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## Assessment of Osteocalcin Level in Association with Type 2 Diabetic Patients.

Arshad Noori Ghani Al-Dujaili\*, and Hadeel Haider Salih Al-Dujaili.

Biology Department, Faculty of sciences, University of Kufa, Iraq.

### ABSTRACT

The study is intended to assess serum levels of Osteocalcin, fasting blood glucose in Type 2 diabetic patients, also correlation between Osteocalcin and fasting blood glucose in both males and females. The study was conducted on randomly selected 65 type 2 diabetic patients (35 Females and 30 Males) attending the diabetes mellitus center in Al-Sadder Teaching City in Al- Najaf / Iraqi and a group of 24 apparently healthy subjects (12 Females and 12 Males) were included as a control group. The Study was carried out from September 2014 to February 2015. The patients' age were ranging from 35 - 64 years old. The results have indicated a significant increase ( $p < 0.05$ ) in serum FBG levels in diabetic patients in comparison with control groups. The results also have indicated a significant decrease ( $p < 0.05$ ) in serum OC levels in diabetic patients in comparison with control groups. The results also have revealed no significant differences ( $p > 0.05$ ) in serum FBG levels between males and females in both patients and control groups. The results have indicated a significant decrease ( $p < 0.05$ ) in serum OC level in females than males in both patients and control group. The results have shown significant negative correlation ( $P < 0.05$ ) between OC and FBG in (males and females), males, females DM patients. The present study has concluded that Osteocalcin were marker for detection and diagnosis of diabetic patients type 2.

**Keywords:** Fasting blood glucose (FBG), Osteocalcin (OC), Type 2 diabetic patients.

*\*Corresponding author*

## INTRODUCTION

**Diabetes Mellitus (DM)** is a group of metabolic disorders distinguished by increased levels of blood glucose caused by impaired insulin action or defect or both [1]. There are mainly three types of diabetes [2]: type 1 diabetes is immune –mediated and require daily administration of insulin, where the other common type is type 2 diabetes associated with insulin resistance or relative deficiency [3] it leads to increase the risk of macro-micro complication particularly cardiovascular disease, blindness, limb amputation and kidney failure [4,5]. Gestational diabetes occurs in pregnant women and can be developed after pregnancy into type 2 diabetes mellitus [1,6].

Type 2 diabetes is most commonly associated with obesity in middle-aged individuals. It is due to defects in insulin receptors on the plasma membrane of cells in target tissues, or an abnormal binding of insulin to receptors [7]

Cardiovascular disease is a major macro-complication in diabetic patients where the elevated of blood glucose lead to increase the risk of Ischemic Heart Disease such as (myocardial infarction, atherosclerosis, angina) [8], type 2 diabetes patients also have increased an average of blood pressure, lipid profile, and obesity, which contributes to increase their risk of cardiovascular disease[9].

**Osteocalcin (OC)** is the most abundant non-collagenous protein of bone matrix [10]. It is synthesized exclusively by osteoblasts, formed by 46 to 50 amino acids [11, 12, 13 ].

OC plays important role in inducing insulin secretion and promotes proliferation of pancreatic  $\beta$ -cells and act as hormone [14], it also acts on adipocytes to promote adiponectin secretion that reduces insulin resistance [15]. Even when on a regular diet, the Osteocalcin-deficient mouse phenotype, characterized by impaired glucose tolerance, increased visceral fat mass, elevated circulating fatty acids, and low circulating adiponectin [16]. OC has an important effect on blood vessels because it is a strong inhibitor of vascular calcification where the levels of serum Osteocalcin ware significantly decrease in patients suffering from cardiovascular disease comparing with patients not suffering from cardiovascular disease in middle-aged and elderly [17].

## MATERIALS AND METHODS

### Patients and Control Groups

The study was performed on randomly selected 65 type 2 diabetic patients (35 females and 30 males) attending the diabetes mellitus center in Al-Sadder Teaching City Al- Najaf / Iraqi . The patients' age were ranging from 35 - 64 years old. The study was carried out from September 2014 to February 2015.

Diabetes mellitus was diagnosed by consultant doctors. The information of patients were obtained through a questionnaire consisted of the name, sex, age, weight, height.

A group of 24 apparently healthy subjects (12 Males and 12 Females) were included as a control group. Type 2 diabetic patients are divided into subgroups according to the gender, ages, duration of disease and body mass index.

