias) our work.

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تقييم وظائف البطين الأيسر فى ما قبل السكرى باستخدام الموجات الصوتية التتبعية النقطية ثنائية الأبعاد

الخلفية : يعتبر داء السكرى واحد من أهم عوامل الخطورة التى تؤدى إلى قصور فى الشرايين التاجية وهبوط عضلة القلب. ووجد أن أكثر من ٧٠٪ من المرضى الذين يعانون من ما قبل السكرى يتحولون لمرضى داء السكرى فى النهاية. وقد أظهرت الدراسات أن الأشخاص الذين يعانون من ما قبل السكرى أكثر عرضة لاختلال وظائف البطين الأيسر التى تؤدى إلى هبوط عضلة القلب.

الهدف من الدراسة : وقد هدفت هذه الدراسة إلى تقييم وظائف البطين الأيسر فى الأشخاص الذين يعانون من ما قبل السكرى وذوى ضغط الدم الطبيعى بواسطة الموجات الصوتية التتبعية النقطية ثنائية الأبعاد.

المرضى وطرق البحث : تضمنت هذه الدراسة ٩٠ شخصاً وقد انقسموا إلى مجموعتين المجموعة الأولى تتكون من ٤٥ شخص يعانون من ما قبل السكرى وذوى ضغط الدم الطبيعى ولا يعانون من أى مشا كل فى القلب والمجموعة الأخرى مجموعة التحكم ٤٥ شخصاً من الأصحاء. كل المرضى خضعوا للفحص الإكلينيكى وعمل رسم قلب سطحى وعمل معامل لتحديد مستوى السكر بالدم بطرق مختلفة، تحليل سكر صائم، وتحليل مستوى السكر بالدم بعد الأكل بساعتين، مستوى الهيموجلوبين السكرى، الدهون الثلاثية ووظائف الكلى، عمل موجات صوتية تضمنت موجات صوتية تتبعية نقطية ثنائية الأبعاد.

النتائج : كان متوسط عمر المرضى ٣٤±١٠.٥ ويمثل الجنس الانثوى ٦٠٪ ومؤشر كتلة الجسم ٣٢.٣±٣.٧ كجم ووجد أن المجموعة الأولى تعانى من ارتفاع مستوى الدهون الثلاثية ومستوى مؤشرات السكر فى الدم وانخفاض مستوى الدهون عالية الكثافة HDL. كما وجد أن وظائف البطين الأيسر الانبساطية قد تأثرت حيث طال زمن التباطؤ ($p<0.0$) وارتفعت النسبة E/e' ($p<0.0$) وانخفضت الموجة e' وارتفعت الموجه a' وانخفضت النسبة e'/a'. وقد وجدت الدراسة أيضاً أن وظائف البطين الأيسر الانقباضية قد تأثرت حيث قل مؤشر التوتر الطولى الكلى GLS ($p<0.0$) والموجة s'. وقد أظهرت الدراسة أيضاً وجود علاقة متوسطة ما بين مؤشرات مستوى السكر المختلفة وقياسات الموجات الصوتية المختلفة خاصة مؤشر التوتر الطولى الكلى GLS والنسبة E/e' والموجة e'.

الخلاصة : الأشخاص الذين يعانون من ما قبل السكرى يعانون من تأثر فى وظائف البطين الأيسر الانبساطية والأنقباضية. كما وجودت علاقة متوسط القوة ما بين مؤشرات مستوى السكر المختلفة و قياسات الموجات الصوتية المختلفة خاصة GLS والنسبة E/e' والموجة e'.

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The Association of LPL Gene Variants with Type 2 Diabetes Mellitus and Obesity Susceptibility among Group of Egyptian Patients

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Abstract

Background: Lipid metabolism depends on Lipoprotein lipase enzyme whose variants were reported associated with type 2 diabetes mellitus (T2DM) and obesity. The lipoprotein lipase (LPL) gene was linked to lipoprotein metabolism, while its pathogenic genetic variants were linked to obesity, insulin resistance (IR) and so diabetes.

