Serum and expression profiles of glucose-dependent insulinotropic polypeptide in correlation with cardiometabolic risk factors among patients with systemic lupus erythematosus

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Background
Premature atherosclerosis has been recognized as a major co-morbid condition in systemic lupus erythematosus (SLE). Glucose-dependent insulinotropic polypeptide (GIP) is closely related to cardiovascular (CV) risk factors. We aimed to evaluate GIP expression level in SLE and to explore the possible associations of GIP expression profile with carotid intima-media thickness and insulin resistance (IR).

Patients and methods
A cross-sectional controlled study was conducted, comprising 170 patients with SLE and 120 controls. GIP expression level was measured by multiplex polymerase chain reaction. The carotid intimamedia thickness was measured. Serum GIP levels, homeostasis model assessments (HOMA-IR and HOMA-b), fibrinogen, and homocysteine were measured.

Results
In the patients with SLE with IR, there were significantly higher values of serum GIP (37.99±13.64) compared with patients with SLE without IR (24.61±10.74), as well as the control group (21.7±3.46). In addition, there were significant positive correlations between GIP serum level and cardiovascular risks. Regarding GIP gene expression levels, there were significantly lower levels of GIP gene expression in patients with SLE with IR (1.29±0.72) compared with patients with SLE without IR (2.43±0.61) as well as the control group. Receiver operating characteristic analysis revealed that the diagnostic power of GIP expression was stronger than GIP serum levels in differentiating SLE from control.
In conclusion, in the SLE group, there were lower GIP expression and higher serum levels than control, especially in IR subgroup. GIP expression and serum levels were associated with cardiovascular disease pathogenesis and progression.

Keywords:
cardiovascular, expression, glucose-dependent insulinotropic polypeptide, insulin resistance, systemic lupus erythematosus

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Introduction

Despite tremendous advances in the treatment of systemic lupus erythematosus (SLE), the mortality among patients with SLE is still higher than the general population. Omics studies have indeed demonstrated that the major cause of death in SLE is no longer active lupus but instead cerebrovascular and cardiovascular diseases (CVDs) secondary to accelerated atherosclerosis [1].

Accumulating studies have reported that several factors are associated with high cardiovascular (CV) risk among patients with SLE, for example, microalbuminuria, hyperhomocysteinemia, proinflammatory lipid profiles, and insulin resistance (IR) [2]. Notably, it was revealed that systemic inflammation is the main physiologic link between IR and SLE. Patients with SLE, even with mild disease, show higher levels of inflammatory markers [3].

Glucose-dependent insulinotropic polypeptide (GIP), such as incretin, has glucose-dependent insulinotropic effect by interacting with its receptor and improves β-cell proliferation and survival via its cognate receptor [4]

There are intriguing reports suggesting that farther to the insulinotropic activity of GIP, it can affect the CV system, in light of the fact that, several studies have

implicated the cardioprotective role for glucagon-like peptide-1 [5]. To the best of our knowledge, this study is the first to explore the correlation between GIP expression and serum levels and cardiometabolic risks among patients with SLE.

The pathological link among SLE, IR, and CVD factors is complex and still partially unclear; therefore, further studies are needed to better elucidate the boundaries of this relationship. Thus, we aimed to estimate GIP expression level in SLE and to explore the possible associations of GIP expression profile with cardio-metabolic diseases as well as SLE disease activity.

Patients and methods

This was a cross-sectional controlled study, including 170 patients with SLE and 120 age-matched and sex-matched controls who were consecutively admitted to outpatient clinics of the Rheumatology and Rehabilitation as well as Internal Medicine Departments at the Zagazig University Hospitals. The study protocol was approved by the Ethics Committee of Faculty of Medicine, Zagazig University. All participants signed an informed written consent form before their inclusion and had disease duration of 1 year or greater. We selected the patients with SLE who met the criteria for SLE, according to 2012 Systemic Lupus International Collaborating Clinics classification criteria [6]. Patients were divided into two groups: SLE without IR [homeostasis model assessments of insulin resistance (HOMA-IR) <2.11; $n=100$] and SLE with IR (HOMA-IR >2.11; $n=70$). All participants underwent complete history taking and thorough clinical examination. We excluded patients with a history of myocardial infarction, angina, stroke, drug-induced lupus, and pregnancy. In addition, patients with hepatitis C virus, hepatitis B virus, other connective tissue diseases, as well as the history of propylthiouracil, isoniazid, or hydralazine use were excluded. A history of proteinuria was defined as 500 mg or more per 24 h. Disease activity was measured using the Systemic Lupus Erythematosus Disease Activity Index [7,8].

Blood sampling

Blood samples were drawn from all participants after an overnight fast. Sera were separated after 1 h of standstill and stored at −80°C. We determined fasting plasma glucose (FPG) levels using the glucose oxidase method (Spinreact, Girona, Spain). Total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides levels were measured by routine enzymatic methods (Spinreact). The low-density lipoprotein (LDL) cholesterol level was calculated using the Friedewald formula [9].

