Role of 1α-Hydroxylase Enzyme and 25(OH)D as a Biomarker of Vitamin D Metabolism in Chronic Kidney Disease

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Abstract

Chronic kidney disease is identified as a risk factor for vitamin D deficiency, vitamin D deficiency is common and high in the chronic kidney disease population so it is recognized as a significant public health problem. The objective of this study was to 1) measured 25(OH)D and 1α-hydroxylase levels. 2) Correlation between 25(OH)D and 1α-hydroxylase among patients with vitamin D deficient and CKD. Blood samples were collected, from 50 patients suffer from CKD and have Vitamin D deficiency, and 50 healthy persons as control group. The biochemical results showed that blood urea, creatinine, phosphorus and parathyroid hormone levels a highly significant increase in the level of patients compared with controls. The biochemical results showed that blood urea, creatinine, phosphorus and parathyroid hormone levels were highly significant increase in the patients compared with controls. While the results revealed a highly significant reduction ($P < 0.01$) in 25(OH)D level, calcium and 1α-hydroxylase enzyme among CKD patients when compared with the healthy controls. The biochemical markers showed a highly significantly correlation between serum 25(OH)D and Creatinine, phosphorus and parathyroid hormone ($P<0.01$). While a highly significant reduction correlation was observed between serum 25(OH)D and calcium, serum 1α-hydroxylase enzyme levels in CKD patients.

Keywords: 1α-hydroxylase, 25(OH)D, Chronic kidney disease.

دور انزيم ١ الفا هايدروكسيلز وفيتامين25(OH)D كمؤشر حيوي لايض فيتامين25(OH)D في مرضى الكلى المزمن

اميرة حميد منزل و أ.د. هناء ناجي عبدالله

الخلاصة

تم تحديد مرض الكلى المزمن كعامل خطر لنقص فيتامين25(OH)D ، ويعد نقص فيتامين 25(OH)Dشائع في مرضى أمراض الكلى المزمنة و يمثل مشكلة صحية عامة كبيرة. تهدف هذه الدراسة 1) قياس مستويات فيتامين 25(OH)D و 1الفا هيدروكسيلاز. 2) العلاقة بين فيتامين 25(OH)Dو 1الفا هيدروكسيليز بين المرضى الذين يعانون من نقص

فيتامينD(OH)25 ومرضى الكلى المزمن. تم جمع عينات الدم ، من 50 مريضا يعانون من مرضى الكلى المزم مع نقص فيتامين D(OH)25 و 50 من الأشخاص الأصحاء كمجموعة سيطرة. أظهرت النتائج زيادة (P>0.01) في مستويات اليوريا في الدم والكرياتينين والفوسفور وهرمون الغدة الدرقية في المرضى مقارنة مع الاصحاء. بينما كشفت النتائج عن انخفاض كبير للغاية (P <0.01) في مستوىD(OH)25 والكالسيوم وانزيم 1الفا هيدروكسيلاز بين مرض الكلى المزمن بالمقارنة مع الاصحاء. أظهرت المؤشرات الكيميائية الحيوية وجود علاقة معنوية بين فيتامين (25(OH) D والكرياتينين والفوسفور وهرمون الغدة الدرقية (P >0.01) في حين لوحظ وجود علاقة نقصان كبيرة بين (25(OH) D) والكالسيوم ومستويات إنزيم المصل 1 الفاهيدروكسيلاز في مرضى الكلى المزمن.

الكلمات المفتاحية : ١ الفا هيدروكسيلز ، فيتامين D_3 ، مرض الكلى المزمن .