Aim of Study: To study the association between LPL (rs1801177, rs320) variants and diabetes regarding disease incidence and susceptibility to obesity.

Patients and Methods: This case control study included 280 subjects, T2DM (n=140) compared to healthy control subjects (n=140). Lipoprotein lipase variants (rs1801177, rs320) across the lipoprotein lipase gene were genotyped by real-time PCR using the TaqMan allele discrimination assay.

Results: The studied variant genotypes frequency was significantly different between diabetic and non diabetic subjects, p-value=0.008 & 0.034 for rs1801177, rs320, respectively. A significant association was detected between LPL gene rs1801177 & rs320 variants and BMI, waist circumference and HbA1C, FBG, TG & HDLc. There's a positive correlation between BMI and LPL rs 1801 177 variant (r=0.367, p=0.001), There's a moderate positive correlation between waist circumference and LPL rs1801177 variant (r=0.383, p=0.001), positive correlation with FBG (r=0.342, p=0.001), positive correlation with HbA1c (r=0.405, p<0.001) and a moderate positive correlation between TG level and LPL rs1801177 variant (r=0.464, p=0.025). A weak negative correlation between HDL level and LPL rs320 variant (r=–0.225, p=0.007).

Conclusions: Both variants were significantly associated with parameters related to obesity and T2DM (BMI, waist circumference, FBG, HbA1c, TG & HDLc). Due to the polygenic nature and the high prevalence of obesity & T2DM, further studies with larger study population are needed to assess our results.

Key Words: *Lipoprotein lipase – Obesity – Insulin – Resistance – Type 2 diabetes.*

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Introduction

INCREASED insulin resistance and decreased insulin secretion, the two pathogenic alterations that determine the development of type 2 diabetes mellitus (T2DM), are impacted by a number of variables, including hereditary and environmental ones. Increased adipose tissue storage as a result of dysfunctional lipid metabolism eventually leads to obesity, and consequently increased risk of T2DM development. Lipoprptein lipase (LPL) play a significant role in the transport, storage, and hydrolysis of triglycerides in the form of chylomicrons and low-density lipoprotein plasma **[1]**.

Changes in the LPL normal function could result in an increase serum free fatty acids, metabolic disorders development is limited as long as excess fats can be stored in adipose tissue. Once the adipocyte storage capacities are exceeded, fat accumulates in muscle and liver cells, which could then result in insulin resistance or a loss of insulin sensitivity in tissues, which could speed up the development of type 2 diabetes **[2]**.

The LPL gene is found on the chromosome 8 (8p22) with a 35kb length, that consists of 10 exons. Many variations are found in the coding and non-coding regions of this gene **[3]**.

Number of single-nucleotide variants (snv) in the LPL gene have been described. Some of those variants were related to disturbed lipid concentration. Several gain-of-function mutations in LPL have been associated with lower plasma triglycerides, as well, confirming its role in triglyceride metabolism **[4]**.

Interestingly, several SNVs were identified in the introns and exons of LPL gene and were reported to be associated with triglyceride levels and T2DM in Mongolian, Russian, Brazilian popula-

tions but no association was reported in Chinese population [5].

The purpose of our work is thus to study the association between LPL (rs1801177, rs320) variants and diabetes regarding disease incidence, susceptibility to obesity which will allow us to design a predictive system for the dyslipidemia treatment using gene variation, family history of diseases and anthropometric indicators. To the best of our knowledge, this is the first study in Egypt on those variants regarding diabetes and obesity patients.

