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ORIGINAL RESEARCH

Relationship of Plasma Gremlin 1 Levels with Body Adiposity and Glycemic Control in Saudi Female Type 2 Diabetes Patients

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Objective: Gremlin 1 is a novel adipokine that plays an important role in obesity and type 2 diabetes mellitus (T2DM). In the current study, we aimed to evaluate plasma levels of Gremlin 1 in diabetic and non-diabetic Saudi adult females and its correlation with body composition, glycemic control and lipid profile.

Methods: A case–control study was conducted among 41 T2DM and 31 non-diabetic adult age matched females (controls). All patients underwent body composition by bioelectrical impedance analysis, with a commercially available body analyzer. Fasting venous samples were analyzed for glycemic markers and lipids, while plasma Gremlin 1 was measured by ELISA. The results were compared between the two groups and correlated with other anthropometric and adiposity parameters.

Results: Gremlin 1 levels were elevated in T2DM patients (345 ± 26 ng/mL) when compared to control subjects (272 ± 16 ng/mL, $p < 0.05$). Diabetic patients having poor glycemic control had significantly higher Gremlin 1 levels (382 ± 34 ng/mL) compared to patients with good glycemic control (291 ± 37 ng/mL, $p < 0.05$). Pearson correlation analysis revealed a positive correlation of Gremlin 1 with fat mass ($r = 0.246$, $p = 0.012$), HbA1C ($r = 0.262$, $p = 0.008$) and HOMA-IR index ($r = 0.321$, $p = 0.001$).

Conclusion: Our study demonstrates an important role of Gremlin 1 in glycemic control and body adiposity in the pathophysiology of obesity and T2DM. Gremlin 1 may emerge as a promising biomarker and therapeutic target in obesity and T2DM.

Keywords: Gremlin 1, adiposity, diabetes mellitus, HbA1C, HOMA-IR

Introduction

Obesity and diabetes are major drivers of many diseases including, heart failure, ischemic stroke, kidney failure and cancer.[1,2] The global prevalence of obesity and diabetes has increased in every country and a meta-analysis study reported a 12.6% prevalence of diabetes mellitus in Saudi Arabia.[3]

The association between obesity and diabetes is very strong, as it was seen to account for 80% to 85% of Type 2 diabetes mellitus (T2DM).[4] This happens because excess visceral fat accumulation is susceptible to inflammation and cytokine production that lead to dysregulation of adipokines in the body impairing insulin signaling.[4]

Bone morphogenetic proteins (BMP), signaling molecules which belong to a superfamily of TGF-β, are involved in many biological actions including osteogenesis, adipogenesis, metabolism, and insulin signaling. Human and animal studies suggest that both serum and adipose tissue levels of BMP4 are increased in obesity.[5,6] Elevated levels of BMP4 from mature adipocytes provide a feedback signal to recruit new adipocytes. The activity of BMP is regulated by several antagonists such as Gremlin 1, Noggin and others.[5,7] The BMP2/4 pathway leads to differentiating and browning of white adipose tissue and counteracts obesity.[8] In obesity, endogenous BMP antagonists are increased and BMP2/4 antagonist Gremlin 1 renders preadipocytes resistant to BPM4 signaling.[5] It has been recently reported that Gremlin 1 is the antagonist of insulin signaling in human adipocytes, liver and skeletal muscle cells.[9] In T2DM patients, Gremlin 1 levels were increased compared to healthy people and correlated positively with the percentage of body fat and insulin resistance.[9]

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Thus, Gremlin 1 is a novel adipokine which is implicated in the pathogenesis of many diseases and holds the potential of novel therapeutic agent that can be used to treat obesity and T2DM, insulin resistance and related complications.[9] The cause and effect relationship of Gremlin 1 with insulin sensitivity/resistance is not fully understood. More studies are required to understand the role of Gremlin 1 in the pathophysiology of T2DM. Therefore, in the current study, we aimed to evaluate plasma levels of Gremlin 1 in diabetic and non-diabetic Saudi adult females and its correlation with body composition, glycemic control and lipid profile.