Immunochemical assays

Fasting serum insulin (FSI) levels were determined by high-sensitivity linked immunosorbent assay (ELISA) kit provided by Biosource Europe S.A., Nivelles, Belgium. HOMA-IR and β-cell function (HOMA-β) were calculated. Serum homocysteine levels were analyzed by homocysteine kit (Vers. 5.1, Ref: EIA-2925; DRG Instruments GmbH, a division of DRG International Inc., Hilden, Germany) through ELISA. We measured anti-dsDNA by the anti-dsDNA indirect immunofluorescence Kit NOVA Lite dsDNA CrithidiaIuciliae kit (INOVA Diagnostics Inc., San Diego, California, USA). C-reactive protein (CRP) and complement C3 and C4 were measured using immunoturbidimetric assay on Roche/Hitachi cobas system (c501) autoanalyzer (Roche Diagnostics, Mannheim, Germany). Fibrinogen level was assessed by the clotting method of Clauss using Fibri-Prest reagent (Diagnostica Stago S.A.S, Asnières-sur-Seine, France) on fully automated coagulometer, STA Compact [10]. Twenty-four hour urine samples were tested.

GIP serum assay

Serum GIP [Human GIP (total) ELISA kit, no. EZHGIP-54K; Millipore, St. Charles, Missouri, USA] was analyzed according to the manufacturer's instructions.

RNA extraction and cDNA synthesis

Total RNA was extracted from whole-blood samples using the PaxGene Blood RNA Kit (PreAnalytiX/QIAGEN Inc., Venlo, The Netherlands) following the manufacturer's protocol. Spectrophotometry was used to determine RNA concentrations, and RNA samples were stored at −80°C, before starting the cDNA synthesis. Using the iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, California, USA), cDNA was synthesized. Reverse transcription was then performed on 1 lg of RNA sample by adding iScript reagents, including 4 ll 59 iScript reaction mix, 1 ll iScript reverse transcriptase, and sufficient nuclease-free water to a reaction volume of 20 ll. The reaction was incubated at 25°C for 5 min, 42°C for 30 min, and 85°C for 5 min, and then stored at −20°C.

Quantitative PCR was used to analyze the GIP gene

Expression with SYBR Green qPCR reagents (Stratagene, Cedar Creek, Texas, USA) and a Step One Plus PCR System Applied Biosystems (Foster City, California, USA). Each individual reaction used 10 ll of SYBR Green and 6 ll of nuclease-free water. This was then mixed with 1 ll of the forward primer and 1 ll of the reverse primer. Finally, 2 ll of cDNA was added. Results were analyzed using (Step One Software Applied Biosystems). Cycle threshold (Ct) was defined as the cycle in which the fluorescence had a significant increase. The Ct value was then standardized using the Ct value of the same sample 18S gene as an internal control.

Carotid ultrasonography

Carotid intima-media thickness (CIMT) was determined by one examiner for all patients across all six sites, using high-resolution B-mode ultrasound (M-Turbo; SonoSite, Bothell, Washington, USA) [11].

Statistical analysis

Statistical analyses were performed using the SPSS statistical package, version 21 (SPSS Inc., Chicago, Illinois, USA). The one-way analysis of variance was used to determine the differences among the three groups. Pearson's correlation test was done to analyze the association between GIP expression and serum levels with cardiometabolic risks. Multiple stepwise linear regression analysis among SLE to test the main independent variables against serum GIP levels and GIP expression level Receiver operating characteristic analysis was performed to assess the potential accuracy of serum GIP and expression level for the diagnosis of polycystic ovary syndrome, the area under the curve (AUC), and the cutoff values. We considered *P* to be significant at less than 0.05.

Results

Clinical characteristics and laboratory parameters of patients with SLE

This study was conducted on 170 patients with SLE, and their mean age was 32.04±6.67 years, having a sex distribution of 11% males and 89% females. Mean duration of disease was 6.4±2.465 years, and the mean lupus disease activity according to Systemic Lupus Erythematosus Disease Activity Index was 10.65±5.7. The clinical characteristics of the patients revealed fever (20%), malar rash (35%), oral ulcer (30.7%), alopecia (31.4%), pleurisy (30%), pericarditis (30%), arthritis (35%), vasculitis (15.8), myositis (16.5), retinal change (2.2%), seizures (5.9%), and psychosis (3.6%). Laboratory findings included proteinuria (11.6%), pyuria (12.7%), hematuria (11%), anemia (31%), thrombocytopenia (11%), lymphopenia (11.7%), and leucopenia (232.8%). The C3 level was 59.55±37.6 mg/dl and C4 was 21.8±14.7 mg/dl (Table 1).

