Relationship between subclinical hypothyroidism and serum osteoprotegerin level in type 2 diabetic patients

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Background
Subclinical hypothyroidism (SCH) and osteoprotegerin (OPG) are associated with higher risks of cardiovascular disorders in patients with type 2 diabetes mellitus (T2DM). It is unknown whether diabetic patients with SCH have elevated OPG compared with those with euthyroid and, if so, whether SCH independently associated with OPG level.
Objective
The objective was to study the association between SCH and serum OPG level among Egyptian adults with newly diagnosed T2DM.
Patients and methods
One hundred and fifty patients with newly diagnosed T2DM and 150 healthy controls matched for sex and age were included in the study. Serum OPG, thyroid-stimulating hormone (TSH), free thyroxine, free triiodothyronine, blood glucose, lipid profile, fasting insulin, homeostasis model assessment of insulin resistance (HOMA-IR), and anthropometric measurements were assessed for all participants.
Results
The prevalence of SCH and elevated level of OPG in the diabetic patients were higher than healthy controls (17.3 vs. 4%, $P<0.001$; and 14 vs. 4.7%, $P=0.02$; respectively). Diabetics with SCH demonstrated significantly higher HOMA-IR, serum TSH, and OPG compared with diabetics without SCH. Serum TSH was significantly correlated with total cholesterol ($P=0.05$), fasting insulin ($P=0.01$), HOMA-IR ($P=0.01$), and OPG ($P=0.005$). Moreover, serum OPG was correlated with waist circumference ($P=0.01$), fasting insulin ($P=0.05$), and HOMA-IR ($P=0.02$). Multiple logistic regression analysis revealed that SCH was associated with serum level of OPG independently of the other significant variables ($\beta=2.49$, $P=0.01$).
Conclusion
T2DM patients with SCH demonstrate higher level and independent association with serum OPG than those with euthyroid. This result might add new information about the causal relationship between SCH and cardiovascular disorders in such a population.

Keywords:
cardiovascular disorders, osteoprotegerin, subclinical hypothyroidism, type 2 diabetes

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Background

Development and progression of type 2 diabetes mellitus (T2DM) is considered as a serious global health burden and closely associated with macrovascular and microvascular complications that negatively affected all-cause and cardiovascular (CV) mortality [1–3].

Osteoprotegerin (OPG) is a secretory glycoprotein that is related to the tumor necrosis factor receptor family and included in the regulation of bone metabolism, vascular tone enhancement, endothelium regeneration, and vascular calcification processes [4,5]. It has been identified as an independent risk factor of cardiovascular disorders (CVD) in patients with T2DM, metabolic syndrome, chronic renal disease, and polycystic ovary syndrome [6–9], all of which were closely related to endothelial dysfunctions [10,11].

Patients with T2DM are more likely to have subclinical hypothyroidism (SCH) when compared with the healthy population [12–14]. SCH is usually asymptomatic and diagnosed by elevated serum thyroid-stimulating hormone (TSH) with normal free thyroxine and free triiodothyronine levels [15]. Several clinical studies have shown an independent association between SCH

and CVD [16–18] and between serum level of OPG and CVD in T2DM patients [19–21]; however, whether SCH is associated with serum level of OPG in T2DM has not been studied before. Moreover, because there is no enough data on serum OPG levels in SCH, it is of great importance to evaluate their possible association. Therefore, we aimed to study the association between SCH and serum OPG among Egyptian adults with newly diagnosed T2DM and also their association with other metabolic risk factors.

Patients and methods

This prospective study included a total of 150 patients with newly diagnosed T2DM before starting antidiabetic medications (64 male and 86 female), aged 45.5±5.1 years, selected from the Outpatient Clinics of Diabetes and Endocrinology Unit at Specialized Medical Hospital, Mansoura University, Faculty of Medicine, Egypt, during the period from February 2016 to March 2017. One hundred and fifty healthy controls matched for sex and age with exclusion criteria similar to the diabetic patients were also included. Informed consents were obtained from all participants, and approval was given by the ethics committee of our institution.

Full history taking and clinical examination were performed for all participants. We used standardized techniques in the assessment of height, weight, BMI, and waist circumference (WC). BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Blood pressure was taken in the sitting position using a random-zero sphygmomanometer. SCH was defined as TSH above the high-reference range (4 μU/ml according the method used in the present study). Exclusion criteria included hypertension, heart failure, acute coronary syndrome, or those taking drugs affecting thyroid or vascular functions (e.g. antiplatelet agent, lipid-lowering agents, antioxidant supplements, immunosuppressants, bisphosphonate, amiodarone, or lithium), smoking, hepatic disorders, connective tissue diseases, malignancy, infection, pregnancy, and female on birth control pills or hormone replacement therapy.

