Evaluation of Cubilin and Megalin levels in association with vitamin D status in Iraqi patient with DM2

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Abstract

Megalin is a large multifunctional endocytic clearance receptor for circulating proteins that has been implicated in vitamin D uptake and delivery to the kidney for activation to 1,25(OH)₂D. Knockout of the megalin gene in mice usually is lethal but the few survivors were characterized as having severe rickets. The findings suggested that VDBP may be a ligand for megalin. The model proposes that VDBP, carrying vitamin D, is filtered by the glomerulus and reabsorbed by the “cargo” receptor megalin or the megalin/cubilin complex in tubular cells, thereby preventing urinary loss of vitamin D. In addition to the classical hypothesis of free vitamin D uptake by diffusion in the proximal convoluted tubules at the basolateral site of the epithelium. The aim of this study was evaluate serum levels of megalin and cubilin in relation with vitamin D. This study included Eighty-four participants of both gender (28 are control and 56 T2DM patients).estimation of serum levels of cubilin and megalin was done using commercial ELISA kit of BT/ China, as well as VD3 measurement. Using of HOMA-IR, DM2 patients were sub divided to insulin resistance (IR) and insulin sensitive (IS) groups. There was significant difference between C and IS patient group (0.222± 0.206), (1.222±0.403) , p-value (0.000) respectively . While there was significant difference between C and IR patient group (0.222± 0.206) , (3.688±1.238) , p-value (0.000), also there was significant difference between IS and IR patient group (1.222±0.403), (3.688±1.238) , p-value (0.000) respectively. In conclusion, experimental studies and findings in rare disorders of receptor dysfunction have established the important role of megalin and cubilin in association with vitamin D.

Key word: T2DM, VD3, Cubilin, Megalin, HOMA-IR

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Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disorder and typically results from excess of caloric intake over energy expenditure. It is characterized by a progressive insulin secretory defect due to insulin resistance, which increases the body’s demand for insulin in order to retain glucose homeostasis. If pancreatic β-cells fail to secrete enough insulin to compensate for increasing insulin demand, the blood glucose level will be elevated gradually [1]. Chronic hyperglycemia is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels resulting in increasing levels of morbidity and mortality [2]. T2DM associated with poor lifestyle is a primarily factor leading to the progressive reduction of physical activity and changes of dietary habits. As a consequence, a greater percentage of the population will become overweight and obese. T2DM is the one of the most prevalent chronic diseases worldwide and one of the major public health challenges of the 21st century. The epidemic of T2DM in the United States and the rest of the world continue to grow rap idly; as
many as 20 million people in the United States may have the disease [3]. The vast majority of patients with diabetes suffer from T2DM [4].

Cubilin is an endocytosis receptor that is highly found in brush border of proximal renal tubules as well as that gastrointestinal tract and expressed as intrinsic factor B12 receptor. It is a protein of 460 KDa which have no transmembrane domain and so it still attached to the membrane via attaching to megalin [5].

Together, megalin and cubilin are responsible for the massive majority of reabsorption of protein in the kidneys' cells. Thus, if any of their physiological functions have impaired, like reduced protein expression, it is associated with an increase in the proteinuria, and this indicates a decrease in renal protein reabsorption [6].

Megalin, a glycoprotein receptor (gp) 330 or low density lipoprotein receptor-related protein 2 (Lp2), is an enormous 600 kDa cell surface protein which has been involved in the uptake of vitamin D and delivering it to the kidneys to be activated into 1,25(OH)2D [7].

Proximal tubular dysfunction may reflect in disturbance of the vitamin D metabolism since the activation of 25-hydroxyvitamin D3 (25(OH)D3) in 1, 25 dihydroxyvitamin D3 (1,25(OH)D3) occurs in the proximal cells. 25(OH)D3 is a steroid; thus, it is complexed to vitamin D-binding protein (VDBP) to circulate in the plasma. Because this complex is filtered in the glomeruli, a receptor-mediated uptake is required to prevent loss of 25(OH) D3 in the urine. For this reason, the brush border of the proximal tubule is equipped with megalin and cubilin, multiligand endocytic receptors respectively by uptake of the filtered protein, including 25(OH)D3-associated VDBP [8]. An endocytic vesicle is formed, and it’s fusion with lysosomes releases 25(OH)D3 to be converted to 1,25(OH)D3 by parathormone-regulated 1-α-hydroxylase.

The present study was designed to investigate the effects of cubilin and megalin levels in association with vitamin D status in Iraqi patient with T2DM.

**Materials and methods:**

This study included Eighty-four participants of both gender (28 are control and 56 T2DM patients). From patients who were clinically proved with T2DM from patients and control subjects, in fasting state by venipuncture, using a 5ml syringe between 8 to 9 A.M., 3 ml of blood were obtained and dispensed in a plain tube and left for hour to clot at room temperature (22 °C). Then, it was centrifuged at 3000 rpm for 10 minutes to collect serum. The serum was divided into aliquots in the endopordorff tube. In each collected clinical samples, estimation of serum levels of cubilin and megalin was done using commercial ELISA kit of BT/ China, as well as VD3 measurement. Statistical analysis was done using SPSS (Statistical Packages for Social Sciences- version 16).