Patients and Methods

Study population:

Our study was case control one conducted over 8 months from January 2021 to October 2022. The study including 280 participants; 140 patients with T2DM who were recruited from the Diabetes Outpatient Clinic, Kasr Al-Ainy Hospital and 140 normal healthy age and sex matched subjects as a control group. Patients with T2DM should have either Fasting blood glucose (FBG) levels of ≥126mg/dL and/or postprandial glucose levels of ≥200mg/dL and/or hemoglobin A1c (HbA1c) levels of ≥6.5%, patients with other endocrinological disorders as thyroid disorders were excluded. The study was approved by Ethical committee of the Kasr Al-Ainy, faculty of Medicine (MS-22-2022) and all patients and controls gave a written informed.

Sample size:

G*Power software was used for sample size calculation [6]. Primary outcome measure is the relationship between gene variant and T2DM and obesity. Using two-way ANOVA with two factors (main effects), we will get 2 possible groups (2 levels for factor A * 2 levels for factor B) and an interaction (numerator) degree of freedom of one ([2 - 1] * [2 - 1]). So, it is calculated that a sample size of 140 patients and 140 control (total 280 patients) achieves 80% power to detect a medium effect size (Cohen f) of 0.28 assuming a numerator degree of freedom of 1 and a confidence level of 95% (alpha error of 0.05).

Data collection:

Study participants were subjected to:

- Detailed history, including associated comorbidities and current treatment. Careful clinical examination with anthropometric measurement including: Height, weight and waist circumference measurement.
- Routine laboratory tests including FBG, HbA1c, HDL cholesterol and triglycerides levels.
- DNA Extraction and Genotyping: Three ml peripheral venous blood samples were collected into sterile Ethylene di amine tetra acetic acid (EDTA) vacutainer tubes. According to the manufacturer's recommendations, genomic DNA was isolated from the whole blood using a QIAamp DNA blood mini kit (Qiagen, Germany) and stored at 20°C. Genotyping of the (rs1801177) and (rs320) of LPL gene was done across all participants' sample sets using the TaqMan Allelic Discrimination Assay Kit (probe ID C___8804865_30 and C___1843003_10) respectively, Applied Biosystems, Foster City, CA, USA); and these SNPs were analyzed on StepOne Real-Time Polymerase Chain Reaction System (Applied biosystems CA94404, Foster City, USA).

Statistical methods:

The statistical package for the social sciences (SPSS) (IBM Corp., Armonk, NY, USA) used for quantitative values, standard deviation, mean, and median. Frequencies and percentages used for categorical variables. For comparisons of normally distributed quantitative variables unpaired t test or ANOVA analysis were used, for the multiple comparisons post hoc test was used. When the expected frequency is less than 5, Chi square test was used to compare categorized data [7]. Correlations between quantitative variables were calculated by Pearson correlation coefficient. To identify independent predictors of BPA, linear regression analysis was used. Results with p-values less than 0.05 were defined as Statistical significance.

Results

This study consisted of 280 participants; divided into two groups as the following: 140 diabetic patients, and 140 healthy control subjects.

There's a significant statistical difference between diabetic patients and control subjects regarding BMI, waist circumference, FBG, TG (p-value <0.001).

There's a significant difference between diabetics and control groups regarding both genes variants. LPL rs 1801177 genotypes AA, AG, GG distribution was 84.3%, 9.3%, 6.4% in diabetic patients versus 71.4%, 22.9%, 5.7 in the control group, p-value=0.008. Similarly LPL gene rs 320 AA, AT and TT distribution was 47.1 %, 43.6%

and 9.3% versus 62.1%, 32.9% and 5% in the diabetic and control group respectively, p-value= 0.034.

In diabetic patients, rs 1801177 mutant variant was significantly associated with BMI and waist circumference, HbA1c, FBG, TG, p-value <0.001. There's was also a significant association between gene rs 1801177 variant and HDLc, p-value=0.01. Furthermore, in the control subjects rs1801177 mutant variant was associated with BMI, p-value =0.031 & waist circumference, p-value=0.007 and TG, p-value=0.017.

