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 \(>\) 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. Osteoprotegerin (OPG) increased with highly significant differences (Pd \(<\) 0.01) in osteoporosis and osteomalacia rather than control in males and females but it decreased with no significant differences (P\(\geq\)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 (P\(\geq\)0.05) when compared with control groups. Decreased levels of Calcium with highly significant differences (Pd \(<\) 0.01) in osteomalacia and osteoporosis patients except in males and females of osteomalacia patients with significant differences (Pd \(<\) 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 (Pd \(\geq\)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

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(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
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 expression. 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, Osteoprotegen 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.

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

**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.

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Osteomalacia</th>
<th>Osteoporosis</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>No. of Individuals</td>
<td>30</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>7.9±0.73</td>
<td>7.7±0.74</td>
<td>7.9±0.73</td>
</tr>
<tr>
<td>Of Calcium (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P Value</td>
<td>d’0.01</td>
<td>d’0.01</td>
<td>d’0.01</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>3.7±0.69</td>
<td>3.5±0.73</td>
<td>3.6±0.69</td>
</tr>
<tr>
<td>Of PO₄ (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P Value</td>
<td>e’0.05</td>
<td>e’0.05</td>
<td>d’0.05</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>7.67±1.39</td>
<td>7.80±1.00</td>
<td>7.73±1.19</td>
</tr>
<tr>
<td>of Corrected Calcium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P Value</td>
<td>d’0.01</td>
<td>d’0.01</td>
<td>d’0.01</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>4.03±0.371</td>
<td>3.84±0.349</td>
<td>3.96±0.369</td>
</tr>
<tr>
<td>Of Ionized Calcium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P Value</td>
<td>Pd’0.01</td>
<td>Pd’0.01</td>
<td>Pd’0.01</td>
</tr>
</tbody>
</table>

M= Males, F= Females and PO₄ = phosphorus

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).
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
researches disagreed with our findings. Jabbar et al., (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
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 postmenopausal 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 classified 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 (T Ca 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 albumin serum 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.

**References**