**Results and Discussion:**

Table (1) and Fig (1) show the mean of cubilin in studied group. There was significant difference between C and IS patient group (0.222±0.206), (1.222±0.403), p-value (0.000) respectively. Also there was significant difference between C and IR patient group (0.222±0.206), (3.688±1.238), p-value (0.000), also there was significant difference between IS and IR patient group (1.222±0.403), (3.688±1.238), p-value (0.000) respectively.
Table (2): Comparison of means for the main factor according to Groups:

<table>
<thead>
<tr>
<th>Factor</th>
<th>C</th>
<th>IS</th>
<th>IR</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M±SD</td>
<td>M±SD</td>
<td>M±SD</td>
<td>C: IS</td>
</tr>
<tr>
<td>Cubilin</td>
<td>0.222±0.206</td>
<td>1.222±0.403</td>
<td>3.688±1.238</td>
<td>0.000</td>
</tr>
<tr>
<td>Megalin</td>
<td>0.181±0.082</td>
<td>1.914±0.333</td>
<td>3.737±0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>VD3</td>
<td>20.118±9.371</td>
<td>28.481±7.909</td>
<td>25.666±10.416</td>
<td>0.004</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.231±0.409</td>
<td>2.058±0.527</td>
<td>3.65±1.229</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table (1) and Fig (1) show the mean of megalin in studied group. There was significant difference between C and IS patient group (0.181±0.082), (1.914±0.333) , p-value (0.000) respectively . While there was significant difference between C and IR patient group (0.181±0.082) , (3.737±0.000) ,p-value (0.000), also there was significant difference between IS and IR patient group (1.914±0.333), (3.737±0.000) , p-value (0.000) respectively.

In a study by Pang et al. [9] on ZDF rats, it was found that the variation in megalin expression might be caused from the degree of insulin sensitivity.

![Figure (1) show the cubilin and megalin in studies group](image)

On the other hand, it was mentioned that megalin mediates the uptake of VDBP from glomerular filtrates and that mechanism is principally implicated in converting 25(OH)D3 into 1,25(OH)2D3, the biologically active form of vitamin D [10].

Also, cubilin was found to be additional endocytic receptor to VDBP, and the genetic defects in cubilin might result in loss of VDBP through the urinary tract and hence, the levels of 25(OH)D3 and 1,25(OH)2D3 in plasma were decreased [10].
It was stated that, the megalin and cubilinendocyti c PTC receptors are involved in the metabolism of vitamin D via reabsorption of VDBP from glomerular filtrates. The impaired function of these receptors might possibly be related with the incidence of vitamin D deficiency in diabetic patients [11].

Also, it has been demonstrated that there was a significant elevation in expression of megalin protein was detected in early T2DM. In addition, in rats with early T2DM, expression of renal megalin was found to be up-regulated in a process which might involve elevated glucose and insulin in a PI3K/Akt-dependent manner.

Vitamin D showed a highly significant in IS group (28.481±7.909) compared to control group (20.118±9.371), and p-value (0.004). Also there was non significant in IR group (25.666±10.416) compared to C (20.118±9.371), and p-value (0.075). While there was non significant difference between (IS/IR), (28.481±7.909), and (25.666±10.416), and p-value=(0.361).

HOMA-IR showed a significant elevation in each of IS and IR groups as (2.058±0.527 and 3.65±1.229) respectively compared to control group as (1.231±0.409) and p-value= 0.000. Also, HOMA-IR showed significant elevation in IR compared to IS group as (3.65±1.229) and (2.058±0.527) respectively and p-value= 0.000.

The assesses of HOMA-IR as well as fasting insulin were found to be reduced with high levels of 25(OH)D [12]. The findings of this study have revealed a negative correlation between HOMA-IR and vitamin D3, although it was non-significant (p-value= 0.563). The findings of the current study agree with chun et al…etc[13] who has conducted a study on Chinese people with T2DM, and he found a negative correlation of IR and the related biomarkers with 25(OH)D status. Similarly, an inverse correlation of IR with 25(OH)D concentration has been detected for values of 25(OH)D between (16-36) ng/mL [13].

Correlation between parameters:

There was positive significant correlation between Cubn with meg. (p-value=0.000), also there was negative non significant correlation between Cubn withVD3 (p-value=0.083). As well as positive non significant correlation between Cubn with HOMA-IR (p-value= 0.006).

Table (2): Correlation between cubilin and different parameters:

<table>
<thead>
<tr>
<th>Item</th>
<th>Cubn</th>
<th>Meg.</th>
<th>VD3</th>
<th>HOMA-IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cubn</td>
<td>Pearson Correlation</td>
<td>1</td>
<td>.891**</td>
<td>-.234-</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.000</td>
<td>.083</td>
<td>.006</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>88</td>
<td>88</td>
<td>88</td>
</tr>
<tr>
<td>Meg.</td>
<td>Pearson Correlation</td>
<td>.891**</td>
<td>1</td>
<td>-.343-**</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.000</td>
<td>.100</td>
<td>.073</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>88</td>
<td>88</td>
<td>88</td>
</tr>
<tr>
<td>VD3</td>
<td>Pearson Correlation</td>
<td>-.234-</td>
<td>-.343-**</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.083</td>
<td>.010</td>
<td>.479</td>
</tr>
</tbody>
</table>
Conclusion:

The endocytic PTC receptors megalin and cubilin are involved in the metabolism of vitamin D by reabsorbing VDBP from glomerular filtrates. Dysfunction of these receptors is likely to be associated with the development of vitamin D deficiency in patients with CKD, in particular those with diabetic nephropathy. Therapeutic strategies to protect the functions of these receptors from injury could be investigated to prevent vitamin D deficiency and its related disorders.

Reference: