T.C.

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SCIENCE INSTITUTE

MASTER'S THESIS

THE CORRELATİON BETWEEN VİTAMİN D3 AND SUPER OXİDE DİSMUTASE ENZYME (SOD) İN PATİENTS WİTH KİDNEY PATİENTS

CHEMISTRY DEPARTMENT

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TEZ ONAYI

AHMED HASSAN SALIH ALBO ANAH tarafından hazırlanan **"THE CORRELATİON BETWEEN VİTAMİN D3 AND SUPER OXİDE DİSMUTASE ENZYME (SOD) İN PATİENTS WİTH KİDNEY PATİENTS"** adlı tez çalışması 27/01/2021 tarihinde aşağıdaki jüri tarafından oy birliği ile Çankırı Karatekin Üniversitesi Fen Bilimleri Enstitüsü Kimya Anabilim Dalında Yüksek Lisans Tezi olarak kabul edilmiştir.

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Ahmed Hassan Saleh AL BUANAH

ÖZET

Yüksek Lisans Tezi

Böbrek hastalarında D3 vitamini ve Süper oksit dismutaz enzimi (SOD) arasındaki korelasyon

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Kronik böbrek hastalığı (KBH) büyük önem taşıyan bir hastalıktır. Etkisinin boyutu, yüksek yaygınlık ve yüksek ölüm oranlarına yansır. Çalışmamızın amacı, böbrek hastalığı olan hastalarda D vitamini eksikliğinin kökenini, önerileri takip eden D vitamini takviyesinin oranını ve birkaç serum parametresi üzerindeki etkisini değerlendirmektir: fosfor dengesi, paratiroid hormonu, 25 (OH) D, 1 , 25 (OH) D. Çalışma ayrıca Süper oksit dismutaz enzim (SOD) aktivitesini ölçen bir antioksidan enzim tanımlama sistemi ve enzimatik olmayan bir antioksidan sistemi içeriyordu. Hastaların % 79.59'unun optimal seviyeden daha düşük D vitamini konsantrasyonlarına sahip olduğu gözlemlendiğinde, D3 vitamini yanlış alan hastaların fosfat seviyesi yaklaşık% 20 ve yetersiz miktarda D3 vitamini alan hastaların konsantrasyonları 25'tir. (OH) D yeterince alınanlardan önemli ölçüde daha düşük. 12 hastada paratiroid hormonunda hafif bir artış gözlemledik. Oksidatif stres durumunda, kontrol grubu ile ön tanı arasında önemli farklılıklar tespit edildi. KBH'nin, vitamin D3'ün azalmasının yanı sıra antioksidan enzimler SOD ve katalazın (CAT) azalmış aktivitesinin bir işareti olduğu düşünülmektedir.

Ocak 2021, 94 sayfa

Anahtar kelimeler: Vitamin D3, Kronik böbrek hastalığı (CKD), Süper oksit dismutaz enzimi (SOD)

ABSTRACT

Master Thesis

The correlation Between Vitamin D3 and Superoxide dismutase enzyme (SOD) in patients with Kidney patients

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Chronic kidney disease (CKD) is a disease of great importance. The extent of its impact is reflected in its high prevalence and high mortality rates. Our study aims to evaluate the origin of vitamin D deficiency in patients with kidney disease, the proportion of vitamin D supplementation following the recommendations, and its effect on several serum parameters (phosphorus balance, parathyroid hormone, 25 (OH) D). 1, 25 (OH) D. The study also included an antioxidant enzyme identification system that measures Superoxide dismutase enzyme (SOD) activity and a non-enzymatic antioxidant system. it was observed that 79.59% of patients had vitamin D concentrations lower than optimal, patients who received vitamin D3 incorrectly had a phosphate level of about 20% and those who received insufficient vitamin D3 had a significantly lower concentration of 25 (OH) D than those who received sufficient vitamin D3. We observed a slight increase in parathyroid hormone in 12 patients. Significant differences were found between the control group and pre-diagnosis in the case of oxidative stress. CKD is thought to be a sign of decreased activity of the antioxidant enzymes SOD and catalase (CAT), as well as decreased vitamin D3.

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Kew words : Vitamin D3 , Chronic kidney disease (CKD) , Super oxide dismutase enzyme (SOD)

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To the first beat in my heart, to the soul that possessed my soul without permission, to the most beautiful heart and most beautiful face. Fadhaa

To those whose images and voices live in the most beautiful moments and days. My dear friends.

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Researcher

Ahmed Hassan Saleh AL BOANAH

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1. INTRODUCTION

1.1 Chronic Kidney Disease

Chronic kidney disease (CKD) is a real global public health problem due to the constant increase in its incidence and prevalence rates due to the increase in its main risk factors, namely diabetes mellitus, arterial hypertension, cardiovascular diseases, collagenases and the aging of the population, and the high cost of its management in the terminal stage with disappointing results (Kazancioğlu, 2013). It is defined by the persistence for more than three months of a decrease in glomerular filtration rate (GFR) <60ml / min / 1.73m^2, or by a glomerular filtration rate> 60ml / min / 1.73m^2 associated with more markers of renal impairment. The various epidemiological studies carried out throughout the world estimate a prevalence varying from 10-15%. Projections for 2030 predict that more than 70% of the world population with end-stage chronic renal disease will end up in developing countries, including most of the countries of Arabic world. It should be noted that in 2004 only 5% of this population had access to renal replacement therapy. In the Democratic Republic of Iraq, studies on the prevalence of kidney disease in the city of Baghdad Province have estimated it at 12.4% and 19.8%. In our community, the only study brought to our attention is the one that evaluated the means of affordability of patients with renal failure on hemodialysis as well as the risk factors for their mortality. It was found that for an average monthly hemodialysis cost of $ 1270, 52.8% of patients reported having a monthly income of $ 205; 34% $ 525 and 13.2% $ 750. And that the irregularity of hemodialysis follow-up was the risk factor for death in hemodialysis (Chandrashekar et al., 2014), ie 77% followed by discontinuation of treatment. Faced with this state of affairs, we decided to bring a stone to the knowledge of chronic kidney disease in Iraq (Sharif et al., 2017).

Definition: CKD is a condition characterized by a significant and progressive decrease in kidney function. It is defined according to the Kidney Disease Improvement Global Outcomes (KDIGO) guidelines (Levey et al., 2002), for daily clinical practice of the National Kidney Foundation as: (Schena, 2000)

1. Kidney damage for at least 3 months, defined by structural or functional abnormalities of the kidney with or without decreased glomerular filtration, manifested by: pathological abnormalities or markers of kidney damage, including alterations in the composition of blood or urine and / or alterations in the image tests.

2. Decrease in kidney function with glomerular filtration rate (GFR) <60 ml / min / 1.73 m^2, for at least 3 months, with or without kidney damage. Within CKD there are various stages that stratify the level of progression of the disease. Among the main risk factors for CKD are: advanced age, family history of CKD, high blood pressure, diabetes, reduced kidney mass, low birth weight, autoimmune and systemic diseases, urinary tract infections, lithiasis, obstructive diseases of the lower urinary tract, use of nephrotoxic drugs, and the African race.

3. Epidemiology**:** Chronic Kidney Disease (CKD) is recognized as a global health problem. It has an approximate prevalence of 7.2% in developed countries, and 10% of the world population, and is an independent factor of cardiovascular morbidity and risk.

1.2 Causes of CKD

The main causes of CKD are shown below, although more than a single cause frequently coexists, enhancing kidney damage:

- Diabetic nephropathy.
- Atherosclerotic vascular disease, nephroangiosclerosis and ischemic nephropathy. Which have in common the presence of high blood pressure.
- Primary glomerular disease, or secondary to systemic disease.
- Congenital and hereditary nephropathies.
- Interstitial nephropathies.
- Prolonged obstruction of the urinary tract (including lithiasis).
- Recurrent urinary infections.
- Systemic diseases such as: lupus, vasculitis or myeloma.

1.3 Epidemiology Current situation in our Environment

Prevalence and Incidence in stages 1-4: Most of the data we have to assess the prevalence of CKD come from records of patients who receive renal replacement treatment, therefore, they are patients who are in their most advanced stage. We know less about the epidemiology of the disease in its earlier stages, probably due to the lack of uniformity in measuring kidney function that existed until recently. For example, in a study by Simai et al. In an area of Valladolid, they calculated creatinine clearance using four methods: serum creatinine concentration, ceatinin clearance using 24-hour urine collections, using the Crockoft-Gault formula and applying the abbreviated MDRD formula. The conclusion was that the prevalence of stages 2 and 3 of CKD was influenced by the calculation method used, so that that of stage 3 had a prevalence that ranged from 3.3 to 8.5% depending on the method. In order to study the prevalence of CKD in Iraq, the study was started in 2016 (Al-Khayat et al., 2016) , which also had as secondary objectives, on the one hand, to study factors associated with CKD in our population and, on the other, to evaluate the prevalence of microalbuminuria-

proteinuria. Their data, recently published, have revealed a total prevalence of CKD in stages 3-5 of 6.83%, while in the population group over 64 years, it reaches 24.42%. When the presence of albuminuria / creatinuria was added to the diagnostic criteria, the total prevalence was 9.16%. The prevalence by stage was 0.99% for stage 1, 1.3% for stage 2; 5.4% for stage 3a, 1.1% for stage 3b, 0.27% for stage 4, and 0.03% for stage 5.

Prevalence and incidence in stage 5d: Regarding patients receiving replacement treatment, we have more data thanks to the regional and national Registries of Renal Patients. In 2016 the incidence in Iraq (COSIT), was 125 ppm (patients per million population), although it presented a marked variability according to the age group studied, while it was 35 ppm in the age group 15-44 years, exceeding the 400 ppm in age groups over 75 years. Regarding prevalence, as of December 31, 2007, there were a total of 36,588 patients in substitution treatment, which meant a total prevalence of 1,009 ppm. The treatment modality of prevalent patients varies widely according to the age group studied, while in the 15-44 age group more than 45% of patients were transplanted, in the group older than 75 years more than 85% received Hemodialysis (HD) treatment. In general, the most prevalent technique considering all population groups was HD with a 46.13% prevalence, while the least used treatment modality was Peritoneal Dialysis (PD) with 6.15% in total. Both the incidence and prevalence figures for Iraq in 2018 place it in the intermediate zone of the neighboring countries in Arabic world (Khaleel et al., 2019), in terms of risk of initiating treatment for Terminal Chronic Renal Failure.

1.4 Etiology- Population Characteristics

The justification for these high prevalence and incidence of CKD is found in different causes. On the one hand, the aging of the population, a particularly serious problem in Iraq and in Europe in general, and on the other, the alarming increase in the incidence of type 2 Diabetes Mellitus and consequently of diabetic nephropathy, as well as the increase in the prevalence of hypertension arterial disease associated with aging and insufficient control of it.

The causes of CKD in Iraq have a similar distribution to that of the rest of Asian Countries. In 2020, Diabetic Nephropathy represented the most frequent etiology (23.6%), followed by Renal Vascular Disease associated with Hypertension (14%), although in a high percentage of patients (19.3%) the etiology was unknown (Alwaash et al., 2020).

1.5 Dietary Treatment of CKD

Food care through compliance with nutritional recommendations plays an important role throughout the CKD process, mainly due to the modification of the requirements of macronutrients and micronutrients as the disease evolves depending on the stage of the disease in which the patient is. The KDIGO guideline is more recent and its members represent the global nephrological community while the KDOQI guideline (Kidney Disease Improvement Global Outcome - KDIGO) was devised by American nephrologists(Al-Saedy and Al-Kahichy, 2011). According to the data of Iraqi Renal Registry report almost two hundred thousand of Iraqi adults are suffering from various stages of Chronic Kidney Disease (CKD). Now-a day's silent CKD has been proposed as an outbreak or epidemic by many researcher (Dhaidan, 2018). A better understanding of vitamin D metabolism and the associations between its serum levels and clinical outcomes in various diseases, including CKD, has led to vitamin D supplementation being incorporated into the treatment of these patients (Al-Saedy and Al-Kahichy, 2011).

1.6 The Vitamin D

1.6.1 Vitamin D in chronic kidney disease

There is a high prevalence of vitamin D (25 (OH) D) deficiency in all stages of CKD, with correlation in several observational studies with markers of kidney injury such as albuminuria, CKD progression, cardiovascular disease, vascular calcification, ventricular hypertrophy, in addition to increased morbidity and mortality (5.24). In a

population of chronic kidney patients at stage 3-5 not undergoing renal replacement therapy, deficient concentrations of 25 (OH) D (<20 ng / mL) correlate with a higher mortality rate (p = 0.031), greater progression to CKD stage 5 (p <0.001) and shorter period until hospitalization (p = 0.027), compared to concentrations 20 ng / mL. No statistically significant differences were found between the failure group (30 ng / mL> 25 (OH) D 20 ng / mL) and the sufficiency group (30 ng / mL) (24). A progressive reduction in the levels of 25 (OH) D was shown, proportional to the decrease in GFR, and consequently to the stage of CKD, despite not being congruent in all studies. Several factors can contribute to this deficit, which can be grouped in a decrease in cutaneous production of cholecalciferol and in urinary loss of the 25 (OH) D-VDBP complex.

The production of cholecalciferol depends on UVB irradiation in the skin. Chronic kidney patients, throughout the progression of CKD, suffer a progressive decrease in sun exposure, promoted in part by the decrease in physical activity and installation of sarcopenia. It should be remembered that the prevalence of CKD increases with age, with approximately 60% of patients over 60 years of age (Franca Gois et al., 2018). Thus, it is expected that an older population will have more physical dependence, which leads to a decrease in activities abroad. Age and also uremia cause skin changes with increased pigmentation that also decreases the efficiency of skin conversion to vitamin D. Another of the factors pointed out is the slowing of the metabolism of these patients and malnutrition. Urinary loss of the 25 (OH) D-VDBP complex appears to be one of the main causes of decreased levels of 25 (OH) D, with an association between vitamin D deficiency and albuminuria. The reason for urinary loss appears to be related to the protein megaline. Experimental studies, in cultures of proximal tubular cells chronically exposed to albumin, and in an animal model, showed decreased megalin expression, such as loss of large amounts of vitamin D-VDBP complexes in the urine of megaline knockout mice, which presented hypocalcaemia and osteomalacia. In addition to the decrease in its expression with the apoptosis of tubular cells induced by proteinuria and inflammatory factors, megaline is also occupied by other proteins that would not normally undergo glomerular filtration under normal conditions. On the other hand, given that the kidney is the main production site of 1.25 (OH) 2D, it is also reduced in

CKD, although it is only evident in more advanced stages due to its hormonal regulation mechanisms. In addition to the deficit in its precursor, the decrease in vitamin D activated in CKD is due to the decrease in renal mass, and consequently in GFR, and the inhibition of the enzyme 1□-hydroxylase, which is considered the main factor (Gluba-Brzózka et al., 2018).

In 1932, the German researcher Windaus isolated (DeLuca, 2014), vitamin D2. Five years later, he succeeded in isolating 7-dehydrocholesterol from pig skin, and demonstrated that under the action of UV this substance produced a vitamin D different from D2, he called it vitamin D3. A At the end of the 20th century, the pathways of vitamin D metabolism were elucidated. Over the past ten years, interest in vitamin D has grown with the discovery of numerous extra-osseous pleiotropic effects, and of its autocrine properties.

Origin and metabolism: Native vitamin D, unlike other vitamins only from food, has two major origins: on the one hand, skin synthesis for vitamin D3 (≈80%) and on the other hand 'power supply for D3 and D2 (≈20%).

Endogenous origin of vitamin D3: Native vitamin D3 or cholecalciferol is synthesized in the skin from a cholesterol derivative, 7-dehydrocholesterol (7-DHC). 7-DHC or provitamin D3 is present in all of the layers of the skin, but its concentration is higher in the depths of the epidermis. Under the action of UVB rays (especially those with wavelengths between 295 and 300 nm), provitamin D3 is converted into previtamin D3. Following a thermal isomerization of a few hours, previtamin D3 will give vitamin D3 (Figure 1.1).

Skin formation of vitamin D3 is tightly regulated. Indeed during an exposure to excessive solar UVB, previtamin D3 is transformed into inactive compounds that are lumisterol and tachysterol. In addition, in this situation, vitamin D3 is inactivated into 5,6-transvitamin D3 by photoisomerization. These phenomena explain the reason why vitamin D poisoning is not observed in the event of strong sun exposure. It should be

noted that tachysterol and lumisterol are capable of giving back previtamin D3 in the event of a shortage of the latter.

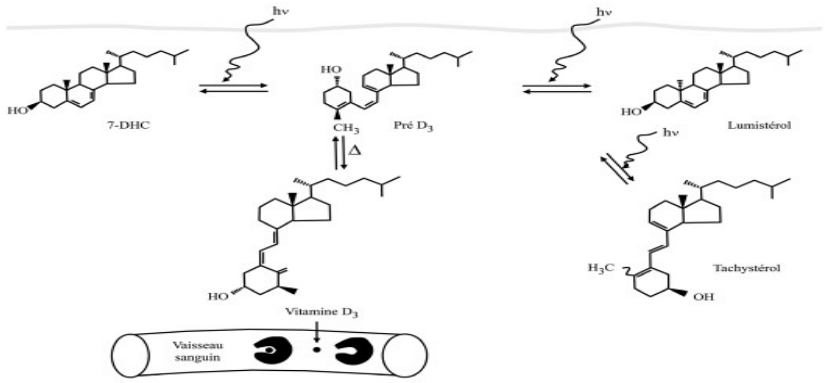

Figure 1.1 Endogenous Synthesis of Vitamin D.

The cutaneous 7-DHC forms under the action of UVB rays previtamin D3. The latter after thermal isomerization gives vitamin D3, which is transported to the blood by the Vitamin D Binding Protein. Pre-vitamin D3 during excessive sun exposure is converted into inactive compounds lumisterol and tachysterol. The endogenous production of vitamin D3 represents 70 to 80% of the total intake of vitamin D. A summer exposure of the arms, torso and legs for half an hour in a white-skinned subject leads to a synthesis of approximately 10,000 to 15,000 Vitamin D3 IU. To cover the necessary vitamin D intakes, Michael Holick, professor of medicine and biophysicist at Boston University recommends an exposure of 2 to 3 days weekly of 0.5 erythemal dose, which is half the time required for an individual suffers a slight sunburn.

Many parameters influence the effects of sun exposure on vitamin D3 synthesis. The quantity of UVB received is in fact dependent on the time of day, and on the year, on the latitude, the altitude, and the pollution. An increase in the solar zenith angle, during the winter period and during the beginnings and ends of the day, is at the origin of an increase in the path of UVB rays in the ozone layer responsible for their absorption (Harinarayan et al., 2013).

In general, below and above latitude 33 ° the synthesis of cholecalciferol is zero during the winter period. Iraq, is at a latitude where the intensity of UVB rays is insufficient most of the year, especially from November to February, to produce an optimal amount of vitamin D. Other parameters not dependent on the environment can disrupt the synthesis of cutaneous vitamin D3. An advanced age due to a decrease in 7-DHC in the skin is a factor favoring vitamin D3 deficiencies. The pigmentation of the skin by melanin constitutes a real UVB filter explaining a lower formation of vitamin D in black populations. The application of sunscreen, as well as clothing habits also have a strong impact. For example, a sun protection factor of 15 can reduce the cutaneous production of cholecalciferol by more than 90%.

Exogenous origin of vitamin D: In our diet two forms of vitamin D exist: cholecalciferol (Vitamin D3) and ergocalciferol (Vitamin D2). However, a small number of foods contain it, and generally in small amounts. For example, around twenty sardines are needed to meet the recommended daily intake. Thus exogenous intakes do not represent more than 20% of the sources of vitamin D in humans. Vitamin D2 comes from a derivative of plant sterol. Its origin therefore comes mainly from plants and certain mushrooms, in particular shiitake, of Asian origin.

Vitamin D3, for its part, is found mainly in fish liver oils such as cod liver oil, in fatty fish such as salmon, herring, sardines, mackerel. Egg yolk is a ten times less rich source of cholecalciferol. Cheese and dairy products fortified with vitamin D3 also represent a significant source. Vitamins D2 and D3 are relatively close in their molecular structures. The difference is a double bond and the presence of a methyl group (Figure 1.2).

