ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by Pharmascope Publications Journal Home Page: www.pharmascope.org/ijrps

Status of serum 25- Hydroxyvitamin D and Leptin in Iraqi diabetic men: Obesity-related study

Gumar O Zamil, Halla Ghazi, Basil O Saleh*

Department of Clinical Biochemistry, College of Medicine, University of Baghdad, Iraq

Article History:

Received on: 22.09.2018
Revised on: 14.12.2018
Accepted on: 16.12.2018

Keywords:

Diabetes mellitus, Obesity, Leptin, 25-hydroxyvitamin D

ABSTRACT

Several works had suggested the high occurrence of low 25-hydroxyvitamin D in type 2 diabetics and found links with central obesity and insulin resistance. Leptin has an important role in natural and acquired immunity. The present study design was aimed to evaluate leptin and 25-hydroxyvitamin D serum levels in a population of Iraqi diabetic men and to identify any correlations that may exist between these parameters and the glycemic indices. This case-control study was achieved at Department of Biochemistry, Medical School, University of Baghdad, and at El-Imam Ali Hospital, Baghdad, Iraq, from July 2017 to January 2018. It consisted of 160 men; 80 types 2 diabetics and 80 aged- and BMI matched healthy men. The patients and controls were also subdivided according to their obesity into subgroups. Investigations included serum measurements of fasting serum glucose (FSG) and lipid profile parameters by using Abbott c4000 automatic biochemical analyzer, 25-Hydroxyvitamin D, leptin and insulin by using ELISA technique in patients and healthy controls. The results revealed that the mean values of FSG of diabetic obese, diabetic overweight, and diabetic normal-BMI patients were significantly increased compared to that of healthy controls (for all; $P<0.0001$). The mean values of serum leptin of diabetic obese, diabetic overweight and diabetic normal-BMI were lower than those of healthy obese, overweight and normal-BMI, but did not reach the significant level. Serum levels of 25-hydroxyvitamin D did not observe any significant change between diabetics and healthy individuals. The findings also revealed the presence of a significant direct relationship between serum 25-hydroxyvitamin D levels and the HOMA values in the diabetic obese group ($r= 0.402$, $P<0.021$). This study concluded that obesity has a significant relation with serum levels of leptin, while the association of diabetes and vitamin D still need further investigations.

* Corresponding Author

Name: Dr. Basil O Saleh
Phone: +96-7904407625
Email: basil_omsal@yahoo.com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v10i1.1779

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INTRODUCTION

Diabetes mellitus (DM) is a complex, chronic illness requiring continuous medical care with multifactorial risk-reduction strategies beyond glycemic control to preventing acute complications and reducing the risk of long-term complications (American Diabetes Association, 2017). These complications cause vast disability, morbidity, and mortality, and even low quality of life. Diabetes is characterized by hyperglycemia and is usually divided into type 1 and type 2 (Guariguata *et al.,* 2014). Central obesity means large amounts of adipose tissues fat due to defect control in consumption and energy expenditure and is regarded as a pathological cofactor in several of chronic diseases like type 2 diabetes (Yao *et al.,* 2015). The immune

complication of obesity initiates insulin resistance by cytokines secreted by infiltrated leukocytes into adipose tissues (Grant *et al.,* 2015). The peptide leptin is predominantly produced by white adipocytes and functions in the brain to control energy production (Dardeno *et al.,* 2010). Hypothalamic centres are considered to be the site of actions of leptin in controlling body weight, temperature and energy (Patterson *et al.,* 2011).

