Differential effect of metformin and/or glyburide on apelin serum levels in patients with type 2 diabetes mellitus: Concepts and clinical practice

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Abstract

Type 2 diabetes mellitus (T2DM) is a metabolic disorder associated with abundant adipocytokine changes which may play an important role in the progression of insulin resistance and micro- and macro-vascular complications. Therefore, the objective of this study was to assess the differential effect of metformin alone or in combination with glyburide on apelin serum levels in patients with T2DM. In this case–control study, fifty patients with T2DM in the age range of 45–65 years and twenty-five healthy controls matched for age and body weight were recruited from single endocrinology center, subdivided according to the diabetic pharmacotherapy into: Group I: healthy controls ($n = 25$), Group II: T2DM patients on metformin ($n = 15$), Group III: T2DM patients on glyburide ($n = 17$), and Group IV: T2DM patients on metformin plus glyburide ($n = 28$). Biochemical and anthropometric variables in relation to apelin serum levels were estimated. Apelin serum levels were low in normal healthy controls compared to T2DM patients ($P < 0.01$). The differential effect of diabetic pharmacotherapy on apelin serum level was statistically significant ($P < 0.01$) compared to the controls, but insignificant when compared among used drugs ($P > 0.05$). Apelin level was high in T2DM compared to the controls; both metformin and glyburide might play a role in this elevation.

Key words: Apelin, glyburide, metformin, type 2 diabetes mellitus

INTRODUCTION

In recent times, type 2 diabetes mellitus (T2DM) has become a serious and common public health problem, since there are about 415 million diabetic patients worldwide in 2015 that will progress to about 642 million diabetic patients by the year 2040.[1] T2DM is a metabolic disorder associated with numerous adipocytokine changes which may play an important role in the progression of insulin resistance and micro- and macro-vascular complications.[2] Most of the adipocytokines are synthesized and secreted by adipocytes such as leptin, omentin-1, and adiponectin[3] in patients with T2DM; there are significant changes in visceral adiposity and gluco-lipid metabolism that *per se* lead to adipocytokine dysregulation.[4]

Apelin is derived from preproapelin 77 amino that cleaved into apelin-36, apelin-17, apelin-13, and apelin-12, which is the active form that binds the apelin receptor. Apelin is found in different tissues such as central nervous system (CNS), heart, lung, mammary gland, and

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gastrointestinal tract. Apelin binds the apelin receptors which are highly expressed in pancreatic islet cells that are involved in the glucose metabolism and stimulation of insulin secretion.[5] Moreover, apelin is an important adipocytokine engaged in the energy metabolism since it improves insulin sensitivity through augmentation of skeletal muscle glucose uptake and provokes cardiac contractility and CNS stimulation.[6] Apelin is mainly produced and secreted from white adipose tissue; hence, apelin serum levels are high in obesity. In addition, apelin synthesis and secretion is stimulated by insulin, so apelin serum levels are elevated in patients with T2DM due to insulin resistance and visceral obesity.[7,8]

The relationship between apelin serum levels and T2DM is still in controversy; human and animal model studies showed apelin at low levels in patients with a newly diagnosed T2DM.[9] Other studies illustrated high apelin levels in obese diabetic patients. On the contrary, a study reported low apelin serum levels in obese diabetic patients.[10]

The differential effect of diabetic pharmacotherapy on apelin serum levels is lacking; some studies exposed the effect of metformin and dipeptidyl peptidase-4 inhibitors on apelin levels in patients with T2DM.[11]

Therefore, the objective of this study was to assess the differential effect of metformin alone or in combination with glyburide on apelin serum levels in patients with T2DM.

MATERIALS AND METHODS

In this case–control study, fifty patients with T2DM in the age range of 45–65 years and twenty-five healthy controls matched for age and body weight (BW) were recruited from a single endocrinology center. All of the enrolled patients and healthy controls gave an informed verbal and written consent for this study and agreement to use their samples in this study. The study was approved by the Scientific Committee Review Board. All measures and procedures were in harmony with the principled standards and Declaration of Helsinki.

Study design
In this study, the diabetic patients were selected according to the grading of American Diabetes Association criteria.[12] Fifty patients with T2DM (31 males + 19 females) and twenty-five healthy controls (13 females + 12 males) matched for age and BW were selected. T2DM patients and healthy controls were subdivided as follows:
- Group I: Healthy controls ($n = 25$)
- Group II: T2DM patients on metformin 500 mg tid ($n = 15$)
- Group III: T2DM patients on glyburide 5 mg twice daily ($n = 17$)

- Group IV: T2DM patients on metformin 500 mg tid plus glyburide 5 mg twice daily ($n = 28$).

