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THE EFFECTS OF TYPE 2 DIABETES MELLITUS ON THE LEVELS OF GLYCATED HEMOGLOBIN, TESTOSTERONE, LEPTIN AND CALCIUM IN IRAQI MALE PATIENTS

Firas Salih Abdul Hadi & Ali Shalash Sultan
College of Science, Dept. Biology, Al-Mustansiriyah University, Iraq

ABSTRACT
The present study was aimed to investigate the effects of T2DM on the levels of HbA1c, testosterone and calcium with relationship of leptin hormone on 45 diabetic male patients and 20 apparently healthy controls that conducted at Specialist Center for Endocrine and Diabetes Diseases in Baghdad province. The age of men ranged 25-54 years. The experiment was started on 1/10/2015 to 15/1/2016. The patients group was divided into two groups according to the BMI value, 25 non-obese (25.94 ±0.38) and 20 obese (33.90 ±0.64). FBG mg/dl showed highly significant (P<0.01) difference (273.16 ±17.73) and (237.05 ±14.94) in both diabetic groups in comparison with control group (81.15±2.01). HbA1% was significantly (p<0.01) high in both groups (9.49 ±0.36); (9.45 ±0.37) respectively in comparison with control group (5.00 ±0.15). Testosterone level ng/ml reduced significantly (p<0.01) in obese T2DM group (2.42 ±0.19) when compared with non-obese group (4.14 ±0.31) and control group (6.10 ±0.44). Leptin µg/l increased significantly (p<0.01) in obese group (12.39 ±1.73) in comparison with non-obese T2DM (5.79 ±1.33) and control group (4.08 ±0.25). Calcium level mmol/l showed significant (p<0.05) decreasing (2.26±0.03) in non-obese T2DM in comparison with control group (2.39±0.03) and obese T2DM (2.30±0.04) but the value was within normal range (2.0-2.6mmol/l). HbA1c% showed significant correlation (P<0.01) with FBG (r=0.81). Leptin showed positive correlation (P<0.05) with FBG (r=0.23) and with BMI (r=0.63) at (P<0.01) level, while negative correlation (P<0.01) with testosterone (r=-0.51).We concluded from our present study that higher correlation of FBG with HbA1c was observed. Testosterone level was reduced more prominent in obese–diabetic group that BMI and leptin level showed significant (P<0.01) correlation(r=0.63) and testosterone showed negative correlation (r=-0.51) with leptin.

KEYWORDS: diabetic, HbA1c, testosterone, leptin.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders characterized by a chronic hyperglycemic condition resulting from defects in insulin secretion, insulin action or both (Njolstad *et al*., 2003). That a chronic hyperglycemic condition may be due to insufficient production of insulin (type 1 diabetes) or the inability of cells to use insulin properly (type 2 diabetes) (Kothandam *et al*., 2012). The World Health Organization (WHO) estimated that in 2009, about 220 million people had diabetes globally, predicting that these numbers would rise to about 366 million by 2030 (WHO, 2009). Glycated haemoglobin (HbA1c) is correlated to glucose levels measurements obtaining that HbA1c could be used as an objective tool of glycemic control (Cramer and Pugh, 2005). One of the complications of type 2 diabetes mellitus (T2DM) is the disturbance of sexual and reproductive functions. Many studies had been shown that T2DM causing low level of testosterone (Onah *et al*., 2013; Asare-Anane *et al*., 2013 and Shahin *et al*., 2015). Testosterone is the primary male sex hormone that is vital for sustaining proper erectile function and libido. It is also critically involved in building muscle, burning fat, supporting endothelial function and bone density (Anawalt and Grant, 2003; Kelly and Jones, 2013). Male hypogonadism is a clinical condition resulting from testicular failure to produce adequate testosterone levels (Nieschlag *et al*., 2004 and Kapoor *et al*., 2006). Men with chronic condition such as T2DM, obesity, hypertension, and hyperlipidemia are more likely to have low testosterone compared to other men (Chandel *et al*., 2008). It is well established that obesity has an impact on the hypothalamus-pituitary-gonadal axis (HPG) that obesity is associated with elevated estrogen in men, activating hypothalamic estrogen receptors triggering inhibition of the HPGaxis (Wang *et al*., 2011). A study on obese males showed that the Body Mass Index (BMI) had a negative correlation with the concentration of testosterone and a positive correlation with estradiol (Vermeulen *et al*., 1993). The most causes of decreasing the level of testosterone in diabetic patients and obese individuals may be resulting from conversion of testosterone to estradiol by the actions of aromatase enzyme located in adipose tissue (Kelly and Jones, 2013). Leptin is a protein hormone composed of 167 amino acids expressed and released by adipocytes (Kumar *et al*., 2015). Lonnqvist *et al*. (1997) showed that serum leptin concentrations were increased in relation to increased body fat content. Leptin plays an important role in regulating of appetite, energy homeostasis, neuroendocrine function; it is also involved in other physiological processes including reproduction (Galic *et al*., 2010; Martins *et al*., 2012).It has been shown that spermatic cells and leydig cells in the testes express receptors for leptin (Banks *et al*., 1999). An increase in