The most common form of diabetes which is an endocrine disorder and usually presented in poor control by excessive hyperglycemia is type 2 (American Diabetes Association, 2009). Active cholecalciferol has essential functions in long glycemic regulation and consequently improvement of diabetes complications (Pittas *et al.,* 2010). The animal form of this vitamin, D_3 is synthesized in human predominantly cutaneous from 7-Dehydrocholesterol by the absorbed UVB solar radiation; 290–315 nm (Zhang *et al.,* 2015); in fact more than 80% of systemic vitamin D derives from epidermis and the other 20% is obtained from animal sources [cholecalciferol (D3)], or plant sources [ergocalciferol (D2)], and through drug supplementations (Fraser *et al.,* 2013). Vitamin D metabolism includes its hydroxylation firstly into 25 hydroxyvitamin D by hepatocytes and secondly by intact renal cells into active form, the 1,25 dihydroxy vitamin D. A number of investigations have found a link between body status of cholecalciferol and the occurrence of diabetes or impairment of glucose metabolism, and that this vitamin by its binding to its receptor (VDR) may help in obesity prevention and compilations. It modifies insulin hormone production and reduces its apoptosis in pancreatic β-cells. Active D upregulate gene expression of the insulin receptor, increasing uptake by these cells (Matyjaszek-Matuszek *et al.,* 2015). Studies have revealed that obesity is accompanied by low serum D levels (Vanlint, 2013). One of the hypotheses explained this association by sequestration of a large quantity of serum D into central adipocytes (Cheng *et al.,* 2010). Another explanation may be due to the fatty complication of obesity-linked downregulation of D production (Targher *et al.,* 2007).

The deficiency and insufficiency of vitamin D are usually attributed to nutritional factor involving UVB exposure and/or dietary availability and also its subsequent metabolism. The functional form of D may aid in upregulation of β-cell function and increasing of insulin secretion and its sensitivity (El-Fakhri *et al.,* 2014). In the state of systemic inflammation, low cholecalciferol may be a contributory factor in the development of T2DM (Nimitphong *et al.,* 2009). This essential vitamin may reduce the systemic inflammatory process by downregulation of gene expression of several pro-inflammatory cytokines, particularly nuclear factor-κB (van Etten *et al.,* 2005), which regulates the transcription of pro-inflammation cytokines included in insulin resistance (Pittas *et al.,* 2004).

SUBJECT AND METHOD

This case-control study was achieved at Biochemistry Department, Medical School, Baghdad University, at El-Imam Ali Hospital and at Research laboratory for the College of Health and Medical Technology/Baghdad, Iraq, from July 2017 to January 2018. It consisted of 160 men subjects; 80 types 2 diabetic men and 80 age- and -BMI matched normal control men. The patients and controls were also be subdivided according to their obesity into different groups: diabetic normal-BMI group which consisted of 28 diabetic men with BMI range (18.5 – < 25 kg/m^2) and age (40 – 65 years), diabetic overweight group consisted of 19 diabetic men with BMI range (25 – < 30 kg/m^2) and age (40 – 65 years), and diabetic obese group which included 33 diabetic men patients their BMI was more than 30 kg/m^2 and age (40 – 65 years).

The blood sample was taken after overnight fasting state from a peripheral vein of each patient and control. The aspirated blood sample allows clotting for 30 minutes, centrifuged at 3000 rpm. The obtained serum samples were divided into two parts; the first one used for fasting glucose and lipid components including total cholesterol, triglyceride, LDL-cholesterol, and HDL-cholesterol measurement within the same day of blood collection by Abbott c4000 automatic biochemical analyzer. The second part of serum samples were frozen at – 20 ^{0}C until the day of estimation of insulin levels using enzyme immunoassay kit (Demeditec, Germany), leptin levels by using a human leptin Demeditec ELISA kit (Germany), and 25(OH)D levels using human 25-hydroxyvitamin D ELISA kit (Human, Germany). HOMA-IR and HOMA-B were derived from fasting glucose and insulin level by using the following equations (Matthews *et al.,* 1985),

A statistical study using SPSS version 24 window was performed to indicate the presence of significant differences and correlation between the studied parameters, with the p-value of less than 0.05 was considered significant.

