

Evaluation of Osteocalcin and Reproductive Hormones in Men with Type 2 Diabetes in Misan Province/Iraq

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Abstract

Background: Diabetes mellitus (DM) is a group of metabolic disorders in which there are high levels of blood sugar over a prolonged period. DM can be accompanied by numerous complications, including different organ systems for instance skeleton and reproductive system. **Objectives:** The aim of the present study was to determine the levels of osteocalcin (OC) and reproductive hormones in men with type 2 DM (T2DM) of Misan province from December 2018 to May 2019. **Materials and Methods:** The study population consists of 110 men aged 35–49 years of whom 66 type 2 diabetic and 44 healthy men; patients with T2DM were divided based on the age into three groups: the first (35–39) years, second (40–44) years, and third (45–49) years; patients with T2DM were also divided based on the body mass index into four groups: normal weight (18.5–24.9), overweight (25–29.9), obesity class I (30–34.9), and obesity class II (35–39.9) groups. **Results:** According to the age, the mean values of fasting blood glucose and glycosylated hemoglobin A1c (HbA1c) were significantly higher in diabetic subjects when compared with the control. OC increases significantly in the second and third groups as compared to control. Luteinizing hormone (LH) of the patients in the third group significantly increases as compared to the first group. Patients of the third group increase significantly compared to the patients in the first group. Follicle-stimulating hormone (FSH) means value no different significantly according to the age. The testosterone (TT) mean value in compare between patients group, the third increase significantly in compare with first group. Estradiol (E2) in the first group increases significantly as compared to control. Prolactin (PRL) in the first group decreases significantly compared to control. According to the BMI, the mean value of fasting blood glucose and HbA1c was significantly higher in diabetic subjects when compared to control. OC value of T2DM significantly increases in the group obesity class II and overweight. LH, TT, and FSH were not significantly significant compared with control. E2 value significant different in compare with control of obesity class I and class II. PRL decreased significantly as compared to control in normal weight group; in patients of obesity class I, the E2 value increases significantly as compared to patients of normal weight. **Conclusions:** This study revealed no relationship between OC and reproductive hormones and no relationship between OC and T2DM.

Keywords: Body mass index, osteocalcin, reproductive hormone, T2DM

INTRODUCTION

Diabetes is a serious metabolic disease, with an increasing prevalence worldwide, and the number of influenced individuals may reach to 693 million in 2045,^[1] of whom 85% will have Type 2 diabetes mellitus (T2DM).^[2] DM is a group of metabolic disorders in which there are high levels of blood sugar over a prolonged period because of insufficient amount of insulin producing from pancreatic β -cell.^[3,4] The prevalence of T2DM has increased along with the increase in obesity resulting from lifestyle changes of modern life.^[5] Patients of T2DM are at critical risk for well-recognized diabetic complications; newly, one more complication has been associated with DM, an augmented risk of fragility fractures which seems to be rather independent of bone mineral density.^[6,7]

Newly established that bone acts as an endocrine organ that secretes at least three hormones: fibroblast growth factor 23, lipocalin 2, and osteocalcin (OC).^[8]

OC is the most abundant protein found in the extracellular matrix of bone synthesized by osteoblasts.^[9] In humans, it is a 49 amino acid with three glutamic acid residues.^[10]

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OC receptor GPRC6A is expressed in different tissues including the liver, skeletal muscle, brain, testis, bone, and pancreatic β -cells.^[11,12]

The study suggests that OC has many roles in deferent tissues, including effect in stimulating testosterone (TT) biosynthesis in Leydig cells.^[13] DM is known to trigger oxidative stress in virtually all tissues of the body comprising male reproductive organs. Several experimental and clinical searches have reported varying degrees of male reproductive impairment in DM. Disruption in the hypothalamic–pituitary–gonadal axis has been reported in DM.^[14]