### Collection of Blood Samples

Five milliliters of venous blood samples were drawn by using disposable needles and plastic syringes from each patients and controls subject. Blood was left at room temperature for 10 minutes to clot, centrifuged 4000 rpm for 10 minutes, and then serum was separated and transported into new disposable tubes.

**Biochemical Measurement**

**Determination of Serum Osteocalcin Level**

Human osteocalcin / Bone gla protein (OT/BGP) ELISA Kit For the quantitative determination of human Osteocalcin concentrations in serum was supplied by CUSABIOBiotech Co., Ltd.USA-Catlog No.CSB-E05128h.

**Determination of fasting blood glucose concentration:**

Glucose kit for quantitative determination of Glucose in human serum was supplied by Bio Merieux, France.

**Statistical Analysis**

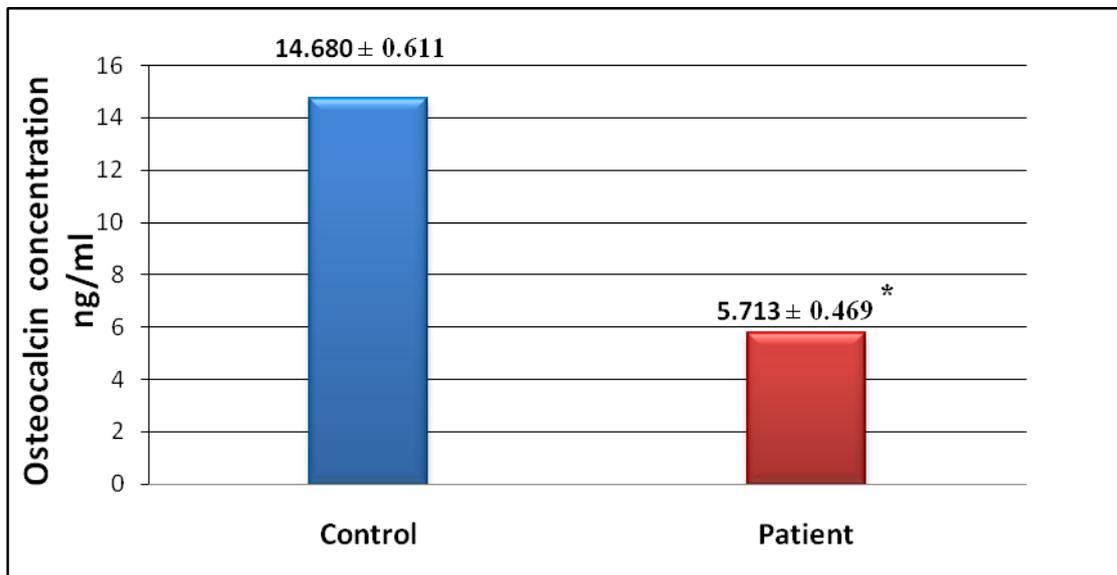
Analysis of data was performed by using megastat (Version 10.12) for excel 2007 in the home computer. The results were expressed as (mean ± standard deviation) . P-value < 0.05 was used as a level of statistically significant [18].

**RESULTS**

**Comparison Between Diabetics Mellitus Patients and Control Groups**

**Osteocalcin Level**

The results shown in Figure (1) have indicated a significant decrease (P<0.05) in serum Osteocalcin level in diabetic patients  $5.713 \pm 0.469$  ng/ml in comparison with control group  $14.680 \pm 0.611$  ng/ml.