leptin levels significantly decreases the production of testosterone from leydig cells (Katib, 2015). Pitter *et al*., (2002); Spat and Pitter, (2004) reported that the role of calcium [Ca2+] in activates the STAR protein facilitating the transport of cholesterol into the mitochondria in the leydig cells. The transported calcium into the mitochondria, enhancing the synthesis of NADH and NADPH. The latter is required by P450 for the cleavage of cholesterol to pregnenolone. Kanchana and Saikumar (2014) reported a negative correlation between serum blood glucose and serum calcium levels that is an increased serum glucose levels was associated with decreased serum calcium levels. The study aim to investigate the effects of T2DM in Iraqi male patients on the levels of glycated (HbA1c%), testosterone and calcium with relationship of leptin.

MATERIALS & METHODS

The study was carried out on (45) Iraqi males diabetic patients aged 31-54 years who visited the Specialist Center for Endocrine and Diabetes at Baghdad province, and apparently healthy control subjects with total number of (20) aged 25-50 years were included in this study and they were diagnosed according to the level of FBG and HbA1c. The study was began on 1/10/2015 to 15/1/2016. Informed consent was obtained from both patients and control group to fill the questionnaire form. Eight milliliters of blood was drawn from each individual after (12-14) hours fasting via vein puncture between (8.30–10.30 A.M). The blood sample was divided into two aliquots; 2 and 6 ml. The first aliquot blood was dispensed in a tube containing Ethylene Diamine Tetra acetic Acid (EDTA) for analysis of HbA1c, While the second aliquot was transferred into plan tubes, centrifuged at 3000 rpm for 10 minutes, sera were used for the determination of FBG, testosterone, leptin and calcium. The BMI was estimated according to (WHO, 2011) with following formula: BMI =W/H^2. FBG was estimated using an Enzymatic colorimetric method according to the kits Biosystems (Trinder, 1969). The HbA1c determination is based on the fluorescence immunoassay technology for hemolyzed whole blood in i-CHROMA^{TM}system (Brooks *et al*., 1999).The quantitative determination of total testosterone concentration in human serum by competitive enzyme-linked immunosorbent assay (ELISA) was done using Monobind Inc kit in accordance with manufacturer's instructions by method of Lashansky (1991). The quantitative measurement of leptin in serum was performed using a leptin enzyme immunoassay or ELISA kit (DRG Diagnostics, Germany), according to the manufacturer's instructions by method of Considine *et al*. (1996). Calcium was determined by using the Randox Kit according to the manufacturer's instructions by method of Sarkar and Chauhan (1967).

Statistical Analysis

The Statistical Analysis System-SAS (2012), using analysis of variance one way (ANOVA) according to Snedcor, and Cochran (1980).

RESULTS & DISCUSSION

TABLE 1: Shows the level (Mean ± SE) of FBG mg/dl; HbA1c%in the study groups

Groups / Normal Values	Parameters	
	FBG (70-110 mg/dl)	HbA1c (4.5-6.5 %)
Control	81.15 ± 2.01 b	5.00 ± 0.15 b
Diabetic: Non-obese	273.16 ± 17.73 a	9.49 ± 0.36 a
Diabetic: Obese	237.95 ± 14.94 a	9.45 ± 0.37 a
LSD value	40.687 **	0.905 **
P-value	0.0001	0.0001

** (P<0.01).

Mean having different small letter in columns are significant.

The level of FBG mg/dl was increased significantly (P<0.01) in diabetic non-obese (273.16 ± 17.73) and diabetic obese (237.95 ± 14.94) in comparison with control (81.15 ± 2.01) (Figure 1).