Laboratory assay

Blood glucose was determined using a commercially available kit, Cobas (Integra-400) (supplied by Roche Diagnostic, Basel, Germany). Serum triglyceride, total cholesterol (TC), and high-density lipoprotein cholesterol were measured using commercially available kits. Low-density lipoprotein cholesterol was estimated according to the method by Friedewald *et al.* [22]. Measurement of fasting serum insulin was done by a solid-phase, enzyme-labeled chemiluminescent immunometric assay using Immulite analyzer (IMMULITE 2000, Diagnostic Products Corp., IL, USA). Homeostasis model assessment of insulin resistance (HOMA-IR) was measured with the following formula: HOMA-IR=fasting insulin (μU/ml)×fasting glucose (mmol/l)/22.5 [23]. Hemoglobin A1c (HbA1c) was measured on a DCA 2000 analyzer, fast ion exchange resin supplied by human (Germany) with the reference range of 4.4–6.4%. Serum free triiodothyronine, free thyroxine, and TSH were assayed by electrochemiluminescence immunoassay, using Elecsys 2010 (Roche Diagnostic). Serum OPG was measured in duplicate by enzyme-linked immunosorbent assay with the Duoset kit (DY805; R&D Systems, Minneapolis, Minnesota, USA) as recommended by the manufacturer. The intra-assay and interassay coefficients of variation were 3–9 and 3–10%, respectively.

Statistical analysis

Data analysis was performed using SPSS statistical package version 17 (SPSS Inc., Chicago, Illinois, USA). Data were expressed as mean±SD for continuous data, proportion, and frequency for categorical data, and median (minimum to maximum) for skewed data. Comparison of two groups was done with Student's *t*-test and Mann–Whitney *U*-test. A χ^2-test was used in categorical data. Relationship between different items was determined using Pearson's and Spearman's correlations. Multiple logistic regression analysis was performed to evaluate the association of SCH with OPG. OPG, fasting insulin, and HOMA-IR were entered in the regression model. *P* value 0.05 or less was considered significant.

Results

Clinical and laboratory characteristics of the 150 newly diagnosed diabetic patients and 150 controls were determined in Table 1. Diabetic individuals had significantly higher WC, BMI, blood pressure, blood glucose, HbA1c, fasting insulin, and HOMA-IR, TC, triglyceride, and low-density lipoprotein cholesterol than controls, whereas high-density lipoprotein cholesterol was significantly lower in diabetics than in controls. Serum levels of TSH and OPG were significantly higher in diabetic patients than in healthy controls (5.3±1.9 vs. 2.4±1.2, $P<0.001$; and 3.04±1.6 vs. 2.5±0.9, $P=0.001$; respectively). Prevalence of SCH in diabetics was 17.3% (26/150) compared with 4% (6/150) in controls ($P<0.001$). The prevalence of elevated level of OPG in diabetic patients was 14% (21/150) compared with 4.7% (7/150) in controls ($P=0.02$).

Table 1 Clinical and metabolic characteristics of all participants

Characteristics	Diabetic patients (*n*=150)	Healthy controls (*n*=150)	*P* value
Sex (female/male)	86/64	80/70	0.26
Age (years)	46.5±5.1	46.0±5.4	0.34
BMI (kg/m^2)	37.8±2.6	29.7±0.7	<0.001*
WC (cm)	99.3±5.9	79.1±1.7	<0.001*
SBP (mmHg)	128.1±7.0	109.2±5.0	<0.001*
DBP (mmHg)	82.2±3.0	74.9±2.0	<0.001*
RBS (mg/dl)	234.5±19.0	123.7±9.6	<0.001*
FI (μIU/ml)	49.9±9.8	8.9±5.0	<0.001*
HOMA-IR	10.8±2.5	2.1±0.3	<0.001*
HbA1c (%)	7.5±0.3	5.6±0.15	<0.001*
TC (mg/dl)	280.0±27.7	170.5±1.8	<0.001*
TG (mg/dl)	198.16±58.2	78.8±13.1	<0.001*
HDL-C (mg/dl)	43.6±9.4	53.5±3.0	<0.001*
LDL-C (mg/dl)	145.8±38.2	81.9±10.0	<0.001*
FT4 (ng/dl)	1.6±0.2	1.5±0.1	0.13
FT3 (pmol/l)	4.6±0.5	4.6±0.5	0.42
TSH (μU/ml)	5.3±1.9	2.3±1.1	<0.001*
OPG (μg/l)	3.04±1.6	2.5±0.9	<0.001*
SCH (%)	17.3 (26/150)	4 (6/150)	<0.001*
High OPG (%)	14 (21/150)	4.7 (7/150)	0.02*