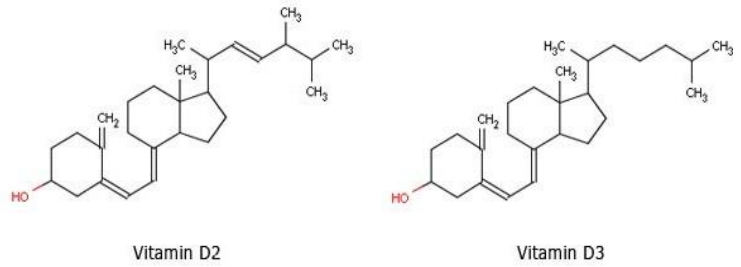

Figure 1.2 Molecular Structure of Vitamin D2 and D3

Metabolism: Vitamins D2 and D3 from food or drug intake are slowly absorbed from the proximal part of the small intestine into the bloodstream. 25 (OH) D and 1,25 (OH) 2 D, due to their higher polarity, are absorbed in the proximal jejunum more quickly and efficiently than vitamin D2 and D3 because they pass directly into the vein door. The absorption of vitamin D is carried out after incorporation into mixed micelles, composed of bile salts of free fatty acids and monoglycerides. After absorption, exogenous vitamin D is mainly integrated into chylomicrons and LDL. Vitamin D3, originating from skin synthesis, is transported by the vitamin D binding protein (DBP) synthesized by the liver. Vitamin D (D2 or D3) needs double hydroxylation to be active. First, the liver carries out a hydroxylation on carbon 25, to form 25 hydroxyvitamin D (25 (OH) D) through 25 OH hydroxylase (CYP2R1). Other cytochromes such as CYP3A4, CYP27A1 and CYP2J2 could also be involved, but to a lesser extent. Schematically, it is often considered that the liver performs all of this hydroxylation, while in fact it only performs about 50%, 25 OH hydroxylase being found ubiquitously. This hydroxylation is not finely regulated and few biological agents are known to influence it. Thus, physiologically, the majority of non-stored vitamin D intake is rapidly hydroxylated to 25 (OH) D. In the general circulation, 25 (OH) D has a long half-life, of the order of 3 weeks, which makes it an excellent marker of the vitamin status of an individual. The 25 (OH) D thus formed is transported in the bloodstream by DBP. The complex is then filtered through the glomerulus and reabsorbed by the epithelium of the proximal tubule of the kidney using megalin. 25 (OH) D is then hydroxylated at carbon 1 by mitochondrial 1-alpha hydroxylase (CYP27B1) to give 1,25 (OH) 2D, a compound with a 500 times higher affinity for VDR than 25 (OH) D. Its half-life is very short, on average 3 to 4 hours. Although predominantly renal, 1-alpha hydroxylase has been found in other tissues. To date, its presence has been determined, among others, in the breast, prostate, pancreas, colon, lungs, skin, parathyroid cells, osteoblasts, monocytes, and blood vessels suggesting ubiquitous synthesis. 25 (OH) D reaching these tissues is internalized and then converted to 1,25 (OH) 2D thus acting in an autocrine or paracrine fashion 37. The blood concentration of 1,25 (OH) 2D is considered to be more than 95% from renal transmission. The remaining 5% corresponds to peripheral hydroxylation. Unlike 25-hydroxylase, renal 1 alpha-hydroxylase is highly regulated to better control the concentration of 1,25 (OH)

2D according to the body's calcium requirements35. Its expression is increased by parathyroid hormone (PTH), is reduced by fibroblast growth factor 23 (FGF23). 1,25 (OH) 2D also regulates it according to the principle of negative feedback. On the other hand, the synthesis of extra-renal 1,25 (OH) 2D does not seem to depend on calcium or on the level of PTH. Vitamin D metabolism involves an inactivation pathway via 24 hydroxylase (CYP24A1), a renal enzyme. On the one hand, it catabolizes 1,25 (OH) 2D into 1,24,25 (OH) 3D, the latter resulting in the formation of inactive calcitroic acid. On the other hand, it hydroxylates 25 (OH) D to 24.25 (OH) 2D, which is also inactive. 24 hydroxylase is inversely regulated with respect to 1,25 (OH) 2D, allowing finer phosphocalcic regulation (Marwaha et al., 2016).

Frequency and origin of CKD deficiency: During the course of CKD, vitamin D deficiency is a very common event. IRC is in fact characterized by both a drop in the concentrations of 25 (OH) D and 1,25 (OH) 2D. Most Iraq (Awad, 2011), 51 observational studies have shown that 25 (OH) D levels decrease with the progression of CKD. This association has not been systematically found. Cunningham and Zehnder noted a prevalence of 75% of insufficiency and 30% of 25 (OH) D deficiency in CRI. Patients (Awad, 2011), on dialysis present a very important vitamin D insufficiency of the order of 81 to 96%.

Many causes can be at the origin of hypovitaminosis D found in the population suffering from CKD. The reduction in serum 25 (OH) D would come from both the low exposure to the sun in patients with CKD, a decrease in the cutaneous capacity to synthesize cholecalciferol, and a decrease in the consumption of rich foods in vitamin D. A recent study conducted by Michaud et al. also highlights a uremic origin, responsible for the reduction of 25 (OH) D by inhibition of 25-Hydroxylase. In addition, the rise in proteinuria during CKD is accompanied by elimination urinary 25 (OH) D bound to DBP. The NEPHROTEST 58 study showed a correlation between blood levels of 25 (OH) D and 1,25 (OH) 2D in CKD patients. In CKD, the frequent vitamin D deficiency therefore has an uncompensated impact on the activity of the VDR. Taken together, these data clearly indicate the installation of an overall vitamin D deficit in the CKD.

1.6.2 Benefit of vitamin D supplementation in CKD

Vitamin D deficiency is therefore frequently found in patients with CKD. It is the source of numerous effects on bone metabolism, and a repercussion on health that can complicate the management in this type of patient. All the beneficial effects of vitamin D have been widely studied through its impact on mortality. A 2008 multivariate study, shows us that a 25 (OH) D concentration of less than 17.8 ng / mL is associated with an increased risk of mortality all causes combined in 26% of cases in the general population. At IRC, a meta-analysis by Pilz et al. described a 14% decrease in mortality for each step increase of 10 ng / mL of 25 (OH) D. However, all mortality studies are retrospective and therefore cannot guarantee a causal link between vitamin D intake and overall mortality. It is, moreover, a limiting agent found for many advanced beneficial effects of vitamin D. Vitamin D could represent a marker of good health linked to better exposure to the sun, and a better diet, not found in patients with multiple diseases.

1.6.3 Nefroprotective effect of vitamin D

Several studies have been developed in order to better understand the role of vitamin D in CKD in addition to phospho-calcium metabolism, considering it a possible therapeutic weapon with enormous potential given its intervention in multiple systems (Gembillo et al., 2019). Due to the lack of effective mechanisms for slowing the progression of CKD, its potential nephroprotective effect is very important. For nephroprotection, several beneficial mechanisms of vitamin D are considered such as suppression of RAAS, decreased systemic inflammation, and decreased proteinuria. The effects of vitamin D on blood pressure and cardiovascular anti-mitogenic effects in chronic kidney patients are also a consequence of these mechanisms, having high importance given their contribution to disease progression and mortality in these patients.

1.6.4 Vitamin D as a drug

supplementation and activated vitamin D analogues Nutritional supplementation of vitamin D includes ergocalciferol, vitamin D2, and cholecalciferol, vitamin D3, both available naturally in some foods, as mentioned above. Studies in healthy subjects have demonstrated a lower efficiency of ergocalciferol in maintaining 25 (OH) D levels within the normal range compared to cholecalciferol after a single oral dose, the difference being 2 weeks to 30 days (de Brito Galvao et al., 2013). However, a recent meta-analysis concludes that the effectiveness of both forms when administered daily is similar. Although nutritional supplements in Portugal are not fully regulated by Infarmed - National Authority for Medicines and Health Products, I. P., according to him, in Portugal only cholecalciferol is available for nutritional supplementation. Calcifediol, 25 (OH) D, available for oral administration in Europe, has the particularity that it no longer requires hepatic hydroxylation. However, this requires attention to dosage, since it has a half-life of 15-18 days, significantly longer than 3-7 days of nutritional vitamin D half-life. This can cause rapid accumulation to serum levels above the upper limit of normal. Pharmacological calcitriol is chemically equal to endogenous, and in addition to hepatic hydroxylation, it also no longer requires hydroxylation by 1□-hydroxylase, being available for direct binding to VDR (Lang et al., 2014).

The active forms of vitamin D have been prescribed since the 1970s to reduce HPTS, they generally have the effect of reducing PTH levels by almost 50%.

In Iraq, only two active forms are available, calcitriol and afacalcidol. The latter is the most prescribed and latest drugs available in Iraq pharmacy given in table 1.1

Table 1.1 The different vitamin D specialties, usable at CKD and available in pharmacies in 2020.

Vitamin type: Cholecalciferol (Vitamin D3)	
ZYMAD 10,000 IU / mL	10 mL vial: 333 drops at 300 IU / drop
ZYMAD Bulbs	Bulbs of 80,000 IU and 200,000 IU
Vitamin D3 B.O.N	200,000 IU ampoule
UVEDOSE and generics	100,000 IU ampoule
ADRIGYL	10 mL vial: 300 drops at 333 IU / drop
Vitamin type: Ergocalciferol (Vitamin D2)	
STEROGYL drops	20 mL vial 1000 drops at 400IU / drop
	Bulb of 600,000 IU drinkable
STEROGYL	Solution for injection 600,000 IU / 1.5 ml (IM)
Vitamin type: Hydroxylated derivative of vitamin D → Calcifediol (25 (OH) D3)	
DEDROGYL	10 mL vial: 300 drops at 5 µg
Vitamin type: Hydroxylated derivative of vitamin D → Calcitriol (1,25 (OH) 2D3)	
ROCALTROL	0.25 µg tablets
Vitamin type: Hydroxylated derivatives of vitamin D → Alfacalcidol	
ONE-ALPHA	10 mL vial: 200 drops at 0.1 µg / drop 0.25 µg, 0.50 µg and 1 µg tablets Solution for injection IV:
	1µg / 0.5mL ampoules
	2 µg / 1mL ampoules

The dosage of serum 1,25 (OH) 2D is very infrequent in Iraq due to the complexity of the dosage, and a less well-established relationship with mortality. It is most often prescribed for the etiological exploration of hypercalcemia, or to analyze a residual endogenous synthesis in CKD in the management of HPTS, or finally to monitor the effectiveness of supplementation by the forms active 55.

1.6.5 Evidence from experimental studies

Several studies have been done regarding the inhibitory effect of SARS. Mice without VDR expression have a 3-fold increased renin expression with a consequent increase in plasma angiotensin II and, clinically, arterial hypertension. In cell cultures of As 4.1 cells, with high renin synthesis, the administration of 1.25 (OH) 2D markedly decreased the activity of the promoter region of the VDR-mediated renin gene, resulting in a decrease in the respective messenger ribonucleic acid (mRNA). In mice lacking 1α-hydroxylase expression, administration of 1.25 (OH) 2D was also effective in decreasing the activation of RAAS and in the treatment of arterial hypertension (Gangula et al., 2013). An experimental study with partially nephrectomized animal models evaluated the renal effect of administering an ACE inhibitor, enalapril, a vitamin D analogue, paricalcitol, or both. It was found that in most of the parameters studied, the combination was more effective than either of the two drugs separately. In addition to lowering blood pressure to normal levels and a lower incidence of glomerulosclerosis, also achieved with enalapril monotherapy, the combination enalapril-paricalcitol allowed less decrease in creatinine clearance, less proteinuria, less interstitial fibrosis, less renal inflammation with decreased macrophages, MCP-1 expression, normalization of TGF-β1 and Smad2. There were no significant changes in calcium and phosphorus, and paricalcitol has a less active bone metabolism than calcitriol (Husain et al., 2015).

Nolan et al. (2015) address the nephroprotective effect of vitamin D through its anti-inflammatory action specifically on the TGF-β1 cytokine. They subjected epithelial cell cultures from proximal renal tubules to 5 ng / mL TGF-β1, after cellular stimulation with 1-2 μM paricalcitol or 0.1% ethyl alcohol, the latter serving as a control. Paricalcitol significantly decreased the action of TGF-β1, increasing the protein expression of E-cadherin and decreasing the gene and protein expression of Jag-1, α-SMA, CTGF and Thrombospondin-1 (TSP-1). The 2 μM dose of paricalcitol also significantly decreased the activation of Smad2, one of the pathways subsequent to activation of the TGF--β1receptor. Some cell cultures were placed in a hypoxic chamber, with no differences from the normoxic ones, with the exception of the

expected HIF-1α stabilization. This stabilization was also observed in normoxic cultures stimulated with paricalcitol, although with less intensity, being dose and time-dependent. Although the role of HIF stabilization is still unclear, some pharmacological hydrolases inhibitors responsible for HIF degradation have shown anti-inflammatory and protective effects in several models of the disease, including kidney damage (Nakuluri et al., 2019).

Paricalcitol has been evaluated in several studies, in addition to those already mentioned here. In an animal model of chronic kidney disease induced by cyclosporine A and gentamicin, it reduces renal inflammation by decreasing the activity of NFkB, by stabilizing its inhibitory subunit, Kappa B inhibitor (IkB). In cell cultures, it also inhibits the tubular expression of RANTES, an important proinflammatory chemokine in renal obstruction. In mice treated with cisplatin, an apoptosis-inducing model, paricalcitol decreases the number of apoptotic cells by attenuating the expression of phospho-p53 and p21, as well as reversing the increase in the Bax / Bcl-2 ratio and caspase-degradation products 3 in an animal model characterized by loss of podocytes and frank proteinuria (9). Studies in animal models and cell cultures appear to support the benefit of active vitamin D in slowing the progression of CKD, suggesting that its association with other RAAS inhibitors is advantageous. However, several important issues arise such as doses, routes of administration, long-term effect, side effects, among others.

1.6.6 Evidence in clinical studies

The nephroprotective potential of vitamin D demonstrated in preclinical studies and its relative safety has aroused interest in clinical studies. However, the population of chronic kidney patients is very heterogeneous, both in terms of aetiology and the stage of CKD patients are in, making comparisons difficult and there are several possible confounders. As a result, few clinical studies have been found regarding the nephroprotective effect of vitamin D.

The Vital study (2010) (De Zeeuw et al., 2010), is a randomized and controlled international study, in which Portugal was one of the participants. Diabetic patients already on therapy with established SARS inhibitors, ACEIs or ARA's II, the effect of adding therapy with paricalcitol, 1 or 2 µg, on albuminuria and TFGe was studied. It should be noted that of the 281 eligible patients, 72% had macroalbuminuria (> 300 mg / day) and 42% had maximum recommended doses of ACEI or ARA's II. The average levels of 25 (OH) D and 1.25 (OH) 2D were quite similar between the groups, being respectively less than 20 ng / mL and approximately 36 pg / mL, both lower than considered sufficient. At week 4 of the study, the group treated with paricalcitol at 2 µg showed a reduction in the urinary albumin-creatinine ratio compared to the placebo group with a difference of -18% (p = 0.053), peaking at the 12th week of study, with a decrease of 28% (60 mg / mmol to 43 mg / mmol, 95% CI). The patients in this group who benefited most from the intervention were those with a higher sodium excretion. At the same time, the average urinary albumin excretion rate in 24 hours also decreased in the 2 µg paricalcitol group, to a 28% decrease in a statistically significant way. Although there was also a reduction in these parameters with 1 µg of paricalcitol, this was smaller and without statistically significant results. In the group with 2 µg of paricalcitol, there was also a decrease in systolic blood pressure and a modest decrease in eGFR from 3 to 5 mL / min / 1.73 m^2 (p = 0.0548). However, this decrease in GFR is attributed to its effect in increasing serum creatinine by increasing production, as previously seen with calcitriol therapy, and not by decreasing its excretion. Although not part of the primary end-points of the study, it should be noted that PTHi values decreased significantly in both groups treated with paricalcitol, and that no significant differences were found in relation to the levels of CRP, fibrinogen, IL-6 , plasma renin activity, aldosterone or TNF-□. Throughout the study, paricalcitol proved to be a safe drug, requiring only control of PTH levels to adjust the dose. In previous observational studies, prolonged use of paricalcitol has been associated with increased survival. After 30 and 60 days of its completion, the repetition of blood tests showed a return to the analytical levels prior to the study, reinforcing the action of paricalcitol in the results. In a post-hoc study of pre-dialysis patients in stage 4 or 5 of CKD, the use of activated vitamin D analogue, mostly alfacalcidol, was independently associated with a lower risk of progression to stage 5 or 50% reduction in CKD TFGe, suggesting a

nephroprotective effect in advanced stages. However, therapeutic doses are not indicated and levels of 25 (OH) D, 1.25 (OH) 2D, among others, have not been evaluated (Williams et al., 2009).

1.6.7 Recommendations for vitamin D therapy in the course of CKD

An effective approach to the treatment of bone and mineral metabolism disorders in CKD, using existing recommendations and clinical and experimental evidence from the Evaluation of Outcome Kidney Disease (KDOQI). Bone and mineral metabolism disturbance should be measured by intact PTH measurement earlier during CKD and treatments with the 'stepped treatment' method should be taken. Then the vitamin D status should be assessed in the step 2/3 CKD if PTH is high and then care should meet current guidelines of practise to administer the vitamin D preparation, such as ergocalciferol, at the doses needed to increase the 25- hydroxyvitamin D level to above 30 ng / ml. In the case of < 30 ng / ml, care should then be carried out in compliance with current practise guidelines. Vitamin D has no substantial biological activity either as D3 or D2, and must be converted into hormone-active 1,25 dihydroxyvitamin D in the body. In comparative studies in CCD patients the discrepancy between the levels of 25-hydroxyvitamin D (ergocalciferol D2) and cholecalciferol (vitamin D3) was not observed (Kawashima et al., 1982).

Phosphorus dietary restriction may be used to regulate hyperparathyroidism in early CKD, but protein constraint should be moderate for the purpose of preventing malnutrition. Other active steps include the use of calcium supplements and the use of phosphate binders and the use of vitamin D vitamins, such as calcitriol, alfacalcidol and doxerkalciterol vitamin D prohormones and analogue paricalcitol vitamin D. The use of active vitamin D sterols to treat hyperparathyroidism is highly beneficial in advanced kidney disease. The 1-α-hydroxyvitamin D3 and 1-α-hydroxyvitamin D2 vitamins are 25-hydroxylated and are 1-25-dihydroxyvitamins D3 and 1-25-dihydroxyvitamin D2 respectively. Vitamin D Vitamin D analogues are active vitamin D molecules with side-chain modifications or A-ring changes implemented in order to eliminate PTH and reduce the effect on levels of calcium and phosphorus. Introducing three analogues

including 19-nor-1,25-dihydroxyvitamin D2, 22, and 26,27-hexafluorocalcitriol have been used. 19-Nor-1,25-dihydroxyvitamin D2 is commonly used in the USA, with significantly lower toxicity than native calcitriol sterol. In stage 3 and in stage 4, the oral outline may be used. No comparative protection and effectiveness studies are conducted on the various vitamin D analogues in CKD patients (Kawashima et al., 1982).

Further studies are required in view of recent findings by Wolf et al. regarding the significance of vitamin D therapy in CKD. In a retrospective cross-sectional study of 825 event hemodialysis populations these authors have explored the association between vitamin D and early mortality levels. The team assessed baseline blood levels of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D and evaluated 90-d death. Vitamin D was deficient, as did 18 pourcent and low 25-hydroxyvitamin D levels, in total, 78 percent of the patient population. Increased mortality. Enabled vitamin D intervention tends to be associated with better outcomes. These primary findings would require confirmation and more randomised , placebo-controlled trials, and more research would be needed if vitamin D sterols are to determine the effects of renal disease progression (Rusińska et al., 2018).

Vitamin D can be acquired in two ways, through diet (vitamin D_2) or through endogenous synthesis induced through the action of ultraviolet B (UVB) radiation in the skin tissue (vitamin D_3). However, in the kidneys, due to the action of the 1-α-hydroxylase enzyme, this metabolite is transformed into the active hormonal form of vitamin D, to 1.25 (OH) D or calcitriol. In recent years, vitamin D has been widely researched and it has been noted that its role goes far beyond its role in bone and calcium metabolism, observing its influence on the cardiovascular and immune systems and on the modulation of pathological and physiological processes, acting in this way, in decreasing the risk of certain chronic diseases, such as some types of cancers (Suh et al., 2010). Clinical and translational studies do not establish the relationship between vitamin D supplementation in patients diagnosed with Vitamin D and CKD. However, this research can contribute to elucidate this issue, in addition to subsidizing the clinical practice of health professionals and assist in the preparation of specific care protocols

for this population, with peculiar clinical characteristics and little discussed in the scope of research. This study aimed to evaluate the results of studies that supplemented with vitamin D, impact of SOD on patients with CKD.

1.6.8 Vitamin D compounds for patients with chronic kidney disease

Patients with chronic renal failure have mortality that greatly exceeds the rate predicted by conventional cardiovascular risk factors. Vitamin D deficiency, highly prevalent in this population, is also independently associated with mortality. A better understanding of the physiology of vitamin D has made it possible to highlight the so-called "extrarenal" functions of this vitamin. Through its cardiovascular effects, for example, vitamin D substitution could functionally participate in the reduction of mortality in renal failure. This article summarizes knowledge about vitamin D in chronic renal failure and offers recommendations on screening and vitamin D substitution in this at-risk population.

Patients with chronic kidney disease have a mortality that far exceeds the rate predicted by "classic" cardiovascular risk factors. The mortality of dialysis patients is thus of the order of 10% per year in our department. The cause of this excess mortality is a subject of active research. Phosphocalcium homeostasis imbalance, an inevitable consequence of loss of kidney function, is a nominee. Several studies have notably demonstrated an independent and strong association between hyperphosphatemia and mortality in patients with CKD (Hruska et al., 2011). Many observational studies have also reported an independent link between vitamin D deficiency and mortality in CKD (Wolf et al., 2007). In a recent metaanalysis, the relative risk of mortality was 0.86 per 25 nmol / l increase in 25-hydroxy-vitamin D3 (25 (OH) D_3) (Pilz et al., 2011).In addition, an improvement in survival was observed in patients dialysis patients receiving active vitamin D therapy (Naves-Díaz et al., 2008). This effect was not explained solely by the suppressive effect on the secretion of PTH (parathyroid hormone). Vitamin D is a central component of phosphocalcic hemostasis and its biosynthesis is directly altered in CKD. Vitamin D could thus constitute the missing link between CRI, hyperphosphatemia and excess mortality.