Regarding LPL rs320 variant in diabetic patients, the mutant variant was significantly associated with BMI (p-value=0.029), waist circumference (p-value=0.037), HbA1C (p-value=0.002), FBG (p-value=0.001), TG (p-value=0.01) and HDLc (p-value=0.02). In Addition, control subjects with the mutant variant of LPL rs 320 had significantly higher FBG (p-value=0.045) and significantly lower HDLc (p-value=0.004).

There's a positive correlation between BMI and LPL rs 1801177 variant (r=0.367, p=0.001), There's a moderate positive correlation between waist circumference and LPL rs 1801177 variant (r=0.383, p=0.001), positive correlation with FBG (r=0.342, p=0.001), positive correlation with HbA1c (r=0.405, p<0.001) and a moderate positive correlation between TG level and LPL rs 1801177 variant (r=0.464, p=0.025). A weak negative correlation between HDL level and LPL rs320 variant (r=–0.225, p=0.007).

Table (1): Characteristics of clinical and biochemical parameters of studied diabetic and control subjects.

	Diabetic patients (No.=140)	Control (No.=140)	p-value
Sex (No., %):			
Male	63 (45%)	66 (47.1%)	0.550 **a**
Female	77 (55%)	74 (52.9%)	
Age:			
(Mean±SD)	49.17±10.96	48.35±10.98	0.535 **b**
BMI:			
(Median, IQR)	36.69, 8.15	33.57, 8.83	<0.001 **c**
Waist circumference:			
(Median, IQR)	96.75, 11	90, 12.75	<0.001 **c**
HbA1 C:			
(Median, IQR)	7.75, 2.07	4.9, 0.6	<0.001 **c**
FBG:			
(Median, IQR)	169.5, 97.75	103, 22.5	<0.001 **c**
Age of onset of T2DM:			
(Median, IQR)	43, 15	–	
TG:			
(Median, IQR)	205, 93.75	107, 21	<0.001 **c**
HDLc:			
(Median, IQR)	27, 11	27.5, 9	0.238 **c**
Oral antidiabetics (No., %)	140 (100%)		–
Combined oral +Insulin (No., %)	86 (61.4%)	–	–

BMI : Body mass index.
HbA1c : Hemoglobin A1 c.
FBG : Fasting blood glucose.
T2DM : Type 2 diabetes mellitus.
TG : Triglycerides.
HDLc : High density lipoprotein cholesterol.
a: Chi-square test.
b: Independent samples *t*-test.
c: Mann-whitney test.

Table (2): Genotype distributions of LPL rs 1801177, and LPL rs320 gene variant in the study groups expressed.

Gene/SNP	Genotype/ haplotype	Diabetic patients (No.=140)	Control non diabetic (No.=140)	p-value*
LPL rs 1801177	Wild (AA)	118 (84.3%)	100 (71.4%)	0.008
	Hetero (AG)	13 (9.3%)	32 (22.9%)	
	Mutant (GG)	9 (6.4%)	8 (5.7%)	
LPL rs320	Wild (AA)	66 (47.1%)	87 (62.1%)	0.034
	Hetero (AT)	61 (43.6%)	46 (32.9%)	
	Mutant (TT)	13 (9.3%)	7 (5%)	

*: Chi-square test.

Table (3): Relation between LPL Rs1801177 gene variants and demographic and biochemical parameters among the study groups.