Methods

This study was conducted in the Department of Physiology, College of Medicine, King Saud University. Seventy-two women were participated, of which 41 were diabetic and 31 were non-diabetic females of ages ranging between 30 and 65 years old, and having different BMIs. Type 2 diabetic patients who had been diagnosed with the condition at least 12 months before the study commenced, according to the American Diabetes Association Guidelines.[10] Patients with a pregnant and/or pre-diabetic status who had impaired fasting glucose levels were excluded. Also, patients who had any disease that could affect the metabolic status of the body and/or the parameters under investigation were also excluded. For example, patients with conditions such as nephrotic syndrome, acute or chronic renal failure, thyroid disorders, acute infections, stroke, diabetic ketoacidosis and non-ketotic hyperosmolar coma were all excluded.

The detailed history was taken and anthropometric parameters such as BMI, waist-hip-ratio (WHR), pulse and blood pressure were recorded. Body composition was analyzed by bioelectrical impedance analysis (BIA) using a commercially available Body Composition Analyzer (Type BC-418 MA, TANITA Corporation Japan). All body compositions were performed in the early morning, with the subjects all in a state of fasting. They were asked to wear light clothing and to have an empty bladder to ensure uniformity in all subjects.

The subject was asked to first wipe the sole of the feet with a wet tissue and then stand over the electrodes of the machine and the results were recorded within 3-5 minutes. The parameters included were height, body weight, body surface area, BMI, obesity degree, protein mass, muscle mass, fat mass and body fat percentage.

Fasting blood sample (5mL) was taken after a 10–12 hours overnight fast. The plasma was separated, aliquoted, and stored at −80°C. Blood samples were analyzed for fasting blood sugar (FBS), total cholesterol, triglycerides, and high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels (GH Ab190811, IGF-1 Ab100545 and IGFBP-2 Ab100540) following the manufacturer's instructions (Abcam, Cambridge, UK).

Plasma Gremlin 1 levels were measured by Human Gremlin 1 ELISA kit (abx151737) kit following the manufacturer's instructions (Abbexa, Cambridge, United Kingdom). Briefly, the plasma samples (diabetic and non-diabetic control subjects) and standard were reacted with a Gremlin 1 specific antibody coated in the 96 well microplates and incubated at 37°C for 90 minutes on a plate shaker. Next, the samples and standard were removed, and 100 µL detection reagent A was added and incubated for 1 hour as before. Following washing, 100 µL detection reagent B was added and incubated for 30 minutes. The microplate was washed and 90 µL of TMB substrate was added. The reaction was stopped by adding 50 µL of stop solution to each well, and the absorbance was read by a microplate reader (EL 800, BioTek Instruments, USA) at 450 nm.

Data were analyzed using SPSS Pc + 21.0 version statistical software. Descriptive statistics (frequencies, percentages, mean and standard deviation) were used to describe the categorical and quantitative variables. Student's *t*-test was used to analyze parametric variables while Mann–Whitney test was used for the non-parametric variables. Spearman correlations were determined to see the relationship of Gremlin1 with HbA1C and fat mass. A linear regression model was computed with plasma Gremlin 1 as a dependent variable, in a univariate analysis and adjusted for age and BMI to see the association of plasma Gremlin 1 with independent predictors. Values of $p < 0.05$ indicate statistical significance.