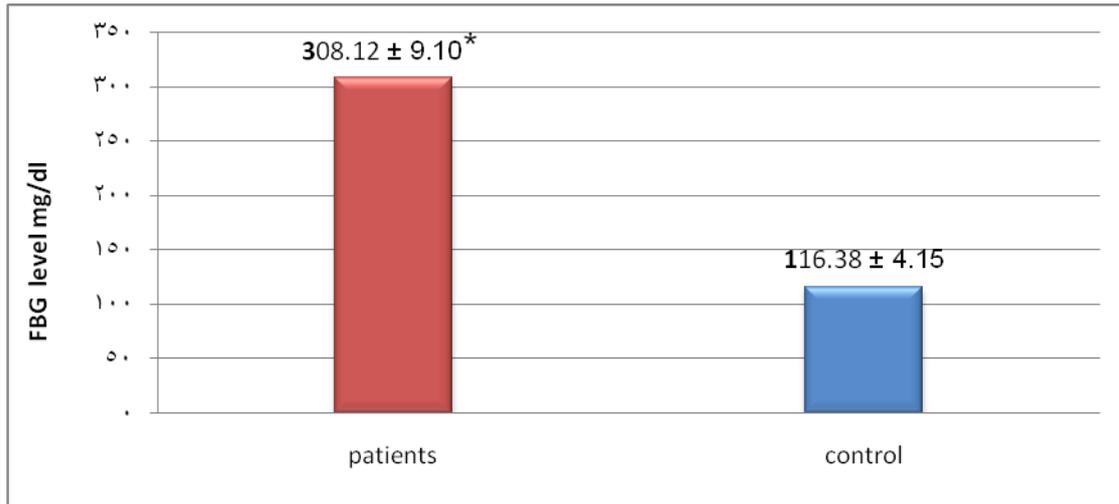


(\*)Statistically significant differences (p<0.05) between patients and control groups

Figure (1):Osteocalcin level in diabetic mellitus patients and control group.

**Fasting Blood Glucose Level**

The results shown in Figure (2) have indicated a significant increase (P<0.05) in fasting blood glucose (FBG) level in diabetic patients  $308.12 \pm 9.10$  mg/dl in comparison with control group  $116.38 \pm 4.15$ mg/dl.



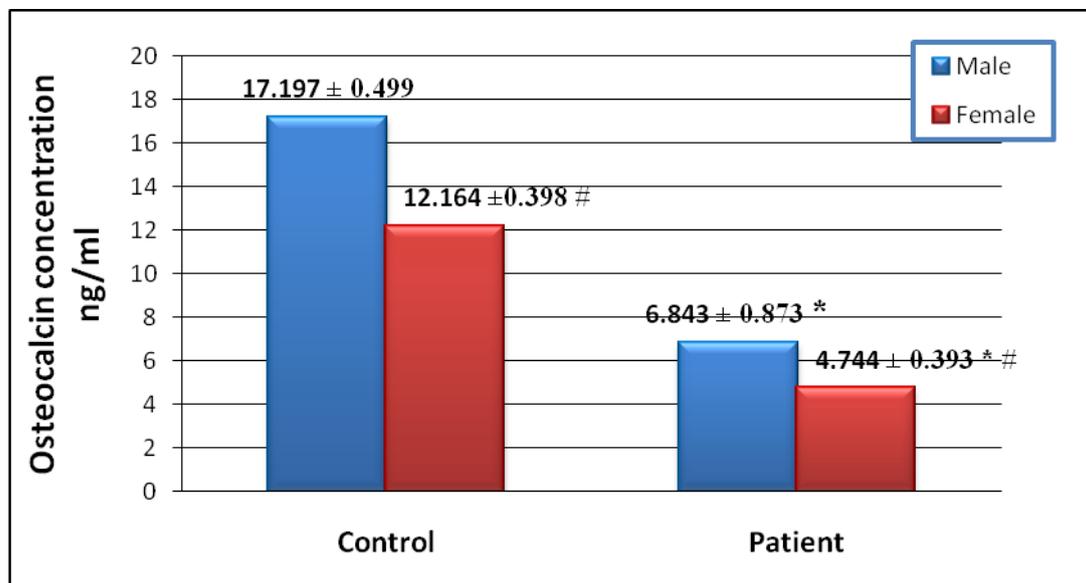
(\*)Statistically significant differences (p<0.05) between patients and control groups.

Figure (2): Fasting blood glucose level in diabetic mellitus patients and control groups.

### Comparison between Diabetic Mellitus Patients and Control Group According to Gender

#### Osteocalcin Level

The results of Figure (3) have indicated a significant decrease (p<0.05) in serum Osteocalcin level in both males and females patients groups 6.843±0.873 – 4.744±0.393 respectively in comparison with males and females of healthy groups 17.197±0.499 – 12.164±0.398 respectively, while the figure shows a significant decrease (p<0.05) in serum Osteocalcin level in females than males in both patients and control groups.



(\*)Statistically significant differences (p<0.05) between males and females patients and control groups

(#)Statistically significant differences (p<0.05) between males and females patients at same groups

Figure (3): Osteocalcin Level in Both Gender of Diabetic Mellitus Patients and Control Group.