Introduction

Chronic kidney disease (CKD) can be defined as abnormalities either in kidney structure or function by decreasing glomerular filtration rate (GFR) of less than 60 mL/min/1.73 m^2 for at least 3 months [1 ،2]. It is currently estimated that nearly 10–15% of the world's population suffer from CKD and accounts for 4% of the deaths worldwide and has progressively increased from 17- 21years [3]. Certainly, the prevalence of vitamin D deficiency or insufficiency is high among patients with CKD [4]. Vitamin D is a biologically inactive fat soluble pro-hormone that is converted primarily in the kidney and found in two groups, namely vitamin D2 and vitamin D3. It results from 7-dehydrocholesterol in the human skin by the action of UV rays found in sunlight with wavelength of 270-290 nm or derived from animal tissues [5]. Vitamin D metabolism in individuals is affected not only by exposure to sunlight or by dietary intake of vitamin D, liver, kidney and other tissue production, but also by genetic variations in genes associated with vitamin D metabolism [6]. There are three main steps in vitamin D metabolism: 1) 25-hydroxylation, 2) 1α-hydroxylation and 3) 24 hydroxylation which are all performed by cytochrome P450 (CYPs) mixed function oxidases [7]. 1α-hydroxylase plays a main role in converting Vit D3 to its active form which is called 1, 25-dihydroxy vitamin D3, and therefore, is essential in regulating the level of biologically active vitamin D and calcium homeostasis [8]. In CKD progression, decreased renal mass limits the amount of 1α-hydroxylase in renal proximal tubular cells. In addition, decreases in GFR and low megalin content contribute to impaired 25(OH) D uptake and protein reabsorption, and increases levels of serum level of phosphate (P) and fibroblast growth factor 23 (FGF23) [9]. The objective of this study, to measured 25(OH)D and 1α-hydroxylase levels, correlation between 25(OH)D and 1α-hydroxylase among patients with vitamin D deficient and CKD.

Materials and Method

Samples collection

This study was carried out in the Tropical-biological Research Unit- College of science / Baghdad University during the period from the first of October 2017 to June 2018. A total of 100 samples (ages ranged between 1- 46 years) were included, (50) CKD patients at the predialytic stage and, (50) healthy individuals as a control group. During sampling, each patient was subjected to a questionnaire form including age, gender and type of drugs like vitamin D, calcium and phosphorus therapy. Patients who were receiving such medications were excluded from our study.

Blood samples were collected from each person in sterile conditions. Each sample was divided into two parts: the first part was kept in EDTA tube as a whole blood for detection of Blood urea, while the other part was clotted and spun by a centrifuge to obtain serum which was kept in plane tubes to perform biochemical tests such us blood urea, serum creatinine, phosphorus, serum calcium, vitamin D3 and parathyroid hormone.

Ethical Consideration

Sampling was done with the consent of the patient

Methods

A cross-sectional study comparing vitamin D deficient CKD patients and healthy control groups.

Measurement of Biochemical parameters

1-Mineral metabolism analysis: calcium (Ca), and phosphorus (P).

2-Renal function on parameters: Blood urea and serum creatinine.

3-Enzyme: 1α-hydroxylase was measured by ELISA.

4-Hormone: 25(OH)D and PTH were measured by ELISA.

Statistical analysis

Data were analyzed by using IBM SPSS statistical program version 25, and expressed as mean ± SD, Chi square tables, T-test tables, and Pearson's correlation. P refers to possibility whereas ($P<0.05$, $P<0.001$and $P>0.05$) were considered as significant, highly significant and non-significant respectively.

Results

In the current study, blood samples were collected from 100 individuals, with their ages ranged between 1- 46 years ,50 vitamin D deficient CKD patients (19 males and 31 females), and 50 healthy control. The results in table showed that the mean age group of the patients was significantly higher than the mean age group of the controls (P<0.05). Gender results showed that there was a highly significant variation between males 19(38%) and females 31(62%) within the CKD patients group, (table 1).

Table(1): Demographic and biochemical characteristics among studied

Characteristics	CKD patients	control group	P value
Age (yrs)	30.9±10.0	23.4±11.6	P< 0.05
Gender Male Female	 19(38%) 31(62%)	 14(28%) 36(72%)	 P<0.01
Blood urea (mg/dl)	151.1 ± 59	7.3 ± 3.5	P < 0.01
Serum creatinine(mg/dl)	31.5 ± 5.0	0.7 ± 0.2	P < 0.01
Calcium(mg/dl)	7.6 ± 0.8	8.9 ± 0.3	P < 0.01
Phosphorus (mg/dl)	6.3 ± 2.8	4.2 ± 0.8	P < 0.01
PTH (pg/dl)	121.8 ± 120.3	79.9 ± 66.2	P< 0.05
25(OH)D level (ng/ml)	19.4 ± 7.4	45.7 ± 12.2	P < 0.01
1α-hydroxylase(ng/ml)	0.5±0.17	38.5±1.60	P < 0.01

The collected laboratory tests and normal range were: creatinine (0.5-1.0) mg/dl, blood urea (10-40) mg/dl, calcium (8.8-10.3) mg/dl, phosphorus (2.5-5) mg/dl, PTH (9.0-94) pg/dl), 25(OH)Vitamin D Insufficient (20–30 ng/mL), total levels and Human 25-Hydroxyvitamin D-1Alpha Hydroxylase, (CYP27B1) (0.78- 50.0) ng/ml.