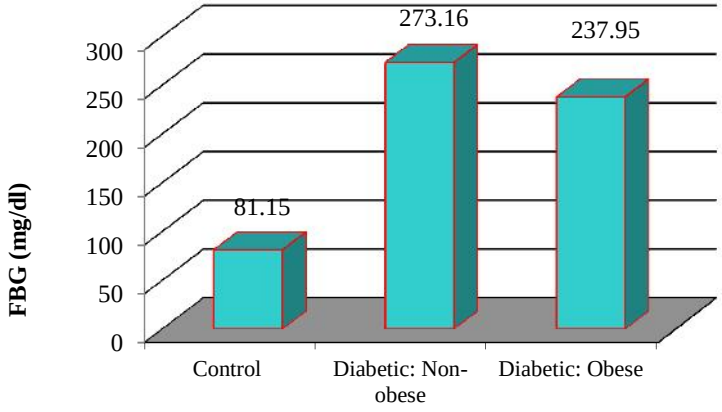

FIGURE 1: showing the levels of FBG in the study groups

The increasing in the level of FBG was in agreement with many researchers (Njostad *et al*., 2003; Hussein and Al-Qaisi, 2012) that chronic diabetes is a group of metabolic diseases characterized by hyperglycemia. The elevation in FBG level may be resulting from defects in insulin secretion, insulin action or both (ADA, 2014). FBG test is directly proportional to the severity of the diabetes mellitus (Rother, 2007; Ngugi *et al*., 2012). That diabetic (non-obese and obese) patients groups showed high level of FBG when compared with the control group,so the increase in the level of FBG in our results was also in agreement with that reported by (ADA, 2015) that diabetic patients showed FBG level ≥ 126 mg/dl. The level of HbA1c % in both diabetic groups non- obese (9.49 ± 0.36); obese (9.45 ±0.37) was increased significantly (P<0.01) in comparison with control group (5.00 ± 0.15) (Figure2).

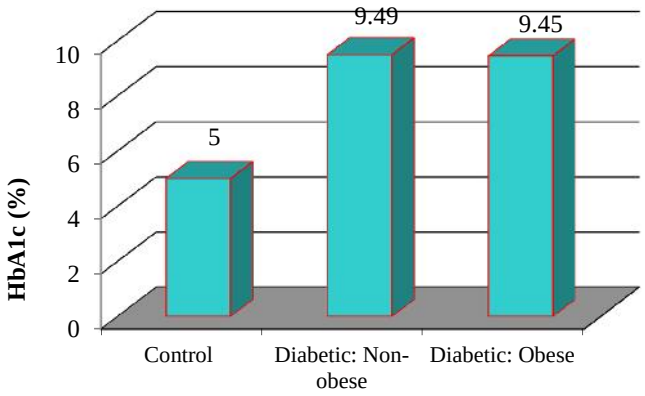

FIGURE 2: Effect of difference group in HbA1c

The rises in the level of HbA1c was associated with the increasing level of FBG in both two diabetic groups, that testing HbA1c is attracting as measures chronic glycaemia in diabetic patients. It has been used as objecting marker of average glycemic control in the monitoring of patients with diabetes (d' Emden, 2014), that the major consequences of hyperglycemia are excessive non-enzymatic glycosylation of various body proteins like hemoglobin, albumin. The significant (p<0.01) positive correlation which was found in our study between FBG and HbA1c was(r = 0.81); (Figure 3). This indicates that the higher level of FBG the higher glycosylation hemoglobin (Akinloye *et al*., 2007), and was in agreement with that reported by Ahmed *et al*., 2013 who found strong relationship between FBG and HbA1c (r = 0.55),and with that reported by Hammed *et al*., 2012 (r=0.58) in Iraqi T2DM patients.