The essential role of the kidney in the production of the active form of vitamin D, 1,25-hydroxy-vitamin D3 (1,25 (OH) D3), is an old concept. The loss of renal 1-α-hydroxylase activity in advanced CKD is a central element in the pathophysiology of secondary hyperparathyroidism and bone metabolic disorders related to renal failure (subsequently abbreviated "renal bone disease"). Over the past fifteen years, however, many scientific advances have profoundly changed the understanding of the physiology of vitamin D. In particular, it appeared that the vitamin D receptor (VDR) was expressed much more broadly than the bone effects " 'traditional' vitamin D treatments did not predict it. In addition, the coexpression of VDR with 1-α-hydroxylase, the activating enzyme of vitamin D, in many peripheral tissues, has given rise to a new concept: the active form of vitamin D, 1,25 (OH) D3, can be synthesized in extrarenal sites and generate local autocrine effects (Cunningham and Zehnder, 2011). Vitamin D thus plays a physiological role in many tissues such as the myocardium, muscles, pancreas, endothelium, brain and even immune cells (Thacher and Clarke, 2011).

Vitamin D has thus become an essential subject already discussed in two excellent reviews published in this journal (Bonny and Burnier, 2008). This review article attempts to describe more specifically the state of knowledge on the roles of vitamin D in CKD, both in connection with phosphocalcic metabolism and its extra-osseous effects. The first part describes the biosynthesis of vitamin D and briefly reviews its role in the pathophysiology of phosphocalcic metabolism in CKD. The second part looks at the extra-osseous functions of vitamin D involved in kidney disease. Finally, a final practical part offers recommendations on screening for and replacing a native vitamin D deficiency in patients with kidney disease. The terms "native vitamin D", vitamin D or cholecalciferol will be used synonymously and will refer to cholecalciferol. The term "active vitamin D" will refer to calcitriol (1,25 (OH) D3). Calcidiol or calcifediol is the intermediate form of vitamin D after hepatic 25-hydroxylation (25 (OH) D3).

1.6.9 Role of vitamin D in the physiopathology of kidney failure

Vitamin D biosynthesis: The name "vitamin D" is not correct because it is a hormone. This is because, unlike other vitamins, vitamin D can be synthesized by the body in the

skin. The origin of circulating vitamin D is twofold: exogenous, food (ergocalciferol, cholecalciferol, present for example in certain plants or fatty fish) and endogenous (by skin synthesis). Dietary vitamin D accounts for 10-20% of the circulating form, the majority coming from skin synthesis. When the skin is exposed to the sun, 7-dehydrocholesterol is converted by UVB rays into previtamin D3 then isomerized into so-called “native” vitamin D3 (cholecalciferol) (figure 1.3) (Jones, 2007). A first hydroxylation takes place at the hepatic level (25-hydroxy-vitamin D3, 25 (OH) D3, calcidiol or calcifediol) and a second takes place in the kidney (1,25-hydroxy-vitamin D3, 1,25 (OH) D3, “active” vitamin D or calcitriol). The enzyme responsible for this second hydroxylation (1-alpha-hydroxylase) is mainly expressed in the proximal renal tubular cells which are the main source of the circulating 1,25 (OH) D3 fraction. This enzyme is also expressed in a large number of other peripheral tissues (see paragraph "extra-osseous" effects of vitamin D). 25 (OH) D3 has a long half-life (two to three weeks) and represents the main circulating form of vitamin D. Its dosage is thus the best reflection of the vitamin stock of the organism (Holick et al., 2011). 1.25 (OH) D3 is the activated form of vitamin D3 responsible for the biological effects of the vitamin. On the other hand, it has a shorter half-life of four to six hours.

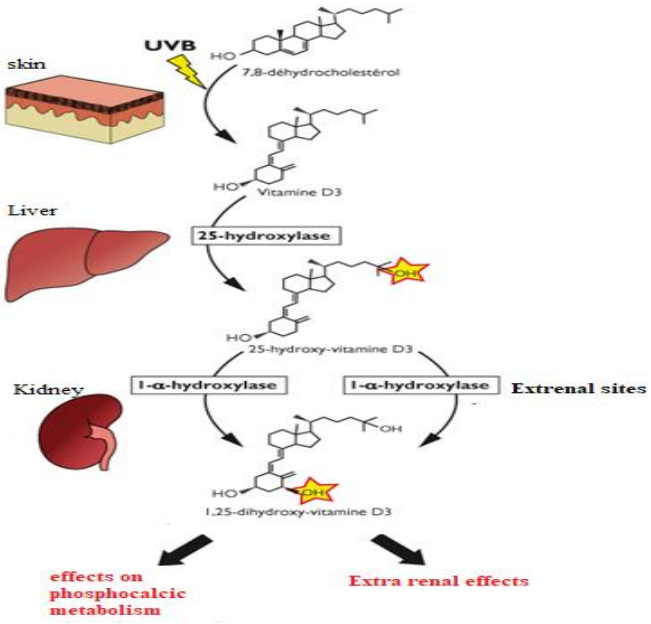

Figure 1.3 Vitamin D and phosphocalcic metabolism in chronic renal failure.

Vitamin D3 has long been known for its bone effects and its role in kidney bone disease is clearly established. Active vitamin D acts directly on the increase of intestinal absorption and renal tubular reabsorption of calcium, increases bone resorption and inhibits the secretion of PTH. Prolonged vitamin D deficiency is the cause of rickets in children and osteomalacia in adults. In CKD, the loss of nephronic mass is associated with a decrease in renal activity of 1-α-hydroxylase and therefore in the circulating level of 1,25 (OH) D3. This results in a negative calcium balance and a secondary elevation of PTH (secondary hyperparathyroidism), responsible for kidney bone disease. This effect appears late and is conventionally described during stage 3 of CKD (glomerular filtration rate <30 ml / min).

Decreased renal function is also associated with early onset phosphate retention (from stages 1-2). Adaptive mechanisms are being set up which also influence the metabolism of vitamin D (Figure 1.4). In particular, FGF23 and Klotho are two proteins whose central phosphaturic role has just been recently described in IRC. FGF23 and its coreceptor Klotho increase renal phosphate excretion by inhibiting the expression of the phosphate cotransporter (NaPi) in proximal tubular cells. From stage 1 of CKD, a decrease in Klotho expression is observed and could participate in phosphate retention (Kuro-o, 2011). At the same time, the expression of FGF23 increases from stage 2 of CKD, promoting elimination renal phosphate. This early adaptive mechanism preserves phosphocalcic homeostasis by preventing plasma phosphate elevation in the early stages of CKD. In advanced stages of CKD, the very high concentration of FGF23 could be deleterious acting as a uremic toxin (Jüppner, 2011).

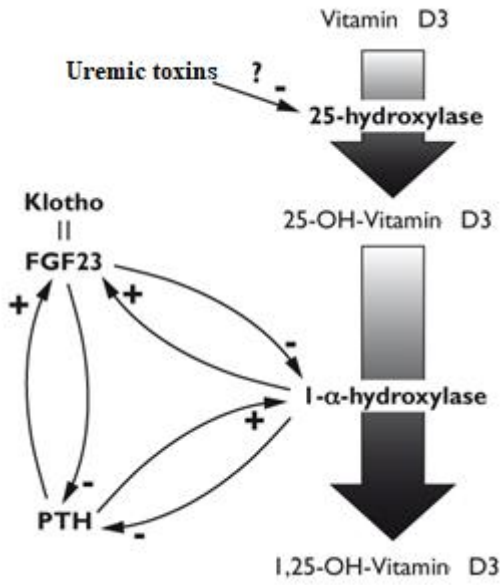

Figure 1.4 Adaptive mechanisms and influence of the metabolism of vitamin D

In addition, FGF23 inhibits 1-α-hydroxylase and the secretion of PTH. Conversely, the increase in 1,25 (OH) D3 possibly has an inducing effect on the expression of Klotho. And closing the loop, 1,25 (OH) D3 has a well described inhibitory effect on PTH secretion. Finally, PTH stimulates the production of 1,25 (OH) D3 and FGF23. Uremia could also inhibit hepatic hydroxylation of vitamin D decreasing the production of 25 (OH) D3 (Michaud et al., 2010). Klotho, FGF23, PTH and 1-α-hydroxylase activity thus interact in a complex manner to maintain a serum calcium levels and limit the plasma phosphate rise (Figure 1.5). They thus early influence the metabolism of vitamin D in CKD. As renal failure progresses, these mechanisms are nonetheless overwhelmed and phosphatemia rises (Figure 3). Persistent hyperparathyroidism is responsible for the negative consequences of CKD on bone mineralization. Hyperphosphatemia and vitamin D deficiency are two candidates that may explain the excess mortality in CKD patients.

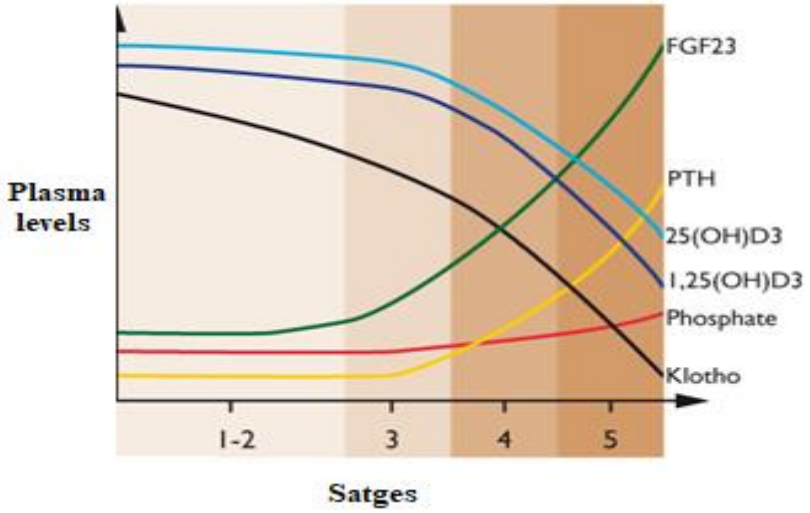

Figure 1.5 Chronic renal failure and vitamin D deficiency.

IRC from its earliest stages is also an important risk factor for 25 (OH) D3 deficiency. The prevalence of vitamin D insufficiency and deficiency is thus of the order of 75% and 30% respectively in chronic renal failure, but can be much higher in certain series (Bouchard et al., 2009). The prevalence of a vitamin deficiency D increases with worsening kidney function regardless of age, sex, weight and race(Chonchol and Scragg, 2007). The decrease in 25 (OH) D3 correlates with the decrease in 1.25 (OH) D3 regardless of the stage of renal failure. The decrease in 25 (OH) D3 may participate in the decrease in 1,25 (OH) D3 observed in advanced stages of renal failure, not in due to the decrease in the activity of 1-α-hydroxylase, but simply by a decrease in the substrate of this enzyme (Dusso and Tokumoto, 2011).

The causes mentioned are a shorter exposure to the sun of a sick population, a diet depleted in vitamin D, or even a less efficient production of vitamin D3 by the skin, like people over 70 years of age (Goldsmith and Cunningham, 2011). Recently, a study also suggests a decrease in hepatic 25-hydroxylase activity linked to uremia (Michaud et al., 2010). Nephrotic syndrome is also a high-risk situation for vitamin D3 deficiency, independent of the loss of renal function. The high proteinuria in these patients is indeed accompanied by a urinary leakage of 25 (OH) D3 linked to the vitamin D binding protein (DBP). Severe vitamin D deficiency can then develop (Vaziri, 1993). This

mechanism is likely to occur in any patient with significant proteinuria, for example secondary to diabetic nephropathy.

The consequences of 25 (OH) D3 deficiency are still only partially understood in kidney disease. 25 (OH) D3 deficiency may contribute to bone complications of CKD. But it is undoubtedly its extra-osseous effects that are likely to explain part of the excess mortality of CKD patients.

1.7 Oxidative Stress

Oxidative stress is increasingly studied both in the field of research and in human medicine. It has been defined as a pronounced imbalance between the antioxidant and oxidant elements in favor of the latter and their potentially harmful effects. In addition, recent research suggests that oxidative stress is involved in the pathophysiology or in the genesis of certain pathological entities. The effects of oxidative stress at the vascular level are well known, it is an endothelial dysfunction and an increase in intracellular calcium which leads to an increase in contractility, then hypertension. In addition, many studies have shown that patients with chronic renal failure undergo deleterious changes in the structure of proteins and lipids secondary to the loss of antioxidant defenses and increased oxidative stress

The imbalance between reactive oxygen species (ROS) and the body's antioxidants, due to factors such as inadequate nutrition and physical inactivity, can lead to a condition called oxidative stress (EO). EO is one of the factors that trigger pathological conditions, such as cardiovascular diseases, neurological disorders, lung diseases and premature aging (Di Rosanna and Salvatore, 2012).

The body's enzymatic defense for ROS are the enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), among other reducing enzymes. SOD is known as the first line of defense, catalyzing the dismutation of superoxide ions into hydrogen peroxide, later transformed into water and oxygen by CAT, GPX or others.

In eukaryotic organisms we can find two intracellular forms of SOD: Cu-Zn-SOD and Mn-SOD (found mainly in the mitochondrial matrix), in addition to the extracellular form of the enzyme, EC-SOD. The presence of SOD in different compartments is important due to the inoxide's inability to permeate membranes and, therefore, needs to be eliminated in the same compartment in which it was formed. Extracellular superoxide dismutase (SOD; EC 1.15.1.1) (EC-SOD or SOD-3) controls the level of superoxide in the extracellular space, catalyzing the dismutation of superoxide in hydrogen peroxide and molecular oxygen. In addition, EC-SOD can react with hydrogen peroxide in a peroxidase reaction, which is known to discontinue its activity - this being the mechanism for controlling enzyme activity. It is mainly expressed in the lung and vascular musculature, but also in the heart, kidneys and placenta(Acharya and Ghaskadbi, 2010).

There are different methods for the quantification or determination of EC-SOD activity, including the method developed by (Misra and Fridovich, 1972)' based on the enzyme's ability to inhibit adrenaline auto-oxidation. Despite the existence of more modern techniques, some works still use the method based on the auto-oxidation of adrenaline, such as that developed by Dries (2011), and (Bhatia et al., 2003), mainly due to the low cost analysis.

(Zhou and Prognon, 2006), believe that there will be a growing interest in the field of enzyme determinations, especially in relation to antioxidants and anti-stress, which increases the need for knowledge of the levels of SOD and other antioxidant enzymes in the population and confirm that studies in this area are fundamental. To have an understanding of the reducing processes, it is necessary to have quantitative information on the generation and removal of superoxide and hydrogen peroxide in cells and tissues (Wagner et al., 2013).

Some studies suggest that oxidative stress or decreased antioxidant protection is related to the progress and pathology of various diseases, including schizophrenia, type 2 diabetes mellitus, where, in addition to the decrease in SOD levels, glycation of enzyme bound to erythrocytes, leading to low enzyme activity.

Our intention was to assess the oxidation of the different molecular lines (proteins, lipids and genetic material) and to discern if any of them would behave as a better oxidative marker in a population grouped in an advanced stage of kidney disease, but without the influence of possible factors contributed by dialysis techniques.

1.7.1 Superoxide Dismutase (SOD)

Superoxide Dismutase (SOD) is a major antioxidant enzyme found in all living organisms. Its function is to neutralize oxygenated free radicals, converting it into non-toxic molecules. It thus exerts a regulatory role of oxidative stress which is responsible for senescence and numerous pathologies. It is one of the most powerful enzymes to fight against oxidation, along with catalase and glutathione peroxidase. In the body, SOD is linked to trace elements. We distinguish 3 types of functions according to its place of activity and the metal with which it is associated:

Superoxide dismutase (SOD) destroys free radical superoxide by transforming it into peroxide which in turn can be destroyed by CAT or GPX reactions. SOD converts the highly reactive superoxide radical to less reactive H_2O_2. In humans, there are three forms of SOD: cytosolic SOD (Cu and Zn dependent), mitochondrial SOD (Mn dependent), and extracellular SOD.

Intracellular SOD, linked to copper and zinc (Cu-Zn), protects polyunsaturated fatty acids from oxidation extracellular SOD, linked to copper and zinc, has a role in protecting proteins in the cell matrix intracellular SOD, linked with manganese (Mn), transforms oxygenated free radicals and protects from apoptosis, cell death (Matés and Sánchez-Jiménez, 2000).

These are mitochondrial superoxide dismutase (SOD Mn), an antioxidant enzyme, and the protein DNA-Damaged Binding 2 (DDB2). The participation of SOD Mn in tumor development is currently poorly understood and depends on the origin of the tumor cell, as well as the regulation of constitutive expression of its gene. In contrast, the biological involvement of DDB2 in tumor growth has never been described in the literature.

These impacts can be explained by the ability of SOD to fight oxidative stress. Indeed, intense or prolonged physical exercise generates free radicals mechanism. In patients with diabetic nephropathy, hyperglycemia is capable of causing an increase in ROS that leads to a decrease in manganese-like superoxide dismutase (mSOD). This molecule in turn is responsible for the maintenance of superoxide dismutase (SOD) at the mitochondrial level. This leads to a decrease in mitochondrial SOD leading to an increase in ROS at the mitochondrial level creating a vicious cycle that contributes to mitochondrial dysfunction. The increase in ROS production in turn causes an activation of the inflammatory cascade and mitogenic processes in the kidney. These changes lead to fibrotic remodeling of the kidney and a decrease in glutathione peroxidase (GPx), SOD, catalase (CAT), and nitric oxide synthase (NOS). These molecules are the main antioxidant enzymes in the kidney and lead to increased ROS production at the kidney level. These changes have been demonstrated in the early stages of CKD (Figure 1.6).

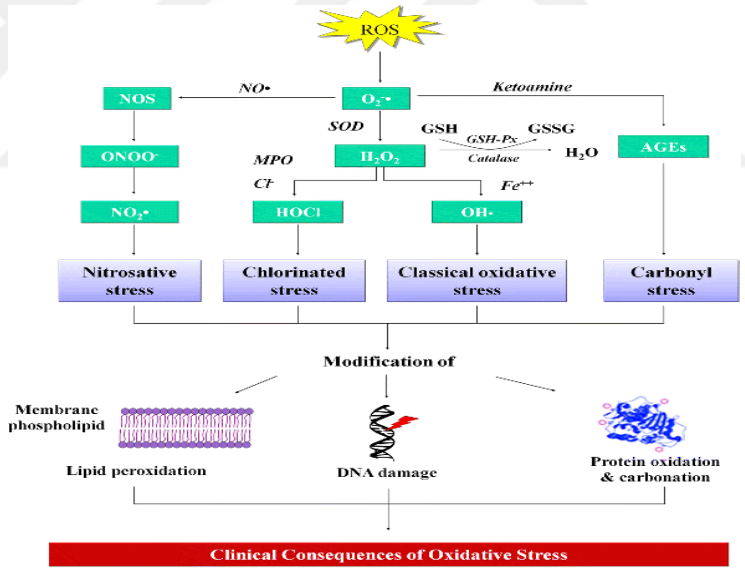

Figure 1.6 Clinical consequences of oxidative stress

Knowledge of the biological production of superoxide radical, and therefore the existence of superoxide dismutase, dates back more than three decades. The ubiquity of this enzyme in aerobic organisms suggests that it constitutes an important defense against oxygen toxicity. However, although one would expect to find superoxide dismutase in all oxygen tolerant organisms, some exceptions have been found

(Lactobacillus plantarum, Mycoplasma pneumoniae, and a widespread strain of Neisseria) lacking this enzyme.

There are different isoforms of this metalloenzyme, differing in their structure and depending on whether one or another metal ion contains in its active center, separating into two phylogenetic phases depending on the similarity in the amino acid sequence, but all of them catalyze the same chemical reaction below with comparable efficacy. It includes enzymes located in organelles, subcellular compartments, and in small amounts in extracellular fluids.

Excluding some exceptions, the different superoxide dismutases are distributed in eukaryotic cells. Cu, Zn-SOD of eukaryotic origin, although it is generally described as a cytoplasmic enzyme, has also been located in the intermembrane space in the mitochondria, in the nucleus, lysosomes and in peroxisomes; and Mn-SOD of prokaryotic origin, although it is located essentially in the mitochondrial matrix it is also found in the cytosol, where according to (Weisiger and Fridovich, 1973), its biosynthesis takes place, being then transported in some way to the interior of the mitochondria. Cu, Zn-SOD is distributed mainly in tissues with high metabolic activity (liver, kidney ...), and Mn-SOD in those with high respiratory processes (liver, kidney and myocardium). There is another type of superoxide dismutase, closely related to Fe-containing Mn-SOD (Fe-SOD), of prokaryotic origin, but which is only found in some species of bacteria and plants, together or not, with Mn-SOD. Another is the extracellular superoxide dismutase (EC-SOD), which also has copper and zinc, but is very different from the intracellular form. It is secreted into plasma and interstitial fluid, where it is by far the predominant isozyme. Unlike Cu, Zn-SOD and Mn-SOD, it shows a different distribution pattern, which is not related to the metabolic activity of the tissues248. In mammals, 90-99% of EC-SOD is localized in tissues, probably mostly bound to proteoglycans heparan sulfate on the cell surface (mainly the endothelium), on the basement membrane and in the tissue matrix connective, which determines the distribution and retention of the enzyme in vivo. However, in some cases, it can be stored intracellularly in secretory vesicles, without being certain that it contributes to protection against the superoxide radical. Therefore, the scope of action of superoxide

dismutase is very wide, it can do so at the extracellular level, but mainly at the intracellular level in the mitochondria and cytosolic compartments. The regulation of production is sensitive both to the amount of tissue oxygen and to the production of intracellular superoxide radical. Increased synthesis under high oxygen tensions has been demonstrated in rats. It catalyzes a dismutation reaction that consists of the oxidation of a superoxide radical to molecular oxygen and the reduction of another superoxide radical to hydrogen peroxide:

$$2O_2^{\cdot -} + 2H^+ \underset{\rightarrow}{SOD} H_2O_2 + O_2$$

It can occur spontaneously at pH 7.4 in which case it is a second order reaction dependent on the concentration of superoxide radical, or be accelerated by approximately 10,000 times by the action of superoxide dismutas, the latter being a first-order reaction, so the half-life of the superoxide radical is independent of its concentration. Spontaneous dismutation between two superoxide anions is probably so slow, because electrostatic repulsion prevents the approach that would allow electron transfer. Therefore, the simple catalytic mechanism of reaction would involve alternating reduction and oxidation of the catalyst (SOD) during successive encounters with the superoxide radical. The catalyst in this way would be able to transfer an electron from one superoxide radical to another without the need for the two anions to come together. In the case of Cu, Zn-SOD, copper is the one that participates in the catalytic cycle, and fluctuates between the cupric and cuprous state, while zinc seems to have only a structural function. The trivalent and divalent states of metals are those involved in the catalytic cycle of Mn-SOD and Fe-SOD. Consequently, it acts by protecting against the toxicity of the superoxide radical, reducing it or eliminating it, either directly or derived from the interaction with other reactive oxygen species. A peroxidase activity of Cu, Zn-SOD has also been described, which can have harmful effects on cells by catalyzing the formation of free radicals using anionic scavengers and hydrogen peroxide as substrates.

Due to the rapid removal of superoxide radical, superoxide dismutase prevents hydroxyl radical production.

1.8 Justification

CKD is a pathology of great importance at the global, regional and national levels. The magnitude of its impact is reflected in its high prevalence, its high mortality rates, and the high costs of its treatment, the years of disability and the bio psychosocial affectation that it produces. CKD is the fourth leading cause of age-adjusted mortality above diabetes and cancer.

In Iraq, in addition to its epidemiological impact, CKD produces great problems derived from health care. Among the main ones are: late referral or in advanced stages, absence of early identification programs in people with risk factors for CKD, lack of specialist doctors, limitation of therapeutic options to better control CKD complications and lack of documents clear guidelines focused on patients with kidney disease. The important socioeconomic impact of CKD should be considered, taking into account the direct cost of renal replacement therapies, as well as indirect costs such as absence from work and the economic burden. Additionally, the social and family impact should not be forgotten, as well as the psychological consequences that a person affected by CKD can have, especially in the more advanced stages. The challenge and complexity that the management of chronic kidney disease represents has led to several different scientific institutions to develop clinical practice guidelines (CPG) for the optimal management of CKD, based on a rigorous systematic review process of the available evidence.

1.9 Hypothesis

The working hypothesis will be based, on the confirmation that in chronic kidney disease there is an imbalance between oxidative and antioxidant factors generating a state of oxidative stress and that, if there are significant differences between the different CKD situations (Predialysis (Pre-D), HD and DP), it will be possible to

quantify and try to establish the influence of dialysis techniques in the control of oxidative balance. Another Hypothesis is to determine the correlation between the Vitamin D3 and CKD patients from Iraq.

1.10 Objectives

- This project aims to take stock of the origin of vitamin D deficiency in CKD, to discuss the renal and extra renal consequences of such a deficiency, as well as the importance of vitamin supplementation in the context of CKD.

- The objective of our study is to evaluate in a hospitalized population suffering from CKD, the percentage of supplementation with Cholecalciferol (vitamin D3) which follows the recommendations and their impact on several serum parameters: phosphocalcic balance, PTH, 25 (OH) D , 1,25 (OH) 2 D. We also investigated whether the pharmaceutical analysis of prescriptions had a positive impact on the application of recommendations.

- To assess, through the analysis of pro-oxidant and antioxidant parameters, the amount of oxidative imbalance in three different populations of chronic renal failure, and the effect that renal replacement techniques have on it.

- The influence of age on the development of oxidative stress.

2. REVIEW OF LITERATURE

2.1 Relation Between Vitamin D With CKD

It is an integrative review, according to the steps proposed by Bellucci and Matsuda (9), and based on the guiding question: "What is the relationship established in the literature about vitamin D supplementation in patients with kidney disease and SOD Vs CKD?" The bibliographic survey was carried out in the databases: PubMed, Lilacs, WOS, Science Direct, SciELO and MedLine, in December 2018-19, through the crossing of Health Sciences Descriptors (DECS) and their respective translations according to the terms Medical Subject Headings (MESH): kidney insufficiency / kidney failure, vitamin D deficiency /vitamin D deficiency and dietary supplement / nutritional supplements/Correlation between Vitamin D3, correlation between SOD etc., The inclusion criteria for the studies were scientific research; available online in full in article format/Abstracts format also, published in the last five years, in English and excluded articles not related to the proposed theme and studies of methodological design that did not allow to identify the proposed objective.

Mora than 100 articles were found initially. After rigorous evaluation in the literature, regarding the relationship between vitamin D supplementation in patients with CKD and Vitamin D, 17 were selected for complete reading, of which 7 were selected according to the objective of this research.

When analysing the journals in which the manuscripts were published, it was found that 3 (42.8%) studies were published in the same journals, in addition to 57.2% being published in reputed journals.

The literature shows different positive repercussions when it comes to vitamin D supplementation, such as, for example, reduced visceral fat, decreased fasting glycemia and the incidence of type I diabetes mellitus, reduced lipid rates and blood pressure, as well as of mortality from cardiovascular disease after supplementation (Prasad and

Kochhar, 2016). In patients with CKD, vitamin D supplementation has promising prospects (Inda Filho and Melamed, 2013).

(Kim et al., 2014),study the results showed an inversely proportional relationship between vitamin D supplementation and proteinuria values, systemic blood pressure, as well as a positive GFR response. This result is in agreement with the literature, since previous studies have shown that patients with hypovitaminosis D present higher values of proteinuria when compared to the control group in which the 25 (OH) D levels were satisfactory.

A study that carried out monitoring with hypertensive elderly people determined that patients with satisfactory levels of vitamin D maintained lower blood pressure values compared to patients with hypovitaminosis (Neves et al., 2012). In contrast, a study with children using a group with vitamin D supplementation and another placebo did not show significant differences between blood pressure values (Kelishadi et al., 2014).

Study of (Morvová Jr et al., 2014), indicates a reduction in intracellular calcium concentration after vitamin D supplementation, as well as a previous published study (Lajdova et al., 2009). Research previously carried out on the cellular metabolic profile, showed that CKD is associated with a significant increase in the concentration of intracellular calcium and that this increase can cooperate for the multiple organ dysfunction of patients on hemodialysis. However, regarding the reduction of intracellular calcium, it is not clear the mechanism in which vitamin D acts causing intracellular ionic reduction (Barbosa et al., 2013). (Garcia-Lopes et al., 2012), (Marckmann et al., 2012), (Delanaye et al., 2013), showed similar results, establishing the following relationship: patients supplemented with vitamin D after reaching satisfactory serum levels of this vitamin, showed a reduction in PTH levels. The literature states that, in calcium deficiency, the organism has physiological signaling pathways to release PTH, which in turn, performs bone resorption, releasing calcium into the plasma, in order to maintain the homeostasis of this ion, a state that is known as secondary hyperparathyroidism.

Studies of (Garcia-Lopes et al., 2012), (Marckmann et al., 2012), (Delanaye et al., 2013), together with study of (Lajdova et al., 2015), also showed that increasing serum vitamin D levels reduces the amount of ionizable calcium. High levels of ionized calcium are associated with higher mortality in patients with CKD (28-29). In a study carried out in London, with a population of 63 patients with CKD, vitamin D supplementation and an increase in serum levels of 25 (OH) D and 1.25-di (OH) D were associated with a reduction in values of albuminuria, corroborating with article from (Kim et al., 2011). Another study carried out in a Brazilian reference university center, with 125 patients with CKD undergoing conservative non-dialysis treatment, also proved the presence of an inverse correlation between the levels of 25 (OH) D and proteinuria.

Few studies address vitamin D supplementation in patients with CKD and vitamin D. Due to this fact, it is understood that the number of researchers involved in this topic is still reduced. However, after analysing the studies included in this integrative review, it was possible to conclude that there is scientific evidence that corroborates vitamin D supplementation in these patients, considering the positive aspects of this therapy, such as reduced BP, increased GFR, reduced PTH, decreased ionized calcium, among others, factors that contribute to a better therapeutic management of these patients.

This study enabled a better understanding of vitamin D supplementation in CKD. In this sense, it is expected that it contributes to the healthcare and clinical practice of health professionals and helps in the development of protocols for this population.

Chronic kidney patients are a population in which the prevalence of vitamin D (25 (OH) D) deficiency is quite high, being associated with an increase in mortality, cardiovascular morbidity and mineral and bone disease associated with CKD. There also appears to be a correlation between 25 (OH) D levels and the rate of glomerular filtration (GFR), suggesting that its correction may alter the pathophysiology of kidney disease and, therefore, alter its progression (Doorenbos et al., 2009).

In a clinical trial, (Stubbs et al., 2010), evaluated the effect of cholecalciferol supplementation on the modulation of the inflammatory response in seven hemodialysis patients who had serum levels of 25 (OH) D <25 ng / mL. Therapy with cholecalciferol resulted in increased levels of 25 (OH) D, increased expression of the vitamin D receptor in monocytes, in addition to reducing circulating levels of interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor-α (TNF-α). (Bucharles et al., 2012), investigated the effect of cholecalciferol supplementation in 30 patients on hemodialysis. An increase in serum 25 (OH) D was observed after three months of supplementation, with a concomitant reduction in C-reactive protein and IL-6, suggesting that the correction of hypovitaminosis D has an anti-inflammatory effect. However, few trials Controlled clinicians investigated whether restoring the nutritional status of vitamin D could reduce the inflammatory response of patients on dialysis. (Marckmann et al., 2012) and (Seibert et al., 2013), failed to demonstrate the beneficial effect of cholecalciferol supplementation on inflammation markers in dialysis patients. Therefore, there are still uncertainties regarding the effect of vitamin D replacement on the inflammatory response of patients with CKD.

2.1.1 Studies on the influence of vitamin D3 on morbidity and mortality an patients with chronic kidney disease

However, the available evidence on the influence of the administration of vitamin D3 on the survival of patients with CKD is not very consistent and is derived from observational studies; Most of the controlled studies carried out do not have the primary objective of assessing survival, but rather assessing changes in analytical parameters in the short or medium term. Nor is there, at the present time, evidence that clearly demonstrates the superiority of one form of active vitamin D3 over another.

In recent years, several systematic reviews and meta-analyzes have been published that analyze the usefulness of these drugs in CKD. The results of these systematic reviews are summarized below.

There are still no studies that consistently show that these treatments are capable of slowing the progression of kidney disease or reducing cardiovascular mortality. There are also no studies that have shown superiority of some active vitamin D molecules over others. Therefore, it is necessary to have clinical evidence to answer some very relevant questions for CKD patients: is morbidity and mortality reduced with the use of nutritional or active vitamin D? Should the plasma concentration of calcidiol be normalized before administering active vitamin D? Are there clinically relevant differences between the different types of active vitamin D?

2.1.2 Active vitamin D and mortality in chronic kidney disease

The observational studies reviewed in a study comparing nutritional and active vitamin D (Kalantar-Zadeh and Kovesdy, 2009), indicate that the administration of any dose or active vitamin D compound is associated with longer survival in CKD patients, whether or not they require dialysis. However, the two Cochrane reviews mentioned, which only include controlled clinical trials, do not find differences in mortality in patients treated with active vitamin D or with placebo, whatever their stage of CKD. They also found no differences in the progression of CKD in patients not on dialysis, or in other clinical variables (fractures, bone pain, or need for parathyroidectomy) in CKD patients (both dialyzed and not dialyzed).

In patients with CKD, the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) were shown to be reduced in erythrocytes. This reduction would be one of the factors that could lead to an increase in lipid peroxidation in the erythrocyte membrane structure with consequent hemolysis and anemia (Durak et al., 1994).

The increase in the production of ROS in uraemia has been proposed as a possible contributing factor for anemia and atherosclerosis in chronic renal failure. Evaluating the oxidative stress of 40 patients with chronic renal failure undergoing haemodialysis, by measuring lipid peroxidation (measured by MDA), antioxidant enzymes and trace elements, an increase in lipid peroxidation levels and changes in fluid fluidity were

observed. Erythrocyte membrane, which could be contributing to the increase of cardiovascular diseases and anaemia in patients with chronic renal failure. In fact, patients on hemodialysis have increased plasma lipid peroxidation and decreased SOD activity, changes that seem to contribute to the occurrence of oxidative disorders in these patients (McGrath et al., 1995). (Hasanoğlu et al., 1994), also observed a reduction in Zn levels and inhibition of SOD activity in the erythrocyte of dialysis patients, which could also contribute to anemia in these patients.

(Chen and Young, 1992), evaluated the reduced glutathione (GSH), Zn content and SOD activity, as well as the formation of MDA, in 25 patients with CKD and 24 controls. They observed that in patients, the levels of GSH, SOD and plasma Zn were reduced, with a greater formation of MDA in the platelets. The decrease in GSH correlated with plasma Zn in these patients. The results of this research suggest that uremic toxins may influence the content of GSH and SOD.

(Rud'ko et al., 1995), evaluated lipid peroxidation through the levels of malonaldehyde and the antioxidant defense system in erythrocytes of patients with CRF without clinical signs of the disease (group 1), under conservative treatment (group 2a), in terminally ill patients (group 2b) and in healthy individuals. They observed that the level of MDA was elevated in patients in group 2b, correlating positively with serum creatinine. CAT and GSH-Px were at normal levels, but SOD was reduced in groups 2a and 2b. It is believed that the progression of CKD maximizes the activation of lipid peroxidation and deterioration of antioxidant defense. A lower activity of CAT, SOD and GSH-Px enzymes has been observed in patients with CRF, under conservative treatment and continuous home peritoneal dialysis (CAPD), which suggests that the antioxidant defense mechanisms are reduced in patients with chronic renal failure.

2.2 SOD Vs CKD

Few studies have investigated the function of SOD in patients with CKD, one of which is the current Akiyama et al study (Akiyama et al., 2005), published in this Nephron

Clinical Practice issue. The author has found that the SOD production in leukocytes has increased significantly in CKD patients, but that growth only affects Cu / Zn-SOD. In addition, the expression of Cu / Zn-SOD mRNA in leukocytes was considerably higher compared to controls. The latter finding is consistent with the previous study (Schettler et al., 2003), which also showed an increase in SOD expression in white blood cells in CKD patients. In comparison to findings of several studies that reported a decrease of erythrocytic SOD activity in CKD patients, the increase of SOD activity was nevertheless different (Bonnefont-Rousselot et al., 2000). We must also be cautious when comparing the various studies, since different cell sources have been used. Given that erythrocytes in CKD patients can be longer due to anaemia, it may explain the disparity in SOD levels between these studies in this shorter life span. This discrepancy raises the issue of the ideal blood factor to determine the operation of SOD in patients with CKD. The role of SOD and its various isoforms in the antioxidant defence mechanism is currently unclear in CKD patients. With myeloperoxydase and chlorinated stress in patients with CKD (Witko-Sarsat et al., 1998), and in the general population, it seems of vital importance to test the SOD function and chlorinated stress markers in tandem, so as to assess how much chlorination results from SOD dysregulation. In Akiyma et al (2005) report, it is not possible to establish whether or not Cu / Zn-SOD inductions and leucocyte activities are sufficiently significant to protect CKD patients against oxidative stress, since the authors did not measure chlorinated stress markers. The position of SOD activation is still unknown as a therapeutical technique.

3. MATERIALS AND METHODS

3.1 Monitoring Vitamin D3 in Kidney Patients in Babylon_ Iraq

The use of vitamin D in CKD therapy has been known for nearly sixty years. Cholecalciferol and calcidiol were the first derivatives used, followed by calcitriol in the 1970s (de Brito Galvao et al., 2013). Currently, many forms are present on the world market such as paricalcitol, oxacalcitrol, doxercalciferol. Until the 2000s, nephrologists used to prescribe calcitriol to correct PTH levels, not native vitamin D. Recent studies have shown the effectiveness of vitamin D2 and D3 in reducing PTH levels in CKD. Faced with low serum 25 (OH) D concentrations, the prescription of native vitamin D at all stages of CKD has become something common in daily practice. The active forms are reserved in hyperparathyroidism resistant to native forms and calcimimetics in the context of dialysis. The previous chapters have highlighted the important role of vitamin D in the overall management of nephrology patients. We therefore hypothesized that patient compliance with vitamin D (D3) supplementation would improve their general condition, and that biological monitoring of phosphocalcic homeostasis would reflect this improvement. In the literature, there are only a few publications concerning compliance with vitamin D supplementation in nephrology patients. As part of the optimization of patient care, we therefore decided to observe and analyze the reality of current prescriptions in the nephrology department of the Marjan Teaching Hospital of Babylon, Iraq.

3.1.1 Study population

As part of our study, we recruited all hospitalized patients with chronic renal insufficiency in the nephrology department at Marjan Teaching Hospital in Babylon in 2020. We indifferently included both men and women. Criteria for non-inclusion were patient rejection, inability to understand the protocol, hypercalcemia, vital diagnostics, patients taking long-term vitamin D intake, and patients from other hosted nephrology services.

3.1.2 Data collected

On entering the service, an initial biological assessment was carried out including the determination of calcium level, phosphatemia, DFG MDRD, PTH, 25 (OH) D and 1,25 (OH) 2 D serum. All the assays were carried out at the Marjan Teaching Hospital in Babylon, in the Human Biology Center (biochemistry laboratory, and bone and endocrine biology laboratory). According to their needs, the patients follow a supplementation by ampoules of cholecalciferol according to the protocol of the Hospital. After supplementation, a final assessment is carried out in town 30 days after taking the last ampoule of the protocol. The MVDN protocol is broken down into three main stages: an initial biological assessment, the prescription of cholecalciferol supplementation according to the Hospital protocol, and a final biological assessment at D + 30 after the last intake of cholecalciferol. To facilitate prescribing, a support document was given to the physicians in the department, as well as prescribing assistance tools comprising standard prescriptions to be given to patients upon discharge. All the patients were called in order to check that the protocol was properly followed. The information transmitted by the patient was verified by contacting the pharmacies of the patients in order to precisely determine the number of ampoules delivered in town. The city medical analysis laboratories have also been called in to ensure that no transmission is forgotten on their part. After four calls (separated by a week's interval) without a response from the patients, we considered them lost to follow-up.

3.1.3 Evaluation criteria

The main endpoints were:

- The proportion of supplementation adapted to the initial 25 (OH) D concentrations measured in each patient.
- The number of total ampoule consumed.

The secondary endpoints were:

- Multivariate analysis of clinical and biological factors that may influence proper compliance with supplementation.
- Analysis of the proportion of supplementation which made it possible to reach normal 25 (OH) levels.
- Modification of other parameters of the biological assessment.
- Multivariate analysis of clinical and biological factors that may influence supplementation.

3.1.4 Analysis of bilirubin, sugar, PCV, GOT, GPT, HbA1c in diabetic patients

- Serum bilirubin determined by the vanadate oxidation method as described by Chung et al (2014).
- Fasting blood glucose (sugar) was measured using a hexokinase method.
- Serum PCV, GOT and GPT values are obtained by the standard procedures. given in the literature (Lin et al , 2010).

  HbA1c value was measured by fine care ID chips.

3.1.5 Bio Statistical Tools

We used several tests and biostatistical means to analyze the results of our study:

- The paired Student's test for the comparison of means between initial assessment and final assessment.
- The Chi 2 and Z-test independence test for frequency comparison.
- The univariate Spearman Correlation test.
- Multivariate logistic regression by step-by-step descending method (Wald test).
- The threshold of statistical significance was set at 5%. If the risk was lower than this threshold, we concluded that there was a significant difference in the parameters tested or a significant correlation. The graphical results are represented in average in frequency +/- SD depending on whether the variable is quantitative or qualitative. The tests were performed using SPPS software.