RESULTS

Table 1 shows the mean (±SD) values of demographic data of diabetic (obese, overweight, normal-BMI) patients and healthy (obese, overweight, normal-BMI) controls. The results revealed That the mean (±SD) values of age had no significant difference between diabetic obese (51.33±6.42 years) and healthy obese (51.53±6.20 years), diabetic overweight (50.47±6.92 years) and healthy

Table 1: Mean (±SD) Values of Clinical Data of Diabetic Patients and Healthy Controls Groups; Obese, Overweight, Normal-BMI

Parameter	D. obese (n=33)	H. obese (n=33)	D. over (n=19)	H. over (n=19)	D. norm (n=28)	H. norm (n=28)
Age (year)	51.33± 6.42^{NS}	51.53± 6.20	50.47± 6.92^{NS}	50.0± 6.05	52.82± 5.42^{NS}	52.71± 7.11
BMI (Kg/m^2)	33.78± 3.42^{NS}	33.5± 3.32	27.7± 1.19^{NS}	27.49± 1.29	23.0± 1.78^{NS}	23.11± 2.09
Waist Circumference (inch)	44.21± 3.31^{NS}	43.73± 3.16	38.47± 2.04^{NS}	37.32± 1.97	33.82± 2.23^{NS}	33.43± 2.43
Duration of DM (Months)	57.36±62.58		96.80±12.69		95.79±53.92	

D. obese: diabetic obese, H. obese: Healthy obese, D. over diabetic overweight, H. over healthy overweight, D. norm: diabetic normal weight, H. norm: healthy normal weight. NS: non-significant differences between diabetic and related healthy individuals.

Table 2: Mean (±SD) values of FSG, Insulin, HOMA-IR, and HOMA-β in Diabetic Patients and Healthy Controls Groups (Obese, Overweight, Normal-BMI)

Parameter	D. obese (n=33)	H. obese (n=33)	D. over (n=19)	H. over (n=19)	D. norm (n=28)	H. norm (n=28)
FSG (mmol/l)	10.21± 4.11$^{\bullet}$	5.32± 0.49	10.81± 4.10$^{\bullet}$	5.1± 0.57	13.92± 3.89$^{\bullet}$	4.76± 0.76
Insulin (µIU/ml)	22.12± 17.54^{NS}	22.38± 17.30	13.99± 5.31$^{\bullet\bullet}$	8.11± 3.61	7.48± 3.21^{NS}	8.22± 3.06
HOMA-IR	10.15± 9.22$^{\bullet\bullet\bullet}$	5.49± 4.61	6.92± 4.42$^{\bullet\bullet\bullet}$	1.85± 0.85	4.87± 3.90$^{\bullet\bullet\bullet}$	1.76± 0.77
HOMA-β	103.50± 107.8$^{\bullet\bullet\bullet\bullet}$	256.9± 150.0	59.42± 61.50$^{\bullet\bullet\bullet\bullet}$	120.48± 79.36	26.46± 29.80$^{\bullet\bullet\bullet\bullet}$	139.23± 67.56

D. obese: diabetic obese, H. obese: Healthy obese, D. over: diabetic overweight, H. over: healthy overweight, D. norm: diabetic normal weight, H. norm: healthy normal weight. t-test revealed; ●significant differences between diabetic and corresponding healthy individuals in FSG (p=0.0001), ●●between diabetic overweight and healthy overweight in insulin level (p=0.0001), ●●●between diabetic obese and healthy obese (p=0.012), between diabetic and corresponding healthy individuals (p=0.0001) in HOMA-IR, ●●●●between diabetic overweight and healthy overweight (),p=0.012), diabetic and corresponding normal weight (p=0.0001) in HOMA-β. NS: non-significant differences between diabetic and related healthy individuals.

overweight (50.00±6.05 years), diabetic normal-BMI (52.82±5.42 years) and healthy normal-BMI (52.71±7.11 years). Similarly, there was no significant change in waist circumference among the studied groups.