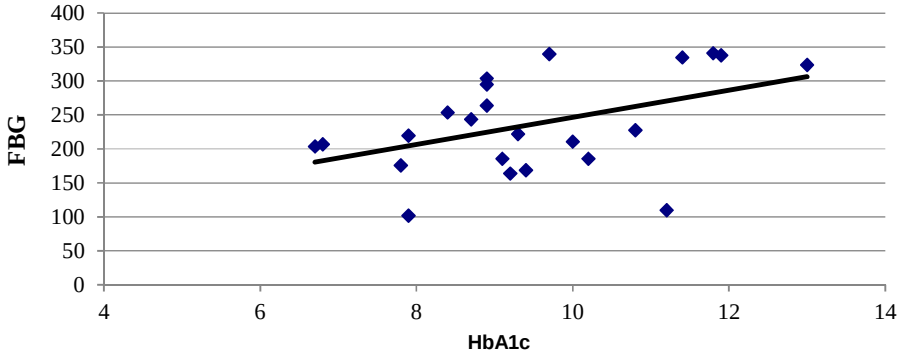

FIGURE 3: Relationship between HbA1c and FBG

TABLE 2: Shows the levels (Mean ± SE) of Testosterone ng/ml, Leptin µg/L and Calcium mmol/l in the study groups

Groups / Normal Values	Parameters		
	Testosterone (2.8-10 ng/ml)	Leptin (3.84 ± 1.79 µg/L)	Calcium (2.0-2.6 mmol/l)
Control	6.10 ± 0.44 a	4.08 ± 0.25 b	2.39 ± 0.03 a
Diabetic: Non-obese	4.14 ± 0.31 b	5.79 ± 1.33 b	2.26 ± 0.03 b
Diabetic: Obese	2.42 ± 0.19 c	12.39 ± 1.73 a	2.30 ± 0.04 ab
LSD value	0.949 **	3.661 **	0.0942 *
P-value	0.0001	0.0001	0.0272

* (P<0.05), ** (P<0.01).

Mean having different small letter in columns are significant.

The level of testosterone hormone (ng/ml) was decreased significantly (P<0.01) in diabetic non-obese (4.14 ± 0.31) but the range was within the normal value. The diabetic obese group (2.42 ± 0.19) showed highly significant (P<0.01) decrease in comparison with the control (6.10 ± 0.44)(Figure 4).

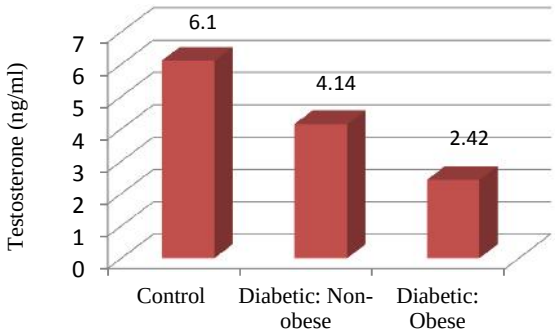

FIGURE 4. Effect of difference group in Testosterone

The decrease in the testosterone hormone level in diabetic patients groups in our study was in agreement with that reported by AL-Jaff *et al*., 2010; Onah *et al*., 2013; Asare-Anane *et al*., 2013 and Shahin *et al*., 2015. While it was disagreement with that reported by Esmaeel, (2013) who recorded non-significant in testosterone levels between ten healthy men aged (25-53 years) and ten diabetic men in the same age in Babylon province. The reduced levels of testosterone may be resulted from the conversion of testosterone to estradiol by the aromatase enzyme located in adipose tissue (Lee *et al*., 2013). Calle and Kaaks, (2004) reported that increased aromatase activity in obese males led to more androgens converting to estrogens, resulting in a higher level of estrogen and a decline of androgen in the plasma. That obesity is associated with elevated estrogen in men, activating hypothalamic estrogen receptors triggering inhibition of the hypothalamic pituitary gonadal axis (Wang *et al*., 2011). A study in 35 hypogonadism of obese men showed that the BMI had a negative correlation with the concentration of testosterone and a positive correlation with estradiol (Vermeulen *et al*., 1993). The decrease in the level of testosterone in our study in both diabetic patients may be related to the high level of leptin more effect in obese diabetic group, that leptin receptors are present on the leydig cells and when circulating leptin levels are high inhibit the testosterone level, thus the elevated leptin levels, commonly found in obese males could alter the HPG axis and contribute to the decreased testosterone production affecting luteinizing hormone(LH) and follicle stimulating hormone(FSH) release showing importance of leptin in reproductive function and have suggested direct effects of leptin is the pituitary level (Katib, 2015). Leptin also was found directly suppresses the stimulatory action of gonadotrophins on the leydig cells in the testis of 28 obese men to reduce testosterone production; therefore, elevated leptin levels in obesity may further diminish androgen status (Isidori *et al*., 1999).Leptin in our study showed negative correlation (r=-0.51) at level (P<0.01) with testosterone hormone (Figure 5).