DBP, diastolic blood pressure; FI, fasting insulin; FT3, triiodothyronine; FT4, free thyroxine; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; OPG, osteoprotegerin; RBS, random blood sugar; SBP, systolic blood pressure; SCH, subclinical hypothyroidism; TC, total cholesterol; TG, triglyceride; TSH, thyroid-stimulating hormone; WC, waist circumference. **P* is significant if ≤0.05.

Patients with newly diagnosed T2DM were divided into two groups: diabetics with SCH and diabetics without SCH. Diabetics with SCH are characterized by significantly higher fasting insulin, HOMA-IR, serum TSH (6.75±1.9 vs. 3.2±0.9 mU/ml, *P*<0.001), and serum OPG levels (2.98±1.09 vs. 2.41±0.95, *P*=0.01) than diabetics without SCH, whereas WC, BMI, blood pressure, blood glucose, HbA1c, and lipid profile were not different in the two groups (Table 2).

Serum TSH was significantly associated with fasting insulin (*r*=0.19, *P*=0.01), HOMA-IR (*r*=0.22, *P*=0.01), TC (*r*=0.17, *P*=0.05), and OPG (*r*=0.19, *P*=0.01) (Table 3). Moreover, OPG level was significantly correlated with WC (*P*=0.01), fasting insulin (*P*=0.05), HOMA-IR (*P*=0.02), and TSH level.

Using multiple logistic regression analysis, we found that SCH was associated with OPG independent of the other significant variables: fasting insulin and HOMA-IR (*β*=2.49, odds ratio: 4.63, 95% confidence interval: 1.46–14.75, *P*=0.01) (Table 4). Furthermore, there was an insignificant association between TSH and serum OPG in healthy controls (*r*=0.06, *P*=0.4).

Table 2 Comparison between diabetics with and without subclinical hypothyroidism

Characteristics	Diabetics with SCH (*n*=26)	Diabetics without SCH (*n*=124)	*P* value
BMI (kg/m^2)	36.5±2.85	35.9±2.6	0.32
WC (cm)	100.5±9.0	98.9±8.5	0.14
SBP (mmHg)	124.0±8.8	123.65±4.8	0.71
DBP (mmHg)	83.0±4.0	82.0±3.8	0.24
RBS (mg/dl)	230.9±17.6	229.6±17.0	0.35
FI (μIU/ml)	48.9±3.1	30.5±2.8	<0.001*
HOMA-IR	11.9±0.9	6.9±0.9	<0.001*
HbA1c (%)	7.0±0.2	7.0±0.2	0.26
TC (mg/dl)	220.1±38.9	219.0±37.1	0.41
TG (mg/dl)	153.4±47.4	139.95±35.7	0.22
HDL-C (mg/dl)	44.9±3.9	45.9±5.6	0.13
LDL-C (mg/dl)	189.9±13.0	189.35±14.5	0.83
TSH (μU/ml)	6.75±1.9	3.2±0.9	<0.001*
OPG	2.98±1.09	2.41±0.95	0.01*

DBP, diastolic blood pressure; FI, fasting insulin; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; OPG, osteoprotegerin; RBS, random blood sugar; SBP, systolic blood pressure; SCH, subclinical hypothyroidism; TC, total cholesterol; TG, triglyceride; TSH, thyroid-stimulating hormone; WC, waist circumference. **P* is significant if ≤0.05.

Table 3 Correlation of thyroid-stimulating hormone with the studied variables and osteoprotegerin in diabetics

Variables	TSH		OPG	
	r	*P* value	*r*	*P* value
Age (years)	0.11	0.23	0.002	0.97
BMI (kg/m^2)	0.16	0.07	0.02	0.83
WC (cm)	0.15	0.08	0.22	0.01
SBP (mmHg)	0.11	0.14	0.11	0.22
DBP (mmHg)	0.04	0.71	0.02	0.83
RBS (mg/dl)	0.12	0.13	0.003	0.95
HbA1c (%)	0.005	0.93	0.11	0.22
FI (μU/l)	0.19	0.01*	0.17	0.05*
HOMA-IR	0.22	0.01*	0.18	0.02*
TC (mg/dl)	0.17	0.05*	0.11	0.22
TG (mg/dl)	0.14	0.12	0.02	0.64
LDL-C (mg/dl)	0.05	0.59	0.01	0.72
HDL-C (mg/dl)	–0.16	0.07	–0.07	0.42

DBP, diastolic blood pressure; FI, fasting insulin; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; OPG, osteoprotegerin; RBS, random blood sugar; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; TSH, thyroid-stimulating hormone; WC, waist circumference. **P* is significant if ≤0.05.