A study by Ducy *et al.*^[15] revealed the existence of a parallel endocrine pathway acting on the endocrine function of the testis, which involves OC. The OC effects on the TT by stimulate TT production from the testes.^[16] In clinical practice, there was also a positive correlation between TT and OC.^[17]

The study by Oury *et al.*^[16] on male mice found that OC regulate the synthesis of TT by Leydig cells, while TT is also reported to modulate the osteoblastic expression of OC in male rats.^[18]

MATERIALS AND METHODS

This study was carried out at the Al-Sadder Teaching Hospital and Center for Endocrinology and Diabetes Specialist in Misan province. The population of this study consists of 110 men with average aged 35–49 years of whom 66 type 2 diabetic with fasting blood glucose and glycosylated hemoglobin A1c (HbA1c) values of 205.03–210.61 and 9.27–9.55, respectively and other 44 healthy men (control group). The study sample was divided by age into three groups: the first (35–39) years, second (40–44) years, and third (45–49 years). Study subjects were also divided by the body mass index (BMI) into four groups, the normal weight (18.5–24.9), overweight (25–29.9), obesity class I (30–34.9), and obesity class II (35–39.9). The BMI was estimated according to the World Health Organization^[19] with the following formula: $BMI = W/H^2$.

Blood sampling

Venous blood sample (6 ml) draws from each fasting patient and control at (8–10 AM). The blood sample was divided into two parts; 2.5 ml was transferred into EDTA tube for HbA1c determination, and other fraction of the blood sample was left for 15 min to clot at room temperature, and then, the serum was separated by centrifugation at 3000 rpm for 5 min, to measure glucose. The last serum was transferred into labeled plain tube and stored at -20°C until used for the evaluation of hormones (OC, luteinizing hormone [LH], follicle-stimulating hormone [FSH], TT, estradiol [E2], and prolactin [PRL]).

Measurement of serum osteocalcin

Serum OC was determined according to the previous study^[20] by the kit supplied from (Shanghai Company, China).

Measurement of reproductive hormones

Reproductive hormones measurement was done using Cobas e411 kit supplied from Elecsys Roch diagnostic GmbH, Mannheim, Germany).

Statistical analysis

Data were analyzed by *t*-test to compare between patients and control, and one-way ANOVA to compare among patients using SPSS version 23 statistic program (SPSS).^[21]

RESULTS

According to the age

Osteocalcin level

OC level in the patients of the second (2.01 ± 0.31 ng/ml) group and the third (1.83 ± 0.31 ng/ml) increases significantly ($P < 0.05$) in comparison with the control of the second (0.97 ± 0.22 ng/ml) and the third (0.55 ± 0.18 ng/ml) groups. There are no significant differences in the OC level between the patients (1.03 ± 0.21 ng/ml) and the controls (1.08 ± 0.15 ng/ml) of the first group. The results showed that the OC level among patients with T2DM increased significantly ($P < 0.05$) in the second group in comparison with the first group, while there are no significant differences between the second and third groups. Furthermore, there are no significant differences between the third and first groups of T2DM [Table 1].

Reproductive hormone

As shown in Table 2, there are no significant differences ($P < 0.05$) in the level of LH in the patients of the first (5.00 ± 0.31 m.lu/mL) and second (6.13 ± 0.52 m.lu/mL) groups when compared to the control in the first (5.22 ± 0.55 m.lu/mL) and second (6.65 ± 0.68) groups, respectively.

While the level of LH in the patients of the third (6.62 ± 2.34 m.lu/mL) group increases significantly ($P < 0.05$) compared to the control in the third (4.90 ± 0.41 m.lu/mL) group. The results of LH level among patients of T2DM which increased significantly ($P < 0.05$) in the third group as compared to the patients of the first group. There are no significant differences ($P < 0.05$) between the third and second

Table 1: The values of osteocalcin in control and patient with type 2 diabetes mellitus (according to age)