Exclusion criteria
Patients with end-stage kidney disease, liver cirrhosis, malignancy, thyroid disorders, psychic and mental disorders, connective tissue diseases, sepsis, shock, and any T2DM not on metformin or glyburide were excluded from the study.

Sample processing
Ten ml of venous blood was drawn after an overnight fasting, 7 mL for glucose, lipid profile and other routine investigations; 3 mL of blood was transferred into specific tubes while containing ethylenediaminetetraacetic acid centrifugated at 3000 rpm for estimation of serum apelin and which fasting insulin by specific enzyme-linked immunosorbent assay (ELISA) kit methods.

Biochemical measurements
Insulin serum level was estimated by ELISA kit method (ACS-180 Mu/L, CIBA CORING DIAGNOSTIC, MEDIFIED, USA), and serum apelin was estimated by ELISA kit method (human apelin EIA, Q9ULZ1, EIA-APC-1, Ray Biotech, Inc., USA).

The Homeostasis Model Assessment-Insulin resistance (HOMA-IR) estimates steady-state β-cell function (β%) and insulin sensitivity (s%) as follows:

$$\text{HOMA-IR} = \frac{\text{GLUCOS} \times \text{INSULIN}^{[13]}}{405}$$

Insulin sensitivity was estimated by Quantitative Insulin Sensitivity Check Index (QUICKI equation) as follows:

$$\text{QUICKI} = \frac{1}{\log(\text{fasting insulin } \frac{1}{4}\text{U / mL} + \log(\text{fasting glucose mg / dL})}^{[14]}$$

Fasting glucose in mg/dL was measured by glucometer, while glycated hemoglobin (HbA1c) was measured by ELISA Kit, MB845, BIOVISION, China.

Lipid profile was measured according to the Friedewald formula as follows:[15]

Atherogenic index = log (triglyceride [TG]/high-density lipoprotein [HDL]); low risk <0.11, intermediate risk 0.11–0.21, and high risk >0.021.

Atherogenic coefficient = Total cholesterol − HDL/HDL.

Cardiac risk ratio = Total cholesterol/HDL and very low-density lipoprotein = TG/5.[16]

Anthropometric measurements
BW and height were measured by stadiometer and then body mass index (BMI) was calculated as

BW (kg)/height (m^2).[17] Blood pressure measurements were also determined at supine position during investigation period.

Statistical analysis

Statistical Package for the Social Sciences software (SPSS Inc., Chicago, IL, USA) was used. Data were presented as mean ± standard deviation, and the variables were tested by Pearson's correlation test and one-way ANOVA test followed by *post hoc* test regarding $P < 0.05$ as statistically significant.

RESULTS

Consort flow diagram of the present study illustrated that five patients were excluded because they do not meet the inclusion criteria, and thus fifty patients with T2DM and twenty-five controls continued this study [Figure 1].

Characteristics of the recruited diabetic patients in the present study showed that age of patients was within 53.86 ± 8.12 years, 62% of them were males, and 38% were females, most of those patients were of White race (96%), and only 4% were of Black race. Regarding the current diabetic pharmacotherapy, 30% of them were on metformin, 34% on glyburide, and 56% on metformin plus glyburide. The associated diseases in those patients were hypertension (78%), hyperlipidemia (96%), ischemic heart disease (IHD) (46%), and diabetic peripheral neuropathy (44%); other characteristics are summarized in Table 1.
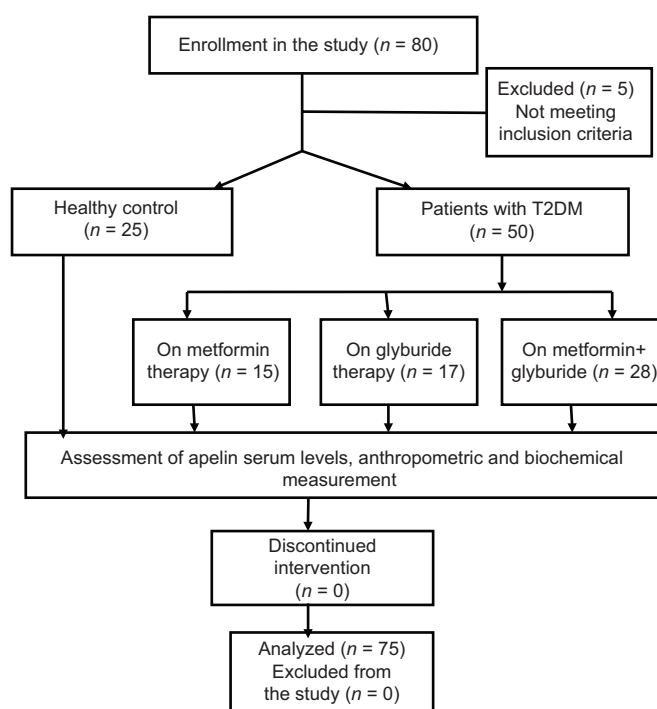
Figure 1: Consort flow diagram of the present study

