Plant Archives Vol. 19 No. 1, 2019 pp. 1199-1207 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

CORRELATION BETWEEN CALCIUM SENSING RECEPTOR WITH OTHER CALCIUM REGULATORS IN OSTEOPOROSIS AND OSTEOMALACIA PATIENTS

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Abstract

Bone turnover helps accomplish long-term correction of the extracellular calcium (Ca^{+2}) homeostasis by the actions of osteoblasts and osteoclasts. These processes are highly regulated by the actions of hormones, most prominently parathyroid hormone (PTH), the release of which is a function of the Ca^{+2}, and is regulated by the action of the Calcium sensing receptor (CaSR) in the parathyroid gland. This study was a cross sectional which included physiological and biochemical comparative between calcium regulators in bone metabolic diseases included Osteoporosis and Osteomalacia patients who conducted in Dijla for medical rehabilitation hospital in Tikrit city. The study started from January 2018 to August 2018 on study population age ranged from (18 - 68) years old. The total of subjects were 90 individuals, 30 (15 male and 15 females) individuals control group and 60 individuals patients groups which divided into two main groups: Osteoporosis and Osteomalacia (10 males and 20 females) for each group. In the present study, Ca SR levels decreased with no significant differences ($P \geq 0.05$) in osteoporosis patients whereas decreased in osteomalacia patients with highly significant differences ($Pd \leq 0.01$) except in male with no significant differences rather than control group. Osteoprotegerin (OPG) increased with highly significant differences ($Pd \leq 0.01$) in osteoporosis and osteomalacia rather than control in males and females but it decreased with no significant differences ($Pe \geq 0.05$) in male of osteoporosis patients rather than in male of control group. Sclerostin (SOST) slightly increased in osteoporosis and osteomalacia patients with no significant differences ($Pe \geq 0.05$) when compared with control groups. Decreased levels of Calcium with highly significant differences ($Pd \leq 0.01$) in osteomalacia and osteoporosis patients except in males and females of osteomalacia patients with significant differences ($Pd \leq 0.05$) when compared with control group. Phosphorous levels decreased with significant differences ($Pd \leq 0.05$) in osteoporosis and osteomalacia female patients rather than male with no significant differences ($Pe \leq 0.05$) when compared with control group. Corrected Ca levels decreased with highly significant differences ($Pd \leq 0.01$) in osteoporosis and osteomalacia patients when compared with control group. CaSR positively correlated with OPG and SOST but negativity with Calcium, Phosphorus, corrected calcium and ionized calcium, they have an important role in the regulation of calcium level at osteoporosis and osteomalacia patients by inhibition of bone turnover process.

Key words : Osteoporosis, Osteomalacia, Calcium Sensing Receptor, Osteoprotegerin, Sclerostin, Calcium, Corrected Calcium, Ionized Calcium.

Introduction

Osteoporosis (OP) is a systemic disease characterized by decreased bone mass and microstructural deterioration of bone tissue, with consequent increase of fragility and susceptibility to fracture (Garnero, 2009). When a person is affected by osteoporosis, the bone cavity spaces become larger which makes the bone less elastic and more likely to break (Datta *et al.*, 2008; Lee *et al.*, 2016). It is a progressive disease with varying stages. The average peak age of bone density is around twenty-five years old but this varies between individuals. Bone density can also vary between different genetic groups and environmental factors that may be present (Nikander *et al.,* 2010). According to data from the World Health Organization (WHO), OP affects more than 75 million people in the United States (USA), Europe and Japan, accounting for more than 8.9 million fractures annually around the world, with more