Variable	Group	LPL Rs1801177			*p*-value
		Wild	Mutant	Hetero	
Sex:					
Male	G1	57 (48.3%)	4 (44.4%)	9 (69.2%)	0.786 **a**
Female		61 (51.7%)	5 (55.6%)	4 (30.8%)	
	G2	60 (54.1%)	3 (33.3%)	11 (55%)	0.478 **a**
		51 (45.9%)	6 (66.7%)	9 (45%)	
Age:	G1	49.59±11.14	47.55±12.5	46.46±8.16	0.422 **c**
(Mean±SD)	G2	48.08±10.69	53.11±14.93	47.75±10.74	0.406 **c**
BMI:	G1	36.01, 8.06	40, 6.33	39, 11.5	<0.001 **b**
(Median, IQR)	G2	33.9, 8.25	27.2, 9.46	29.39, 7.6	0.031 **b**
Waist circumference:	G1	95.5, 9.526	109, 22.5	105, 6	<0.001 **b**
(Median, IQR)	G2	91, 10	83, 12.5	89.5, 11.5	0.007 **b**
HbA1 C:	G1	7.8, 2.15	8, 1.2	7.3, 1.25	<0.001 **b**
(Median, IQR)	G2	4.9, 0.57	5.1, 0.5	4.8, 0.7	0.214 **b**
FBG:	G1	169.5, 110.25	185.5, 80.5	165, 58.5	<0.001 **b**
(Median, IQR)	G2	105, 16	100, 32.5	100, 17	0.488 **b**
Age of onset of DM: (Median, IQR)	G1	43, 15	45, 10.5	42, 10.25	0.817 **b**
TG:	G1	198, 66	380, 164.5	250, 132	<0.001 **b**
(Median, IQR)	G2	107, 21	100, 2	107, 18.75	0.017 **b**
HDL:	G1	27, 11	14, 8.5	30, 9	0.01 **b**
(Median, IQR)	G2	27, 9	30, 3.75	28, 9.5	0.054 **b**

BMI : Body mass index. T2DM : Type 2 diabetes mellitus. **a**: Chi-square test. G1: Among diabetic group.
HbA1 c : Hemoglobin A1c. TG : Triglycerides. **b**: Kruskal-wallis test. G2: Among control group.
FBG : Fasting blood glucose. HDLc : High density lipoprotein cholesterol. **c**: One way ANOVA test.

Table (4): Relation between LPL Rs320 gene variants and demographic and biochemical parameters among all study groups as expressed.

Variable	Group	LPL Rs320			*p*-value
		Wild	Mutant	Hetero	
Sex:					
Male	G1	31 (47%)	5 (38.5%)	34 (55.7%)	0.366 **a**
Female		35 (53%)	8 (61.5%)	27 (44.3%)	
	G2	47 (54%)	4 (57.1%)	24 (52.2%)	0.797 **a**
		40 (46%)	3 (42.9%)	22 (47.8%)	
Age:	G1	48.5±10	50.6±13.56	49.5±11.5	0.758 **c**
(Mean±SD)	G2	48.93±10.58	43.71±10.78	47.97±11.78	0.541 **c**
BMI:	G1	36.19, 8.32	39, 4.12	35.5, 10.05	0.029 **b**
(Median, IQR)	G2	34.78, 8.6	37.46, 9.5	31.4, 8.2	0.918 **b**
Waist circumference:	G1	97.25, 12	105, 13.5	95, 8	0.037 **b**
(Median, IQR)	G2	90, 13	92, 10.75	89, 5	0.191 **b**
HbA1 C:	G1	7.75, 2.72	11, 6.15	7.4, 1.2	0.002 **b**
(Median, IQR)	G2	4.9, 0.7	4.8, 0.8	4.9, 0.525	0.231 **b**
FBG:	G1	165, 107.5	300, 166	155, 80.5	0.001 **b**
(Median, IQR)	G2	103, 24	103, 36	101, 15.25	0.045 **b**
Age of onset of DM: (Median, IQR)	G1	42, 14.25	45, 16	44, 14.5	0.894 **b**
TG:	G1	233, 111.25	241, 192.5	190, 73	0.010 **b**
(Median, IQR)	G2	107, 19	98, 23	102, 10	0.558 **b**
HDL:	G1	29, 12.25	21, 13	27, 11	0.020 **b**
(Median, IQR)	G2	33, 3	24, 9.25	27, 9	0.004 **b**

BMI : Body mass index. T2DM : Type 2 diabetes mellitus. **a**: Chi-square test. G1: Among diabetic group.
HbA1 c : Hemoglobin A1c. TG : Triglycerides. **b**: Kruskal-wallis test. G2: Among control group.
FBG : Fasting blood glucose. HDLc : High density lipoprotein cholesterol. **c**: One way ANOVA test.