Groups	Parameter (ng/ml)	Control	Patient	P
First group (35-39) years	OC	$n=16$ 1.08 ± 0.15	$n=22$ 1.03 ± 0.21^b	0.84
Second group (40-44) years	OC	$n=14$ 0.97 ± 0.22	$n=22$ $2.01 \pm 0.31^{*a}$	0.01
Third group (45-49) years	OC	$n=14$ 0.55 ± 0.18	$n=22$ $1.83 \pm 0.31^{*a,b}$	0.01

LSD (OC)=0.98. *Significant at the ($P < 0.05$). Values represent mean $\pm$ SE. Different letters refer to a significant differences among patient groups. Similar letters refer to nonsignificant differences among patient groups. OC: Osteocalcin, SE: Standard error, LSD: Least Significant Difference

groups, LH level in patients of second group increased significantly compared to the patients of first group [Table 2].

There are no significant differences ($P < 0.05$) in the values of FSH in the patients of the first (4.33 ± 0.53 m.lu/mL), second (6.20 ± 0.76 m.lu/mL), and third (5.95 ± 0.58 m.lu/mL) groups in comparison to the control in the first (4.24 ± 0.31 m.lu/mL) group, second (4.63 ± 0.52 m.lu/mL), and third (4.85 ± 0.67 m.lu/mL) groups, respectively. Furthermore, there are no significant differences ($P < 0.05$) of FSH values among patient of T2DM in all three age groups [Table 2].

The values of TT showed no different significantly ($P < 0.05$) in the patient of first (2.86 ± 1.45 ng/mL), second (3.64 ± 0.20), and third (3.89 ± 0.29 ng/mL) groups in comparison to the first control (3.05 ± 0.26 ng/mL), second control (3.39 ± 0.19 ng/mL), and third control (3.47 ± 0.26 ng/mL) with the third patient [Table 2].

The value of TT among patients of T2DM increased significantly ($P < 0.05$) in the third group in comparison with the first group. There are no significant differences ($P < 0.05$) between the third and second groups, and also there are no significant differences ($P < 0.05$) between the first and second groups [Table 2].

The value of E2 in the first patient (22.66 ± 2.49 pg/mL) increases significantly ($P < 0.05$) in comparison with the control first (14.92 ± 2.07 pg/mL) group, while no significant differences ($P < 0.05$) between the second patient (14.86 ± 1.84 pg/mL) and second the control (10.78 ± 2.21 pg/mL). There are no significant differences ($P < 0.05$) between the third patient (18.07 ± 2.58 pg/mL) and third control (21.58 ± 2.37 pg/mL). The value of E2 among the patients of T2DM increased significantly ($P < 0.05$) in the first group compared to the second group, while there are no significant differences ($P < 0.05$) between the first and third patient groups. Furthermore, there are no significant differences ($P < 0.05$) between the second and third patient groups [Table 2].

The value of PRL in patients of the first (9.40 ± 0.93 ng/mL) group decreases significantly ($P > 0.05$) in comparison with first control (13.01 ± 1.36 ng/mL) group. There are no significant differences ($P < 0.05$) between the second patient (9.94 ± 0.80 ng/mL) and second control (11.08 ± 1.49 ng/mL). Further, no significant differences ($P < 0.05$) between the third patient (8.56 ± 0.88 ng/mL) and third control (9.82 ± 0.76 ng/mL) are found. There are no significant differences ($P < 0.05$) in the PRL value among all patients [Table 2].

According to body mass index Osteocalcin level

OC level increased significantly ($P < 0.05$) in the patients of overweight (1.77 ± 0.24 ng/ml) and obesity class II (1.27 ± 0.35 ng/ml) groups compared to the control in overweight (1.00 ± 0.23 ng/ml) and obesity class II (0.29 ± 0.14 ng/ml), respectively. There are no significant differences in the OC value in the patients of normal weight (1.07 ± 0.25 ng/ml) and obesity

Table 2: The values of reproductive hormone in control and patient with type 2 diabetes mellitus (according to age)