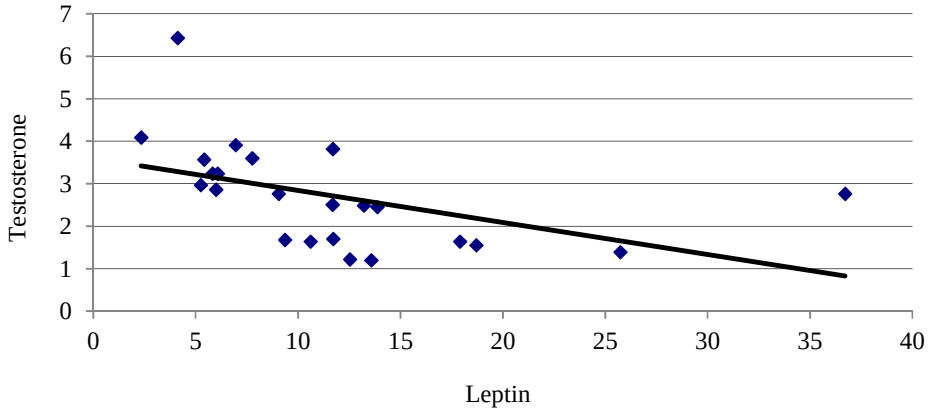

FIGURE 5. Relationship between Leptin and Testosterone

Leptin hormone level (µg//L) showed highly significantly (P<0.01) increase in diabetic obese group (12.39 ± 1.73) in comparison with control group (4.08 ± 0.25) and in diabetic non obese group (5.79 ± 1.33) respectively (Table 2); (Figure 6).

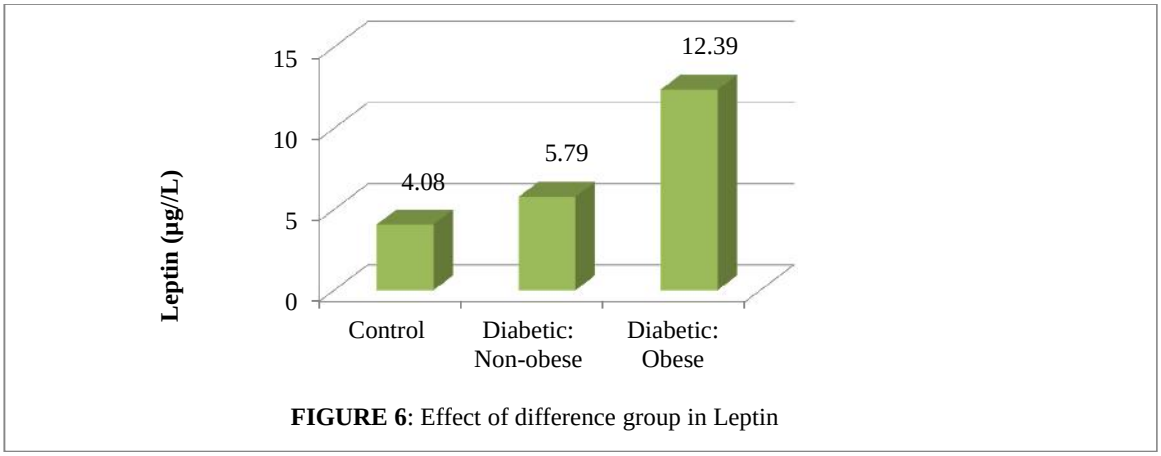

FIGURE 6: Effect of difference group in Leptin

Serum leptin levels in our results increased non significantly in non-obese T2DM and were higher in obese T2DM patients that supported by the findings of several studies (Fischer *et al*., 2002; Al-Daghri *et al*., 2003 and Kumar *et al*., 2015) who observed that elevated leptin levels could confound an association with diabetes, that leptin may play a role in the pathophysiology of diabetes possibly by suppressing insulin secretion. Leptin decrease pre-proinsulin mRNA expression in β-cells thus decreases the synthesis of insulin. It also reduces the release of insulin from human pancreatic β-cells, which leads to the development of T2DM (Peterson *et al*., 2002). Our study showed strong positive correlation (P<0.01) r=0.63 of leptin with BMI levels in obese and non-obese diabetic patients than normal healthy subjects. In spite the significant statistically decrease in the calcium level in non-obese diabetic group (2.26±0.03) in our results but it was within normal range (2.0-2.6 mmol/l) such study by Kanchana and Saikumar, (2014) reported a negative correlation between FBG and serum calcium levels that is an increased blood glucose levels was associated with decreased serum calcium levels. Mosaad *et al*., 2006 showed that the levels of zinc, calcium and magnesium decreased in the blood of both types1, 2 of DM, that might be attributed to impaired absorption and/or the excess excretion of these metals in urine (glycosuria) which may induce a deficiency or marginal state of these minerals in the blood of 55 with non-insulin-dependent diabetes mellitus (NIDDM) and from 40 with insulin-dependent Diabetes mellitus (IDDM) Egyptian patients as well as from 20 healthy volunteers. From other side, the role of calcium [Ca2+] in activates the STAR protein facilitating the transport of cholesterol into the mitochondria. The transported calcium into the mitochondria, enhancing the synthesis of NADH and NADPH. The latter is required by P450 for the cleavage of cholesterol to pregnenolone (Pitter *et al*., 2002; Sp¨at and Pitter, 2004).In this context the increase in [Ca2+]*i* has a dual stimulatory effect on steroid production: (1) acting on the supply of the main substrate for testosterone synthesis; and (2) modifying the redoxstate of the mitochondria. So the statistically decrease of testosterone (4.14 ±0.31) ng/ml in non-obese diabetic group in our study may be related in marginal decrease of calcium level in that group when compared with control group(6.10 ±0.44), while obese diabetic group didn't show decrease in calcium level (2.42 ±0.19) in that group; the mostly causes in decreasing of testosterone level in obese T2DM group may be related to the higher level of leptin (12.39 ±1.73µg/l) that associated with increasing BMI (Kumar *et al*., 2015).

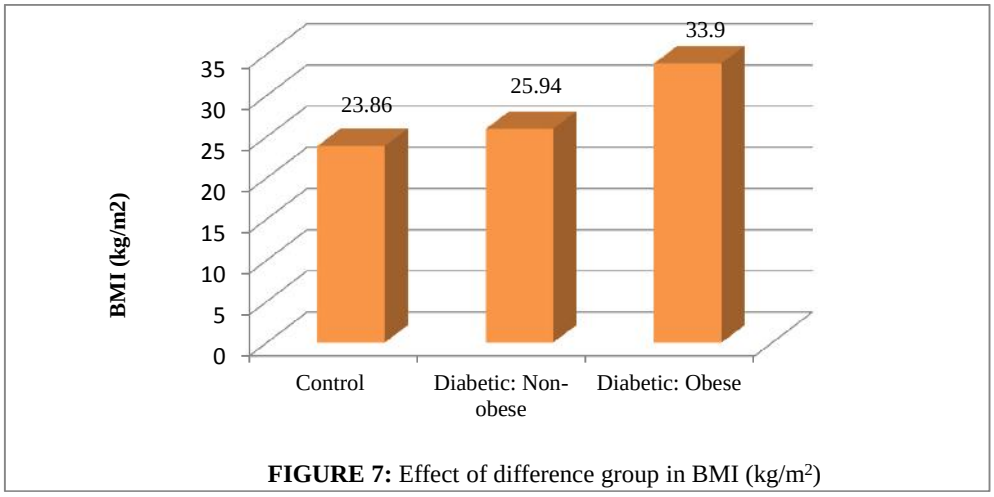

FIGURE 7: Effect of difference group in BMI (kg/m^2)

Body mass index (BMI) kg/m^2 was increased significantly (P<0.01) in diabetic non obese (25.94 ±0.38) and in diabetic obese (33.90 ±0.64) in comparison with control group (23.86 ±0.25).The diabetic obese (33.90 ±0.64)

showed highly significantly increase when compared with the diabetic non obese (25.94 ±0.38) (Figure 7).The non-obese T2DM group was over weight (BMI >25.0) while in obese T2DM group was (BMI >30) in our results; that risk of T2DM increased progressively and significantly with increasing levels of initial BMI, and also with the duration of overweight and obesity. Studies have shown the critical importance of overweight and obesity, particularly of long duration, in the development of T2DM (Rai and Jeganthan, 2013).

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