Table (5): Correlation between LPL Rs1801177, and LPL Rs320 gene polymorphism and demographic and biochemical parameters among diabetic group.

Variable	Genotype	*r**	*p*-value
Age	LPL Rs1801177	–0.088	0.3
	LPL Rs320	0.05	0.558
BMI	LPL Rs1801177	0.367	0.001
	LPL Rs320	0.074	0.384
Waist circumference	LPL Rs1801177	0.383	0.0001
	LPL Rs320	0.011	0.896
FBG	LPL Rs1801177	0.342	0.001
	LPL Rs320	0.109	0.199
HbA1C	LPL Rs1801177	0.405	0.001
	LPL Rs320	0.107	0.208
TG	LPL Rs1801177	0.464	0.001
	LPL Rs320	–0.096	0.257
HDLc	LPL Rs1801177	0.007	0.936
	LPL Rs320	–0.225	0.007

BMI : Body mass index. *: Spearman correlation.
HbA1c : Hemoglobin A1 c.
FBG : Fasting blood glucose.
T2DM : Type 2 diabetes mellitus.
TG : Triglycerides.
HDLc : High density lipoprotein cholesterol.

Discussion

Obesity is a highly prevalent health issue with great impact on the society, health facilities, it's associated with increased risk of other comorbidities including insulin resistance and T2DM **[8]**.

Various variants of LPL gene have been linked to changes in TG and HDL-C levels in obese individuals. Previous studies have also demonstrated that people with different BMIs had variable LPL activity in addition to variable insulin level and activity. Collectively, these studies could explain an interaction between LPL genotype and obesity, insulin resistance & T2DM **[9,10]**.

Our study was aiming to investigate whether or not LPL gene (rs 320) and (rs 1801177) variants were associated with association T2DM and obesity susceptibility.

We detected a statistically significant difference between diabetic and non diabetic subjects in LPL rs 1801177 genotypes AA, AG, GG distribution, as the frequency was 84.3%, 9.3%, 6.4% in diabetic patients versus 71.4%, 22.9%, 5.7 in the control group, p=0.008. Similarly LPL gene rs 320 AA, AT and TT distribution was 47.1%, 43.6% and 9.3% versus 62.1%, 32.9% and 5% in the diabetic and control group respectively, p=0.034.

In our study, LPL (rs 1807711) was associated with dyslipidemia with triglyceride-rich lipoprotein metabolism and lipoprotein remodeling including HD, as patients with the mutant variant had statistically significant higher levels of TG, lower levels of HDL than other variants. Interestingly, this difference was also evident in the TG levels in non dyslipidimic control subjects. Similar to our study significant association was found in the study done by Marateb et al. **[11]**.

While in the study of Izar et al. **[12]**, the lipid parameters in diabetic patients were not affected by LPL (rs 1801177) variant.

We found significant association between LPL (rs 1801177) variant with high fasting blood sugar and HbA1C in diabetic patients having the mutant variant (p<0.001).

Lotta et al., **[13]** findings were similar to ours where LPL(rs1801 177) minor allele (MAF=1.9%) in the patients was associated with lower insulin sensitivity, higher fasting glucose, higher levels of liver markers and higher risk of type 2 diabetes (p=0.0086).

Regarding LPL (rs320) variant our study revealed a significant association between the mutant variant and BMI of the patients (p=0.029). As well as waist circumference (p=0.037), high HbA1C level (p=0.002) and high FBG (p<0.001). The mutant variant was also associated with higher TG, Lower HDL in diabetic patients (p=0.01, p= 0.02) respectively. Further more control subjects with the mutant variant had lower HDL levels (p=0.003).