Clinical characteristics and laboratory parameters of studied groups

In the present study, we classified the studied groups into three groups: a control group (n=120), and two SLE groups (n=170). The groups were matched regarding age and ethnicity. To evaluate IR among SLE group, we classified SLE group into SLE without IR (n=100) and SLE with IR (n=70). The cutoff of IR was 2.1 calculated by HOMA-IR.

Notwithstanding, the pathological link among GIP, SLE, and CV risk factors is complex and still partially unclear. Consequently, to better elucidate the boundaries of this relationship, we investigated the CV risks in all studied groups. In this regard, our study revealed that in SLE groups, patients had statistically significant higher values of systolic blood pressure (SBP) and diastolic blood pressure (DBP) than the control group. Regarding anthropometric measures, BMI, waist circumference, waist/hip ratio values were statistically significantly higher in the SLE group compared with the control group. In addition, the lipid profile in the form of total cholesterol, triglyceride (TG), and LDL of patients with SLE was higher than the control group. Furthermore, the glycemic profile (FPG and FSI) was statistically significantly higher in the SLE group compared with the control group. Even more importantly, the nontraditional CVR risk factors, fibrinogen, uric acid, high-sensitivity CRP, as well as CIMT, were statistically significantly higher in the SLE group compared with the control group ($P<0.001$*), as shown in Table 1.

Interestingly, regarding the effect of IR among SLE groups, our results observed that patients with SLE with IR had statistically significant higher values of SBP and DBP than patients with SLE without IR. Regarding anthropometric measures, BMI, waist circumference, and waist/hip ratio values were statistically significantly higher in patients with SLE with IR compared with patients with SLE without IR. In addition, the serum levels of LDL and TG in patients with SLE were higher than patients with SLE without IR. Furthermore, the glycemic profiles (FPG and FSI, HOMA-IR, and C peptide) were statistically significantly higher in

patients with SLE with IR compared with patients with SLE without IR. Even more importantly, the nontraditional CV risk factors, as well as CIMT, were statistically significantly higher in patients with SLE with IR compared with patients with SLE without IR. In contrast, HOMA-β and HDL were statistically significantly lower in patients with SLE with IR compared with patients with SLE without IR and control group ($P<0.001$*), as shown in Table 2. Nonetheless, there was a nonsignificant difference between studied groups regarding homocysteine levels ($P>0.005$; Table 1).

Comparison of serum GIP (pg/ml) and GIP gene expression level in the studied groups

In the patients with SLE with IR, there were significantly higher values of serum GIP levels (37.99±13.64) compared with patients with SLE without IR (24.61±10.74) as well as the control group (21.7±3.46) ($P<0.001$*; Table 1).

Regarding GIP gene expression levels, there were significantly lower levels of GIP gene expression in patients with SLE with IR (1.29±0.72) compared with patients with SLE without IR (2.43±0.61) as well as the control group (4.59±0.72) ($P<0.001$*; Table 1).

Correlation between serum GIP (pg/ml) and GIP gene expression levels and cardiometabolic risk factors as well as SLEDAI among patients with SLE

In the SLE group (n=170), serum GIP levels were significantly positively correlated with SBP and anthropometric measures, that is, BMI and waist/circumference. Furthermore, serum GIP levels were significantly positively correlated with glycemic parameters as well as TG and LDL. Even more interestingly, serum GIP levels were significantly positively correlated with non-traditional cardiometabolic factors, that is, fibrinogen, uric acid, and high-sensitivity CRP, as well as CIMT. On the contrary, serum GIP levels were negatively correlated with HDL ($P<0.01$*; Table 2).

Regarding relative expression levels of GIP, there was a significantly negative correlation with SBP and anthropometric measures, that is, BMI and waist/circumference. Furthermore, serum GIP levels were significantly positively correlated with glycemic parameters, that is, TG and LDL (traditional CV risks). Interestingly, among nontraditional CV risk factors, only serum uric acid was significantly negatively correlated with expression levels of GIP. On the contrary, the expression levels of GIP were positively correlated with HDL ($P<0.01$*) (Table 2).

Table 1 Clinical, anthropometric, and laboratory characteristics of the studied groups