The results of blood urea and creatinine levels showed a highly significant increase in the level of patients compared with controls (151.1 ± 59 *vs* 31.5 ± 5.0); (7.3 ± 3.5 *vs* 0.7 ± 0.2, respectively). The result demonstrated a highly significant (P < 0.01) reduction in serum calcium level in CKD patients when compared with the control group (7.6 ± 0.8 vs. 8.9 ± 0.3), respectively). While phosphorus concentration showed that there was a highly significant increase in the patients' group levels when compared with the controls (6.3 ± 2.8 vs. 4.2 ± 0.8). There was a marked increase in PTH in the patient group compared to the controls (121.8 ± 120.3 vs. 79.9 ± 66.2), as shown in (Table 1). In addition, the results in table (1) revealed a highly significant reduction (P < 0.01) in Vitamin D level among CKD patients when compared with the healthy controls (19.4 ± 7.4 vs. 45.7 ± 12.2, respectively). There was a higly significant

reduction in 1α-hydroxylase enzyme in the patient's group compared to the controls (0.5±0.17vs. 38.5±1.60, respectively).

Correlation between 25(OH)D and some biochemical parameters

Among the vitamin D deficient CKD patients, there was a significant inversely correlation between low 25(OH)D levels and each of blood urea ($p< 0.001$), serum creatinine ($p < 0.001$), phosphorus ($p < 0.001$), and PTH ($p <0.001$). While a highly significant reduction correlation was observed between low serum 25(OH)D and calcium ($P<0.001$), serum 1α-hydroxylase enzyme levels ($P<0.001$) (Figure 1).