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than 4.5 million occurring in the Americas and Europe (Pinheiro *et al.*, 2010). Osteomalacia is a bone disease characterized by the inability of newly formed bone matrix to undergo mineralization, as well as low bone density. Hence, the ratio of mineralized bone to matrix is reduced and the amount of non-calcified matrix is increased (SEMT 2014; Varim *et al.*, 2016).The calcium sensing receptor (CaSR) is ~120 kDa protein, consisting of 1078 amino acid, with 612 amino acids in the extracellular domain (ECD), 250 amino acids of which comprise seven transmembrane spanning domains (TM), intracellular (ICL) and extracellular loops (ECL), and 216 amino acids of a long C-terminus cytoplasmic tail (ICD) (Ziegelstein *et al.,* 2006). In bone, CaSR inhibits osteoclast activity and stimulates osteoblast activity, causing a diminished release of Ca^{2+} (Dvorak *et al.*, 2007). Mineral bone disorders, including secondary hyperparathyroidism (SHPT), are closely associated with cardiovascular complications and are the main cause of morbidity and mortality in haemodialysis (HD) subjects (USRDS 2013). CaSR expression is decreased in human uremic parathyroid glands to nearly 60% of normal expressioná. Calcimimetics, allosteric activators of CaSR, provide an effective means of reducing parathyroid hormone (PTH) secretion in such patients 8. Serum PTH concentrations and other features of SHPT (Yokoyama *et al.*, 2002; Debelle *et al.*, 2013). Osteoprotegerin (OPG) is expressed as a circulating glycoprotein of 401 amino acids with seven structural domains. Among them, domain 7 contains a heparin-binding region as well as the free cysteine residue that is required for disulphide bond formation and allows OPG to interact and combine with another molecule of OPG to form a dimeric ligand. Therefore, circulating OPG can be found either as a free monomer of 60 kD or as a disulphide bond-linked homodimer form of 120 kD, which is usually biologically more active than the monomeric one. Moreover, OPG can also circulate while bound to its ligands, which are RANKL and TRAIL (Stella *et al.*, 2017). OPG is a protein that belongs to the tumor necrosis factor (TNF) superfamily, which was identified by three independent groups (Hofbauer and Schoppet, 2004). OPG plays a critical role in the regulation of bone turnover. OPG specifically inhibits osteoclastic bone resorption and vascular calcification by interfering with binding of the RANK ligand to Rank, as well as promotes the survival of endothelial cells (Morony *et al.,* 2008; Kim *et al.,* 2016). Sclerostin (SOST) is a 190-residue with a molecular weight approximately 22.5 kDa secreted glycoprotein that is predicted to contain a cysteine-knot motif and is a member of the DAN/Cerberus protein family (Veverka *et al.,* 2009).The observable variability of serum sclerostin levels affects BMD of both cortical and cancellous bone in the general population (Sapir-Koren and Livshits, 2014). The molecular effect of sclerostin levels and BMD were studied by Reppe *et al.,* (2015), they showed that the genetic and epigenetic changes in SOST influence its mRNA expression in bone and serum sclerostin levels in postmenopausal women. Body Ca exists in two major compartments: skeleton (99%) and extracellular fluid (1%). Total blood Ca in the extracellular fluid is present in three forms in equilibrium with one another; ionized Ca (Ca^{+2}) represents about 50-65% of total Ca, Ca bound to plasma protein mainly albumin (Alb) represents about 30-45% of total Ca and Ca complex with an ion as citrate represents about 5-10% of total Ca (Beckett *et al.,* 2005).

Materials and methods

This was a cross sectional study which was conducted in Dijla for medical rehabilitation hospital in Tikrit city. The study started from January 2018 to August 2018 on study population age ranged from (18- 68) years old The total subjects were 90 individuals (60 bone diseases patients and 30 control) had hormonal assay (calcium sensing receptor, Osteoprotegren and Sclerostin by using Enzyme Linked Immunosorbent Assay (ELISA) kits (Sunlong company) and biochemical assay calcium, phosphorus, Ionized Calcium and corrected calcium by spectrophotometer using biolabo kits, who agreed to participate in the study were recruited and separated to three groups as following:

Osteoporosis Group

Included a total of 30 patients (10 males and 20 females); their ages ranged from 30-68 years. These group was diagnosed by measured the Bone Mineral Density (BMD) obtained at the lumbar spine (L2-L4) and right and left femurs, using dual energy X-ray absorptiometry (DXA) machine (Dexxum) in Dijla for medical rehabilitation Hospital.

Osteomalacia Group

This group consist of 30 osteomalacia patients (10 males and 20 females) which aged range of (66-18).

Control Group :

It included a total of 30 subjects (15 males and 15 females); their ages ranged from 18-68 years.They were apparently healthy.

The statistical analysis was carried out by using statistical program (SPS, 2001) and comparison between groups which were made by using one-way analysis of variance (ANOVA), and tried out the arithmetic means for parameters by using test of Duncan multiple ranges. Correlation coefficient (r) between CaSR and other

parameters was reported by using regression plots. The level of statistical significance was taken at (P≥0.05).

Results

The mean ± SD of CaSR levels for total, males and females of osteoporosis patients were (168.3±29.5, 163.6±23.9, 170.7±32.2) pg/ml, osteomalacia patients (138.1±34.4, 160.5±36.7, 126.9±27.9) pg/ml and control (178.5±43.7, 181.4±46.2, 175.6±42.5) pg/ml respectively as shown in table 1 and fig. 1, while the mean ± SD of OPG levels for total, males and females of osteoporosis patients were (225.3±103.9, 163.7±63.8, 256.1±107.4) pg/dl, osteomalacia patients (310.9±88.5, 358.8±33.0, 286.9±98.1) pg/dl and control (160.7±103.4, 202.8±96.5, 118.6±95.1) pg/dl respectively as shown in table (1) and fig. 2, but the mean ± SD of SOST levels for total, males and females of osteoporosis patients were (23.9±8.9, 25.1±10.4, 23.4±8.3) ng/ml, osteomalacia patients (23.5±7.2, 27.3±6.7, 21.7±6.8) ng/ml and control (22.6±5.0, 24.1±4.3, 21.0±5.3) ng/ml respectively as shown in table 1 and fig. 3, and the mean ± SD of Calcium levels for total, males and females of osteoporosis patients were (8.8±0.42, 8.7±0.38, 8.7±0.45) mg/dl, osteomalacia patients (7.9±0.73, 7.7±0.74, 7.9±0.73) mg/dl and control (9.2±0.59, 9.3±0.60, 9.1±0.57) mg/dl respectively as shown in table 2, but the mean ± SD of Phosphorous levels for total, males and females of osteoporosis patients were (3.8±0.14, 3.8±0.51, 3.8±0.51) mg/dl, osteomalacia patients (3.6±0.69, 3.5±0.73, 3.7±0.69) mg/dl and control (4.0±0.49, 3.9±0.53, 4.1±0.48) mg/dl respectively as shown in table 2, while the mean ± SD of Corrected Ca levels for total, male and female of osteoporosis patients were (8.17±0.629, 8.34±0.416, 7.99±0.762) mg/dl, osteomalacia patients (7.73±1.19, 7.67±1.39, 7.80±1.00) mg/dl and control (9.03±0.958, 9.03±0.678, 8.96±1.19) mg/dl respectively as shown in table 2, and the mean±SD of iCa levels for total, male and female of osteoporosis patients were (4.44±0.251, 4.44±0.260, 4.44±0.253) mg/dl, osteomalacia patients (3.96±0.369, 3.84±0.349, 4.03±0.371) mg/dl and control (4.56±0.336, 4.57±0.301, 4.55±0.375)mg/dl respectively as shown in table 2.

In osteoporosis patients, there was a positive correlation between CaSR and (OPG and SOST) with correlation coefficient (r=0.05 and 0.286) respectively that showed by regression plots in figures (4 and 5), but there was a negative correlation between CaSR and (Ca,

Table 1 : Levels of Calcium regulators in bone diseases patients and control.

Group	Mean ± S.D. Of CaSR	P value	Mean ± S.D. Of OPG	P value	Mean ± S.D. Of SOST	P value
Control Total	178.5±43.7		160.7±103.4		22.6±5.0	
Male	181.4±46.2		202.8±96.5		24.1±4.3	
Female	175.6±42.5		118.6±95.1		21.0±5.3	
Osteoporosis Total	168.3±29.5	Pe"0.05	225.3±103.9	Pd"0.05	23.9±8.9	Pe"0.05
Male	163.6±23.9	Pe"0.05	163.7±63.8	Pe"0.05	25.1±10.4	Pe"0.05
Female	170.7±32.2	Pe"0.05	256.1±107.4	Pd"0.01	23.4±8.3	Pe"0.05
Osteomalacia Total	138.1±34.4	Pd"0.01	310.9±88.5	Pd"0.01	23.5±7.2	Pe"0.05
Male	160.5±36.7	Pe"0.05	358.8±33.0	Pd"0.01	27.3±6.7	Pe"0.05
Female	126.9±27.9	Pd"0.01	286.9±98.1	Pd"0.01	21.7±6.8	Pe"0.05