These data are consistent with the results obtained by others such as Bushueva et al., **[14]** in the Russian population, Javorsky´ et al. **[15]** in the Slovak population, and Vardarl et al. **[16]** in the Turkish population. They showed that the mutant genotype of the rs320 locus of the LPL gene is a marker for the development of T2DM and dyslipidemia without possible relationship with gender was identified.

Similar results were also detected by Hubacek et al., and Kahn et al., who reported that LPL (rs320) variant was associated with hypertriglyceridemia and T2DM among Russian and Mexican population **[17,18]**.

On the other hand, Kochetova et al., **[10]** studied LPL (rs320) variant in 930 Russian individuals and they didn't detect an associations with anthropometric parameters. However, similar to our

results the mutant variant is a risk factor for the development of T2DM in individuals with normal body weight.

In contradiction, Durgawale et al., showed no association between LPL (rs320) variant and the occurrence T2DM **[1]**.

Opposite to our findings were He T et al., who suggested that the risk allele for LPL (rs320) variant was the T allele **[19]** and that the G/A (minor alleles) allele exerts a protective role in the populations of Asia, but not Europe, since Europeans have a reverse relationship with the G allele. As we have found.

Similar studies in Saudi and Chinese Han patients failed to report any association with development of T2DM **[20,21]**. Another interesting study among Polish and Russian athletes showed that rs320 SVP has variable phenotypic effects of T2DM depending on the type and intensity of physical activity **[22]**.

Conclusion:

In our study, LPL (rs1801177) and LPL (rs320) variant distribution were significantly different between diabetic and non diabetic subjects. Both variants were significantly associated with parameters related to obesity and T2DM (BMI, waist circumference, FBG, HbA1c, TG & HDLc). Even in the non diabetic subjects, the studied variants were associated with some of these parameters. Due to the polygenic nature and the high prevalence of obesity & T2DM, further studies with larger study population are needed to assess our results.

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ارتباط المتغيرات الجينية لانزيم ليباز البروتين الدهنى مع داء السكرى من النوع الثانى والقابلية لحدوث السمنة بين مجموعة من المرضى المصريين

يعتمد التمثيل الغذائى للدهون على إنزيم ليباز البروتين الدهنى الذى تم الإعلان عن ارتباط متغيراته بداء السكرى من النوع الثانى وقابلية الأفراد للسمنة. تم ربط جين ليباز البروتين الدهنى بعملية التمثيل الغذائى للبروتين الدهنى، فى حين تم ربط المتغيرات الجينية المسببة للأمراض بالسمنة ومقاومة الأنسولين ومرض السكرى.

قمنا بعمل بحث لدراسة الارتباط بين متغيرات إنزيم ليباز البروتين الدهنى (rs 1801177 وrs 320) ومرض السكرى فيما يتعلق بالإصابة بالمرض وقابلية المرضى للإصابة بالسمنة.

فى دراستنا، كان توزيع المتغير إنزيم ليباز البروتين الدهنى (rs 1801177 وrs 320) مختلفاً باخلاف ذو دلالة إحصائية فى وتوزيعه بين الأشخاص المصابين بداء السكرى من النوع الثانى وغير المصابين لداء السكرى.

ارتبط كلا المتغيرين بشكل كبير بالمعايير المتعلقة بالسمنة ومؤشر كتلة الجسم ومحيط الخصر.

حتى فى الأشخاص غير المصابين بالسكرى، ارتبطت المتغيرات المدروسة ببعض هذه المعايير. ولكن نظراً للطبيعة المتعددة للجينات والانتشار الواسع للسمنة وداء السكرى من النوع الثانى، هناك الحاجة إلى مزيد من الدراسات تشمل عدد أكبر من المرضى لتقييم نتائجنا.