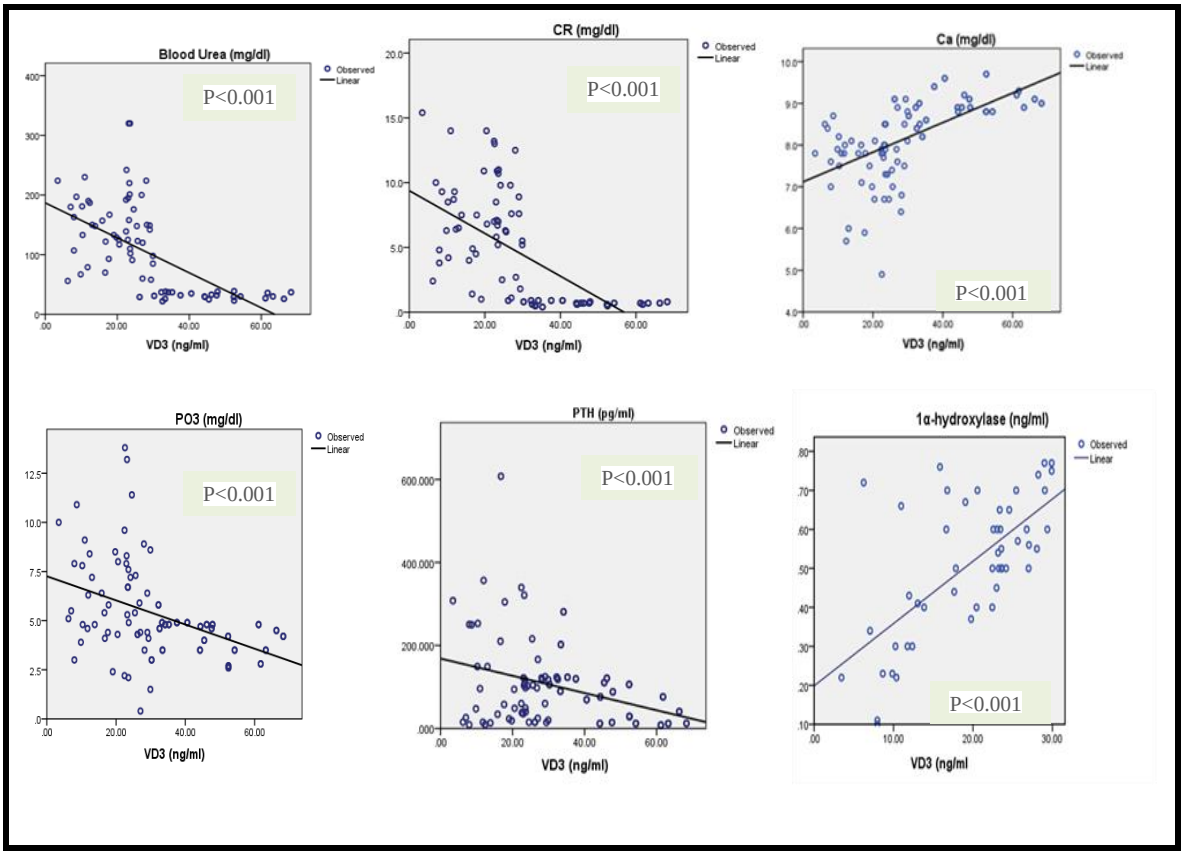

Figure (1): Association between 25(OH)D levels and blood urea, serum creatinine, calcium, phosphorus, PTH and serum 1α- hydroxylase among vitamin D deficient in CKD patients.

Discussion

The presence of vitamin D deficiency in general population and in patients with CKD has been estimated. This clinical problem has always been seen as large in our country, since most of its territory is located in a tropical region, where the incidence of sun light is excessive.

This result was concordant with a local study performed by Merzah and Hasson, who recorded a significant increase ($P< 0.001$) in urea and creatinine levels in CKD patients when

compared with the control group [10]. Creatinine is filtered by the glomerulus and thus, serum creatinine level is considered as an indirect measure of glomerular filtration. Diminishing of glomerular filtration rate results in the rise of plasma concentrations of serum creatinine and urea. This rise indicates the progression of kidney disease and thus serum creatinine has greater prognostic ability compared to urea for predicting the adverse outcomes [11]. While, this study was not consistent with the results obtained by Nisha and Jagatha [12]. As well as,this results were in agreement with Inaguma *et al*. who reported low calcium levels in CKD compared to the control group[13].

Another results were similar to this results in finding elevated phosphorus levels among Korean chronic kidney patients [9]. The results obtained by Nadkarni and Uribarri were also consistent with this results [14]. Increased consumption of canned foods with phosphate additives may have negative effects on patients with chronic kidney disease, in particular, as shown by Chang and Anderson [15]. On the other hand, this study differed from Pires *et al*. study who indicated that phosphorus levels are slow to progress with the development of chronic kidney disease [16]. The natural range of absorbed phosphorus increases by 0.1 mg/dl from 3.3 to 4.5 mg/dl. As well as, Results of this study agreed with the findings of Al- Jasim and Yaseen who stated that PTH was highly significantly increased ($P<0.001$) in the sera of CKD patients compared with the control group[17]. In CKD, there is a reduction in calcium receptors expression in vascular smooth muscle cells. Consequently, this leads to a decrease in calcium levels in serum indicating a relationship between PTH and calcium levels in chronic kidney disease. Parathyroid glands release higher concentrations of PTH in response to a low level of calcium blood. In this regard, PTH is the most important hormone that contributes to the regulation of phosphate in the kidney [17]. It has been shown that a higher level of serum phosphate adjusted for creatinine clearance was associated with mortality in chronic kidney disease. While the study disagreed with the results obtained by Melamed and Thadhani who showed that the level of PTH was in a decline in CKD [18]. The reason is most likely related to giving patients vitamin D as a treatment so the level of PTH dropped.

This study was consistent with the study performed by Batacchi, *et al.* who showed that the mean Serum 25(OH)D levels of CKD patients and the control group were (19.4± 7.4 vs. 45.7 ±12.2, respectively) with a highly significant difference ($p >0.001$)[19]. Many factors may contribute to low 25(OH)D levels in CKD, including loss of vitamin D binding proteins in the urine, ineffective vitamin D synthesis in the skin on exposure to UV-B radiation and possibly reduced sun exposure and nutritional intake. Moreover, an increase in the level of f FGF23, which can directly suppress the activity and expression of 1a-hydroxylase, decreases vitamin D