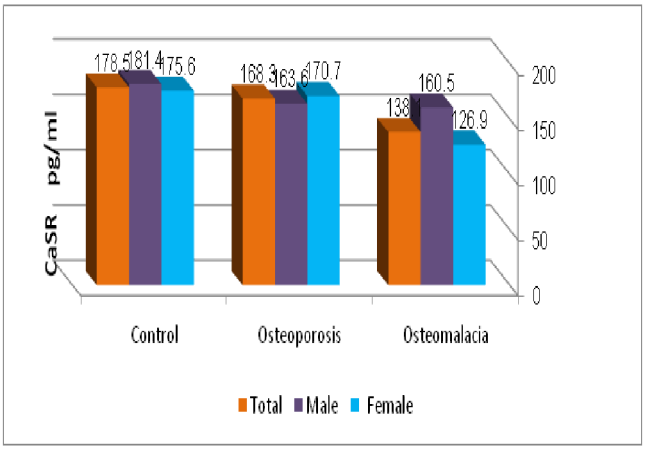

Fig. 1 : Levels of CaSR (pg/ ml) in bone diseases patients and control

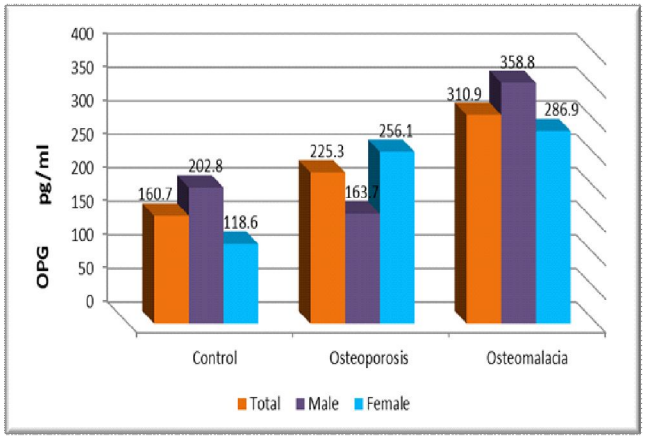

Fig. 2 : Levels of Osteoprotegren (pg/ dl) in bone diseases patients and control

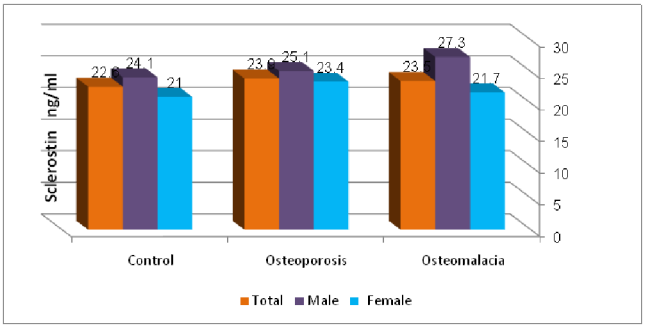

Fig. 3 : Levels of Sclerostin (ng/ml) in bone diseases patients and control

correlation coefficient (r= 0.361 and 0.397) respectively that showed by regression plots in fig. (10 and 11), but there was a negative correlation between CaSR and (Ca, Po4, corr.ca and ica) with correlation coefficient (r= -0.184, -0.019, -0.276 and -0.295) respectively that showed by regression plots in fig. (12, 13, 14 and 15).

Discussion

In the present study, CaSR levels decreased with no significant differences (Pe≤0.05) in osteoporosis patients whereas decreased in osteomalacia patients with highly significant differences (Pd≥0.01) except in male with no significant differences rather than control group. These results are agreed with Cetani *et al.,* (2003), Nisio *et al.,* (2018).

Table 2 : Levels of Total Calcium, Phosphorus, corrected calcium and ionized calcium in bone diseases patients and control.

Groups	Osteomalacia			Osteoporosis			Control		
	Total	M	F	Total	M	F	Total	M	F
No. of Individuals	30	10	20	30	10	20	30	15	15
Mean ± S.D. Of Calcium (mg/dl)	7.9±0.73	7.7±0.74	7.9±0.73	8.7±0.45	8.7±0.38	8.8±0.42	9.1±0.57	9.3±0.60	9.2±0.59
P Value	d”0.01	d”0.01	d”0.01	d”0.05	d”0.05	d”0.01			
Mean ± S.D. Of PO^4 (mg/dl)	3.7±0.69	3.5±0.73	3.6±0.69	3.8±0.51	3.8±0.51	3.8±0.14	4.1±0.48	3.9±0.53	4.0±0.49
P Value	e”0.05	e”0.05	d”0.05	e”0.05	e”0.05	d”0.05			
Mean ± S.D. of Corrected Calcium (mg/dl)	7.67±1.39	7.80±1.00	7.73±1.19	7.99±0.762	8.34±0.416	8.17±0.629	8.96±1.19	9.03±0.678	9.03±0.958
P Value	d”0.01	d”0.01	d”0.01	d”0.01	d”0.01	d”0.01			
Mean ± S.D. Of Ionized Calcium (mg/dl)	4.03±0.371	3.84±0.349	3.96±0.369	4.44±0.253	4.44±0.253	4.44±0.251	4.55±0.375	4.57±0.301	4.56±0.336
P Value	Pd”0.01	Pd”0.01	Pd”0.01	Pe”0.05	Pe”0.05	Pe”0.05			

M= Males, F= Females and PO_4 = phosphorus

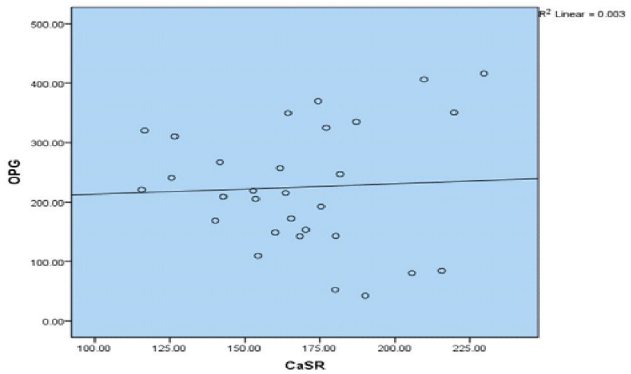

Fig. 4 : Correlation between CaSR with OPG in osteoporosis patients.