Results

Anthropometric and Biochemical Analysis

Demographic and body composition analysis of 72 female participants are shown in Table 1. Anthropometric analysis showed that diabetic patients had a significantly higher fat mass (non-diabetic 31.53 ± 1.48 vs diabetic 35.90 ± 1.49 Kg)

Table 1 Comparison of Demographic and Body Composition Between Non-Diabetic and Diabetic Groups

Parameter	Participants		p value
	Non-Diabetic (n=31)	Diabetic (n=41)	
	Mean±SEM		
Waist	91.83±1.42	100.59±1.71	<0.001
Hip	109.82±1.60	117.54±1.81	0.002
WHP Ratio	0.84±0.01	0.85±0.009	0.372
BMI	32.16±5.77	34.02±5.42	0.097
BMR (KJ)	5747.41±87.55	5937.14±94.86	0.145
BMR (kcal)	1372.95±20.88	1418.81±22.62	0.140
Fat %	40.82±0.95	43.26±0.77	0.049
Fat Mass (Kg)	31.53±1.48	35.90±1.49	0.041
FFM	43.16±0.91	45.11±1.00	0.154
TBW (Kg)	31.88±0.57	33.94±0.62	0.017
VFR	10.15±0.47	11.69±0.42	0.017
Trunk (Fat %)	38.18±0.99	39.61±0.88	0.286
Trunk (Fat mass	16.01±0.66	17.31±0.73	0.195
Trunk (FFM)	24.78±0.35	25.71±0.36	0.067
Trunk (PMM)	23.71±0.33	24.57±0.34	0.076

Note: Values of $p < 0.05$ indicate statistical significance.
Abbreviations: FFM, Fat Free Mass; PMM, Predicted Muscle Mass; Fat%, Body Fat Percentage; TBW, Total Body Water; BMR, Basal Metabolic rate; BMI, Body Mass Index.

Table 2 Comparison of Biochemical Profiles Clinical Parameters Between Non-Diabetic and Diabetic Groups

Parameter	Participants		p value
	Non-Diabetic (n=31)	Diabetic (n=41)	
	Mean±SEM		
FBS (mmol/L)	5.2±0.08	9.19±0.58	<0.001
HbA1C (%)	5.26±0.057	8.35±0.30	<0.001
Cholesterol (mmol/L)	5.03±0.07	4.73±0.14	0.051
Triglyceride (mmol/L)	1.20±0.10	1.45±0.10	0.096
LDL (mmol/L)	3.04±0.09	2.73±0.14	0.058
HDL (mmol/L)	1.37±0.06	1.26±0.09	0.337
Insulin (mIU/L)	12.82±0.69	18.40±1.97	0.003
HOMA-IR	2.77±0.16	7.48±1.05	<0.001

Note: Values of $p < 0.05$ indicate statistical significance.
Abbreviations: FBS, Fasting Blood Sugar; HbA1C, Hemoglobin A1C; HDL, High-density Lipoprotein; LDL, Low-density Lipoprotein; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance.

and visceral fat ratio (control 10.15 ± 0.47 vs diabetic 11.69 ± 0.42, $p < 0.05$). Table 2 shows the biochemical profiles clinical parameters between non-diabetic and diabetic groups. There was a significant increase in FBS (from 5.2 ± 0.08 to 9.19 ± 0.58 mmol/L unit [mean±SEM]), hemoglobin A1C (HbA1C) (from 5.26 ± 0.057 to 8.35 ± 0.30 mmol/L unit), insulin (from 12.82 ± 0.69 to 18.40 ± 1.97, mIU/L) and HOMA-IR index (from 2.77 ± 0.16 to 7.48 ± 1.05), $p < 0.001$ in diabetic patients compared to non-diabetic control subjects. Total cholesterol was significantly lowered, from 5.03 ± 0.07 to 4.73 ± 0.14 in diabetic patients ($p = 0.051$), however there were no significant differences in triglycerides and LDL and HDL levels in diabetic and non-diabetic participants ($p > 0.05$).