Groups	Parameter	Control	Patients	P
First group (35-39 years)	LH (m.lu/mL)	n=16 5.22±0.55	n=22 5.00±0.31 ^b	0.71
	FSH (m.lu/mL)	4.24±0.31	4.33±0.53	0.89
	TT (ng/mL)	3.05±0.26	2.86±1.45 ^b	0.64
	E2 (pg/mL)	14.92±2.07	22.66±2.49 ^{*a}	0.02
	PRL (ng/mL)	13.01±1.36	9.40±0.93 [*]	0.03
Second group (40-44 years)	LH (m.lu/mL)	n=14 6.65±0.68	n=22 6.13±0.52 ^a	0.54
	FSH (m.lu/mL)	4.63±0.52	6.20±0.76	0.09
	TT (ng/mL)	3.39±0.19	3.64±0.20 ^{a,b}	0.38
	E2 (pg/mL)	10.78±2.21	14.86±1.84 ^b	0.16
	PRL (ng/mL)	11.08±1.49	9.94±0.80	0.50
Third group (45-49 years)	LH (m.lu/mL)	n=14 4.90±0.41	n=22 6.62±2.34 ^{*a}	0.01
	FSH (m.lu/mL)	4.85±0.67	5.95±0.58	0.23
	TT (ng/mL)	3.47±0.26	3.89±0.29 ^a	0.30
	E2 (pg/mL)	21.58±2.37	18.07±2.58 ^{a,b}	0.29
	PRL (ng/mL)	9.82±0.76	8.56±0.88	0.32
LSD	LH (1.62), TT (1.03), E2 (7.8)			

*Significant at the ($P < 0.05$). Values represent mean±SE. Different letters refer to a significant differences among patient groups, Similar letters refer to nonsignificant differences among patient groups. SE: Standard error, LH: Luteinizing hormone, FSH: Follicle-stimulating hormone, TT: Total testosterone, E2: Estradiol, PRL: Prolactin, LSD: Least Significant Difference, TT: Total testosterone

class I (1.35 ± 0.29 ng/ml) groups as compared to the control of normal weight (0.89 ± 0.19 ng/ml) and obesity class I (1.00 ± 0.19 ng/ml) groups, respectively. However, there are no significant differences in controls and patients of the third group. There are no significant differences in the OC value among patients of T2DM in different BMI groups [Table 3].

Reproductive hormone

As shown in Table 3, there are no significant differences ($P < 0.05$) in the value of LH in the patients of normal weight (6.51 ± 0.49 m.lu/mL), obesity class I (4.79 ± 0.41 m.lu/mL), and obesity class II (5.82 ± 0.43 m.lu/mL) groups when compared with control of the normal weight (7.62 ± 1.19 m.lu/mL), obesity class I (5.31 ± 0.71 m.lu/mL), and obesity class II (6.75 ± 0.86 m.lu/mL).

The patients of overweight (5.90 ± 0.40 m.lu/mL) group increase significantly ($P < 0.05$) comparison with the control (4.63 ± 1.04 m.lu/mL) of overweight group. There are no significant differences in insulin value among patient of T2DM in different BMI groups [Table 3].

As shown in Table 3, there are no significant differences ($P < 0.05$) in the value of FSH in the patients of normal weight (6.13 ± 1.43 m.lu/mL), overweight (5.46 ± 0.45 m.lu/mL), obesity class I (4.09 ± 0.80 m.lu/mL), and obesity class II (6.14 ± 0.87 m.lu/mL) compared to control of normal weight (4.09 ± 0.76 m.lu/mL), overweight

Table 3: The values of osteocalcin in control and patient with type 2 diabetes mellitus (according to body mass index)

Groups	Parameter (ng/ml)	Control	Patients	P
Normal weight (18.5-24.9)	OC	n=8 0.89±0.19	n=10 1.07±0.25	0.57
Overweight (25-29.9)	OC	n=16 1.00±0.23	n=36 1.77±0.24*	0.02
Obesity class I (30-34.9)	OC	n=12 1.00±0.19	n=13 1.35±0.29	0.34
Obesity class II (35-39.9)	OC	n=8 0.29±0.14	n=7 1.27±0.35*	0.03