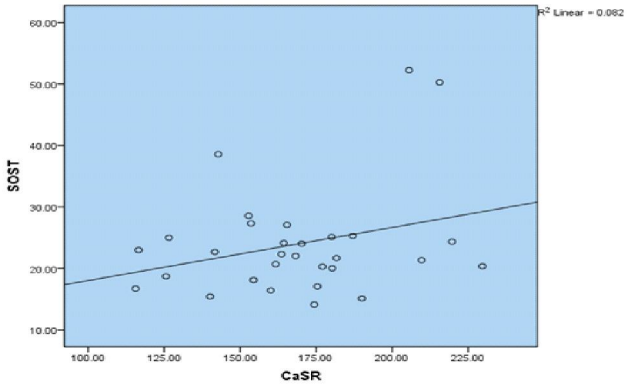

Fig. 5 : Correlation between CaSR with SOST in osteoporosis patients.

Po4, corr.ca and ica) with correlation coefficient (r=-0.238, -0.422, -0.245 and -0.242) respectively that showed by regression plots in fig. (6, 7, 8 and 9).

In osteomalacia patients, there was a positive correlation between CaSR and (OPG and SOST) with

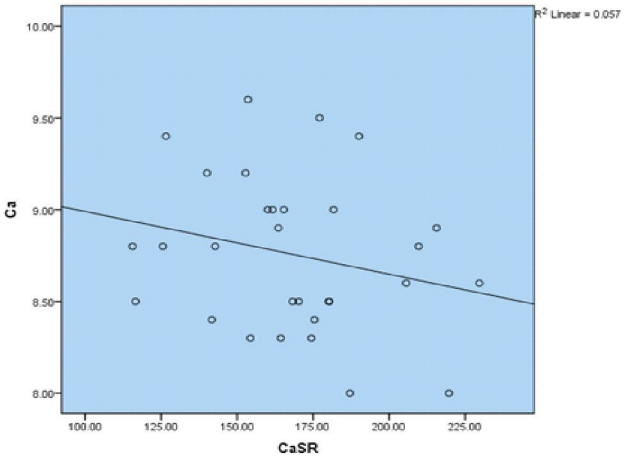

Fig. 6 : Correlation between CaSR with Ca. in osteoporosis patients.

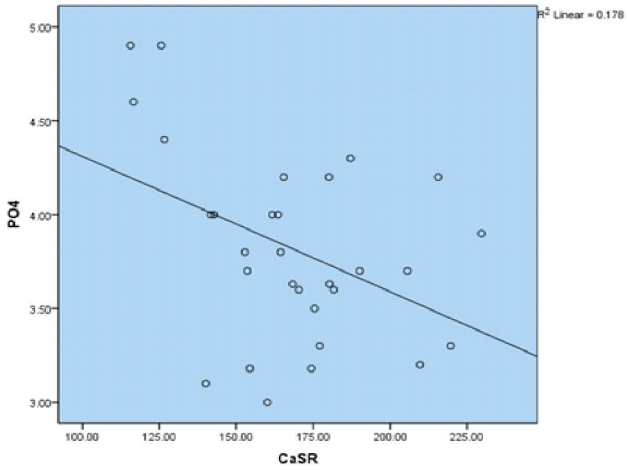

Fig. 7 : Correlation between CaSR with PO4 in osteoporosis patients.

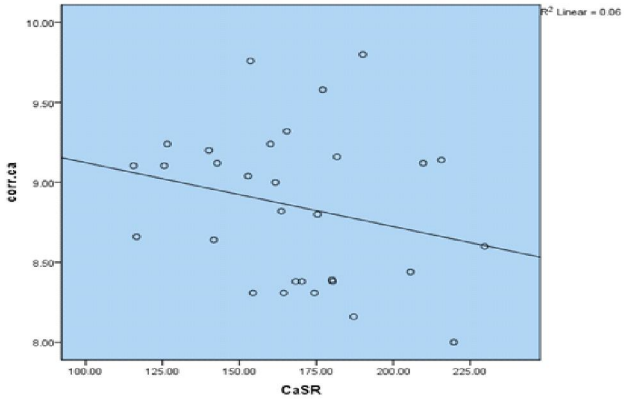

Fig. 8 : Correlation between CaSR with Corr.ca in osteoporosis patients.