levels during the course of the disease [20]. Several mechanisms might explain the 25(OH)-VD deficiency in the CKD population. First, most patients with CKD have restricted protein and caloric intake, so vitamin D is relatively low. Second, many CKD patients have limited outdoor physical activities with reduced exposure to sunlight. Finally, greater loss of urinary vitamin D metabolites occurs in patients with overt proteinuria [21]. In addition, 1-alpha hydroxylase activity decreases due to reduced function of renal mass and an elevation in circulating FGF-23 levels, which potently suppress 1-alpha hydroxylase expression [22]. Whether sufficient non-renal 1-alpha hydroxylase activity is available to activate 25-hydroxy vitamin D in end-stage CKD patients is unknown. In clinical practice, activated vitamin D agents are prescribed to chronic dialysis patients and those with end-stage CKD under the assumption of inadequate 1-alpha hydroxylase activity in this setting. The current results were also consistent with the results of Ko, *et al.* who reported a significant negative correlation between low serum 25(OH)D levels and blood urea ($P<0.037$) [20]. The development of chronic kidney disease which causes a reduced renal mass that limits the amount of 1α-hydroxylase in renal proximal tubular cells and reduced in glomerular filtration rate is associated with a concomitant rise in the blood urea [23]. As well as, our result in this study agreed with Liu *et al.* who found a highly significant negative correlation between creatinine and low 25(OH)D ($P<0.001$) [24]. An elevation in the serum creatinine concentration usually means a reduction in the glomerular filtration rate and vitamin D become slight [4]. In addition, this result was in agreement with the results of Restrepo, and Aguirre who found a decrease in the levels of calcium accompanied with vitamin D decline [25]. Another study conducted by Narayanasamy, *et al*., agreed with this result when they found a highly significant positive correlation between serum vitamin D and Ca ($P< 0.0001$) [26]. The results obtained in this study were also consistent with the results of Molina, *et al*., who reported a significantly correlation between serum vitamin D and Calcium ($P<0.027$) [27].

The active vitamin D metabolite, 1,25-dihydroxyvitamin D (1,25(OH) 2D) stimulates the active calcium transport through the intestinal wall. Vitamin D in chronic kidney disease and dialysis patients [28]. Furthermore, current result agreed with the study conducted by Rajbhandari, *et al*. who revealed a significant increase correlation between serum vitamin D and Phosphorus ($P<0.045$) [29]. Moreover, hyper-phosphatemia causes reduced synthesis of calcitriol and secondary hyperparathyroidism which causes increased degradation of vitamin D with decreased substrate for calcitriol synthesis. Although direct effect of serum phosphorus on vitamin D is not well known, inverse relation of serum phosphorus and vitamin D in CKD has been documented in a recent study [29].

The result in this study agreed with Oh, *et al*., (2017) and Ardehali, et al. who indicated a highly significant correlation between low serum 25(OH) D levels and serum concentrations of PTH[31]. These findings demonstrated that as the CKD patients' renal function deteriorates, a series of events leads them to diminish their solar exposure. Low protein diets, containing low amounts of vitamin D are often prescribed for this type of patients, a fact that probably contributes to lower serum levels of vitamin D3[25]. For vitamin D to exert its endocrine effects, it is critically important for the kidney to activate both nutritional and sunlight-derived vitamin D. In the kidney disease, the activation of vitamin D is abrogated; this occurs early in the course of kidney disease, which leads to reduction in calcium absorption from the gut, increases PTH levels from the parathyroid gland, and culminates the secondary hyperparathyroidism. To countervail these effects, calcitriol, a vitamin D receptor activator, has been synthesized and administered to treat secondary hyperparathyroidism among patients with CKD. Low vitamin D may identify risk of CKD complications. In addition, low serum 25(OH)D concentration was strongly associated with hyperparathyroidism, perhaps because 25(OH)D concentration reflects the extent to which vitamin D metabolism is deranged in CKD. Furthermore, a highly significantly reduction correlation was observed, which reveals a positive correlation between serum 25(OH)D and 1α-hydroxylase levels in CKD patients. There were also scarce reports to study this investigation. However, the detailed mechanism should be studied in the further.

Conclusions

The conclusion that 1α-hydroxylase enzyme and vitamin D levels were reduced in patients with CKD, and vitamin D levels were positively correlated with 1α-hydroxylase levels. As well as, low vitamin D may identify risk of CKD complications. Low serum 25(OH)D concentration was strongly associated with hyperparathyroidism, perhaps because 25(OH)D concentration reflects the extent to which vitamin D metabolism is deranged in CKD.

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