*P<0.05 is significant. OC: Osteocalcin

Table 4: The values of reproductive hormone in control and patient with type 2 diabetes mellitus (according to body mass index)

Groups	Parameter	Control	Patient	P
Normal weight (18.5-24.9)	LH (m.lu/mL)	n=8 7.62±1.19	n=10 6.51±0.49	0.41
	FSH (m.lu/mL)	4.09±0.76	6.13±1.43	0.42
	TT (ng/ml)	3.39±0.14	3.64±0.27	0.42
	E2 (pg/mL)	16.24±3.37	17.72±3.35	0.76
	PRL (ng/ml)	15.50±2.39	7.35±1.08 ^{ab}	0.01
Overweight (25-29.9)	LH (m.lu/mL)	n=16 4.63±1.04	n=36 5.90±0.40*	0.01
	FSH (m.lu/mL)	4.40±0.43	5.46±0.45	0.10
	TT (ng/ml)	3.16±0.20	3.59±0.26	0.19
	E2 (pg/mL)	14.12±2.36	16.10±1.64	0.49
	PRL (ng/ml)	9.42±0.72	9.33±0.58 ^{ab}	0.91
Obesity Class I (30-34.9)	LH (m.lu/mL)	n=12 5.31±0.71	n=13 4.79±0.41	0.53
	FSH (m.lu/mL)	4.83±0.23	4.09±0.80	0.39
	TT (ng/ml)	2.92±0.30	3.06±0.49	0.81
	E2 (pg/mL)	12.40±2.46	22.89±3.49*	0.02
	PRL (ng/ml)	12.47±1.49	11.68±1.52 ^a	0.71
Obesity Class II (35-39.9)	LH (m.lu/mL)	n=8 5.82±0.43	n=7 6.75±0.86	0.36
	FSH (m.lu/mL)	4.95±1.22	6.14±0.87	0.42
	TT (ng/ml)	4.02±0.36	3.54±0.27	0.31
	E2 (pg/mL)	31.29±4.17	14.31±4.10*	0.02
	PRL (ng/ml)	9.54±1.03	9.83±2.31 ^{ab}	0.91

LSD (PRL)=4.33. LH: Luteinizing hormone, FSH: Follicle-stimulating hormone, TT: Total testosterone, E2: Estradiol, PRL: Prolactin, LSD: Least Significant Difference. *Significant at (P<0.05). ^{ab}Different letters refer to a significant differences among patient groups. Similar letters refer to non-significant differences among patient groups.

(4.40±0.43 m.lu/mL), obesity class I (4.83±0.23 m.lu/mL), and obesity class II (4.95±1.22 m.lu/mL) groups, respectively. There are no significant differences in the FSH value among patients of T2DM in different BMI groups.

The results showed that the TT value of patients showed no significant differences (P<0.05) in normal weight (3.64±0.27 ng/ml), overweight (3.59±0.26 ng/ml), obesity

class I (3.06±0.49 ng/ml), and obesity class II (3.54±0.27 ng/ml) when compared with controls of normal weight (3.39±0.14 ng/ml), overweight (3.16±0.20 ng/ml), obesity class I (2.92±0.30 ng/ml), and obesity class II (4.02±0.36 ng/ml). There are no significant differences in TT value among patients of T2DM in different BMI groups [Table 3].

The results showed that the value of E2 in obesity class I (22.89±3.49 pg/mL) and obesity class II (14.31±4.10 pg/mL) increases significantly (P<0.05) in comparison with control obesity class I (12.40±2.46 pg/mL) and obesity class II (31.29±4.17 pg/mL) groups. The results showed no significant differences (P<0.05) in the E2 value of the patients in normal weight (17.72±3.35 pg/mL) and overweight (16.10±1.64 pg/mL) when compared with the controls of normal weight (16.24±3.37 pg/mL) and overweight (14.12±2.36 pg/mL). There are no significant differences in the E2 value among patients of T2DM in different BMI groups [Table 3].

The value of PRL in the patients of normal weight patient (7.35±1.08 ng/ml) decreases significantly (P>0.05) in comparison the controls of normal weight (15.50±2.39 ng/ml) group. There are no significant differences (P<0.05) in the patients of overweight (9.33±0.58 ng/ml), obesity class I (11.68±1.52 ng/ml), and obesity class II (9.83±2.31 ng/ml) when compared with the controls of overweight (9.42±0.72), obesity class I (12.47±1.49 ng/ml), and obesity class II (9.54±1.03 ng/ml) groups, respectively [Table 3].

The value of PRL in the patients of obesity class I group increases significantly (P<0.05) as compared to the patients of normal weight. However, there are no significant differences (P<0.05) among patients of normal weight, overweight, and obesity class II groups. Further, there are no significant differences (P<0.05) found in comparison among patients of overweight, obesity class I, and obesity class II groups [Table 3].

DISCUSSION

The results of this study showed that the OC level increased significantly (P<0.05) in the patients with T2DM in both second and third age groups compared to the control groups and also showed that the OC level of the patients in the second and third group increases significantly compared to the patients of first group. The study by Ingram *et al.*^[22] showed that reported OC increases with age, while the result was disagreement with the study by Alfadda *et al.*^[23] on men who have T2DM aged ≥40 years, which reported that OC levels are drop in T2DM subjects. Hwang *et al.*^[24] in the study on patients aged 25–60 years reported no association between T2DM and OC.

The results showed no significant difference between patients and control in the first age group. Hwang *et al.*^[24] in the study on patients aged 25–60 years reported no association between T2DM and OC.

The results of the present study showed that the level of LH in men with T2DM in the third age group (45–49 years) increased significantly ($P < 0.05$) compared to control agreement with the studies by Ali *et al.* and Natah *et al.*^[25,26] that found the LH level increases significantly in the patients of T2DM. The results also showed that LH level in the second and third age groups of T2DM increased significantly ($P < 0.05$) compared to the first age group of T2DM; these results may be due to LH increased with age.

Davidson *et al.*^[27] reported in the study on men aged 42–93 years that LH increases with age. A Japanese study by Fukui *et al.*^[28] on men of T2DM aged 40–69 years found that the LH level increased with age. The results showed no significant difference in FSH; these results are in agreement with the study by Achemlal *et al.*^[29] that found no significant difference in the FSH level in T2DM.

The results showed TT level of the patients in the first, second, and third age groups is not significantly different ($P < 0.05$) compared with control. This result agrees with the study by Natah *et al.*^[26] which found that the TT concentration in T2DM is not significantly different compared with control. In addition, the results are in agreement with that reported by Esmaeel,^[30] which recorded no significant difference in the TT levels between ten healthy men aged 25–53 years and ten T2DM men in the same age in Babylon province, while TT of the patients with T2DM in third age group increases significantly ($P < 0.05$) compared to men with T2DM in the first age group. This result may be related to a hypothalamic defect and/or to an absence of pituitary response to GnRH agreement with Payne and O'Shaughnessy,^[31] which reported TT in males is principally synthesized in the Leydig cells, the number of Leydig cells in turn is controlled by LH and FSH, and in addition, the amount of TT generated by the present Leydig cells is under the control of LH which regulates the expression of 17-B hydroxyl steroid dehydrogenase.

In this study, the E2 level increased significantly ($P < 0.05$) when compared with control in the first group. This results may be because the enzyme aromatase, which converted TT to E2. This result is in agreement with the study by Al-Aaraji^[32] done in Baghdad on T2DM men of age 37–66 years, which reported increased E2 level in T2DM due to enzyme aromatase which associated with obesity in turn its associated with T2DM. However, in other groups, there are no significant different; this result agrees with the study by Yadav *et al.*^[33] that found in the study on men aged 45–75 years no significant difference in E2 levels in T2DM and controls and showed no correlation between E2 and HbA1c levels.