The differences between controls and T2DM patients were highly significant in all metabolic and anthropometric variables ($P < 0.01$) except of height ($P > 0.05$). BMI was 29.80 ± 5.86 in diabetic patients compared to controls (26.90 ± 3.45) ($P = 0.02$). Apelin serum levels were low (51.83 ± 6.82 ng/L) in normal healthy controls compared to 77.43 ± 7.44 ng/L in T2DM patients ($P < 0.01$) [Table 2].

Apelin serum levels in patients with T2DM were highly correlated with fasting insulin levels ($P < 0.001$) and with HOMA-IR ($P < 0.01$) [Figures 2 and 3]; also it showed significant correlations with the parameters of cardiac-metabolic profile [Table 3].

The differential effect of diabetic pharmacotherapy on apelin serum level was statistically significant ($P < 0.01$) compared to the controls, but insignificant when compared among used drugs ($P > 0.05$); the difference was high between controls and diabetic patients [Table 4].

Thus, apelin serum levels were low in healthy controls compared to the diabetic patients ($P < 0.01$), whereas there were insignificant differences in apelin serum levels in diabetic patients regarding specific diabetic pharmacotherapy [Figure 3].

**Table 1: Metabolic and clinical characteristics of recruited type 2 diabetes mellitus patients**

Characteristics	Mean±SD, *n* (%)
Age (years)	53.86±8.12
Gender, male:female ratio	31:19 (62:38)
Race, White:Black ratio	48:2 (96:4)
Duration of T2DM (years)	14.87±2.73
Diabetic pharmacotherapy	
Metformin	15 (30)
Glyburide	17 (34)
Metformin plus glyburide	28 (56)
Concomitant diseases	
Hypertension	39 (78)
Hyperlipidemia	48 (96)
IHD	23 (46)
Diabetic peripheral neuropathy	22 (44)
Other pharmacotherapy	
Statins	31 (62)
Fibrates	19 (38)
Anticoagulants	12 (24)
Antiplatelets	40 (80)
ACEIs	30 (60)
CCBs	20 (40)
Tonics	47 (94)
Analgesics	41 (82)
Smoking	22 (44)

Data are expressed as mean±SD, *n* (%). IHD: Ischemic heart disease, ACEIs: Angiotensin-converting enzyme inhibitors, CCBs: Calcium channel blockers, SD: Standard deviation, T2DM: Type 2 diabetes mellitus

Table 2: Anthropometric and biochemical changes in relation to apelin serum levels in patients with type 2 diabetes mellitus compared to the healthy volunteers

Variables	Control (*n*=25)	T2DM (*n*=50)	95% CI	*t*	*P*
Weight (kg)	81.33±4.11	89.12±3.84	5.87-9.70	8.09	0.0001*
Height (cm)	173.83±9.95	172.81±8.99	−5.56-3.52	−0.44	0.65
BMI (kg/m²)	26.90±3.45	29.80±5.86	0.36-5.43	2.28	0.02†
SBP (mmHg)	111.54±12.89	164.77±13.85	46.61-59.84	16.04	0.0001*
DBP (mmHg)	73.44±3.87	94.33±8.31	17.39-24.38	11.91	0.0001*
FBG (mg/dL)	88.45±3.64	143.69±12.34	50.20-60.27	21.84	0.0001*
HbA1c (%)	5.80±1.22	9.66±2.65	2.74-4.97	6.90	0.0001*
FSI (μ/L)	5.41±1.52	8.99±2.63	2.44-4.71	6.28	0.0001*
HOMA-IR	1.18±0.022	3.19±0.23	1.91-2.10	43.45	0.0001*
QUICKI	0.37±0.01	0.32±0.02	−0.058-0.041	−11.75	0.0001*
TC (mg/dL)	145.32±11.57	186.64±12.45	35.37-47.26	13.86	0.0001*
TG (mg/dL)	133.59±11.22	223.24±13.34	83.45-95.84	28.85	0.0001*
VLDL (mg/dL)	26.71±6.74	44.64±9.39	13.72-22.13	8.50	0.0001*
HDL (mg/dL)	53.66±5.90	32.66±4.88	−23.55-18.44	−16.34	0.0001*
LDL (mg/dL)	64.94±9.34	109.33±11.32	39.16-49.61	16.92	0.0001*
AI	0.036±0.001	0.475±0.002	0.438-0.439	1032.37	0.0001*
AC	1.70±0.022	4.71±1.11	2.56-3.45	13.51	0.0001*
CRR	2.70±0.33	5.71±1.99	2.20-3.81	7.48	0.0001*
Apelin (ng/L)	51.83±6.82	77.43±7.44	22.06-29.13	14.43	0.0001*

Data are expressed as mean±SD. *$P<0.01$, †$P<0.05$. BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, FBG: Fasting blood glucose, HbA1c: Glycated hemoglobin, FSI: Fasting serum insulin, HOMA-IR: Homeostasis Model Assessment-Insulin Resistance, QUICKI: Quantitative Insulin Sensitivity Check Index, TC: Total cholesterol, TG: Triglyceride, VLDL: Very low-density lipoprotein, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, AI: Atherogenic Index, AC: Atherogenic coefficient, CRR: Cardiac risk ratio, T2DM: Type 2 diabetes mellitus, CI: Confidence interval, SD: Standard deviation
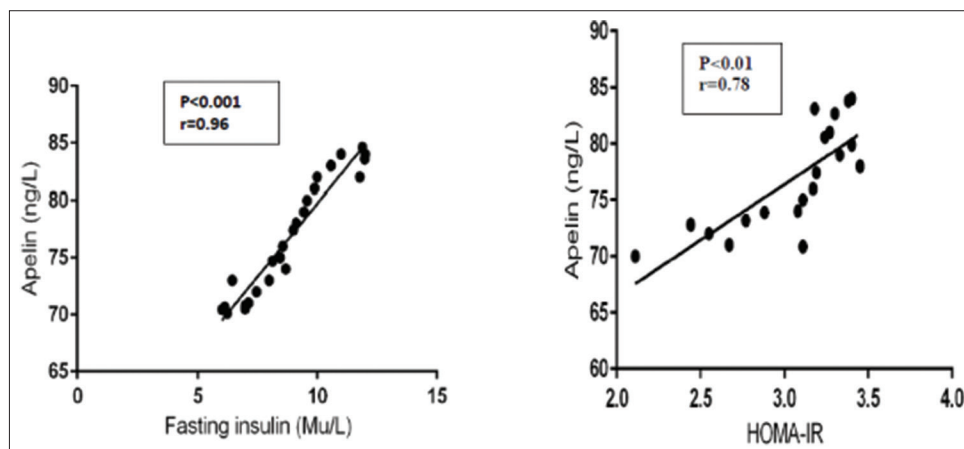
Figure 2: Correlations of apelin levels with fasting insulin and Homeostasis Model Assessment-Insulin resistance in patients with type 2 diabetes mellitus

Moreover, the present study exposed insignificant differences in apelin serum levels in sex of both controls and diabetic patients [Figure 4].

DISCUSSION

In T2DM, many adipokines participate in the control of blood glucose homeostasis and body energy; among them, a new peptide called apelin is widely expressed in human tissues. Apelin therapy leads to significant reduction of insulin resistance and improves insulin sensitivity.[18] Furthermore, apelin regulates adipose tissue lipolysis, since administration of apelin leads to significant reduction of adiposity. Apelin acts as a regulator of insulin secretion, but the remarkable point is the controversy about apelin serum levels in T2DM.[19]