3.2 Study of Systemic Oxidative Stress in (CKD) Patients

3.2.1 Methodology

The pre-dialysis group consisted of 26 men and six women, with an average age of 65±15 years. The causal nephropathy was vascular in 21 cases (65.7%), in eight cases (25%) it was diabetic, in two cases (6.2%) tubulointerstitial nephritis and in one case (3.1%) renal polycystic disease undergoing hemodialysis at the Nephrology Unit of the Marjan Teaching Hospital of Babylon, Iraq. The evaluated patients underwent three weekly HD sessions with an average duration of 4.2 ± 0.3 hours. The average HD treatment in patients was 45.2 ± 40 months. Exclusion criteria were patients with liver disease, known chronic inflammatory processes, known heart disease, smoking and alcoholism. As there are no predicted normal values for oxidative stress variables, it was decided to analyse a group of people without any known pathology and non-smokers. For this purpose, the blood of 18 donors, four women, with a mean age of 34.8 ± 10.1 years, of the Blood Bank of Marjan Teaching Hospital of Babylon, Iraq. This group of individuals was called the control group. The study was evaluated and approved in its ethical and methodological aspects by the Scientific Committee and by the Health Research and Ethics Committee, Iraq. Prior to the evaluations, all patients signed an informed consent form.

Clinically stable patients in the previous six months were included and those with neoplastic disease, active bleeding, active inflammatory or infectious disease, and those who had received intravenous iron treatment in the previous three months were excluded from the study.

3.2.2 Blood collection

Blood collection from HD patients was performed at two different times: at the beginning of the HD session (pre-HD), when the patients were connected to dialysis machines, and at the end of the session (post-HD). The collections were performed in the arterial portion of the arteriovenous fistula. For the determinations of oxidative stress parameters, 14 ml of blood in a tube with EDTA as an anticoagulant were extracted by vacutainer. These samples were immediately centrifuged, separating serum and plasma. The blood was stored in a vacuum collection tube containing anticoagulant heparin (10 mL). To measure uric acid in plasma samples, a Uric Acid kit – Liquiform was used. The evaluation of oxidative stress was carried out at the Cardiovascular Physiology Laboratory of the Physiology Department of the teaching hospital. Blood collection in the control group, carried out to analyze oxidative stress, was made at the end of the donation.

3.2.3 Blood preparation for assessment of oxidative stress

The blood preparation for the evaluation of oxidative stress was imperatively performed on the day of collection. After collection, blood stored in a tube with heparin anticoagulant was centrifuged for 20 minutes at 3000 rpm in a refrigerated centrifuge (Remi Made, India), the plasma was removed, aliquot in 2.0 ml Eppendorf tubes and stored at a temperature of negative -80ºC. The erythrocytes were washed with saline (0.9% NaCl) three times. After washing the red cells, part (75 □L) was diluted in 500 □L of saline to measure lipoperoxidation by chemiluminescence and hemoglobin concentration. These measurements were performed on the day of collection. The remaining erythrocytes were aliquoted and stored in a freezer at -80 ºC. These erythrocytes were stored in a solution of 1 mM acetic acid and 4 mM magnesium

sulphate. These samples were used for protein analysis and activity of antioxidant enzymes, catalase (CAT) and superoxide dismutase (SOD). Plasma was used to assess protein oxidation using the carbonyl method and to determine nitrates and nitrites.

3.2.4 Haemoglobin measurement

Haemoglobin was measured in blood samples prepared with saline, used to measure lipoperoxidation. For analysis, a mixture of cyanides was used, thus obtaining Drabkin's reagent, which interacts with haemoglobin, forming cyanomethaemoglobin, measured in a spectrophotometer. The spectrophotometer used in this work was Varian, model Cary. For dosages, 5 mL of Drabkin's solution and 20 µL of sample were placed in a test tube, allowed to react for five minutes and the absorbance was read on the spectrophotometer at a wavelength of 546 nm. The results were expressed in milligrams per millilitre.

3.2.5 Measurement of lipoperoxidation (LPO) - chemiluminescence (CL)

CL was measured in a beta counter (Figure 1) (CL-960i model, Mindray Made, India) with disconnected matching circuit and using the tritium channel. The determinations were performed in a dark room, in glass vials kept in the dark to avoid phosphorescence activated by fluorescent light. The reaction medium in which the test was performed consisted of 4 mL of a 140 mM KCl regulatory solution, 20 mM phosphates, pH 7.4, to which 10 mL of red blood cells diluted in saline were added. Then, an initial reading was performed, considering the baseline light emission. The tertbutyl hydroperoxide, at a concentration of 400 mM, was added to the reaction medium (30 µL), for a final concentration of 3 mM. Then, the light emission was measured and, from this, the basal emission was deducted for calculations.

Figure 3.1 Cl 960i chemiluminescence analyzer

3.2.6 Total antioxidant capacity (TRAP)

To measure the total antioxidant capacity - Total Reactive Antioxidant Potential (TRAP) (Neto et al., 2020), a technique was used based on the formation of a radical that emits light, and detected by a beta counter (Figure 2) (Hitachi AccuFLEX LSC-8000 model) with the disconnected coincidence circuit and using the tritium. The measurements were performed in a dark room and in glass vials kept in the dark to avoid phosphorescence activated by fluorescent light. The reaction medium consisted of 3.0 mL of an AZO solution, to which the amount of 10 µL of luminol was added, making a baseline reading. Then, the sample was added (around 10 µL). To establish the standard curve, two readings were carried out containing Trolox (water-soluble vitamin E), in quantities of 5 µL and 10 µL. The first reading was performed with ABAP and luminol; in this, the formation of free radicals was verified, subsequently making a standard curve with the use of Trolox as an antioxidant, and the antioxidant capacity of the samples was measured by observing the time that the sample inhibits the formation of the radicals of luminol. The results were expressed in mM of trolox (Moniruzzaman et al., 2012)

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Figure 3.2 Hitachi accuFLEX LSC-8000 liquid scintillation counter

3.2.7 Antioxidant enzymes

The technique used to determine SOD is based on inhibiting the reaction of the superoxide radical with pyrogallol. The superoxide radical is generated by the autooxidation of pyrogallol when in a basic medium. The SOD present in the sample competes for the superoxide radical with the detection system. The oxidation of pyrogallol leads to the formation of a colored product, detected spectrophotometrically at a wavelength of 420 nm. SOD activity was determined by measuring the rate of formation of oxidized pyrogallol (Marklund and Marklund, 1974). The results were expressed in U SOD / mg protein. The activity of the antioxidant enzyme catalase (CAT) was determined by measuring the absorbance reduction at 240nm in the presence of hydrogen peroxide (H2O2). The activity is expressed in nanomols of H2O2, reduced per minute per milligram of protein. The protein concentration, used in calculating the activity of antioxidant enzymes, was measured by the method of Lowry *et al* ., Using bovine serum albumin as a standard ((Pomory, 2008).

3.2.8 Determination of nitrates and nitrites

Nitrate levels were estimated by the Griess reaction according to the procedure described by (Granger et al., 1996). Plasma samples (50 µL) were incubated with enzymatic cofactors and nitrate reductase, so that nitrate could be reduced to nitrite. Griess reagent (1 g sulfanilamide, 0.1 g naphthylene and 2.3 mL of 85% phosphoric acid) was added. The absorbance was read at 540 nm. The results were evaluated by comparing with a standard curve made using 1 mM sodium nitrate and the results were expressed as µM nitrate.

3.2.9 Protein oxidation - carbonyl method

Plasma samples were incubated with 2.4 dinitrophenylhydrazine (DNPH 10 mmol / L) in 2.5 mol/L HCl solution for one hour at room temperature. The samples were shaken every 15 minutes. Then, 20% TCA solution was added to the sample tubes, which were kept on ice for 10 minutes and centrifuged for five minutes. Then, the precipitated proteins were collected. The same procedure was performed using a 10% TCA solution. The pellet was washed three times with ethanol: ethyl acetate (1: 1) (v / v). At the end, the precipitate was dissolved in 6 mol / L of guanidine solution, the samples were stirred for 10 minutes in a bath at 37 ºC and read at 360 nM. The results were expressed in nmol / mg prot (Maxwell et al., 2003).

3.2.10 Statistical analysis

After all tests were performed, the averages and standard errors of the mean were calculated for each of the measurements performed and for each of the groups studied. For statistical analysis of the pre- and post-HD, pre-HD and control data, Student's *t* test was used, with significant differences with $p < 0.05$.

4. RESULTS

Number of patients included in the study: For 4 months, 120 patients were hospitalized in the Nephrology department, 15 patients were not included (12 patients presented with hypercalcemia, and 2 followed monthly treatment of Uvedose® for life). In all, 120 patients and 40 controls could therefore be included in the study.

4.1 Patient Characteristics

Overall characteristics: The characteristics of this population in Table 4.1:

Table 4.1 Initial characteristics of the patients included (n = 120).

Characteristics of the population	Frequency or mean +/- standard deviation
Gender (M)	47 / 47.96%
Age (Years)	67.22 +/- 15.83
Weight (Kg)	74.75 +/- 18.34
Size (m)	1.658 +/- 0.098
BMI (Kg / m2)	26.69 +/- 6.67
calcemia (mmol / L)	2.117 +/- 0.251
Phosphatemia (mmol / L)	1.404 +/- 0.549
PTH (ng / L)	250.9 +/- 158.71
25 (OH) D (ng / mL)	19.83 +/- 15.77
1,25 (OH) 2 D (pg / mL)	19.60 +/- 18.64
DFG MDRD (mL / min / 1.73m2)	25.83 +/- 20.77

We can observe that the number of men and women is close. The patients are on average 67 years old and have a tendency to be overweight (mean BMI of 26.69). At the biological level we observe a tendency to a slight hypocalcemia (2.117 mmol / L on average, for normal values between 2.2 and 2.5 mmol / L), to a high phosphatemia (N:

0.80-1, 45 mmol / L), and at a high PTH level (N: 15-65 ng / L). Low mean concentrations of 25 (OH) D are noted while the mean concentrations of 1,25 (OH) 2D are within normal values (N: 16-56 pg / mL). These parameters are consistent with the advanced stage of CKD in patients with an MDRD GFR of 25.83 mL / min on average. A significant correlation is also observed between the GFR and several parameters including serum calcium (r = 0.214; p = 0.035), phosphatemia (r = -0.578); p <0.001), PTH (r = -0.455; p <0.001), 25 (OH) D (r = -0.233; p = 0.021).

Age:

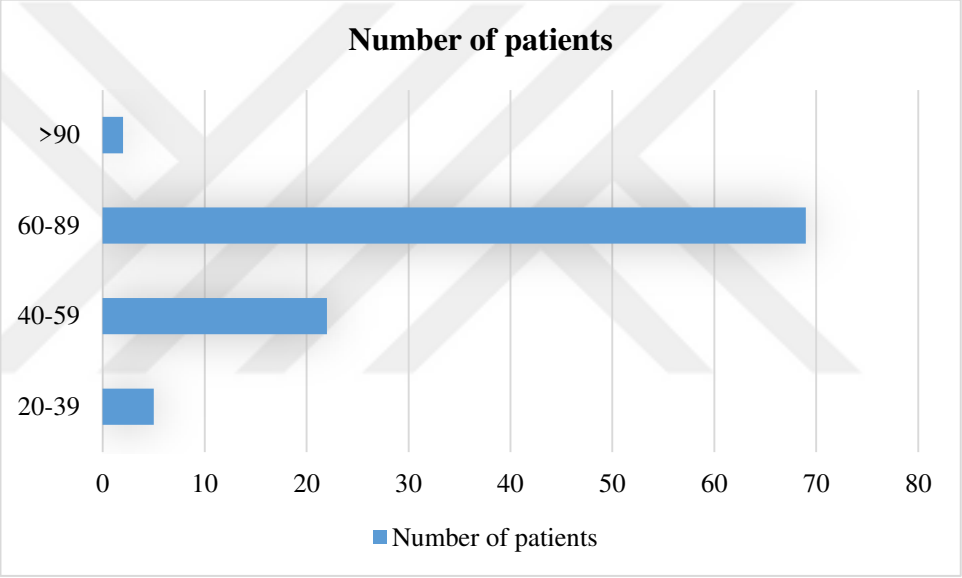

Figure 4.1 Age group of patients followed.

Number of patients according to their age group (N = 120). The majority of patients are in the 60-89 age group. There are only 5 patients aged 20 to 39 years and only 2 above 90 years (Figure 4.1).

GFR:

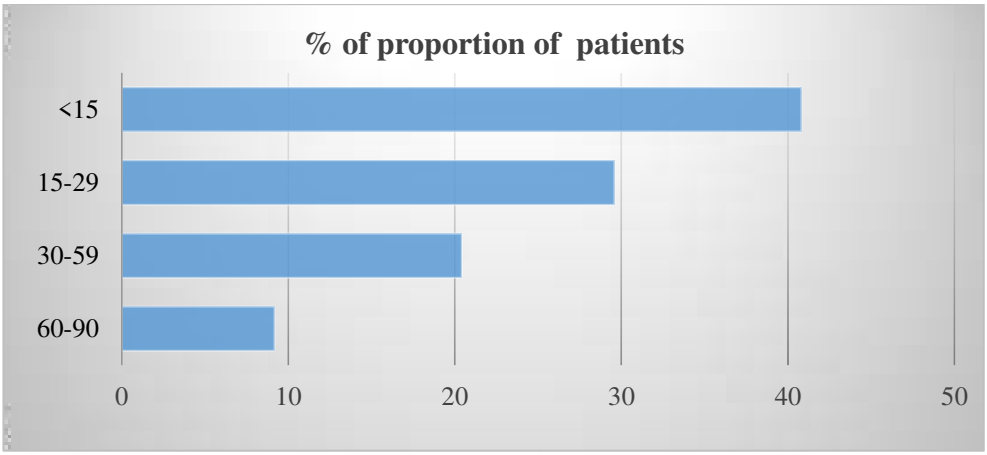

Figure 4.2 Distribution of patients (%) according to their GFR.

Proportion of patients (%) based on MDRD GFR in mL / min (N = 120). We note that all stages of CKD are represented with increasing proportions with the advancement of the disease. The patients in the study are at a very advanced stage of CKD, 29 or 29.59% of our sample have severe renal failure, and 40 or 40.82% of our sample have end-stage renal disease (Figure 4.2).

25 (OH) D level:

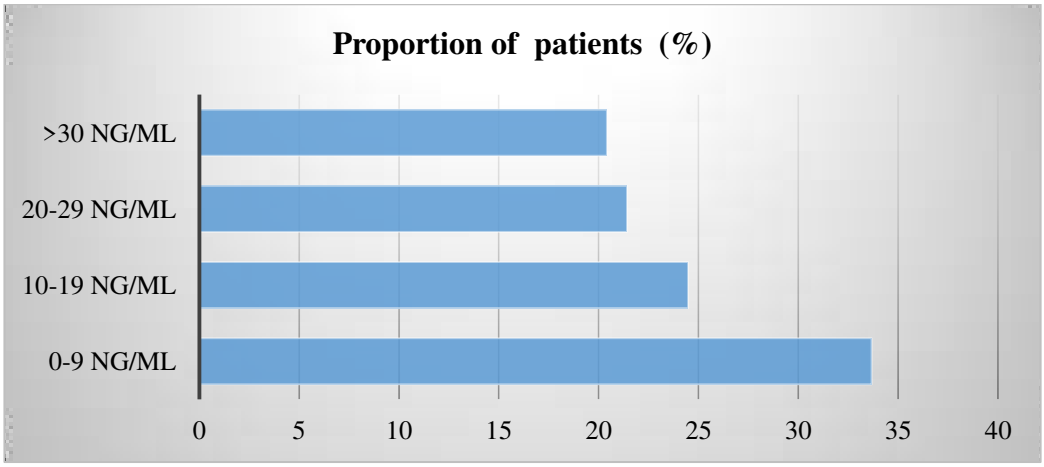

Figure 4.3 Distribution of 25 (OH) D concentrations.

Percentage of patient (%) according to the serum 25 (OH) D concentration groups in ng /mL (N = 98) (Figure 4.3). 0-9 ng / mL, 10-19 ng / mL, 20-29 ng /mL correspond respectively to the stages of severe deficiency, moderate deficiency, and vitamin D insufficiency according to the Endocrine society 140. Values of 25 (OH) D greater than 30 ng / mL correspond to those recommended in the patient with CKD. The serum 25 (OH) D concentrations encountered are in low values. Only 20.41% of patients have concentrations> 30 ng / mL, a threshold considered normal or ideal. This therefore means that 79.59% of patients or a large majority have suboptimal concentrations, respectively 21.43% at the stage of insufficiency (20-30 ng / mL) and 58.16% of patients at the stage of deficiency. (<20 ng / mL).

4.1.1 Pathologies and Associated Treatments:

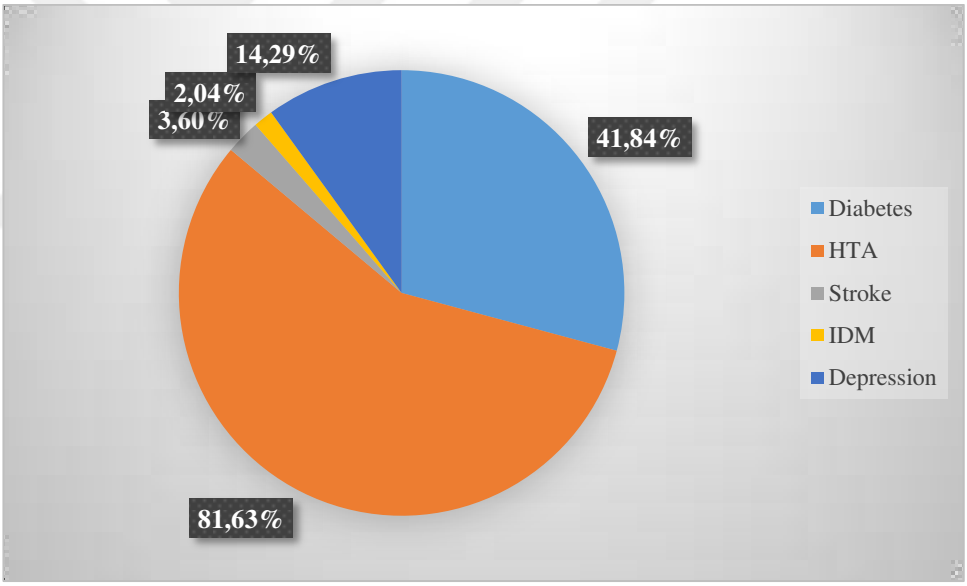

Figure 4.4 Frequency of recorded pathologies present in our patients (N = 120).

In our population hypertension and diabetes represent the two main comorbidities. The distribution frequency of the pathologies recorded is detailed in (Figure 4.4). In terms of prescribed treatments, the taking of antidepressants, antidiabetics and antihypertensive drugs are frequent, and are linked to the pathologies presented by patients. PPIs are also heavily prescribed. This can potentially be explained by the large number of drugs taken. There is indeed a significant correlation between the number of treatments

prescribed and the presence of PPI in the personal treatment of our population (r = 0.35; p <0.001). In addition, 65% of the patients in the study follow a treatment comprising more than 5 drugs and can be considered as poly-drugs.

4.1.2 Patient status during the study

There were 120 CKD patients entered into nephrology, 120 of which were followed. Of these patients, 20 did not require supplementation, 27 were lost to follow-up, 36 had a missed protocol, and 15 patients had a full protocol. The complete protocol was defined by the taking of all the prescribed ampoules accompanied by the realization of the final biological assessment. Based on their serum concentrations, 78 patients required cholecalciferol supplementation. Of these, 27 were lost to follow-up, 36 did not follow the planned protocol. Only 15 patients performed the entire protocol with respect for supplementation and carrying out the post-supplementation biological assessment. In all, 104 ampoules of Uvedose® were delivered to 56 patients not lost to follow-up, including 64 ampoules in hospital. These results correspond to an average number of 1.86 ampoules per patient, ie a value lower than the minimum quantity specified in the protocol of Hospital. If the protocol was fully respected for these 56 patients, 156 ampoules should have been delivered and administered. Two patients among the protocols not respected had for computer prescription "1 ampoule per day for 180 days" probably related to an awkwardness during the computer prescription on the hospital software. In fact, they received 1 ampoule per day for 2 days for one and 3 days for the other before the prescription was stopped following one of our pharmaceutical interventions. We decided to exclude these patients from the analyses concerning the respect of the number of ampoules taken.