Variables	Control (n=120)	SLE without IR (n=100)	SLE with IR (n=70)	P_1	P_2	P_3
Age (years)	32.67±6.63	33.04±6.17	31.126±7.55	0.176	0.082	0.524
SBP (mmHg)	120.05±12.1	138.7±8.46	142.2±7.1	<0.001*	<0.001*	<0.001*
DBP (mmHg)	76.6±15.65	98.6±8.65	100.3±9.1	<0.001*	<0.001*	0.405
BMI (kg/m^2)	28.9±1.07	29.07±3.93	36.78±3.76	0.743	<0.001*	<0.001*
Waist circumference (cm)	86.9±1.07	92.02±3.9	109.5±4.06	<0.001*	<0.001*	<0.001*
Waist/hip ratio	0.86±0.01	0.93±0.04	1.2±0.054	<0.001*	<0.001*	<0.001*
CIMT (mm)	0.77±0.009	1.13±0.051	1.44±0.054	<0.001*	<0.001*	<0.001*
TC (mg/dl)	141.1±9.06	185.7±25.5	235.4±24.1	<0.001*	<0.001*	<0.001*
TG (mg/dl)	128.1±9.1	191.3±26.6	238.2±45.7	<0.001*	<0.001*	<0.001*
LDL c (mg/dl)	116.8±9.1	178.6±27.5	222.3±42.2	<0.001*	<0.001*	<0.001*
HDL c (mg/dl)	51.2±4.81	36.3±5.473	28.46±3.3	<0.001*	<0.001*	<0.001*
FPG (mg/dl)	90.2±9.44	101.1±38.5	121.1±58.6	<0.001*	<0.001*	<0.001*
FSI (IU/ml)	8.945±1.07	7.52±1.8	25.58±11.6	<0.001*	<0.001*	<0.001*
HOMA-IR	1.8±0.35	1.84±0.90	7.75±5.763	0.901	<0.001*	<0.001*
HOMA-β	116.2±13.9	79.1±15.83	66.3±15.73	<0.001*	<0.001*	<0.001*
C-peptide	2.2±0.26	2.3±1.583	6.3±2.27	0.392	<0.001*	<0.001*
Fibrinogen (mg/dl)	291.3±13.5	366.1±41.2	404.2±59.5	<0.001*	<0.001*	<0.001*
Homocysteine (mmol/l)	9.23±1.18	9.2±0.74	9.51±0.87	0.915	0.094	0.121
hs-CRP (μg/ml)	2.98±0.35	6.99±3.56	10.5±4.56	<0.001*	<0.001*	<0.001*
Uric acid (mg/dl)	4.45±0.595	6.34±2.13	8.3±2.28	<0.001*	<0.001*	<0.001*
Serum GIP (pg/ml)	21.7±3.46	24.61±10.74	37.99±13.64	<0.001*	<0.001*	<0.001*
Relative GIP gene expression	4.59±0.72	2.43±0.61	1.29±0.72	<0.001*	<0.001*	<0.001*

BMI, body mass index; CIMT, carotid intima-media thickness; DBP, diastolic blood pressure; FPG, fasting plasma glucose; FSI, fasting serum insulin; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessments of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; P_1 control vs SLE without IR; P_2 control vs SLE with IR; P_3 SLE without IR vs SLE with IR; SBP, systolic blood pressure; SLE, systemic lupus erythematosus; TC, total cholesterol; TG, triglyceride. *$P<0.05$, statistically significant.

Multiple stepwise linear regression analysis among SLE to test the main independent variables against serum GIP levels and GIP expression level

In SLE group, linear regression analysis revealed that only FPG, HOMA-β, and C peptide were the main predictors of serum GIP levels among other cardiometabolic risk factors ($P<0.01^*$). However, only FPG, FSI, HOMA-β, and C peptide were the main predictors of serum GIP levels among other cardiometabolic risk factors ($P<0.01^*$) (Table 3).

Logistic regression analysis test for evaluation of the main independent variables associated with cardiovascular risks among SLE

Among patients with SLE, after adjustment of age, sex, clinical and laboratory characteristic of SLE, the only variables associated with IR was GIP expression [95% confidence interval (CI)=0.024 (0.005–0.117)] and serum GIP levels [95% confidence interval=1.221 (1.064–1.402)] ($P<0.01^*$). However, other CV risks were nonsignificantly associated with IR (PË 0.05).

The accuracy of serum levels and expression levels of GIP for discriminating SLE from the control group by ROC analysis

The power of serum GIP levels to diagnose SLE among studied participants was evaluated using receiver operating characteristic analysis. The AUC was 0.926 (95% CI=0.893–0.959), with sensitivity of 98% and specificity of 53.8%, at a cutoff value of 23.42 (Fig. 1).

Regarding relative expression levels of GIP, the AUC to diagnose SLE among studied participants was 0.995 (95% CI=0.989–1.000), with sensitivity of 99.3% and specificity of 98.3%, at a cutoff value of 3.307 (Fig. 2).

Table 2 Correlations between serum glucose-dependent insulinotropic polypeptide (pg/ml) and expression levels with cardio metabolic risk factors of systemic lupus erythematosus systemic lupus erythematosus