Analysis of Plasma Gremlin 1 Levels in Diabetic and Non-Diabetic Subjects

Plasma Gremlin 1 levels were increased in diabetic patients (345 ± 26 ng/mL) as compared to non-diabetic subjects (272 ± 16 ng/mL), [mean±SEM] ($p < 0.05$) Figure 1. Segregation of diabetic patients into having good and poor glycemic control, according to American Diabetes Association criteria (7.5% and above), revealed that patients with poor glycemic control exhibited significantly higher Gremlin 1 levels (382 ± 34 ng/mL) as compared to patients with good glycemic control (291 ± 37 ng/mL, $p < 0.05$), Figure 1. Pearson correlation analysis revealed a positive correlation of Gremlin 1 with fat mass ($r = 0.246$, $p = 0.012$, Figure 2), HbA1C ($r = 0.262$, $p = 0.008$, Figure 3). Anthropometric and biochemical parameters of patients with good and poor glycemic control are described in Tables 3 and 4.

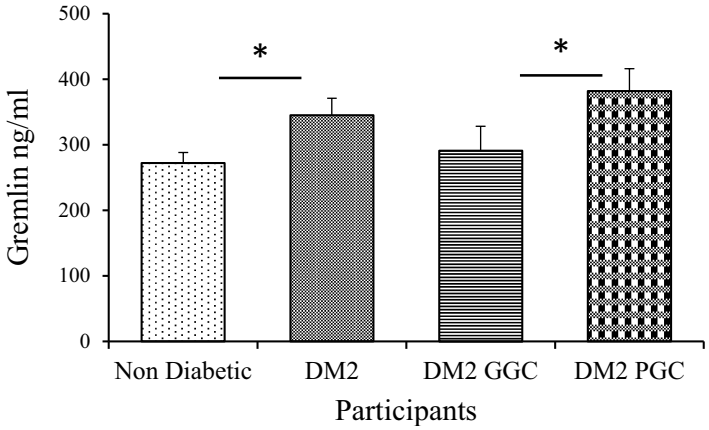

Figure 1 Analysis of Gremlin 1 levels in diabetic and non-diabetic females. Gremlin 1 levels in diabetic and non-diabetic females were analysed by ELISA. The error bars represent standards error of the mean. *Indicates statistical significant differences between non-diabetic (n=31) vs diabetic females (n=41) and between patients with good glycemic control (n=17) and poor glycemic control (n=24). Values of $p < 0.05$ indicate statistical significance.
Abbreviations: DM2, Type 2 diabetes mellitus; GGC, Good glycemic control; PGC, Poor glycemic control.