### Correlation between Osteocalcin and Fasting Blood Glucose Levels

The results of correlation and linear regression between Osteocalcin and FBG levels in patients are indicated by :

1-The presence of a significant negative correlation ( $P < 0.05$ ) between Osteocalcin and FBG concentrations of DM patients (males and females), ( $r = -0.825$ , Osteocalcin =  $-0.043 + 19.24 \text{ FBG}$ ), (Figure 4).

2- The presence of a significant negative correlation ( $P < 0.05$ ) between Osteocalcin and FBG concentrations in females of DM patients, ( $r = -0.882$ , Osteocalcin =  $-0.025 + 12.81 \text{ FBG}$ ), (Figure 5).

3- The presence of a significant negative correlation in males DM patients between Osteocalcin and FBG concentrations ( $P < 0.05$ ), ( $r = -0.909$ , Osteocalcin =  $-0.07 + 28.62 \text{ FBG}$ ), (Figure 6).

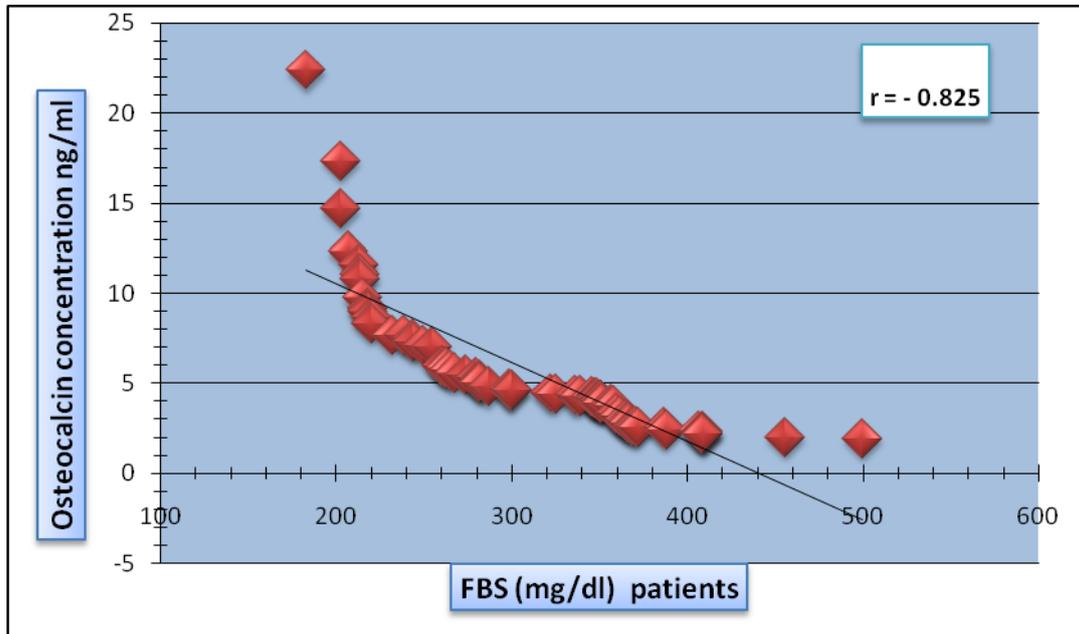


Figure (4):Correlation Between Osteocalcin and Fasting Blood Glucose in Type 2 DM Patients.

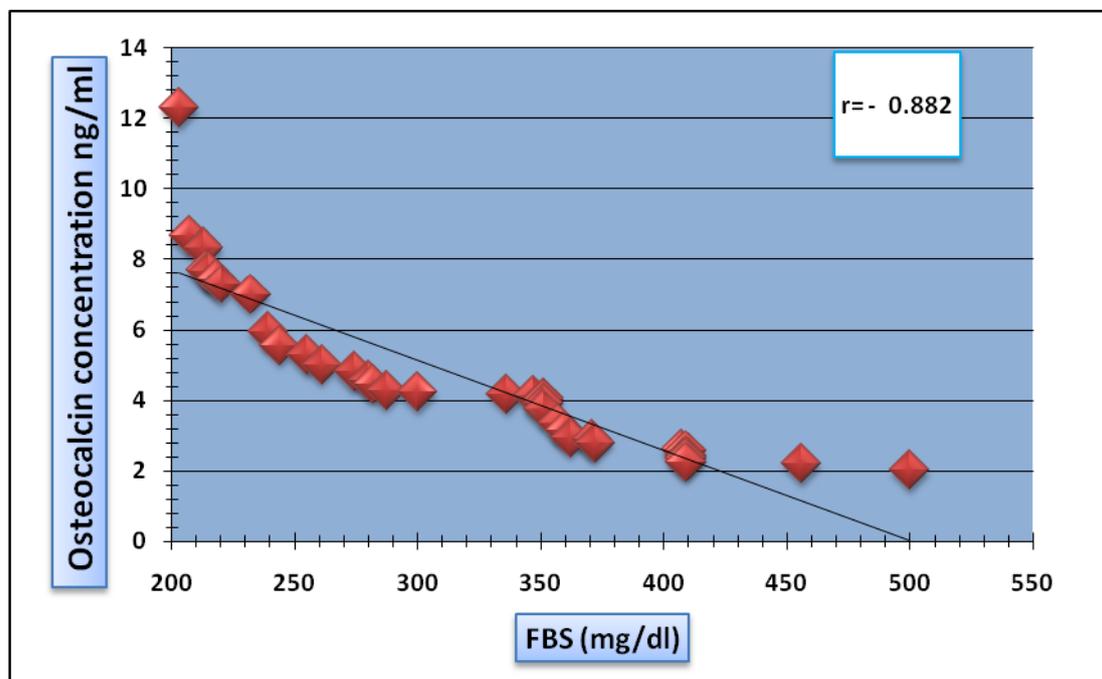


Figure (5):Correlation Between Osteocalcin and Fasting Blood Glucose in Type 2 DM Females Patients .

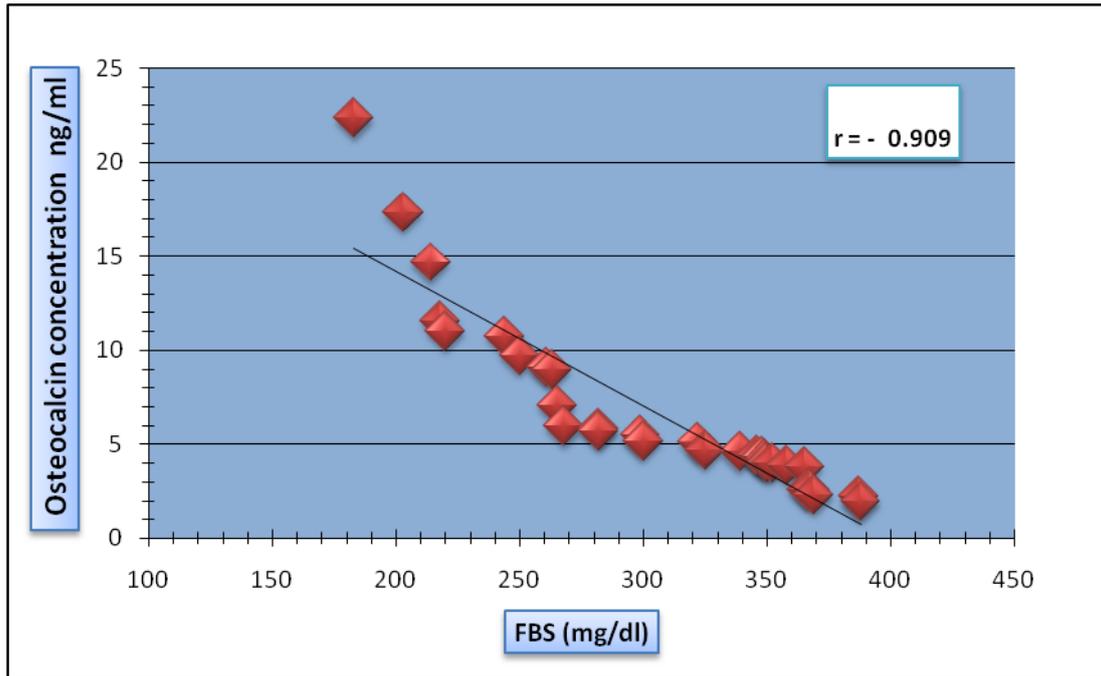


Figure (6):Correlation Between Osteocalcin and Fasting Blood Glucose in Type 2 DM Males Patients.

## DISCUSSION

### Osteocalcin Level and Fasting Blood Glucose in all Patients

#### Osteocalcin Level

The present study revealed a significant decrease ( $p < 0.05$ ) in serum Osteocalcin level in diabetic patients in comparison with control group as presented in Figure (1)

The results were agreement with [19;20]. Insulin resistance result from defect of Osteocalcin secretion [21]. In patients of Type 2 diabetes were found to significantly increased level of both IL-6 and hs-CRP. Moreover, serum Osteocalcin level was significantly and negatively associated with IL-6 and hs-CRP. Our findings are supported by some previous studies [22; 19; 23] that indicated a possible anti-inflammatory effect of Osteocalcin .