Variables	Serum GIP		GIP expression	
	r	*P*	*r*	*P*
SBP (mmHg)	0.181	<0.001*	–0.211	<0.001*
DBP (mmHg)	0.045	0.588	–0.087	0.290
BMI (kg/m^2)	0.402	<0.001*	–0.344	<0.001*
Waist circumference (cm)	0.475	<0.001*	0.529	<0.001*
Waist/hip ratio	0.004	0.971	–0.006	0.954
CIMT (mm)	0.484	<0.001*	–0.586	<0.001*
TC (mg/dl)	0.402	<0.001*	–0.344	<0.001*
TG (mg/dl)	0.315	<0.001*	0.176	<0.001*
LDL-C (mg/dl)	0.319	<0.001*	–0.148	0.071
HDL-C (mg/dl)	–0.295	<0.001*	0.512	<0.001*
FPG (mg/dl)	0.210	<0.001*	–0.183	<0.001*
FSI (lU/ml)	0.606	<0.001*	–0.279	<0.001*
HOMA-IR	0.440	<0.001*	–0.021	0.796
HOMA-β	–0.065	0.432	0.093	0.259
C-peptide	0.719	<0.001*	–0.358	<0.001*
Fibrinogen (mg/dl)	0.329	<0.001*	–0.043	0.602
Homocysteine (mmol/l)	0.023	0.781	–0.086	0.927
hs-CRP (μg/ml)	0.354	<0.001*	–0.013	0.876
Uric acid (mg/dl)	0.364	<0.001*	–0.143	<0.001*

CIMT, carotid intima-media thickness; FSI, fasting serum insulin; DBP, diastolic blood pressure; FPG, fasting plasma glucose; GIP, glucose-dependent insulinotropic polypeptide; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessments of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride. *Statistically significant ($P<0.05$).

Discussion

Despite the growing evidence that the survival rate of patients with SLE has risen significantly in recent decades, the prevalence of mortality among patients with SLE is higher than healthy control as it reaches threefold higher than control [12]. Patients with SLE have significantly higher risks of CVD than the general population especially premature atherosclerosis [13].

GIP had insulinotropic activity in addition to its effects on the CV system. Previous studies have evaluated the cardioprotective role for glucagon-like peptide-1; nevertheless, the actual role of GIP on CVS is still unexplored. Thus, we aimed in the current study to investigate the GIP expression level in SLE and to explore the possible associations of GIP expression profile with cardiometabolic diseases as well as SLE disease activity.

The main finding of the present study is that the frequency of IR with cutoff greater than 2.1 among patients with SLE was 41.2%. Emerging evidence confirmed that inflammation is the main contributors of IR among patients with SLE in addition to obesity [14] and glucocorticoids therapy [15].

Omics studies have indeed demonstrated the role of inflammatory biomarkers in the pathogenesis of IR [2]. Based on these observations, a study conducted by Gazareen *et al.* [16] revealed that patients with SLE had a higher IR than healthy control. Noteworthy, similar to our study, this research study used FSI, HOMA-IR, and HOMA-β for estimation of IR.

In this study, we attempted to pierce out the association between inflammation and immunity with CVD. The current study observed that patients with SLE with IR had statistically significantly higher values of traditional risk factors in the form of blood pressure, obesity indices, hyperglycemia, and dyslipidemia

Table 3 Multiple stepwise linear regression analyses in patients with systemic lupus erythematosus to test the influence of the main independent variables against serum glucose-dependent insulinotropic polypeptide and glucose-dependent insulinotropic polypeptide expression levels (dependent variable)

Model	Unstandardized coefficients		Standardized coefficients	*t*	*P*	95% CI	
	B	SE	*β*			Lower bound	Upper bound
1							
Serum GIP							
(Constant)	5.388	9.004		0.598	0.550	–12.34	23.118
SLEDAI	0.067	0.035	0.064	1.942	0.053	–0.001	0.135
FPG (mg/dl)	0.105	0.021	0.543	5.001	<0.001*	0.064	0.147
FSI (IU/ml)	–0.327	0.222	–0.522	–1.47	0.143	–0.765	0.111
HOMA-IR	0.001	0.003	0.008	0.371	0.711	–0.006	0.008
HOMA-β	0.039	0.010	0.150	3.946	<0.001*	.020	0.058
Uric acid	0.215	0.258	0.057	0.831	0.407	–0.294-	0.723
Homocysteine	0.160	0.163	0.022	0.985	0.326	–0.160	0.481
Fibrinogen	–0.035	0.038	–0.271	0–.90	0.369	–0.110	0.041
C peptide	5.538	0.383	1.309	14.45	<0.001*	4.784	6.293
GIP expression							
(Constant)	2.440	1.473		1.656	0.099	–0.461	5.341
SLEDAI	–0.000	0.006	–0.015	–0.611	0.541	–0.015	0.008
FPG (mg/dl)	0.057	0.003	1.392	16.628	<0.001*	0.051	.064
FSI (IU/ml)	–0.208	0.036	–1.569	–5.722	<0.001*	–0.280	–0.136
HOMA-IR	0.000	0.001	0.007	0.425	0.671	–0.001	0.001
HOMA-β	0.016	0.002	0.298	10.157	<0.001*	0.013	0.020
Uric acid	0.053	0.042	0.067	1.260	0.209	–0.030	0.136
Homocysteine	–0.028	0.027	–0.012	–1.056	0.292	–0.081	0.024
Fibrinogen	–0.012	0.006	–0.437	–1.886	0.060	–0.024	0.001
C-peptide	0.342	0.063	0.381	5.448	<0.001*	0.218	0.465

CI, confidence interval; FPG, fasting plasma glucose; FSI, fasting serum insulin; GIP, glucose-dependent insulinotropic polypeptide; HOMA-IR, homeostasis model assessments of insulin resistance; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index.

profiles compared with patients with SLE without IR. Even more importantly, the non-traditional CV risks, as well as CIMT, were statistically significantly higher in patients with SLE with IR compared to patients with SLE without IR.