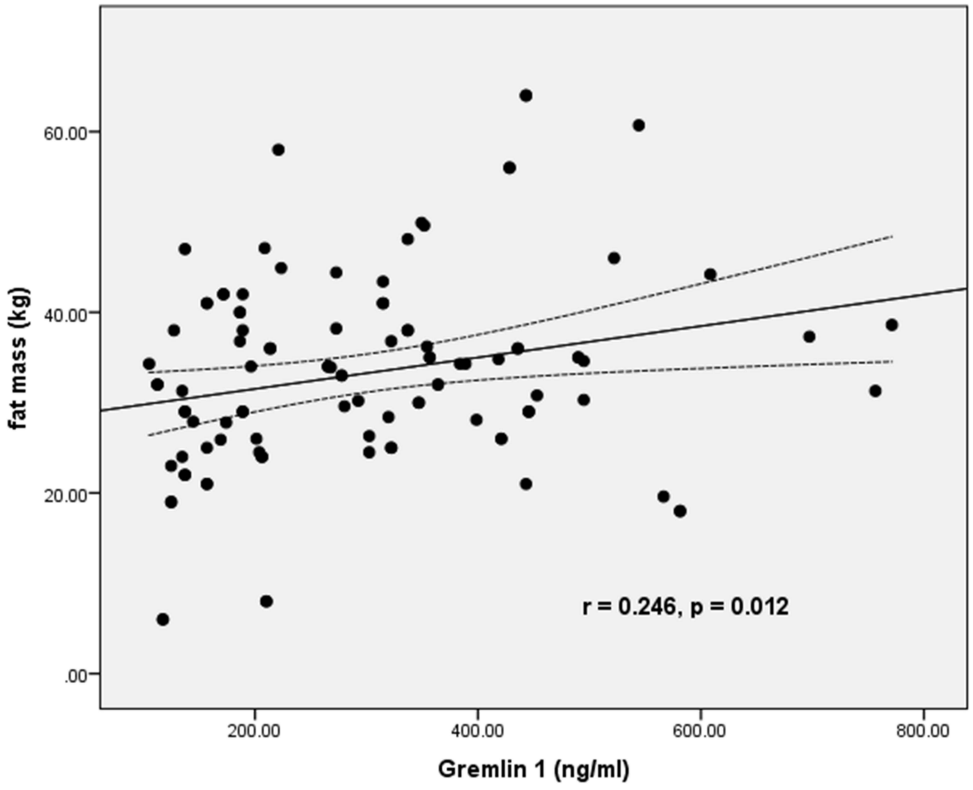

Figure 2 Pearson's correlation of Gremlin 1 with fat mass in all subjects.

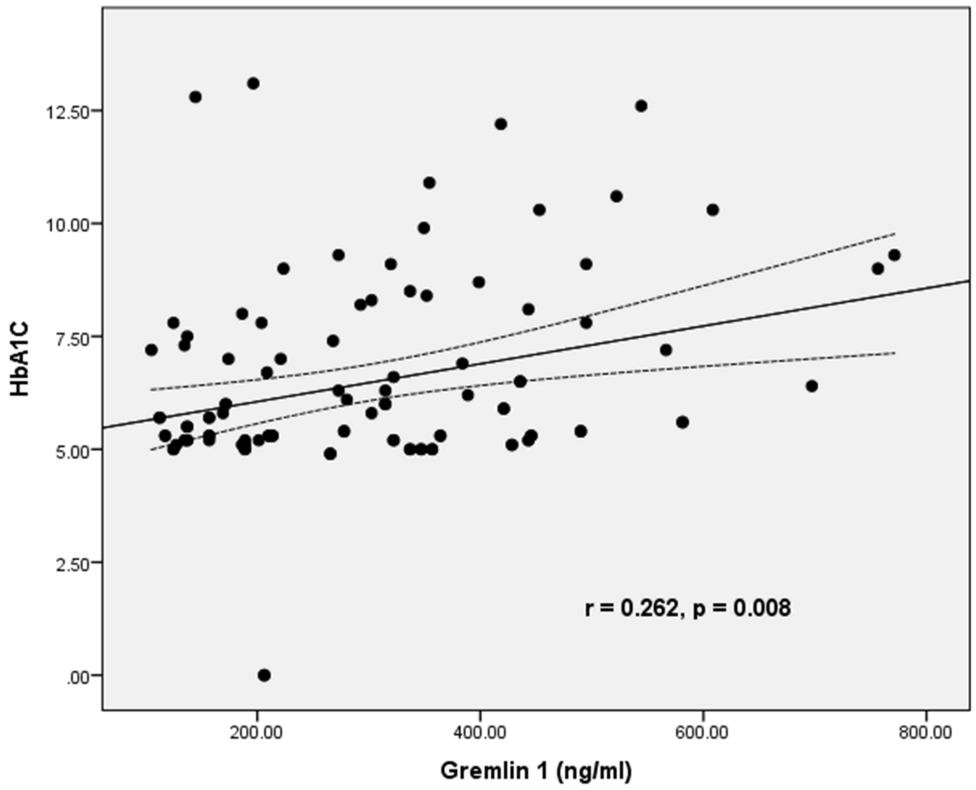

Figure 3 Pearson's correlation of Gremlin 1 with HbA1C in all subjects.
Abbreviation: HbA1C, hemoglobin A1C.

A linear regression model was computed with plasma Gremlin 1 as a dependent variable, in a univariate analysis and adjusted for age and BMI to see the association of plasma Gremlin 1 with independent predictors. Significant predictors were HbA1C and HOMA-IR (Table 5).