#### Fasting Blood Glucose Level

The results presents of study have indicated significant increase ( $p < 0.05$ ) in serum blood glucose concentration in comparison with control group as presented in Figure (2).

Blood glucose is tightly controlled by two key processes: insulin secretion by pancreatic  $\beta$ -cells in response to a nutrient challenge and insulin action on major target organs, i.e., skeletal muscle, liver and adipose tissue. T2DM is often associated with obesity and results from insufficient insulin production / secretion and IR [24].

The insulin in most people of diabetic patients who are unable to use its glucose for energy and due to a problem with the body insulin receptors and to problems with the chemical make up of insulin itself; and this condition is called insulin resistance [25; 26; 24].

#### Osteocalcin Level in Diabetic Patients According to Gender

The results of present study have indicated a significant decrease ( $p < 0.05$ ) in serum Osteocalcin level in females than males as presented in Figure (3)

They were in agreement with other reports like [27; 28] who refer to higher concentration of osteocalcin in children than in adults and during puberty than adult levels. Most studies show a higher concentration of Osteocalcin in adult males than in adult females. At a time of menopause former studies showed that a higher concentration of OC and ALP in white Caucasian women [29; 30]. A higher bone markers have been noted in adult males than female [31]. Steroid hormones differences have a predictor of bone markers in both gender; previous studies revealed that testosterone levels in boys are more predictor of bone formation than estradiol in girls [32 ; 33; 34].

### The Correlation Between Osteocalcin and Fasting Blood Glucose

The results of correlation represent a negative significant correlation ( $p < 0.05$ ) between Osteocalcin and fasting blood glucose.

These results are agree with [35] who showed the inversely relation between OC and higher glucose level. Recently , a negative relationship between OC and insulin resistance has been reported [19; 36]. Hwang *et al* declared that plasma OC level is inversely associated with the development of DM [20]

Glucose metabolism was an important aspect of regulation of energy expenditure and previous studies indicated that Osteocalcin was strongly related to glucose metabolism by altering pancreatic beta cell proliferation and insulin sensitivity [16; 37]. Other researchs suggest that although Osteocalcin levels are lower and bone formation is decreased in type 2 diabetes, diabetic patients are not susceptible to bone resorbation [38].

Lee *et al.* (2007) showed that gene expressions in mice with deficient Osteocalcin have decreased in a mass of beta-cell while Osteocalcin in mice engineered with increased proliferation of beta-cell and increased insulin levels were increase [16].

Recently, a novel function of the skeleton on energy metabolism have been described. OC is an osteoblast-derived hormone regulating insulin secretion, insulin sensitivity in peripheral tissues, and energy expenditure [39]. It has been established that bone is an important endocrine organ to regulate glucose/ lipid metabolism through increasing insulin secretion from the pancreas. Insulin signaling in osteoblasts regulates both bone acquisition and bone resorption [40].

Suppression of Osteocalcin secretion and synthesis with reduction in osteoblast mass function *In vivo* or *In vitro* have indicated in hyperglycemic states [41]. Other studies focusing on certain markers and using other plant extract have been conducted by other researchers. Aldujali and colleagues [44] showed that there is a significant Correlation between Cardiovascular Diseases in Obese Men with The Inflammatory Markers Dyslipidemia, C-Reactive Protein and Tumor Necrosis Factor- $\alpha$ . Further studies by Aldujali and colleagues [45,46,47] showed significant relationship Between Adipocyte Fatty Acid-Binding Protein In Obese Men With Cardiovascular Diseases and the Effect of Methanolic Leaf Extract of *Moringa oleifera* on some Biochemical Markers in obesity induced rats. On the other hand they conducted a research between the effect of Methanolic Extract of *Moringa oleifera* and Exogenous Ghrelin on Lipid Profile in Atherogenic Rats and have found a significant impact of moringa extract on the reduction of lipid profiles in Atherogenic Rats [47].

### CONCLUSIONS

Osteocalcin is a good biomarker for prediction and diagnosis for type 2 diabetes mellitus and the study also proved a negative relation between Osteocalcin and fasting blood glucose.

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