Our findings are in concordance with reports explored by Feingold *et al*. They found dyslipidemia especially hypertriglyceridemia in rats injected with inflammatory markers, tumor necrosis factor-α [17]. Likewise, another research reported improvement in lipid profile following immune intervention [18,19]. These studies together with the mentioned evidence support the role of inflammation in the pathogenesis of IR.

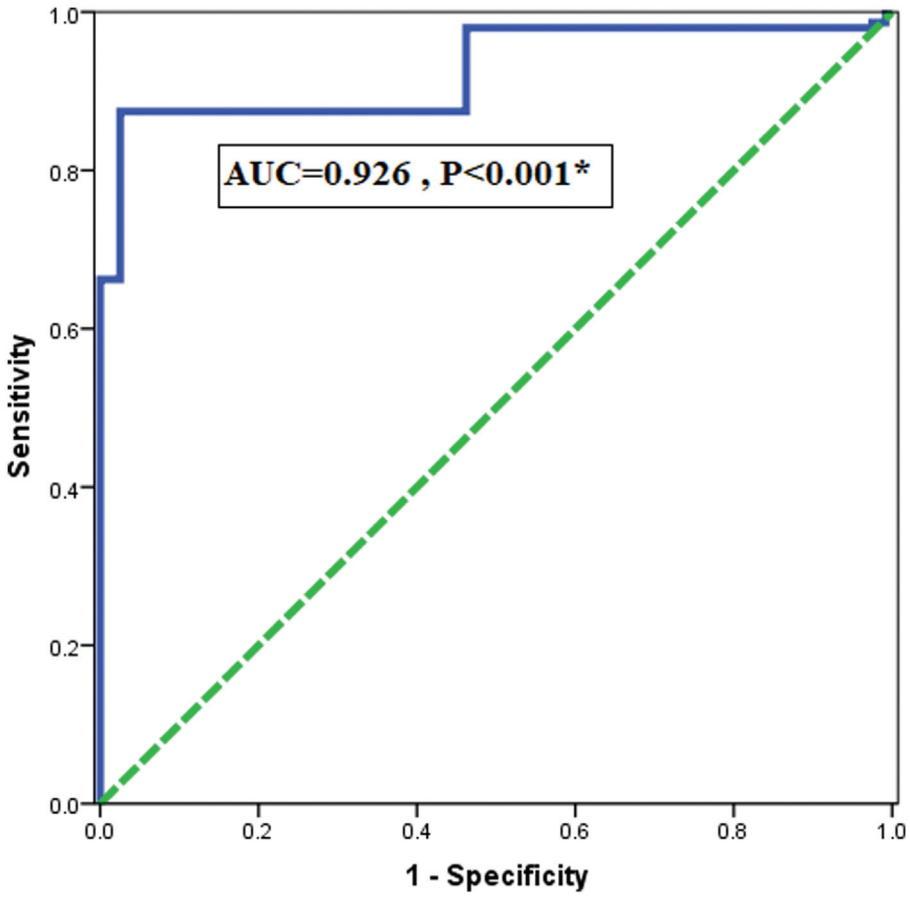
To better elucidate the levels of dyslipidemia among patients with SLE our reports confirmed dyslipidemic profile in SLE compared with control. Similar results were observed by Wierzbicki [20] and Chung *et al*. [21].

On the contrary, a study by Tso and Huang [22] did not find any association between SLE and lipid profile. Similar to this result, a study conducted by Ormseth *et al*. [23] found IR among SLE but they did not detect hyperlipidemia in SLE.

The results presented here are innovative, as this study was the first study that investigated the possible association of GIP expression profile and GIP serum level in SLE. We found higher levels of GIP serum levels in SLE compared with healthy control, especially in IR subgroup of patients with SLE. On the contrary, GIP expression profile was lower in SLE compared with healthy control. Interestingly, the GIP expression level was lower in IR subgroup of SLE.However, the precise molecular mechanisms that regulate insulin secretion and expression remain unclear. Accumulating studies have reported that GIP levels increased in diabetic and IR, and these finding could be owing to GIP resistance, which impairs the glucose regulation and the stimulating effect on adipogenesis [24].

Our results showed that despite the diversity and heterogeneous nature of GIP, both

Figure 1



Receiver operating characteristic curve of serum glucose-dependent insulinotropic polypeptide (pg/ml) level for prediction of systemic lupus erythematosus.

associated with traditional and nontraditional CVD risk factors. Regarding the GIP serum levels, the correlation was positive; however, the correlation between GIP expression and traditional and nontraditional CVD risk factors was negative.

In agreement with our results, in an experimental study conducted by Nagashima *et al.* [25], the plaques in the aorta in rat injected with GIP were smaller than the control group, as GIP could decrease the mRNA expressions of adhesion molecules and suppress the formation of foam cells.

Our results revealed that among other cardiometabolic risk factors, only FPG, HOMA-β, and C-peptide were the main predictors of GIP expression and serum levels by using linear regression analysis to analyze our data.

Surprisingly our results showed that the diagnostic power of GIP expression was stronger than GIP serum levels in differentiating SLE from control.

The interesting results of the study conducted on Chinese populations observed that genetic variations in the GIP gene may be predisposing risk factors for CAD, especially in patients with T2D, through its gene expressions and/or molecular function impairment [26].

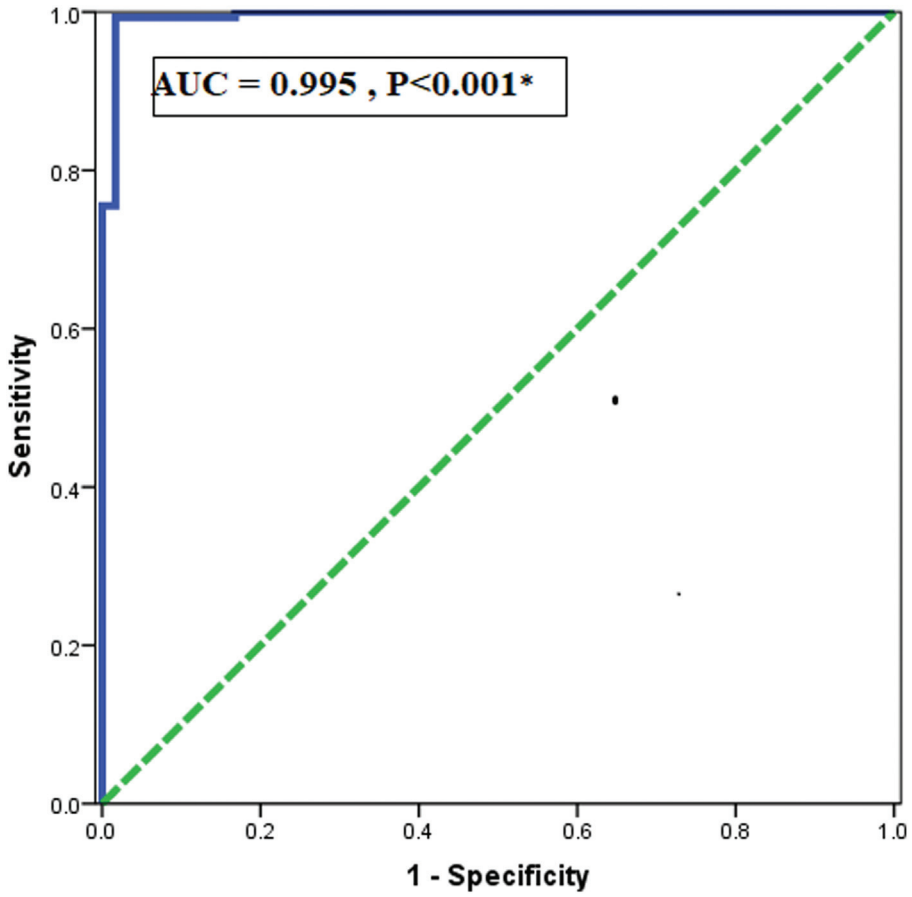
Conclusion

In summary, the results of this intriguing study that identified dysregulated GIP expression and higher serum levels in SLE than control can discriminate IR among patients with SLE. Importantly, these results provide new insights into the dynamic role of GIP expression and serum levels associated with CVD pathogenesis and progression.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

Figure 2



Receiver operating characteristic curve of glucose-dependent insulinotropic polypeptide gene expression for prediction of systemic lupus erythematosus.

References

1 Gladman DD, Urowitz MB. Morbidity in systemic lupus erythematosus. J Rheumatol 1987; 14(Suppl 13):223–226.

2 Kaplan MJ. Premature vascular damage in systemic lupus erythematosus: an imbalance of damage and repair?. Transl Res 2009; 154:61–69.

3 Escarcega RO, Garcia-Carrasco M, Fuentes-Alexandro S, Jara LJ, Rojas-Rodriguez J, Escobar-Linares LE, *et al.* Insulin resistance, chronic inflammatory state and the link with systemic lupus erythematosus-related coronary disease. Autoimmun Rev 2006; 6:48–53.

4 Campbell JE, Drucker DJ. Pharmacology, physiology, and mechanisms of incretin hormone action. Cell Metab 2013; 17:819–837.

5 Ussher JR, Drucker DJ. Cardiovascular biology of the incretin system. Endocr Rev 2012; 33:187–215.

6 Petri M, Orbai AM, Alarcón GS, Gordon C, Merrill JT, Fortin PR, *et al.* Derivation and validation of the systemic lupus international collaborating clinics classification criteria for SLE. Arthritis Rheum 2012; 64:2677–2686.

7 Parameswaran A, Attwood K, Sato R, Seiffert-Sinha K, Sinha AA. Identification of a new disease cluster of pemphigus vulgaris with autoimmune thyroid disease, rheumatoid arthritis, and type I diabetes. Br J Dermatol 2015; 172:729–738.

8 Marinou I, Walters K, Dickson MC, Binks MH, Bax DE, Wilson AG. Evidence of epistasis between interleukin 1 and selenoprotein-S with susceptibility to rheumatoid arthritis. Ann Rheum Dis 2009; 68:1494–1497.

9 Frieldewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without the use of the preparative ultracentrifuge. Clin Chem 1972; 18:499–502.

10 Clauss A. Rapid physiological coagulation method in determination of fibrinogen. Acta Haematol 1957; 17:237–246

11 Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, *et al.* Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. J Am Soc Echocardiogr 2008; 21:93–111.

12 Souza DC, Santo AH, Sato EI. Mortality profile related to systemic lupus erythematosus: a multiple cause-of-death analysis. J Rheumatol 2012; 39:496–503.

13 Chiu CC, Huang CC, Chan WL, Chung CM, Huang PH, Lin SJ, *et al.* Increased risk of ischemic stroke in patients with systemic lupus erythematosus: a nationwide population-based study. Intern Med 2012; 51:17–21.

14 Carr MC, Brunzell JD. Abdominal obesity and dyslipidemia in the metabolic syndrome: the importance of type 2 diabetes and familial combined hyperlipidemia in coronary artery disease risk. J Clin Endocrinol Metab 2004; 89:2601–2607.

15 Simard JF, Mittleman MA. Prevalent rheumatoid arthritis and diabetes among NHANES III participants aged 60 and older. J Rheumatol 2007; 34:469–473.

16 Gazareen S, Fayez D, El-ajjar M, Dawood A, Essa E, El-zorkany K. Study of insulin resistance in patients with systemic lupus erythematosus and rheumatoid arthritis. Menoufia Med J 2014; 27:215–225.

17 Feingold KR, Soued M, Staprans I, Gavin LA, Donahue ME, Huang B, *et al.* Effect of tumor necrosis factor (TNF) on lipid metabolism in the diabetic rat: evidence that inhibition of adipose tissue lipoprotein lipase activity is not required for TNF-induced hyperlipidemia. J Clin Invest 1989; 83:1116–1121.

18 Stagakis I, Bertsias G, Karvounaris S, Kavousanaki M, Dimitra Virla, Amalia Raptopoulou. Anti-tumor necrosis factor therapy improves insulin resistance, beta cell function and insulin signaling in active rheumatoid arthritis patients with high insulin resistance. Arthritis Res Ther 2012; 14: R141.

19 Papa C, Netea MG, Radstake T, Van derMeer JWM, Stalenhoef AFH, van Riel PLC, *et al.* Influence of anti-tumour necrosis factor therapy on

cardiovascular risk factors in patients with active rheumatoid arthritis. Ann Rheum Dis 2005; 64:303–305.

20 Wierzbicki AS. Lipids, cardiovascular disease and atherosclerosis in systemic lupus erythematosus. Lupus 2000; 9:194–201.

21 Chung CP, Long AG, Solus JF, Rho YH, Oeser A, Raggi P, *et al.* Adipocytokines in systemic lupus erythematosus: relationship to inflammation, insulin resistance and coronary atherosclerosis. Lupus 2009; 18:799–806.

22 Tso TK, Huang WN. Elevation of fasting insulin and its association with cardiovascular disease risk in women with systemic lupus erythematosus. Rheumatol Int 2009; 29: 735–742.

23 Ormseth MJ, Swift LL, Fazio S, Linton MF, Raggi P, Solus JF, *et al.* Free fatty acids are associated with metabolic syndrome and insulin resistance, but not inflammation in SLE patients. Lupus 2012; 22:26–33.

24 Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R Creutzfeldt W. Preserved incretin activity of glucagon-like peptide 1 [7–36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. J Clin Investig 1993; 91:301–307.

25 Nagashima M, Watanabe T, Terasaki M, Tomoyasu M, Nohtomi K, Kaneyama JK, *et al.* Native incretins prevent the development of atherosclerotic lesions in apolipoprotein E knockout mice. Diabetologia 2011; 54:2649–2659.

26 Ma X, Huang J, Lu D, Gu N, Lu R, Zhang J, *et al.* Genetic variability of the glucose-dependent insulinotropic peptide gene is involved in the premature coronary artery disease in a chinese population with type 2 diabetes. J Diabetes Res Vol 2018.