Table 2 shows the means of FSG and insulin in studied individuals. In diabetic obese, the mean of FSG levels was significantly elevated (10.21±4.11 mmol/l) when compared to that of healthy obese (5.32±0.49 mmol/l p< 0.0001). Moreover, the mean value of FSG of diabetic overweight (10.81±4.10 mmol/l) was significantly higher than in healthy overweight (5.10±0.57 mmol/l, p<0.0001). In addition, there was a significant increase of FSG mean value in diabetic normal-BMI (13.92±7.09 mmol/l) when compared to that of healthy normal-BMI (p<0.0001). The mean (±SD) value of insulin levels had no significant difference in diabetic obese (22.12±17.74 µIU/ml) compared to that of healthy obese (22.38±17.25 µIU/ml). However, the mean (±SD) value of insulin of diabetic overweight (13.99±5.31 µIU/ml, p<0.0001) was significantly increased in comparison with that of healthy overweight (8.11±3.61 µIU/ml). But, insulin mean value did not observe significant change between diabetic normal-BMI (7.48±3.21 µIU/ml) and healthy normal-BMI (8.22±3.06 µIU/ml).

Table 3 shows that the serum leptin concentration did not change significantly in diabetic obese (12.67±7.90 ng/ml) when compared to that of healthy obese (14.76±8.06 ng/ml). Also, between diabetic overweight (7.11±3.89 ng/ml) and healthy overweight (7.77±3.77 ng/ml) and between diabetic normal weight (2.80±1.67 ng/ml) and healthy normal weight (3.62±2.16 ng/ml). However, diabetic obese had significantly elevated leptin levels than that of diabetic overweight and

diabetic normal weight (p < 0.0001). Also, the mean value of serum leptin of diabetic overweight was significantly higher than that of diabetic normal weight (p < 0.006).

Table 3: Mean (±SD) Values of Leptin and 25-hydroxyvitamin D in Diabetic patients and Healthy Controls Groups (Obese, Overweight, Normal-BMI)

Parameter	D. obese (n=33)	H. obese (n=33)	D. over (n=19)	H. over (n=19)	D. norm (n=28)	H. norm (n=28)
Leptin (ng/ml)	12.67± 7.90$^{NS,\bullet}$	14.76± 8.06	7.11± 3.89$^{NS,\bullet}$	7.77± 3.77	2.80± 1.67^{NS}	3.62± 2.36
25- Hydroxyvitamin D (ng/ml)	17.22± 4.23^{NS}	19.10± 5.69	19.78± 4.84^{NS}	18.98± 5.02	17.80± 7.12^{NS}	20.31± 6.70

D. obese: diabetic obese, H. obese: Healthy obese, D. over diabetic overweight, H. over healthy overweight, D. norm: diabetic normal weight, H. norm: healthy normal weight. the t-test revealed; ● significant differences between D. obese and each of D. overweight and D. normal weight (p=0.0001), between D. overweight and normal weight (p=0.006) NS: non-significant differences between diabetic and related healthy individuals and among diabetic men.

The mean value of serum 25(OH) D in all participants of this study was 18.87 ng/ml. Mean values of serum 25-hydroxyvitamin D concentrations of diabetic obese (17.22±4.33 ng/ml), diabetic overweight (19.78±4.84 ng/ml), and diabetic normal weight (17.80±7.12 ng/ml) were comparable to those of healthy controls; obese (19.10±5.69 ng/ml), overweight (18.98±5.02 ng/ml), and normal weight (20.31±6.70 ng/ml). Also, the mean value of D concentrations of diabetic overweight was on borderline of significant increase than that of diabetic normal weight ($p < 0.054$). Figure 1 shows the significant direct relationship between HOMA-B value and serum 25-hydroxyvitamin D levels in the diabetic obese group ($r= 0.402$, $p<0.021$).