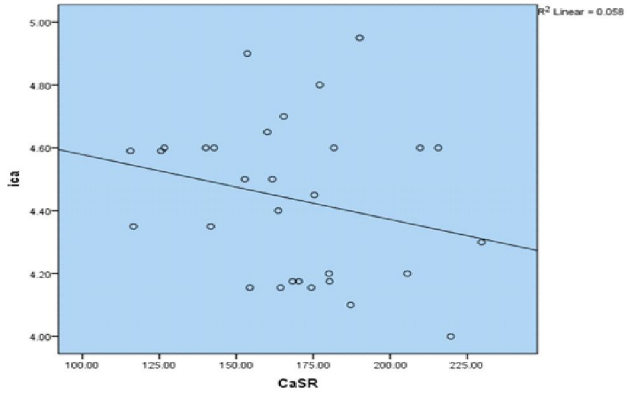

Fig. 9 : Correlation between CaSR with ica. in osteoporosis patients.

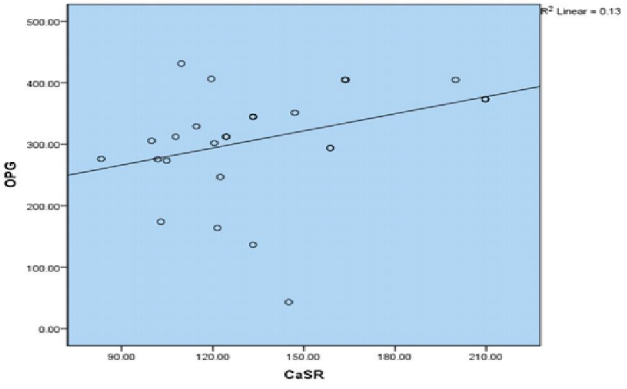

Fig. 10 : Correlation between CaSR with OPG in osteomalacia patients.

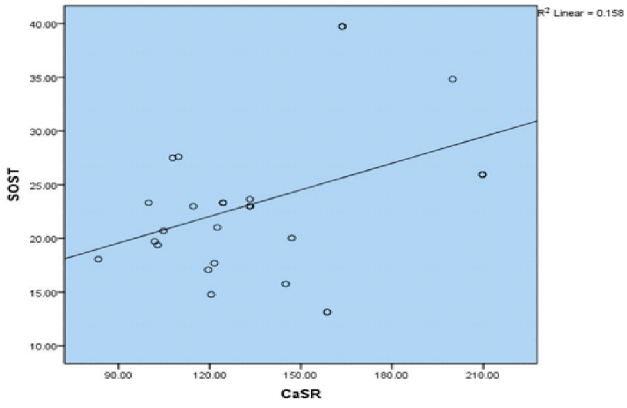

Fig. 11 : Correlation between CaSR with SOST in osteomalacia patients.

CaSR, a G-protein coupled receptor activated by Ca^{2+}, is abundantly expressed in the parathyroid glands, bone and kidneys. In the parathyroid glands, CaSR expression is upregulated by vitamin D, and CaSR activation inhibits synthesis and secretion of PTH. In bone, CaSR inhibits osteoclast activity and stimulates osteoblast activity, causing a diminished release of Ca^{2+} (Dvorak *et al.,* 2007; Tejwani and Qian, 2013).

OPG increased with highly significant differences (Pd≤0.01) in osteoporosis and osteomalacia rather than control in male and female but it decreased with no significant differences (Pe”0.05) in male of osteoporosis patients rather than in male of other group in compare with male in control group. These results were agreed with Zhneg *et al.,* (2012) and Kim *et al.,* (2016), but no

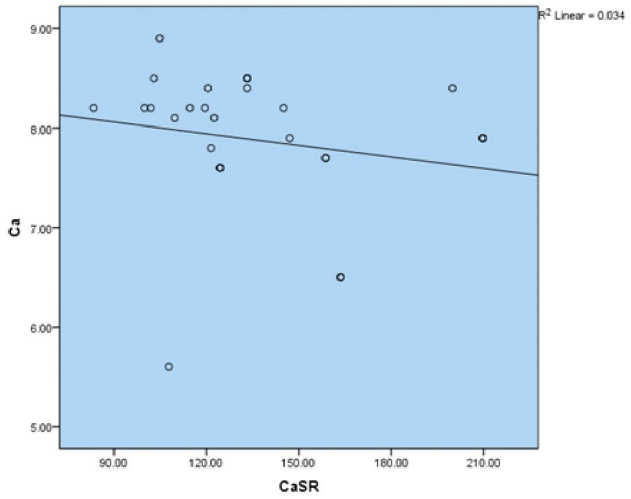

Fig. 12 : Correlation between CaSR with ca. in osteomalacia patients.

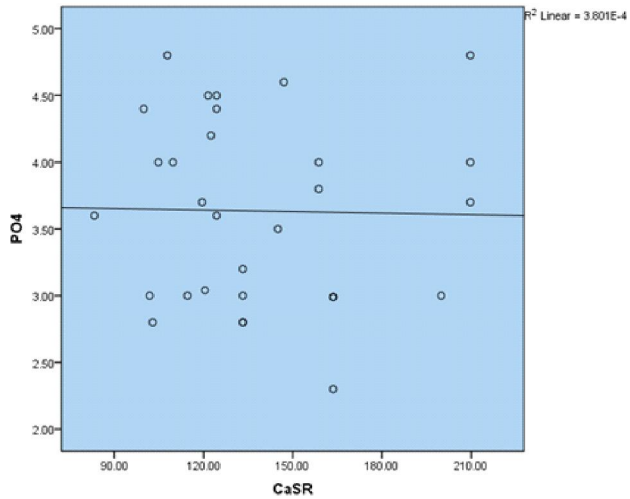

Fig. 13 : Correlation between CaSR with PO4 in osteomalacia patients.

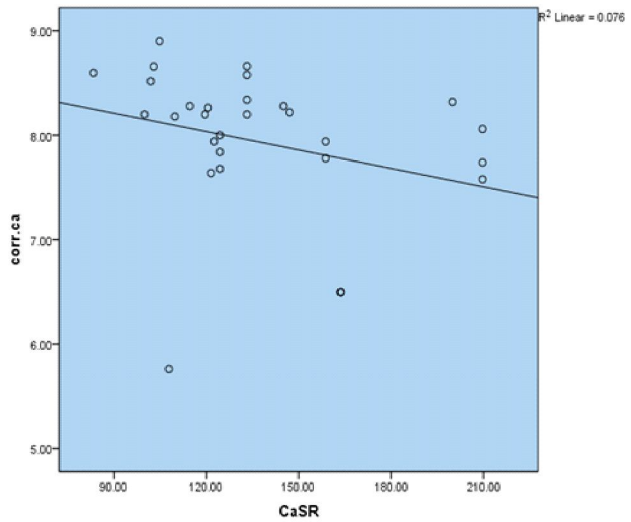

Fig. 14 : Correlation between CaSR with corr.ca in osteomalacia patients.

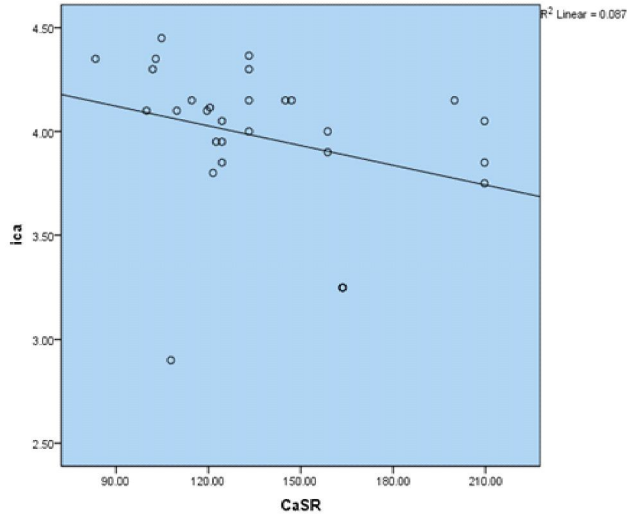

Fig. 15 : Correlation between CaSR with ica. in osteomalacia patients.

researches disagreed with our findings.

Jabbar*etal.,* (2011) suggested that in postmenopausal women with osteoporosis, the plasma OPG levels were inversely related to spine and femoral neck BMDs even after adjustment, and were shown to contribute to the development of osteoporosis. In addition, serum OPG levels were negatively correlated with lumbar spine and femoral neck BMDs for middle-aged men (Oh *et al.,* 2005). However, higher levels of OPG were associated with higher BMDs at the lumbar spine, femoral neck, and total hip for women using estrogen, but not for non-users; and higher levels of OPG were associated with higher BMD at the lumbar spine for men (Stern *et al.*, 2007; Bucur *et al.*, 2015).

The elevated level of OPG theoretically should be a protective factor for bone metabolism; however, conflicting results were produced. So Jian-Qing *et al.,* (2011), speculated that the increasing of OPG should be a compensatory response to counteract the bone loss.