A study by Natah *et al.*^[26] found no different in the E2 concentration of T2DM in the study done in Babylon province including 120 diabetic patients with T2DM (60 males and 60 females) aged 35–65 years, most of them were on oral hypoglycemic drugs.

PRL concentration decreases significantly ($P < 0.05$) in the patients with T2DM in the first group; this result is in

agreement with the study done by Balbach *et al.*^[34] that reported decreased PRL level in T2DM.

The results of the present study showed that OC level increases significantly ($P < 0.05$) in overweight and obesity class II, in agreement with the study by Küçükler *et al.*^[35] This result may be due to insulin has an anabolic effect on osteoblasts,^[36] so our study suggests that insulin resistance causes decreased bone formation and increased bone absorption and OC released into blood.

Our results disagree with the study by Bao *et al.*^[37] who reported that serum OC concentration in the metabolic syndrome patients was significantly decreased than in control group. On the other hand, Kindblom *et al.*^[38] revealed that OC level was significantly and negatively correlated with weight, BMI, and fat mass.

Our study showed that LH concentration in patients with overweight increase significantly ($P < 0.05$) compare with overweight control group, this result agreement with the study by Dhindsa *et al.*,^[39] which reported that one-third of men with obesity and T2DM have subnormal TT concentrations and gonadotropin concentrations are inappropriately normal.

Previous study by Kapoor *et al.*^[40] showed that about one-third of T2DM men have low serum TT levels because of a high prevalence of symptomatic hypogonadism. TT levels in the obese decline with increasing body weight due to the declined levels of sex-hormone binding globulin; these hormone changes get worse in massively obese men, creating a relative hypogonadal state, which seems to be closely related to insulin resistance.^[41,42] The Leydig cell responsiveness to exogenous gonadotropin stimulation was attenuated in men with obesity and insulin resistance.^[43]

While E2 increases significantly ($P < 0.05$) in obesity class I as compared with control, this result may be due to adipose tissue that expresses the enzyme aromatase, which converts TT to E2,^[44–46] while E2 level decreases significantly in obesity class II compared with control. The study by Dhindsa *et al.*^[47] including men with T2DM reported that E2 concentrations were directly related to TT but not to BMI or age; agree with this study due to the data of our study shown declined TT in obesity class II but this declining not reach to significant ($P < 0.05$).

In obese subjects, high E2 levels may be a product of peripheral androgen aromatization; estrogen excess is one of the factors involved in the decline of LH levels in massive obesity.^[48]

A study on obese males showed that the BMI had a negative correlation with the concentration of TT and a positive correlation with E2.^[49] The most causes of reducing the concentration of TT in diabetic patients and obese people may be resulting from conversion of TT to E2 by the activities of aromatase enzyme found in adipose tissue.^[50] PRL level in normal weight decreases significantly as compared to control also; its decrease in the patients of normal weight

compared patients' groups. This result agreed with the studies by Ling *et al.* and Hogan and Stephens,^[51,52] who found that human adipose tissue produces prolactin and also expresses prolactin receptors. Brandebourg *et al.*^[53] reported that PRL increases with BMI.

PRL also has a crucial role in regulating whole-body insulin sensitivity and glucose metabolism by increasing β -cell proliferation and promoting cumulative insulin secretion.^[54,55]

CONCLUSIONS

This study revealed increasing OC level with BMI and age in the patients with T2DM and increasing E2 levels of T2DM patients with increased BMI.

Ethical clearance

The study was carried out with patients verbal and analytical approval before sample was taken. Permission to conduct this study was issued by the Health institutional; AL Sadder Teaching Hospital and Center for Endocrinology and Diabetes Specialist in Misan province/Iraq.

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Conflicts of interest

There are no conflicts of interest.

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