Table 3 Anthropometric Measurements of Participants with Good and Poor Glycemic Control

Parameter	Participants		p value
	GGC (n=17)	PGC (n=24)	
	Mean±SEM		
Waist	98.94±12.25	101.72±10.37	0.433
Hip	117.76±13.13	117.40±11.01	0.923
WHP Ratio	0.84±0.05	0.86±0.05	0.160
BMI	34.07±5.25	33.99±5.63	0.965
BMR (KJ)	5852.88±552.93	5994.44±658.34	0.456
BMR (kcal)	1398.88±132.17	1432.36±156.91	0.460
Fat %	43.48±5.08	43.12±5.08	0.824
Fat Mass (Kg)	35.59±9.33	36.11±10.05	0.865
FFM	45.22±3.89	45.04±7.88	0.921
TBW (Kg)	33.07±2.78	34.53±4.69	0.215
VFR	11.88±2.73	11.56±2.83	0.160
Trunk (Fat %)	39.64±6.04	39.58±5.62	0.974
Trunk (Fat mass)	17.17±4.78	17.41±4.89	0.878
Trunk (FFM)	25.49±2.20	25.86±2.46	0.610
Trunk (PMM)	24.34±2.06	24.73±2.33	0.572

Note: Values of $p < 0.05$ indicate statistical significance.
Abbreviations: GGG, Good Glycemic Control; PGC, Poor Glycemic Control.

Table 4 Clinical Parameters of Participants with Good and Poor Glycemic Control

Parameter	Participants		p value
	GGC (n=17)	PGC (n=24)	
	Mean±SEM		
FBS (mmol/L)	6.80±1.06	10.80±4.19	<0.001
HbA1C (%)	6.68±0.55	9.56±1.64	<0.001
Cholesterol (mmol/L)	4.57±0.87	4.83±0.95	0.372
Triglyceride (mmol/L)	1.35±0.65	1.54±0.49	0.286
LDL (mmol/L)	2.57±0.90	2.83±0.80	0.328
HDL (mmol/L)	1.38±0.61	1.27±0.33	0.434
Insulin (mIU/L)	14±6.50	21.40±15.07	0.036
HOMA-IR	4.36±2.33	7.41±3.01	0.001

Note: Values of $p < 0.05$ indicate statistical significance.
Abbreviations: GGC, Good Glycemic Control; PGC, Poor Glycemic Control.

Table 5 Linear Regression Model of Plasma Gremlin 1 with Age and BMI as a Dependent Variable

	Unstandardized Coefficients Beta	Standardized Coefficients Beta	t	Sig.	95% Confidence Limits	
					Lower Bound	Upper Bound
HbA1C	19.624	0.255	2.616	0.010	4.741	34.507
HOMA-IR	13.955	0.256	2.626	0.010	3.411	24.499
Total cholesterol	7.469	0.037	0.376	0.708	−31.991	46.928
Triglycerides	18.943	0.082	0.824	0.412	−26.689	64.576
LDL	4.848	0.023	0.236	0.814	−35.901	45.596
HDL	−38.876	−0.101	−1.010	0.315	−115.266	37.513

Discussion

The present study demonstrates plasma Gremlin 1 levels in Saudi adult females with T2DM and its associations with clinical and anthropometric parameters. Gremlin 1 is a highly conserved soluble extracellular secreted glycoprotein and is involved in limb morphogenesis, organogenesis and bone development.[11–13] Gremlin 1 is also implicated in pathological conditions such as cancer, renal fibrosis, diabetes and obesity.[14]