Number of ampoules:

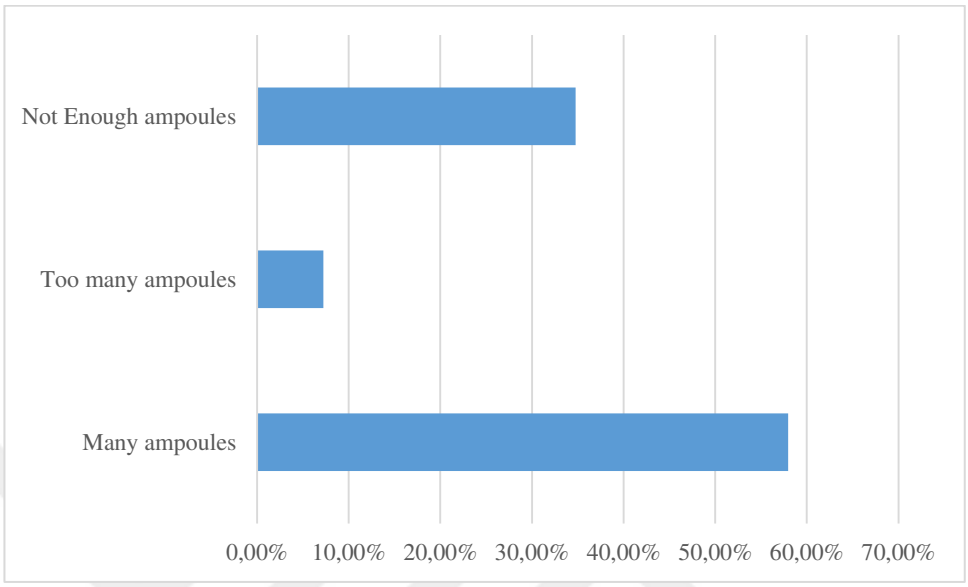

Figure 4.5 Respect of the number of ampoules (N = 69).

The 69 patients analyzed correspond to the 98 patients followed, cut out of the 27 patients lost to follow-up and the 2 patients who received their ampoules on a daily basis.

The number of ampoules taken by patients in the hospital and in the city, was compared to the theoretical quantity that the patient should have taken, in the event of compliance with the Hospital protocol. Among the 69 analyzable patients, from whom we obtained information regarding the number of ampoules taken, 57.97% obtained the correct number of ampoules, 34.78% did not receive enough ampoules, and 7, 25% had too much (Figure 4.5).

4.2 Causes Of Compliance And Non-Compliance With Supplementation

4.2.1 Univariate analysis: relationship between compliance or non-compliance with supplementation and clinical and biological data

In univariate analysis, the factors significantly associated with insufficient vitamin supplementation are a low concentration of 25 (OH) D ($p < 0.001$), the need for a relay in town ($p < 0.001$), high phosphatemia ($p < 0$, 05), as well as the absence of beta-blocker ($p < 0.05$) (Table 4.2). At the same time, we find that the factors significantly associated with compliance with supplementation are low phosphatemia ($p < 0.001$), a high calcium level ($p < 0.05$), the absence of a relay in town ($p < 0.01$), the absence of taking an anxiolytic ($p < 0.05$) and phosphate binders ($p < 0.05$). Serum 25 (OH) D was correlated with good compliance with supplementation, but only indicatively ($p = 0.064$) (Table 4.2). The multivariate analysis was carried out by integrating the six parameters significantly correlated in univariate into the model. Three factors remain independently correlated with non-compliance with the ampoule intake: phosphatemia ($p < 0.01$), the need for a relay in town ($p < 0.05$) and the taking of anxiolytics ($p < 0$, 05) (Table 4.2).

Table 4.2 Risk factors for compliance or non-compliance with vitd3 supplementation in univariate analysis.

		Respect of the number of ampoules		Too many ampoules		Not enough ampoules	
		Correlation coefficient	*p*	Correlation coefficient	*p*	Correlation coefficient	*p*
Global Features	Sex	0.032	0.791	-0.229	0.059	0.103	0.401
	Age	0.073	0.551	-0.123	0.315	0.003	0.98
	BMI	0.164	0.25	-0.282	0.045	0.006	0.969
Antecedents	Diabetes	-0.006	0.961	-0.162	0.204	0.112	0.382
	HTA	0.017	0.895	0.141	0.271	-0.11	0.391
	Stroke	0.142	0.267	-0.059	0.647	-0.115	0.371
	IDM	0.142	0.267	-0.059	0.647	-0.115	0.371
	Depression	-0.012	0.929	-0.095	0.458	0.074	0.563
Treatments	IEC	0.068	0.577	0.186	0.126	-0.183	0.133
	Beta blockers	0.082	0.501	0.271	0.024	-0.248	0.04
	ARAII	-0.166	0.174	0.144	0.239.	0.068	0.474
	Diuretics	-0.125	0.305	0.013	0.913	0.123	0.314
	Statins	0.067	0.584	-0.117	0.337	0	1
	Anticoagulant / Antiaggregant	0.083	0.498	0	1	-0.087	0.477
	Calcium channel blockers	-0.023	0.85	0.248	0.04	-0.124	0.31
	Anti-inflammatory	0.086	0.484	-0.077	0.532	-0.044	0.721
	Antidepressants	0.017	0.89	-0.127	0.298	0.058	0.635
	Anxiolytics	-0.27	0.025	0.109	0.372	0.217	0.073
	IPP	-0.011	0.927	-0.154	0.206	0.104	0.396
	Insulins	-0.089	0.468	-0.048	0.697	0.121	0.32
	Teen	-0.03	0.805	-0.134	0.271	0.112	0.36
	Azoles	-0.028	0.82	-0.053	0.663	0.061	0.618
	Calcium	-0.028	0.82	-0.053.	0.663	0.061	0.618
	Phosphate binders	-0.291	0.015	0.364	0.002	0.088	0.474
Biology report	Calcemie (mmol/L)	0.243	0.044	-0.142	0.244	-0.17	0.163
	Phosphatemie(mmol/L)	-0.39	0.001	0.222	0.067	0.276	0.022
	PTH (ng/L)	-0.2	0.11	0.357	0.004	-0.009	0.945
	25(OH)D(ng/mL)	0.21	0.084	0.283	0.018	-0.389	0.001
	1.25 (OH)2D (pg/mL)	0.08	0.685	_	_	-0.08	0.685
Other	DFG (MDRD)	-0.155	0.204	-0.282	0.019.	0.006	0.96
	Need a relay in town	-0.322	0.007	-0.385	0.001	0.567	0
	Treatment greater than 5 drugs	-0.042	0.735	0.218	0.072	-0.087	0.477

With Spearman's correlation coefficient (r) and statistical significance (p). If r = -1: negative correlation, if r = 0: no correlation, if r = +1 positive correlation. *p* significant from 0.05.

* p <0.05; ** p <0.01; *** p <0.001.

4.2.2 Multivariate analysis: relationship between compliance with supplementation and clinical and biological data

Table 4.3 Causes of compliance with vit D3 supplementation in multivariate analysis by step-by-step logistic regression (Wald).

Respect of the number of bulbs (ampoules)	Coefficient	Statistical significance
Anxiolytics	-1.213*	0.05
Phosphatemia	-1.620**	0.005
Need a relay in town	-1.407*	0.024
Phosphate binders	Removed from model	0.999
Calcemia	Removed from model	0.908
25 (OH) D	Removed from model	0.705
Constant	4.001	_

The multivariate analysis was carried out by integrating the six parameters significantly correlated in univariate into the model. Three factors remain independently correlated with non-compliance with the ampoule intake: phosphatemia (p <0.01), the need for a relay in town (p <0.05) and the taking of anxiolytics (p <0 , 05) (Table 4.3).

4.2.3 Specific Representations of The Parameters Influencing Supplementation

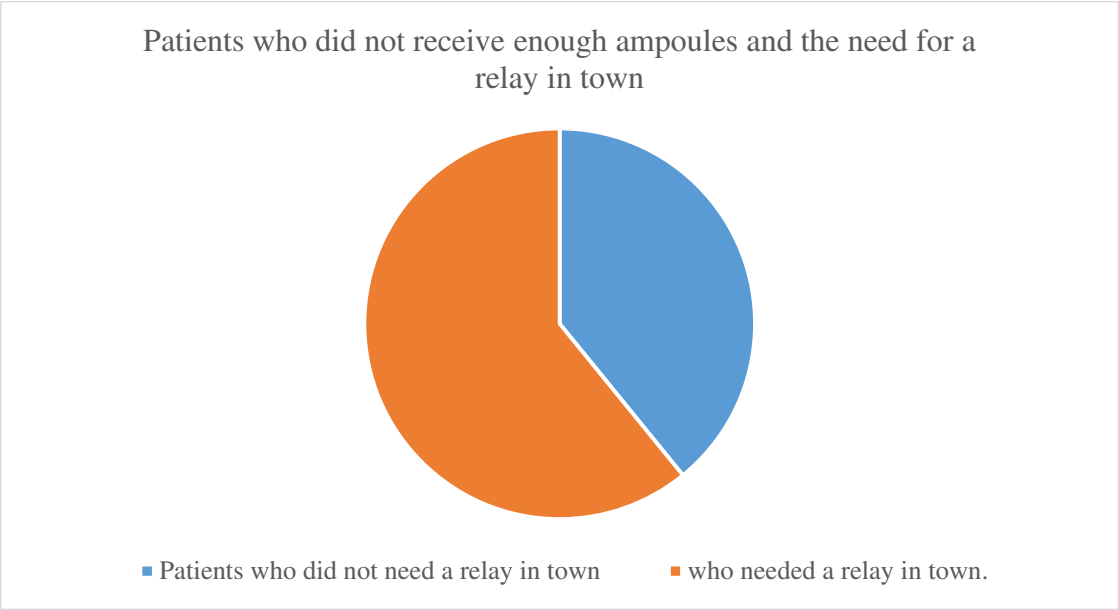

Figure 4.6 Relationship between the number of patients who did not receive enough ampoules and the need for a relay in town.

*** p <0.001 Chi-square test, N:27 patients who did not need a relay in town versus 42 who needed a relay in town.

As expected compared to the univariate analysis, it appears that patients who require a relay in town have a significantly higher risk of not receiving the right number of ampoules. This difference is strongly marked, to the point that only patients needing a relay in town did not receive all of their supplementation (Figure 4.6).

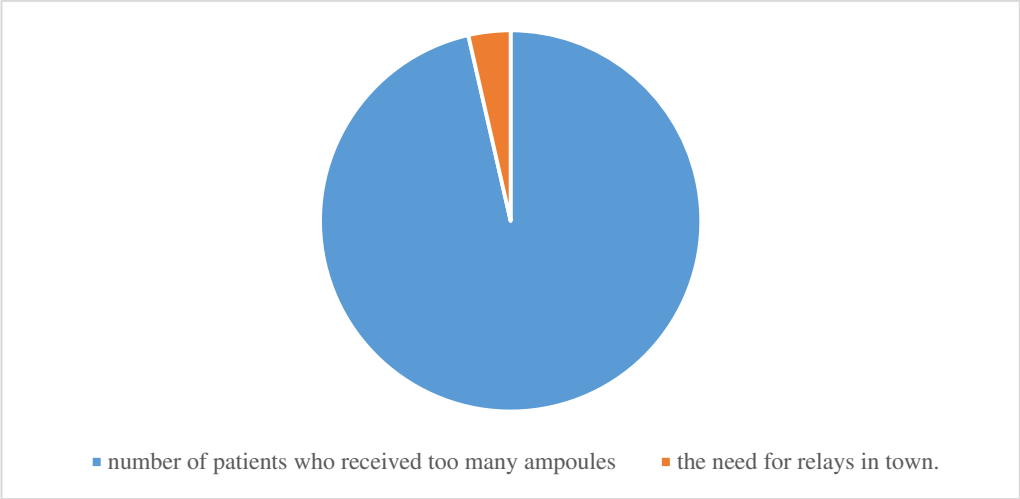

Figure 4.7 Relationship between the number of patients who received too many ampoules and the need for relays in town.

Chi-square test, N: 27 patients who did not need a relay in town versus 42 who needed a relay in town.

On the contrary, patients not requiring any relay in town received too many ampoules (Figure 4.7). These are mainly patients in whom supplementation was started when their initial 25 (OH) D was in the values <30ng / mL.

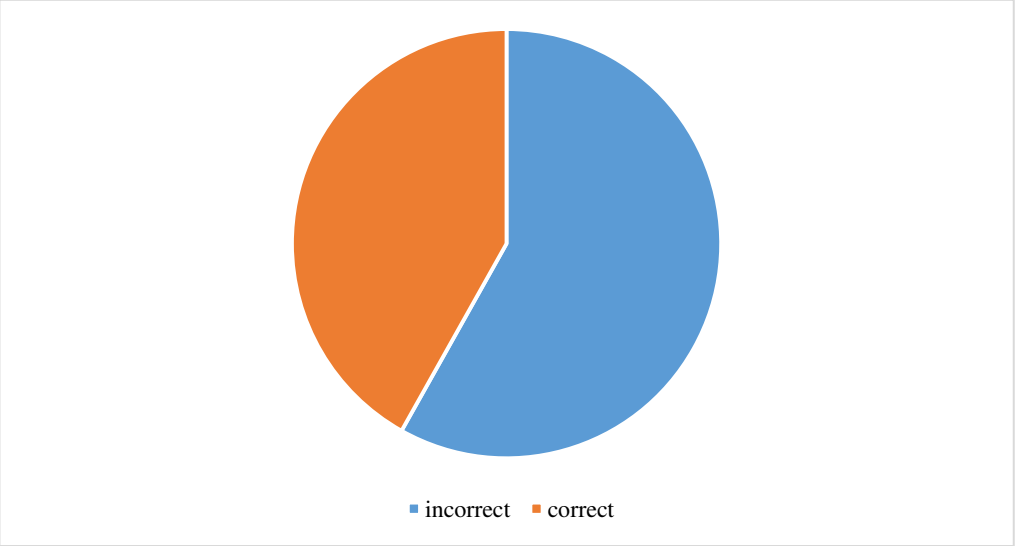

Figure 4.8 Relationship between phosphatemia and compliance with vitamin D3 supplementation.

Correct: Respect of the correct number of ampoules and incorrect (Too many ampoules + not enough ampoules) received compared to the initial 25 (OH) D, * p <0.05, student test, N = 69.

Patients incorrectly supplemented with vitD3 have a significantly higher phosphate level by approximately 20% compared to those correctly supplemented (figure 4.8).

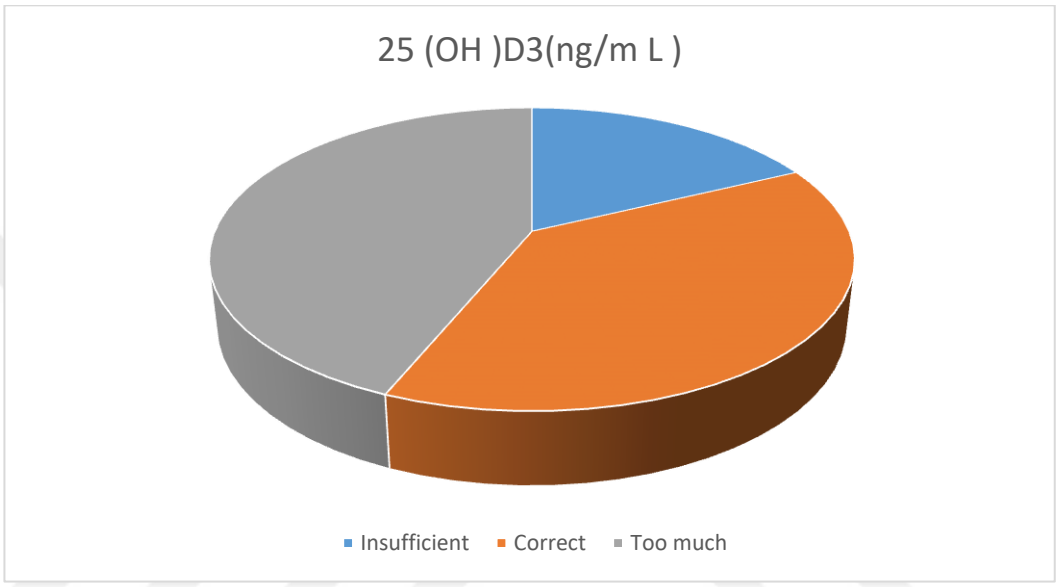

Figure 4.9 Relationship between 25 (OH) D concentration and vitamin D3 supplementation.

** p <0.01 *** p <0.001, ANOVA post Tukey test, N = 69.

Chronic renal failure patients insufficiently supplemented with vitD3 have significantly lower 25 (OH) D concentrations than those adequately supplemented. Over-supplemented patients have 25 (OH) D concentrations exceeding 30 ng / mL (Figure 4.9).

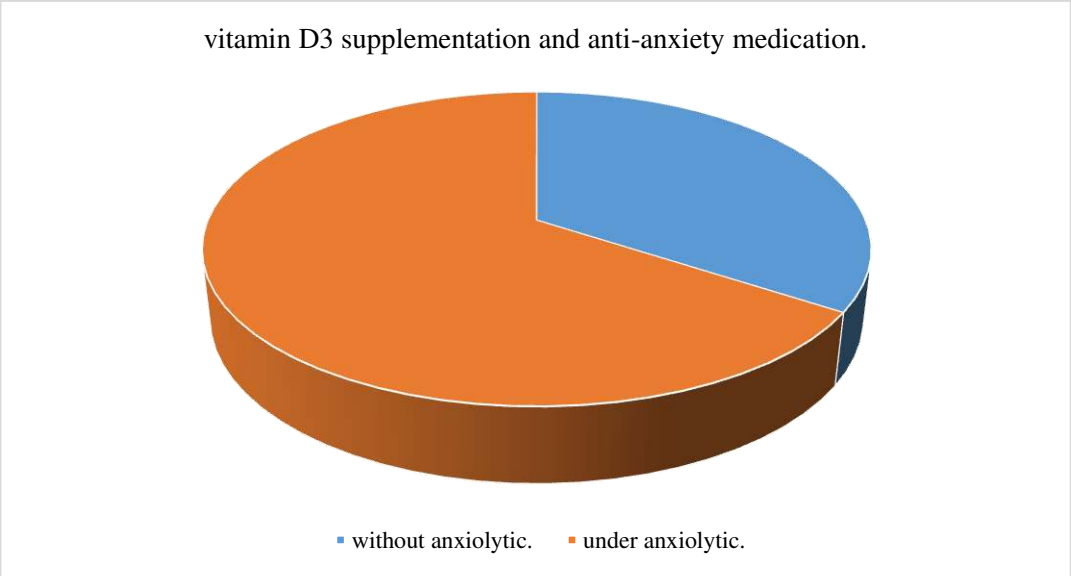

Figure 4.10 Relationship between vitamin D3 supplementation and taking an anxiolytic.

* p <0.05, Chi-square test, N = 69.

Patients on anxiolytics have a significantly greater, almost doubled, risk of having incorrect vitamin D3 supplementation compared to patients without anxiolytics (Figure 4.10).

4.3 Biological Results Post Supplementation

We obtained 15 complete protocols, allowing us to be able to analyse the variations in biological values following supplementation with Overdose, but without being able to perform a subgroup analysis according to the GFR.

4.3.1 Impact on Vitamin D Levels

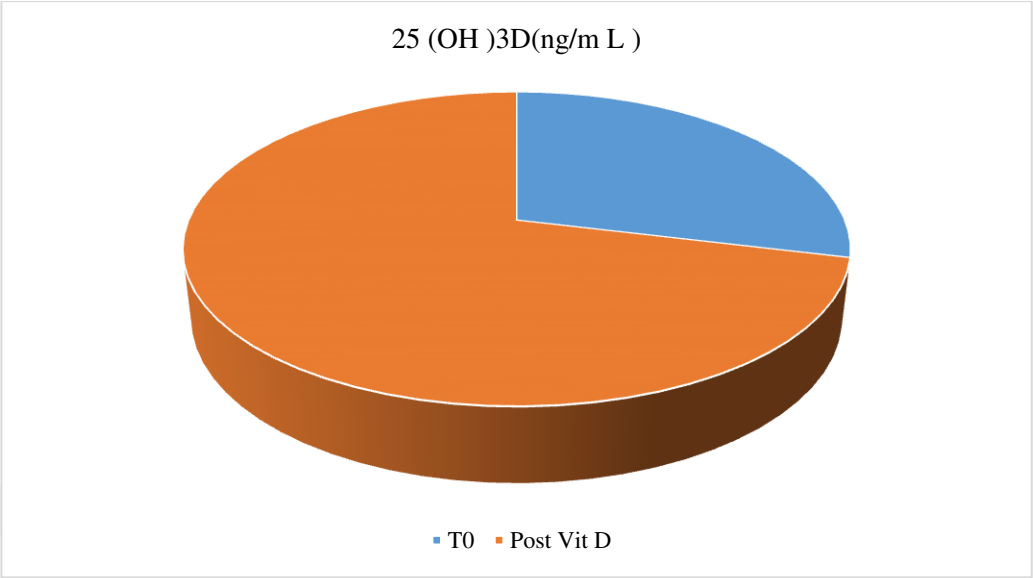

Figure 4.11 Impact of vitamin D3 supplementation on 25 (OH) D.

T0 (Before supplementation), Post-Vit D (After supplementation) *** p <0.001 paired Student test (N = 15).

We note that the MVDN study made it possible to have a very significant increase in the concentrations of 25 (OH) D. They increase by a factor of 2.5, from an average of 13.79 ng / mL before supplementation to one of 36.19 ng / mL (Figure 4.11).