Discussion

This study investigated the association between SCH and serum level of OPG in patients with newly diagnosed T2DM. It has been known that SCH exhibits a close link to CVD in T2DM [16–18], but

Table 4 Multiple logistic regression analysis of thyroid-stimulating hormone with the significant variables

Variables	β	OR	95% CI	*P* value
OPG	2.49	4.63	1.46–14.75	0.01*
FI	0.07	1.08	0.86–1.2	0.14
HOMA-IR	0.39	1.4	0.86–2.1	0.22

CI, confidence interval; FI, fasting insulin; HOMA-IR, homeostasis model assessment of insulin resistance; OPG, osteoprotegerin; OR, odds ratio. *P is significant if ≤ 0.05.

the exact mechanism is still inconclusive. Our main finding of the independent association between SCH and elevated serum level of OPG might add more information about the causal relationship between SCH and CVD in such a population.

The association between T2DM and SCH is well recognized, with a prevalence of 10–24% [12–15]. In the present study, prevalence of SCH in diabetics was 17.3% compared with 4% in controls. We found a significant association between SCH and TC, fasting insulin, and HOMA-IR. These results are in agreement with those of Chubb *et al.* [24] who reported that a slight variation in TSH level could exert a marked effect on lipid concentrations in adults with DM. Furthermore, Maratou *et al.* [25] proved that patients with SCH have insulin resistance that is comparable to that of the patients with hypothyroidism. These findings indicate that patients with T2DM are more prone to have vascular complications when hypothyroidism associates with diabetes, which is in agreement with the finding of many researches [16–18].

Thus, the presence of SCH can aggravate the classical risk factors such as dyslipidemia, hypertension, and insulin resistance and lead to an increased CV risk in such patients. Moreover, the increase in plasma blood glucose and HbA1c leads to glucotoxicity and more atherogenic dyslipidemia, which contributes to two-fold to four-fold excess risk of CVD in diabetics.

Our result of higher prevalence of elevated serum level of OPG in the diabetic patients in this study compared with controls is in agreement with previous researches [19–21]; furthermore, we demonstrated that OPG level was significantly associated with high TC, fasting insulin, and HOMA-IR. These results are in parallel with those of Bernardi *et al.* [10], who observed that serum OPG was correlated positively with insulin resistance in T2DM. Moreover, OPG was found as a powerful marker of macrovascular and microvascular complications and could be utilized for risk stratification in the general population and T2DM with CVD beyond classic CV risk factors [26–28] However, evidence regarding elevated OPG level as a marker of CV events in diabetics without known CV disease still remains inconclusive and requires further investigations [29,30].

In the current study, we found that newly diagnosed diabetics with SCH demonstrated higher serum level of OPG than those with euthyroid. These data were in good agreement with a previous report demonstrating higher serum OPG in hypothyroid patients than in controls [31]. Moreover, multiple logistic regression analysis demonstrated that SCH was independently associated with OPG level. This interaction between lower thyroid function and higher serum level of OPG might be a key determinant for a more CV risk in these populations.

The mechanisms of SCH-related CVD in diabetic patients is suggested to be related to aggravation of the classical risk factors such as hypertension, dyslipidemia, insulin resistance [32], and impaired nitric oxide availability and reduced endothelium-dependent vasodilation [33]. Therefore, the finding of our study suggested that the association between SCH and high serum level of OPG might have an important role in the etiopathological relationship between SCH and development of CVD in patients with T2DM.

The limitation of our study is that analysis of thyroid hormones and serum levels of OPG were measured at a single time point. Follow-up thyroid function are needed to confirm the association between serum level of OPG and the clinical course of SCH. However, the strength of the study is its case control design, which can establish the causal association between SCH and serum OPG level in T2DM.

Conclusion

Our findings suggest that diabetics with SCH revealed an independent association between TSH and serum level of OPG, which might be considered as a causal link between SCH and CVD in T2DM patients. Screening and treatment of SCH may be required and it could help in the management of diabetic vascular complications. Future studies also are required to confirm this association and to test the effect of thyroxin therapy on serum level of OPG in such populations.

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Conflicts of interest
There are no conflicts of interest.

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