In our study, the levels of plasma Gremlin 1 were higher in T2DM patients compared to the non-diabetic control subjects. Our findings are in consistent with a previous study by that reported elevated serum levels of Gremlin 1 in obese male and female subjects with T2DM.[9] Our study population consisted of only females and we are not sure of any gender-based effect of Gremlin 1 secretion in pathological conditions. However, an earlier study reported higher Gremlin 1 levels in males compared to females in patients with pulmonary arterial hypertension.[15] Thus, further studies are required to understand the gender-based effect of Gremlin 1 in different disease conditions. It should be noted that we used commercially available ELISA kit to analyse Gremlin 1 levels while Hedjazifar et al used in-house modified ELISA with noncommercially available antibody in their study.[5]

An additional finding of note in our study was the elevated plasma levels of Gremlin 1 in patients with poor glycemic control compared to patients who had good glycemic control. However, there was no significant difference between T2DM patients with good glycemic control and healthy subjects. Gremlin 1 secreted by adipose tissue has an antagonistic effect to insulin signaling and contributes to insulin resistance.[9] Therefore, it is possible that Gremlin 1 levels are elevated with dysglycemia in our study cohort having poor glycemic control. This also suggests that achieving good glycemic control in diabetes would lead to a positive

adipokine profile. Adiponectin is a cardioprotective adipokine which is significantly lower in obesity and T2DM and so having good glycemic control in T2DM is associated with a better adiponectin profile.[16,17]

We also aimed to find correlations between plasma Gremlin 1 and various anthropometric and clinical parameters. Cumulative analysis of Gremlin 1 from diabetic and control subjects showed positive correlations with fat mass, HbA1C and HOMA-IR index. This was an important observation since adiposity and hyperglycemia both lead to increased insulin resistance. Our study shows that an additive effect of Gremlin 1 on glycemic control and insulin resistance exists in both control and diabetic subjects. Similar findings of a positive correlation between Gremlin 1 and other body indices such as HOMA-IR, body fat and HbA1C have been reported in previous studies.[9,18,19] Gremlin 1 is an antagonist of insulin signaling in skeletal muscle, adipocytes and liver cells, thus making it an important therapeutic target in insulin resistance and T2DM as well as other related complications. Diabetic patients with acute coronary syndrome showed increased levels of Gremlin 1 and Macrophage migration inhibitory factor.[20] An earlier study reported increased urinary excretion of Gremlin 1 in type 1 and T2DM patients with diabetic kidney disease.[21] In an animal model, knockout mice lacking Gremlin 1 gene were protected against the experimentally induced model of type 1 diabetes, whereas over-expression of Gremlin 1 in renal tubules in transgenic mice resulted in glomerular and interstitial injury.[22,23] These studies suggest the potential of Gremlin 1 as a promising biomarker to predict the illnesses.

The additive list of adipokines is increasing day by day with diversity in their functions and effects ranging from protective to pathogenic roles for instance proinflammatory to dysmetabolic activity.[24,25] In this regard some of the friendly adipokines are adiponectin and leptin, while others like visfatin and resistin have inverse effects.[26] The novel adipokine Gremlin 1 can be added to this list as well.

To the best of our knowledge, there is scarce clinical data on the role of Gremlin 1 in adipogenesis and insulin resistance syndromes. We report that fat mass and glycaemia correlates positively with Gremlin 1 levels both in T2DM and healthy subjects. Although this is a cross-sectional study with a small sample size, it unravels the need for further work in this area.

Conclusions

Diabetic patients with poor glycemic control exhibited higher plasma levels of Gremlin 1 than patients with good glycemic control and Gremlin 1 was positively correlated with fat mass and glycemic parameters. Our data highlight the potential of Gremlin 1 as a biomarker and therapeutic target in obesity and T2DM.

Ethical Approval

The study protocol followed the Helsinki Declaration and was approved by the Institutional Review Board, College of Medicine, King Saud University (Ref. No. 20/0977/IRB). Informed consent was obtained from the study participants prior to study commencement.

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Disclosure

Khalid A Al-Regaiey, Syed Shahid Habib and Muhammad Iqbal are senior authors. The authors declare no potential conflicts of interest.

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