The present study illustrated a high apelin serum level in patients with T2DM compared to the healthy controls as revealed by Dray *et al's*. study which showed elevated serum apelin in patients with T2DM.[20] On the contrary, Zhang *et al's*. study showed low level of serum apelin in T2DM,[21] while Telejko *et al's*. study confirmed normal apelin level in patients with T2DM.[22] Raised apelin serum
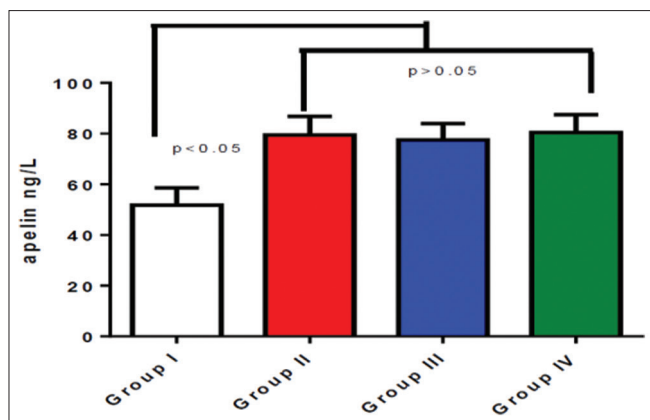
Figure 3: Apelin serum levels in patients with type 2 diabetes mellitus compared to the controls $*P < 0.01$

Table 3: Correlations of apelin with anthropometric and biochemical variables in patients with type 2 diabetes mellitus

Variables	r	P
Weight (kg)	0.51	0.0001
Height (cm)	−0.20	0.16
BMI (kg/m^2)	0.36	0.01
SBP (mmHg)	0.14	0.33
DBP (mmHg)	0.16	0.26
FBG (mg/dL)	0.43	0.001
HbA1c (%)	−0.31	0.02
QUICKI	0.46	0.0007
TC (mg/dL)	0.22	0.12
TG (mg/dL)	0.32	0.02
VLDL (mg/dL)	0.30	0.03
HDL (mg/dL)	0.24	0.09
LDL (mg/dL)	−0.22	0.093
AI	0.36	0.01
AC	0.31	0.028
CRR	0.28	0.04

BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, FBG: Fasting blood glucose, HbA1c: Glycated hemoglobin, QUICKI: Quantitative Insulin Sensitivity Check Index, TC: Total cholesterol, TG: Triglyceride, VLDL: Very low-density lipoprotein, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, AI: Atherogenic Index, AC: Atherogenic coefficient, CRR: Cardiac risk ratio

Table 4: The differences in apelin serum levels among patients with type 2 diabetes mellitus regarding the specific diabetic pharmacotherapy compared to the controls

Apelin (ng/L)	Difference	95% CI	P
Group I versus Group II	27.600	21.69-33.50	<0.01
Group I versus Group III	25.72	20.03-31.40	<0.01
Group I versus Group IV	28.66	23.68-33.63	<0.01
Group II versus Group III	1.88	−8.22-4.52	0.86
Group II versus Group IV	1.06	−4.72-6.84	0.96
Group III versus Group IV	2.94	−2.61-8.49	0.51

Tukey HSD *post hoc* test estimates the mean differences and the significance in apelin serum levels in different treated groups compared to the healthy controls. CI: Confidence interval, HSD: Honestly significant difference
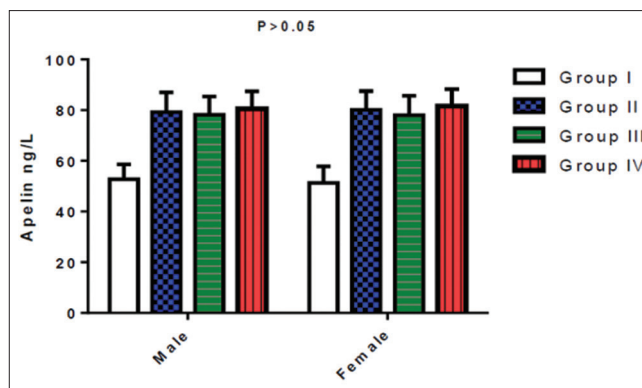
Figure 4: Gender differences in apelin serum levels

levels in the present study might be due to the elevation of BMI and insulin resistance given that most of the recruited patients were obese with significant insulin resistance. These findings correspond with Ba *et al*'s. study which revealed that high apelin serum levels were linked to obesity and level of insulin resistance, since apelin is positively correlated with BMI.[23] Moreover, low apelin serum level was observed following loss of BW as demonstrated by Khajeh *et al.*, study illustrated a rapid reduction in apelin serum levels following partial gastric resection in patients with morbid obesity.[24] Cavallo *et al*'s. cohort study observed that apelin was increased in relation to the level of insulin resistance in diabetic patients independent of BW since; 3 days following bariatric surgery for morbidly obese patients led to significant improvement in cardiometabolic profile and reduction in apelin serum levels independently from weight loss.[25] In addition, a study confirmed that apelin serum levels were increased more in type 1 DM than T2DM, suggesting that obesity is not the chief determinant of apelin levels.[26]

Similarly, apelin improves insulin sensitivity and increases muscle glucose uptake through inhibition of adenylate cyclise and stimulation of AMPK activity; thus, apelin is necessary for insulin sensitivity,[27] which coincided with the findings of the present study.

Our study also showed that elevated apelin serum levels were associated with hyperlipidemia, high atherogenic index, elevated cardiac risk ratio, and other cardiometabolic disturbances as supported by Akinci *et al*'s. study that illustrated a significant association of apelin serum levels with cardiometabolic risk factors in gestational DM.[28]

In addition, apelin serum levels are significantly correlated with most of the cardiometabolic risk factors, serum insulin, and HOMA-IR, but they are negatively correlated with HbA1c; these findings might explain the compensatory elevation of apelin levels to overcome these disturbances. The negative association with HbA1c could indicate that apelin plays an important potential role in the glycemic

balance.[29] Nearly 46% of the recruited patients are associated with IHD; it has been documented that apelin serum levels are reduced in IHD;[30] thus, elevated apelin serum levels in the current study are mainly predicted by the effect of T2DM than the effect of IHD.

In the current study, 44% of the recruited patients were associated with peripheral diabetic neuropathy which may explain high apelin serum levels since Bilir *et al*'s. study confirmed that apelin levels were high in patients with diabetic neuropathy compared to the diabetic patients not associated with diabetic neuropathy; this elevation in apelin levels might be due to the microangiopathic complications.[31]

Regarding the effect of diabetic pharmacotherapy on apelin serum levels in patients with T2DM, metformin and/or glyburide significantly elevates apelin serum levels compared to the controls, but there were insignificant differences between the treated groups. This finding was supported by Fan *et al*'s. study that illustrated the significant effect of metformin in the elevation of apelin serum levels compared to the baseline value due to activation of apelin secretion though 5 monophosphate-activated protein kinase activation.[11] In contrast, a study showed that metformin may play an important role in the reduction of apelin serum levels in women with polycystic ovarian syndrome.[32]

The apelin serum levels were high in patients with T2DM treated with glyburide, but no previous studies have documented these effects, since Araki *et al*'s. study confirmed that glimepiride significantly raised adiponectin serum levels.[33] Besides, gliclazide improves adiponectin serum levels in patients with T2DM;[34] thus, glyburide might increase apelin serum levels through elevation of adiponectin, which is positively correlated with apelin serum levels. A study by Alexiadou *et al*. showed that exogenous insulin administration does not change the apelin serum levels in T2DM;[35] therefore, the positive effect of glyburide on apelin serum levels may not be mediated through provoking insulin secretion; unfortunately, adiponectin serum levels were not assessed in the present study.

Regarding other pharmacotherapy treatments received by patients with T2DM, 62% of the patients were on statins which may increase apelin levels as observed in a study by Kadoglou *et al*. that observed significant effect of atorvastatin in the elevation of apelin serum levels in patients with T2DM.[36]

The present study did not observe any gender differences in apelin serum levels in patients with T2DM, since all the recruited women in this study were at postmenopausal period as supported by Karakoc *et al*'s. study that illustrated insignificant effect of gender in relation to apelin serum levels in patients with T2DM.[37]

Limitations

Limitations of the present study were small sample size, being single-center study, and not involved patients with a newly diagnosed T2DM. Furthermore, HOMA score was used in the present study (which is less accurate method) instead of euglycemic hyperinsulinemic clamp method (which is more accurate method). Indeed, it is extremely difficult to mention normal standard level of apelin due to the presence of different forms (apelin 12, 13, 18, and 36) in the circulation which may lead to cross-reactivity. Therefore, larger -scale studies are recommended to illustrate the effect of diabetic pharmacotherapy on specific apelin serum levels.

CONCLUSION

Apelin level was high in T2DM patients compared to normal healthy controls; both metformin and glyburide might play a role in this elevation.

Ethics committee approval

Ethics committee approval was established for this clinical study from the College of Medicine, Al-Mustansiriyia University (Decision Date/No: 02-01-2016/22).

Informed consent

Written informed consent was gained from all patients and healthy controls in this study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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