In the present study, SOST slightly increased in bone diseases groups included osteoporosis and osteomalacia patients with no significant differences (Pe”0.05) when compared with control groups. In the current results of SOST agreed with Ardawi *et al.* (2011), Deveci *et al.,* (2018).

Sclerostin is a potent negative regulator of bone formation that mediates its effects by inhibiting canonical Wnt signaling pathways (Li *et al.,* 2005; Poole *et al.,* 2005).

Based on its known actions on bone, (Monroe *et al.*, 2012) decreases in sclerostin production would be expected to increase bone formation rates because loss or inhibition of sclerostin is associated with increased bone mass in both animal models and in humans (Balemans *et*

al., 2002 ; Li *et al.,* 2008). Many studies (Mirza *et al.*, 2010) and others (Mödder *et al.*, 2011; Ardawi *et al.*, 2011) have compared the serum sclerostin levels of women premenopause and postmenopause and found that postmenopausal women have significantly higher levels. In addition, there is a negative correlation between age and serum sclerostin that occurs in both premenopausal and postmenopausal women (Mödder *et al.*, 2011; Ardawi *et al.,* 2011). These authors also showed that treatment of postmenopausal women with estrogen for 4 months decreased sclerostin levels in bone marrow plasma, which is a better measure of sclerostin levels in the bone microenvironment. The published data in humans examining levels of sclerostin in serum and bone marrow plasma argue that estrogen inhibits sclerostin production and/or enhances its degradation or clearance (Mödder and colleagues, 2011).

Expression of sclerostin by osteocytes is regulated by mechanical forces and hormones that are known to affect bone metabolism such as parathyroid hormone, calcitonin and glucocorticoids (Sims and Chia, 2012). Studies in vitro and in animal models have shown that parathyroid hormone inhibits the expression of the SOST gene by osteocytes (Bellido *et al.*, 2005). In line with the *in vivo* studies, patients with primary hyperparathyroidism due to chronic elevation of PTH have significantly lower serum sclerostin levels compared with patients who have undergone parathyroidectomy and have normal PTH concentrations, thus confirming the down-regulation of the SOST gene by PTH in humans (Van Lierop *et al.*, 2010). Calcitonin, on the other hand, which inhibits osteoclast resorption, up-regulates sclerostin expression by osteocytes, while it decreases other osteocyte products such as MEPE and DMP (Gooi *et al.*, 2010; Sims and Chia, 2012).

As serum calcium is homeostatically controlled and the integrity of bone may be sacrified to maintain serum calcium within the normal range (Tai *et al.*, 2015). Consequently, serum calcium is a poor predictor of histological features and is not indicative of bone resorption in osteoporotic patient (Harvey *et al.,* 2017).

In most cases, the diagnosis of osteomalacia is suspected by the clinical history and by abnormalities in biochemical tests such as low values of serum and urinary calcium, serum phosphate and 25-hydroxyvitamin D, and high values for alkaline phosphatase and parathyroid hormone (Crandall *et al.,* 2016).

The deficiency in calcium and phosphorous may lead to lowering of formation of hydroxyapatite crystals in osteoporotic women. This as the bone mineralization is reduced, fee osteocalcin may be observed in the blood which explains its high concentration in the serum of post-menopausal women, thereby making it one of the important markers of osteoporosis (Bristow *et al.,* 2014; Kopecky *et al.,* 2016).

Use of albumin-corrected calcium concentrations may lead to inappropriate clinical decisions with withdrawal of vitamin D, calcium containing phosphate binders and reduction of calcium concentration of a patient classiûed as hypercalcaemic (Gøransson *et al.,* 2005).

However, measuring a serum calcium level is not as easy as it may first seem; while total serum calcium (TCa) can be measured, it is the ionized fraction which is not protein bound that is biologically active. Ionized calcium (iCa^{2+}) is neither easily nor routinely measured in all laboratories. Thus a number of formulae have been derived to estimate the iCa^{2+} or the 'corrected' total calcium (TCa corr.) from TCa (Mittal *et al.,* 2017).

A significant relationship between albumin and serum calcium has been reported. There is an inverse relationship between the amount of calcium bound to non-albumin proteins and serum albumin levels. Specifically, there is a greater proportion of calcium bound to non-albumin binding proteins as albumin levels fall (Jain *et al.,* 2008).

Conclusions

OPG and sclerostin, are affected in the presence of Osteoporosis and Osteomalacia, and the CaSR positively correlated with OPG and SOST but negativity with Calcium, Phosphorus, corrected calcium and ionized calcium, they have an important role in the regulation of calcium level at osteoporosis and osteomalacia patients by inhibition of bone turnover process.

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