Among the 15 supplemented patients, 10 patients were able to obtain 25 (OH) D concentrations above the threshold of 30 ng / mL. Thus, 2/3 of the patients were able to obtain a desired 25 (OH) D concentration. By extrapolating to the 10 patients who received all of their ampoules but no biological workup, approximately 7 additional patients would have had their 25 (OH) D concentration rise above the desired threshold of 30 ng / mL. However, by intention to treat (ITT), 24 patients did not receive enough ampoules. Considering in these cases, the supplementation insufficient to reach the desired threshold, the ratio of the number of patients having had a suitable rate after supplementation (17 patients) by the 49 patients not lost to follow-up who needed supplementation, we obtain a supplementation efficiency limited to 34.69%. Regarding 1,25 (OH) 2D we only obtained one dosage post supplementation. The patient's 1,25

(OH) 2D increased from 5 pg / mL to 83 pg / mL. This is insufficient to draw statistical conclusions.

4.3.2 Impact on calcemia

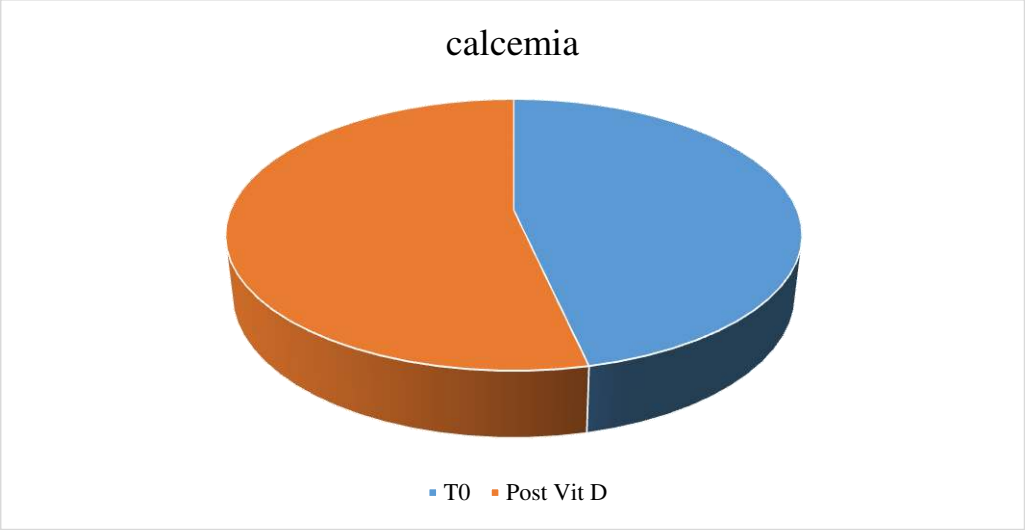

Figure 4.12 Impact of vitamin D3 supplementation on serum calcium.

T0 (Before supplementation), Post-VitD (After supplementation) * p <0.05 paired Student test (N = 15).

By analyzing the results, we found a significant increase in serum calcium after carrying out the Hospital protocol with an increase of 8.53% (Figure 4.12).

4.3.3 Impact on phosphatemia

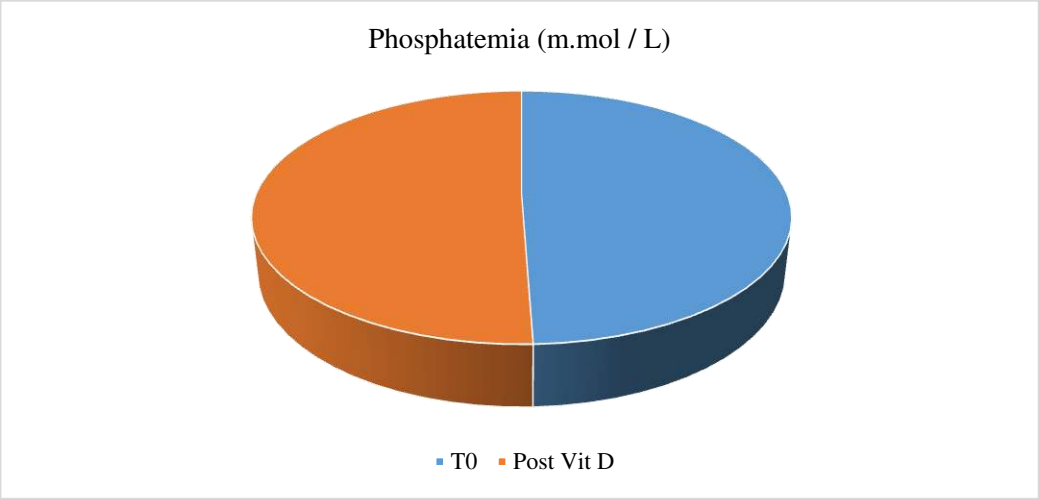

Figure 4.13 Impact of vitamin D3 supplementation on phosphatemia.

T0 (Before supplementation), Post-VitD (After supplementation) paired Student test (N = 15).

No significant difference was observed concerning the phosphatemia, in the patients treated with Overdose (Figure 4.13). This is consistent considering that vitamin D also increases the expression of FGF 23 and its receptor clothe, also promoting phosphaturia. This effect limits the effects of vitamin D on the absorption of phosphate at the digestive level. The final impact depends on the comorbidities, and would seem to compensate for each other in our sample of patients.

4.3.4 Impact on (THA)

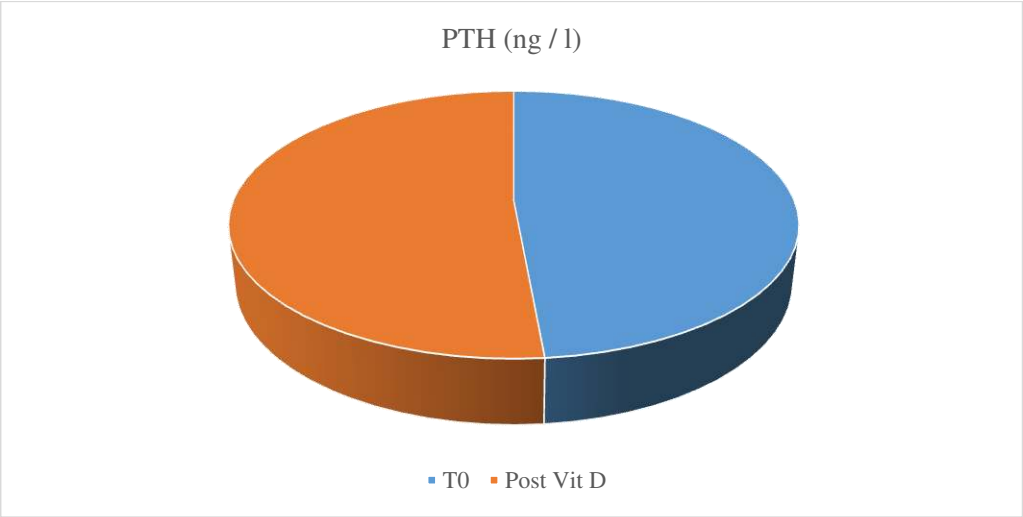

Figure 4.14 Impact of vitamin D3 supplementation on PTH.

T0 (Before supplementation), Post-VitD (After supplementation) paired Student test (N= 12).

We notice a slight increase in PTH in the 12 patients in whom we managed to obtain PTH values after supplementation with cholecalciferol, however this increase is not significant (Figure 4.14) and could correspond to the evolution of hyperparathyroidism in our patients.

Pharmaceutical interventions: During the 3-month follow-up we also analyzed prescription errors and performed pharmaceutical interventions (PI) both by oral transmission and through the computer software used for prescriptions (table 4.2). PIs are defined as all proposals for modifying drug therapy, initiated by a pharmacist and which may be beneficial for the patient.

Table 4.4 Pharmaceutical interventions established during the study.

Nature of the PI	Number of patients
Prescription every 30 days instead of 15 days	2
Insufficient dosage	7
Excessive dosage:	
Prescription period too long.	19
Prescription of an ampoule in patients requiring none.	5
Daily prescription for 180 days.	2

The majority of errors noted relate to too long processing time on the prescription computer software. Prescribers, mostly medical interns, tended to prescribe an ampoule every 15 days for 180 days. This duration is excessive, since the longest supplementation period of the Hospital protocol is only 45 days. Then 5 patients received an ampoule when they had a 25 (OH) D level greater than 30 ng / mL. These prescriptions are of no significant clinical consequence as it is often advisable to give a follow-up ampoule every 3 months to allow 25 (OH) D concentrations to be maintained. However, as the majority of prescriptions were at the rate of 1 ampoule every 15 days for several months, our PIs again made it possible to avoid vitamin D overdoses on the initial prescription. The errors, the most at risk were the two prescriptions of Overdose previously described, at 1 ampoule per day for 180 days, following a computer handling error.

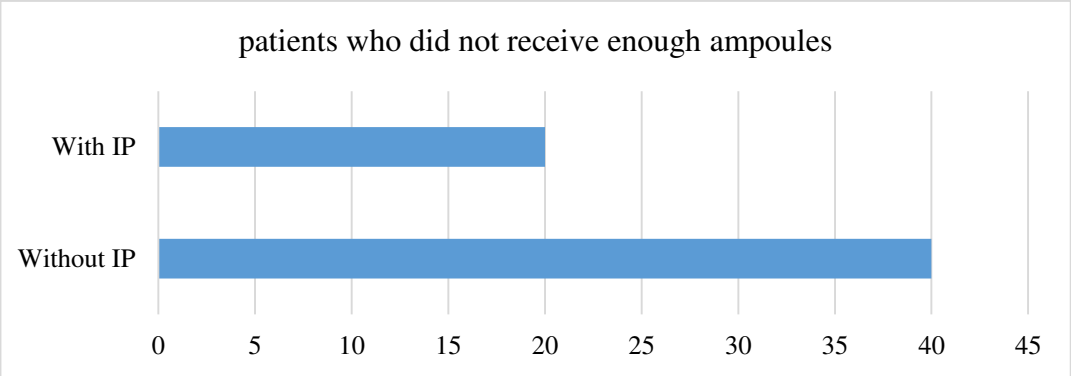

Figure 4.15 Impact of pharmaceutical interventions on vitamin D supplementation, Khi2 test.

We note that the proportion of patients who did not receive enough ampoules is greater in the group without PI than in the group of patients who had PI (Figure 4.15). The difference was indicative ($p < 0.10$) but not significant, probably related to an insufficient number of patients.

4.4 Biochemical Analysis of Diabetic Patents Blood

Table 4.5: Biochemical analysis of the Diabetic patients serum.

Serum bilirubin levels (µmol/L)	1.24
Fasting glucose (mg/dL)	159
Packed red cell volume PCV(%)	36.24
serum glutamic oxaloacetic transaminase (GOT) (u/L)	86.24
Glutamic pyruvic transaminase GPT(u/L)	46.31
HbA1c (%)	7.67

- Serum bilirubin levels (μmol/L): In order to avoid protein glycation, bilirubin can play a key role. The relation between weaker diabetic regulations and low levels of bilirubin may be clarified by that hypothesis in this research (Table 3.5). This literature information indicates a clear inverse connexion between fasting plasma glucose and serum bilirubin. The serum bilirubin values with an average 1.24 μmol/L for the subject, both well regulated and poorly controlled. A statistically important difference in bilirubin was ($p<0.001$). Serum bilirubin was strongly and inversely associated in the Pearson Correlation study with BMI ($r=-0.31$, $p=0.024$), blood glucose fasting ($r=-0.267$, $p<0.001$), and HbA1c ($r=0.0267$, $p<0.001$). The low level of serum bilirubin could warn doctors to worse control in type 2 diabetic subjects. In addition to calculating HbA1c, serum bilirubin assessment may be useful for tracking the diabetic community.
- As a final analysis, the mean of fasting blood glucose of the selected patients, calculated in Table 4.5 (on average), was confirmed, confirming the importance of maintaining the assessment and control of blood glucose levels routinely so that these are positively reflected value, eliminating future risks and complications from poor disease control. The means obtained were fasting blood glucose (159mg/dL) confirming what has already been said, that when a good control of blood glucose levels is maintained, good level control is automatically maintained. Maintaining the average glycemic level between 100 and 125 mg / dL should be a goal for all patients with diabetes, since the average glucose between these values ensures the measurement of glycated haemoglobin (HbA1c) always below 7.0%, fundamental in prevention of risks and complications inherent to the disease.
- Our study shows that in diabetic patients (HbA1c ≥7.5) PCV decreased substantially compared to non-diabetic (HbA1c ≥.6.5). The dehydration and accumulation of protein can cause a reduction in PCV of a diabetic patient.
- Biomarkers of liver function include SGOT and SGPT, and their measurement is a validated toxicology test. In our present study, concentrations of SGOT and SGPT were significantly increased (Table 4.5) in diabetic groups compared to those in the control group. Moderate Serial Rise SGOT and SGPT), Bilirubin

have been reported in higher levels indicates the diabetic status of the selected patients.

- Blood glycemoglobin analysis (HbA1c) offers proof of an individual's mean blood glucose levels over the previous 2 to 3 months, the estimated half-life of red blood cells (RBCs). HbA1c has recently been recommended by ADA for diagnosing diabetes with 6.5 percent cut-off to alternative standards based on fast glucose (FPG ≥7.0 mmol / L) for plasma glucose. It should be remembered that levels of HbA1c are directly proportional to levels of blood glucose. A simple blood glucose test (FGT) is a measure of the concentration of glucose in the blood of a person at a given time.

4.5 Oxidative Stress Levels of (CKD) Patients and Healthy Individuals (Control)

Table 4.6 Oxidative stress levels of CKD patients before (Pre-HD) and after (Post-HD) a hemodialysis session (HD)

Variable	Pre-HD	Post-HD
Carbonyls (nmol / mg protein)	5.62 ± 0.46	5.93 ± 0.52
Lipid Peroxidation - CL (cps / mg Hb)	22.194 ± 1.734	20.080 ± 1.624
Catalase (pmol / mg protein)	5.08 ± 0.19	5.03 ± 0.13
Superoxide Dismutase (SOD) (U / mg prot)	3.1 ± 0.26	3.9 ± 0.65
Total Antioxidant Capacity (U Trolox / mL of sample)	1.476 ± 119	648 ± 87 *
F2 isoprostanos (ng/ml)	821.89 ± 300.47	270 ± 95
GPX (U/mg prot)	60.58 ± 6.35	45.37 ± 7.56
8-oxo-dG (DNAn)/106	2.96 ± 1.85	7.88 ±2.32
8-oxo-dG (DNAmit)/106	13.85 ± 1.44	15.73 ± 2.28
Malondialdehide (MDA)	1.11 ± 0.11	0.7 ±0.34
Lipoprotein a (mg / dl)	23.33 ± 14.72	62.75 ± 41.12
Triglycerides (mg / dl)	93.63 ± 48	128.77 ± 9.78
Carbonylated proteins (nmol / mg prot)	3.63 ±1.12	7.41 ± 0.84
GSSG/GSH	1.39 ± 0.75	6.89 ± 1.91
Colesterol total (mg/dl)	183 ± 22.94	166.97 ± 7.83
Nitrate (μM)	3.35 ± 0.41	3.67 ± 0.33
Nitrite (μM)	0.10 ± 0.01	0.13 ± 0.01

Values expressed as mean ± standard error. Significant difference (*) between Pre and Post-HD using the t test ($p < 0.05$). (CL - Chemiluminescence).

In Table 4.6, data are expressed in chronic renal failure patients obtained before and after a hemodialysis session. It can be seen that there was no significant variation in the enzymes SOD and Catalase after hemodialysis session. There was also no change in the levels of oxidative damage to lipids and proteins, as well as in the levels of nitrates and nitrites after HD.

Table 4.7 Comparison between oxidative stress levels of CKD patients before a haemodialysis session (HD) (Pre-HD) and healthy individuals (Control)

Variable	Pre-HD	control
Carbonyls (nmol / mg prot)	5.62 ± 0.46	3.44 ± 0.22 *
Lipoperoxidation - CL (cps / mg Hb)	22194 ± 1734	12891 ± 398 *
Catalase (pmoles / mg prot)	5.08 ± 0.19	7.94 ± 0.26 *
Superoxide Dismutase (U / mg prot)	3.1 ± 0.26	6.92 ± 0.18 *
Total Antioxidant Capacity (U Trolox / µL of sample)	1476 ± 119	279 ± 28 *
Nitrate (µM)	3.35 ± 0.41	1.35 ± 0.36
Nitrite (µM)	0.10 ± 0.01	0.05 ± 0.01 *
LDL-Col (mg/dl)	113 ± 19.45	94.87 ± 6.57
HDL-Col (mg/dl)	51.13 ± 9.43	47.74 ± 3.92
Creatinine (mg/dl)	0.95 ± 0,09	3.04 ± 0,21
Urea (mg/dl)	38.5 ± 7.54	107.97 ± 0.21*

Values expressed as mean ± standard error. Significant difference (*) between Pre and Post-HD using the t test ($p < 0.05$). (CL - Chemiluminescence).

In Table 4.7, where data from patients on hemodialysis are compared with controls, a significant reduction in the activity of antioxidant enzymes SOD and Catalase is observed in CKD patients. In addition to the activities of antioxidant enzymes being significantly lower in the chronic kidney population, it was observed that oxidative damage to lipids, assessed by OLP by chemiluminescence, and damage to proteins, assessed by Carbonyl levels, were significantly increased in this group of patients. The total antioxidant activity showed a significant reduction after HD session, also observed in the pre-HD (6.15 ± 0.23 mg / dL) and post-HD (4.49 ± 0.20 mg / dL) uric acid levels. ($p < 0.05$).

4.6 Correlation Studies Between Vitamin D3 and (SOD) in (CKD) Patients

This research focused on exploring the relationship between vitamin D (cholecalciferol) concentrations in kidney patients and serum antioxidant enzymes in relation to the corresponding topics. The relationship of these enzymes with fruit and vegetable intake has also been evaluated. Prevalence of extremely deficient vitamin D in kidney patients and their controls were respectively 30 and 11 percent, suggesting that the majority of students had low vitamin D concentrations. As shown in in chapter 3 results , while the period of exposure to sunlight between the groups was significantly different, Serum 25(OH) D levels were not significantly linked to sunlight exposure in these further studies found that, despite plenty of sunlight, low serum 25(OH) D levels are prevalent in all areas of Iranian populations. (Hashemipour et al., 2004) showed no correlation between sunlight exposure and serum Cholecalciferol levels to support our findings in this respect. Vitamin D deficiency is well known by decreased sun exposure in long-term CKD survivors. Although future population studies have shown low level 25(OH) D to be predictive for renal failure, our comparisons with other cross-sectional studies have been restricted. This is not the case with acute renal failure situations. No substantial difference between cases and their controls was found even in the 25(OH)D serum levels. This result is consistent with other research in kidney patients, but is in contrast to the analysis (Poole et al., 2006). In a transverse analysis in Iran, Iranmanesh and Gadari compared serum 25(OH) D levels of 44 first-time patients who had kidney problems with 44 stable elderly ambulant and no serum Vitamin D differences documented in CKD patients. In the later research, however, 77 percent of patients suffering from cholecalciferol had serum 25(OH) D values lower than those of the stable older patients.

(Boshtam et al., 2005), reported that there were no statistical ties in Iranian female tapestry weavers between serum cholecalciferol levels and lipid profile. In our study, while LDL-C levels were higher than those of control in kidney patients ($p = 0.03$), no significant association was observed between Cholecalciferol and LDL-C in both groups. In our research, while LDL-C levels were higher than those of controls, in both groups. Our findings were similar to other CKD and diabetes studies conducted in Iran

(Bonakdaran et al., 2010), but contrasted to (Hirschler et al., 2014). studies in Argentina 2014.

(Cherubini et al., 2000) found that SOD and GPX activity as antioxidant enzymes in patients suffering from the kidney in a plasma were decreased as a result of elevated oxidant stress immediately after an CKD and was shown to increase over time. In the analysis by (Fang et al., 2013), SOD antioxidant activity decreased dramatically following the beginning of heart stroke on gerbbles. The effects of vitamins C and E on the antioxidants in DM rats have been studied by (Chen et al., 2001). SOD is increased after six weeks of treatment. When all the findings are collected, it can be clear that brain ischemia will lead to an increased oxidative stress. It is important to note the findings.

The present research is the first to confirm the hypothesis that cholecalciferol is an effective factor that could boost antioxidant safety against CKD oxidative stress. There has never been a substantial association between serum cholecalciferol levels and SOD and GPX activity. The high prevalence of cholecalciferol deficiency in our subjects may be one of the reasons for that. An additional explanation is that, within 3days, we have taken blood samples, perhaps, if this period were longer, oxidative stress-related complications of the kidney would have been prolonged and then a beneficial link might have been found between cholecalciferol concentrations and antioxidant enzymes. It was found that cholecalciferol adjunction in patients with atopic dermatitis could increase erythrocyte SOD and catalase activity. Furthermore, a positive association between Cholecalciferol concentrations and SOD / GPX activity occurred in (Taheri et al., 2010), studies in the diabetic subject. The possibility has also been highlighted of a complex blend of fruit and vegetables contributing to their preventative effects, as a result of activation of the endogenous anti-oxidant protection system. Epidemic studies have reported that use of fruit and vegetables is correlated with protective effects against age-related diseases.