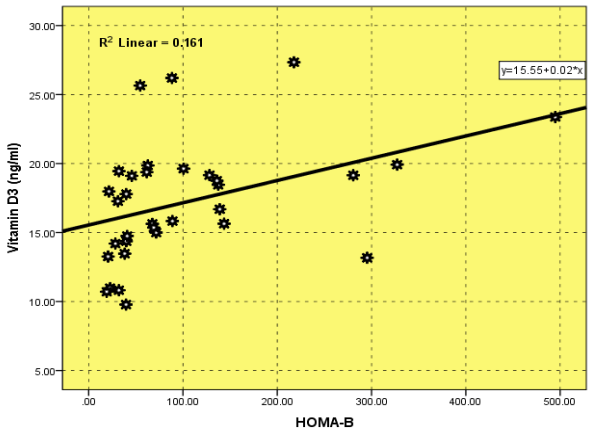

Figure 1: Correlation of serum HOMA-B with Vitamin D (r= 0.402, p<0.021) in the diabetic obese group

DISCUSSION

Also, nearly one-third of the involved Iraqi men had D insufficiency, and two third were deficient. On the basis of the Institute of Medicine (IOM) guidelines for evaluation of D status, deficient was considered when D level less than 20 ng/ml, and insufficient in a range of 21-29 ng/m (Ross *et al.,* 2011). It has been suggested that sequestration of D in excess fat body stores may decline the bioavailability of this vitamin (Holick, 2007). This relation is complex as it results in lowering leptin levels, a suppressing appetite peptide leading to unintentional weight gain and obesity (Klok *et al.,* 2007).

The present findings indicated the absence of a significant correlation between D levels and BMI in diabetics and controls. However, other studies concluded that obesity is associated with D deficiency in Iranian population (Taheri *et al.,* 2012). Vitamin D by its association with intracellular calcium both through with and without PTH may influence insulin secretion and sensitivity. Sustained and persistent elevations of cellular calcium may block insulin-target tissues from sensing the brisk intracellular calcium fluxes necessary for insulin function, like glucose transport (Pittas *et al.,* 2007). Leptin may be classified as a pro-inflammatory cytokine due to its structural similarity with cytokines, and it is proved to exert essential roles in natural and acquired immunity due to this effect (Lee *et al.,* 2006). Furthermore, leptin enhances the production of TNF and IL-6 by monocytes (Santos-Alvarez *et al.,* 1999). Pro-inflammatory cytokines like TNF-α and IL-6 were reported to be associated with insulin resistance in adipose tissue and increase resistin level (Steppan *et al.,* 2002).

Menendez *et al.,* (2001) suggested that D significantly inhibits leptin secretion by adipose tissue. Maggi *et al.,* (2014) suggested that accumulation of D in adipocytes subsequently accompanied by leptin secretion due to reducing the amount of D introduced to the liver for hydroxylation. The hypocalcemia associated with D abnormality induces secondary hyperparathyroidism which enhances lipogenesis and increased stored fat. This later factor leads to hyperleptinemia by adipocytes, stimulating FGF-23 expression in osteoblasts, the inhibitor of the 1α-hydroxylase enzyme in the kidney responsible about the production of 1,25(OH)2D (Adams *et al.,* 2010). Consequently, both obese and T2DM subjects are leptin-resistant, but it may be higher, lower, or similar in diabetics compared to BMI-matched non-diabetic controls (Katsiki *et al.,* 2011).

It is well known that in the pathogenesis of diabetes, hyperglycemia and dyslipidemia are the most important acquired factors that act additively to accelerate the disease progression. Vitamin D may improve hypertriglyceridemia through its stimulatory effect on a luminal surface coated lipoprotein lipase enzyme (Wang *et al.,* 2009). The level of peptide leptin reflects the amount of adipocyte fat. Also, leptin has pro-inflammatory property and block fat accumulation in extra adipocytes (Meek *et al.,* 2016). Permanent hyperglycemia and hyperlipidemia are linked with upregulation of lipogenesis and fatty liver and pancreatic islets damage. This later process causes endoplasmic reticulum stress and inflammation namely lipotoxicity and reduced insulin sensitivity (Kumashiro *et al.,* 2011).

CONCLUSION

The present study revealed a significant effect of obesity on leptin concentrations and minor changes of serum vitamin D. Also, diabetes mellitus has no significant effect on serum leptin and vitamin D concentrations.

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