The intake per week in CKD patients of more than 4 servings of fruit and vegetables was 15% and 28%, which were both lower than controls. Moreover, the activity of SOD

and GPX enzymes and fruit intake in stroke patients have had a strong positive association. Parallel to this observation, (Mahe et al., 2010). used the validated 14-item FFQ to equate the dietary pattern of retinal patients with control subjects. The influence of the anti-stroke supplement on the nutrient intake of kidney patients was lower, in a meta-analysis related to the use of fruit and veg and the risks of stroke. The effect of a supplement with dietary doses of anti-stroke was updated by (Hu et al., 2014). The supplementation did not substantially impact the activity of anti-oxidant enzymes. However, the DNA damage and LDL oxidation resistance has been increased. With respect to CKD factors, future studies have identified multiple risk factors for subtypes of CKD. The kidney risk factors include ageing, HTN, diabetes, smoking, CKD history, auricular fibrilation, left-cell hypertrophy. In our study, patients with HTN have had substantially greater experience, elevated serum LDL-C level or CKD experience than controls. Logistic regression models were used to determine the impact of predicting risk factors. These results are consistent with studies in and around Iraq that emphasise common risk factors. Thus we can identify the order of our patients' most important kidney problems predictors. Further studies on Iraq CKD patients showed that the prevalence of CKD risk factors (HTN, DM, hypercholsterolemia, smoking, valving heart disease respectively) was more than 50%. A cross-sectional multihospital analysis conducted with kidney problems showed that HTN was the most significant risk factor in the development of injury in Iraq. Patients were recruited within 1–3 days of the onset of stroke for a strength point in our research and had no prior history of stroke (sometimes due to decreased sunlight sensitivity in post-stroke patients with cholecalciferol deficiency). This study also revealed that, while there was plenty of sunshine in the area, high levels of cholecalciferol deficiency are considered in the elderly subject. In the end, our research had minimal capacity to establish a transient correlation between Cholecalciferol deficiency and CKD as a cross sectional study. Moreover, total serum concentrations of antioxidant and antioxidant enzymes in RBCs, which could be a target for potential operations, were not measured. Our findings indicate that the incidence of extreme cholecalciferol and lower intakes of fruit and vegetables is higher for patients with kidneys problems. In addition, fruits associate favourably with levels of antioxidants. The best predictors of CKD are elevated blood pressure and LDL-C serum and the history of CKD.

Additional experiments with more subjects and assessment of more complex antioxidant biomarkers are proposed in order to study the potential causal effects in CKD patients of particular antioxidants and levels of Vitamin D3.

Biochemical properties (table 3.5) of Diabetic patients after treatment with Vit D3 gradually decreased and comparatively lesser than the values obtained in Table 3.5 except HbA1c.

5. DISCUSSION

Despite current recommendations, nearly 80% of chronic renal failure patients at the Marjan Teaching Hospital of Babylon still have 25 (OH) D serum values below the recommended threshold of 30 ng/mL. This percentage is close to the 76% found by a previous study carried out by (Kausz and Pahari, 2004) in the same region1. In 6 years, the number of publications on vitamin D has continued to increase without this impacting the level of deficiency in patients in CKD. The characteristics of our population are in agreement with the physiopathology of CKD, with a tendency to hyperparathyroidism and hyperphosphatemia, with a high prevalence of diabetes and hypertension.

Our study found the ability of cholecalciferol to increase 25 (OH) D and 1,25 (OH) 2D, as shown in numerous studies. For 25 (OH) D Alvarez et al found a concentration multiplied by 1.6 and (Chandra et al., 2008), by 2.8 in patients with CRI after supplementation with cholecalciferol. However, the 25 (OH) D concentration seems to decrease gradually, hence the need to resort to supplementation every 3 months thereafter (Del Valle et al., 2011). Concerning 1,25 (OH) 2 D, (Marckmann et al., 2012) observed a mean increase of 13.5 pg / mL in chronic renal failure not on dialysis, following the intake of cholecalciferol. We noticed in MVDN that the assay of 1,25 (OH) 2D was hardly performed in the city laboratory, which can constitute an obstacle to its monitoring. More complex and more expensive, the dosage of 1,25 (OH) 2D has little place in cholecalciferol supplementation to date in the various recommendations. With a relative risk of hypercalcemia of 4.76 according to a meta-analysis of (Battista et al., 2013), have shown that the use of vitamin D3 in patients with secondary hyperparathyroidism leads to a moderate and temporary elevation of serum calcium without clinical signs of toxicity. The toxicity of vitamin D can have potentially harmful consequences, which can lead to extra-osseous calcifications including cardiovascular, as well as urinary lithiasis. While it is complicated to know with certainty the long-term toxic risk, the data suggest that the risk of acute intoxication is limited and would only be observed for daily doses exceeding 40,000 IU.

However, other studies in particular those of (Bolland et al., 2011), have shown no impact on serum calcium following vitamin D3 supplementation. Therefore, if vitamin D3 exposes a risk of hypercalcemia, the latter is relatively low, and less than with active derivatives. Most experts, however, believe that vitamin D should not be used in patients with hypercalcemia. We also noticed a slight elevation in PTH, a result inconsistent with other studies on the subject. The majority of studies versus placebo show that cholecalciferol significantly decreases PTH in dialysis patients or not. This slight increase may be hypothetically linked to the degradation of HPTS in our patients, half of whom had terminal CKD. In addition, our results are limited by the low number of city checks, and therefore more subject to individual variability. The low number of city checks is also associated with an insufficient number of light ampoule delivered. Our study highlighted the fact that nearly a third of patients with chronic renal failure in the nephrology department were insufficiently supplemented with vitamin D. This factor can potentially explain why, despite current recommendations, nearly 80% of our patients are still vitamin D deficient. In addition, 1/3 of the patients in whom we have obtained post-supplementation laboratory results, n 'were able to obtain an optimal 25 (OH) D concentration. In RCT, the success of the supplementation is obtained in 70-80% of cases. This may imply that an additional ampoule could be added to the Hospital protocol for CKD patients.

Several factors have been correlated with the risk of not following the protocol. The 25 (OH) D concentration was significantly correlated in the univariate analysis, but not in the multivariate analysis. Patients with a low concentration of 25 (OH) D require a larger number of ampoules, most often forcing them to follow a relay of the socket in town. The need for relay in town was in fact independently correlated with the risk of not complying with cholecalciferol supplementation. This hospital / city relay is a real problem, which requires considering optimizations of the patient circuit. As most often, it is a question of forgetting a prescription, a pharmaceutical medication conciliation activity would make it possible to identify them and increase the quality of follow-up. In addition, patients with elevated phosphatemia are also independently more at risk of not monitoring their supplementation properly. In theory, these are patients with greater renal impairment or at least with a more marked uremic repercussion. One potential

explanation would be that prescribers could more easily forget the vitamin prescription when patients present complications requiring more extensive treatment. However, our study does not have a specific indicator of the burden of care.

Patients with a low serum 25 (OH) D concentration, and high phosphatemia are more at risk of poor vitamin D management when they are theoretically those who need it the most. These results confirm the need to find means of improving vitamin D management in CKD patients, in order to benefit from its effects.

We also note that patients on anxiolytics have a greater risk of having insufficient vitamin D3 supplementation. This could be explained by poor compliance in this type of patient after the delivery of the discharge order. More specific action should be expected for patients on anxiolytics.

One of the therapeutic options to optimize the success of vitamin D treatment would be to consider systematic daily or monthly supplements. In January 2001, Bosom worth advised patients at risk, including CKD patients, a minimum intake of 2000 IU per day 150 (Jean et al., 2015) reported that a monthly dose of 100,000 IU of cholecalciferol was able to correctly supplement more than 85% of dialysis patients. Other adaptations are possible, in Belgium (Delanaye et al., 2013), have shown that a dose of 25,000 IU every 14 days allowed 75% of dialysis patients to have a 25 (OH) D concentration greater than 30 ng / mL after a year. These treatment regimens would potentially be more subject to a problem of compliance, but less exposed to the risk of forgetting to transcribe. In addition, these regimens have the advantage of being in line with the new social security recommendations on the prescription of vitamin D without prior dosage. However, in Iraq the specialties allowing a daily or every two weeks intake are very limited.

Among the areas for improving vitamin D management, we also observed that the pharmaceutical analysis had a beneficial impact. As we have seen, pharmaceutical interventions have improved the correct prescription of vitamin D3 in the Nephrology department in 25 patients. However, even if the proportion of non-compliance with the

protocol was almost twice as low in patients who had benefited from a PI, this difference remained at the limit of significance. A larger sample size, and a two-arm study would be needed to accurately assess the impact of pharmaceutical analysis. The intervention of the hospital pharmacist can have interesting consequences in terms of the quality of care. Indeed (Leguelinel-Blache et al., 2015),

showed that 79% of hospital pharmaceutical interventions improve patient care. It would therefore be wise for the pharmaceutical analysis to focus particularly on the problems of the hospital / city relay and to target more particularly the patients most at risk of absence or poor adherence to treatment. Techniques such as medication reconciliation would improve communications between the city and the hospital. A pharmacist, a pharmacy intern or a pharmacy extern from the nephrology service could best ensure the follow-up of these patients. Nurses should also be kept informed of the current dosages of this vitamin in order to avoid administration errors, especially the daily intake of vitamin D3 ampoules.

The cytosolic SOD enzyme is copper and zinc dependent. The decrease in these ions in patients receiving hemodialysis may contribute to a decrease in SOD activity (Marreiro et al., 2017), which can also be directly decreased by an increase in the production of superoxide and hydrogen peroxide ions. In addition, the majority of CKD patients receive therapy with erythropoietin and iron, and it is known that iron acts as a catalyst in the generation of active oxygen species, such as the toxic hydroxyl radical, and that ferric species may be involved at different stages of LPO as initiation and propagation (Nuhu and Bhandari, 2018). All of these factors may have influenced SOD activity in these patients, with a consequent decrease in their activity.

Catalase, like SOD, showed no variation in its activity after a hemodialysis session. However, when compared to the control group, both were significantly lower. Exposure to some elements such as aluminum, silicon or iron during dialysis, deficiency of some essential element involved in the activation of some enzymes such as selenium or zinc, in addition to other factors not yet clarified, may contribute to the

low activity of catalase and SOD in erythrocytes of patients undergoing dialysis (Liakopoulos et al., 2017).

The assessment of total antioxidant capacity (TRAP) can provide information regarding the systems' capacity to resist oxidative stress imbalances. The method used in this study allows measuring the capacity of the sample to act as an antioxidant against formed free radicals. The capacity presented by the sample is compared to a standard, in this case the Trolox (water-soluble vitamin E) (Apak et al., 2007). Among the antioxidant substances measured by the technique, are: vitamin D3, uric acid and glutathione, the main water-soluble non-enzymatic antioxidants in plasma. Substances are measured together and not individually.

Levels of vitamin D3 in chronic renal failure patients on HD are, in most cases, lower than those found in healthy people. In addition, a decrease in vitamin D3 levels is observed at the end of dialysis (Jean et al., 2015). As a result of the action of free radicals, vitamin E is converted to α-tocopheryl, a free radical. The free radical a-tocopheryl has no antioxidant properties and can trigger the lipid peroxidation process of the membranes. Vitamin D, one of the antioxidants measured by TRAP, is a substance responsible for the regeneration of the radical a-tocopheryl to tocopherol (vitamin E). This may be an explanation for the reported concomitant reduction in TRAP and vitamin D after a HD session.

(Zare-Mirzaie et al., 2018) observed a marked depletion of antioxidants after a hemodialysis session. The authors also found a decrease in the levels of vitamin D and thiols, and an increase in the levels of the Calcifediol in relation to the level of vitamin D. The antioxidant activity of plasma and uric acid levels were significantly higher in chronic kidney patients. After HD, vitamin D3 levels decreased and the SOD/ vitamin D3 ratio increased, while plasma antioxidant capacity and uric acid level were significantly lower after the dialysis procedure. The increase in the levels of the Calcifediol radical after dialysis shows an increase in the concentration of active oxygen species (Thakkinstian et al., 2015).

(Norris et al., 2018), in an evaluation of the total antioxidant capacity in human plasma, showed that it was formed by the presence of: urate (35 - 65%), 25(-OH)D (0 - 24%), vitamin E (5 - 10%) and proteins (10 - 50%). In this study, there was a parallel decrease in both uric acid and antioxidant status of the plasma after a HD session, as well as a higher TRAP in patients when compared to controls. This higher TRAP has been attributed to an increase in the urate level of these patients, and the results of this work are in line with findings in the literature.

The high antioxidant capacity does not indicate an improvement in antioxidant defenses and does not exclude the deficiency of some antioxidants (Prescha et al., 2018). In serum and saliva samples, TRAP values are emphatically correlated with uric acid due to the high concentration of this antioxidant. This dependence on the values of TRAP with the concentration of an antioxidant in which the concentration is little related to oxidative stress limits the use of this technique as an index of antioxidant status in plasma. Direct measurements of the levels of vitamins and other water-soluble antioxidants could assist in the discussion of these data. Several studies show that oxidative stress markers are increased in chronic renal failure (Kumar et al., 2017). One of the markers evaluated in this study was the determination of oxidative damage to lipids, lipoperoxidation (OLP). OLP has been shown to be involved in the pathogenesis of diseases such as atherosclerosis and cancer, diseases commonly found among CKD patients undergoing HD.

Free radicals attack the polyunsaturated fatty acids of cell membranes, resulting in the formation of OLP products such as conjugated dienes and malondialdehyde (MDA), one of the components measured by TBARS, which is a widespread method, however, with little sensitivity and specificity. It is known that many factors, such as fatty acid composition, amount of fat and antioxidants, can modify this reaction. During HD, there is an influence of several substances including glucose, which can affect the reaction with TBA. Thus, it is necessary to use a more sensitive technique to improve the quality and accuracy in determining lipoperoxidation in chronic kidney disease. The determination of OLP in this study was done using the chemiluminescence (CL) technique, described by (Flecha et al., 1991), This technique has been used to determine

the occurrence of oxidative stress in several pathological situations and is a very sensitive method for measuring OLP. Analyzing the data presented in Tables 1 and 2 , it is observed that the CL values were not modified by the HD session and that they were significantly higher than the control group. Although the technique adopted for measuring damage to lipids is different from the one usually used, the results point to the same path, an important state of oxidative stress in these patients, not being altered by a single hemodialysis session.

It has been established that proteins represent targets for oxidative-mediated injury. The attack of active oxygen species on proteins can lead to functional changes and, in particular, to the progressive loss of their metabolic, enzymatic or immunological properties. Products of protein oxidation and products of DNA base oxidation have also been suggested as important oxidative stress indices. This new line of study indicates that the measurement of oxidized proteins may have some advantages in comparison with lipoperoxidation products because their formation is relatively early and the relative stability of these compounds (Bhattacharyya et al., 2014).

The formation of carbonyls represents an early marker of protein oxidation. They involve cations in the redox cycle such as iron and copper, which have protein binding sites and can transform amino acid residues into carbonyls in the presence of hydrogen peroxide and superoxide anion. The amino acids lysine, arginine, proline and histidine are the most likely to generate carbonyls.

In the present work, protein oxidation in plasma was evaluated using the carbonyl technique. The data presented show the levels of carbonyls in the plasma of chronic renal failure patients on HD, significantly elevated when compared with control subjects. This high rate of damage to proteins, observed in CKD patients, was not modified after a single hemodialysis session. In addition to the evaluation of active oxygen species, measurements were taken regarding the metabolites of NO, an active nitrogen species. NO, also known as an endothelium-dependent relaxation factor, is a hydrophobic gas responsible for controlling vascular tone, promoting vasodilation, inhibiting some processes such as platelet aggregation, leukocyte adhesion to the

endothelium, production of endothelins (peptides with potent vasocontricting action). It also causes variation in the force of contraction and heart rate. NO is released when there is an increase in blood flow, an increase in *shear stress* and increased blood pressure, causing relaxation of the vessels. This is an adaptation mechanism that helps to maintain blood pressure at normal values. An increase in the production of AOS, such as superoxide, hydrogen peroxide and lipoperoxides, in addition to a decrease in NO synthesis, was observed in patients with essential hypertension, when compared to normal individuals. These individuals with arterial hypertension still have decreased concentrations of antioxidants such as vitamin D and SOD (Baradaran et al., 2014).

In hypertension due to chronic kidney disease, inhibition of NO synthesis was observed (Togliatto et al., 2017). However, when these patients are submitted to HD sessions, an increase in NO may be induced by the increase in blood flow and shear stress to which the blood is subjected during the procedure. This increase in NO, together with the loss of volume, seems to be one of the factors responsible for the hypotension presented by some patients during HD. The circulating superoxide can react with NO, inactivating it and forming nitrates, or peroxynitrite (ONOO-), which is a highly oxidizing toxic product. The spontaneous decrease in peroxynitrite can lead to the formation of nitrite and hydroxyl radical. This mechanism may explain the significant increase in nitrite levels observed in renal patients after a hemodialysis session. Although an NO metabolite was increased after HD, there was no increase in the levels of OLP and carbonyls after the procedure. This could be combated by the non-enzymatic antioxidant system, which had an important reduction during the HD procedure. Another hypothesis is that a single HD session, due to the short time, would not be enough to detect the damage caused by these radicals, since, when there is an increase in nitrite levels, there is also an increase in the hydroxyl radical. A set of factors, such as an increase in active oxygen species, a reduction in the activity of antioxidant enzymes and a reduction in the concentration of non-enzymatic antioxidants after HD, can constitute a vicious cycle of oxidative stress in these patients.

Oxidative stress: the values and differences of the oxidative stress biomarkers are listed in Table 4.6.

Significant variations have been identified between the control group and prediagnosis. MDA association with HDL-col ($r = 0.41$; $p<0.05$) was important, but there were no connexions with other parameters of oxidative stress.

Protein oxidation: The values of carbonated protein were positive associations with GSSG / GSH ratios ($r = 0.51$; $p < 0.05$) and 8-oxo-dG ($r = 0.49$; $p<0.05$) and negative with SOD ($r = -0.39$; $p < 0.05$) and GSR ($r = -0.41$; $P < 0.05$). Protein oxidants were positive and negative.

Genetic oxidative damage: There have been substantial variations between the research group and the control group for both nuclear and mitochondrial values of eight-oxo-dG. Significant homocystone correlations ($r=0.31$; $p<0.05$); lipoprotein(a) ($r=0.38$; $p<0.01$); mitochondrial 8 oxodonal proteins ($r=0.40$; $p<0.05$); GSSG / GSH ($r=0.6$; $p<0.001$) and described proteins (compared to above), have been observed at 8-oxodonal dG values. Inverted associations between 8-oxo-dG values are similar: total proteins ($r = -0.25$; $p<0.01$); GSH ($r = -0.65$; $p<0.000$); SOD (-0.5; $p<0.000$), respectively.

Table 4.7 represents the antioxidant defences for predialysis and the antioxidant enzyme activity of the control group. The enzyme of the GSR has had important association with: GSH ($r = 0.56$, $p < 0,05$,), SOD ($r = 0.4$, $p < 0,05$) and reciprocal reaction to nuclear 8-oxo-dG ($r = -0.57$; $p < 0,001$). This molecule of glutathion was substantially reversed to 8-oxo-dG ($r = -0.66$, $p < 0,001$) and GPSG ($r = -0,612$, $p < 0.001$) and Carbony protein ($r = -0.59$, $p < 0.001$). MDA ($r = -0.88$, $p<0.001$) and SOD ($r = 0.45$, $p < 0.005$) had the following correlations.

SODs is related to nuclear 8-oxo-dG ($r=-0.5$, $p<0.05$), MDA ($r= -0.46$, $p<0.01$) and

Conclusion

CKD is a common pathology , It is characterized in particular by a progressive decrease in the activity of 1-alpha hydroxylase, by an overall loss of nephrotic mass and by the accumulation of uremic toxins. All of them are responsible for a decrease in the

synthesis of 1,25 (OH) 2D, the active form of vitamin D and promotes an overall vitamin D deficit. Vitamin D3 has many beneficial pleiotropic effects, particularly in the bone, cardiovascular and renal protection. Vitamin D3 supplementation would therefore have a role to play in the management of the morbidity and mortality in nephrology patients. As part of this thesis, we studied the quality and the biological impact of the prescription of vitamin D3 in the nephrology department of the Marjan Teaching Hospital of Babylon through the MVDN study. Our results highlighted the fact that nearly a third of patients were not properly supplemented, which may partly explain why, despite current recommendations for supplementation, 80% of patients with chronic renal failure do not have an optimal concentration. . The hospital pharmacist could therefore have an important role in optimizing this supplementation. Targeted actions could range from discharge conciliation to supervise the hospital / city relay, to pharmaceutical analysis to optimize the initial prescription defined by an increase in oxidized macromolecules, both lipids, proteins and genetic material, and a decrease in the activity of the main antioxidant enzymes. The population in Pre-D has a worse oxidative profile, with oxidized parameters significantly higher than the population with substitution treatment, which leads us to think that uremic state per se is one of the main causes, given that the other two groups maintained adequate levels of uremic toxin clearance. In dialysis patients.

It is concluded, therefore, that there is a real importance of the nutritional factor on the effectiveness of hemodialysis therapy. This is clear when realizing that specific dietary mediations for the chronic kidney patient, combined with the drug arsenal and hemodialysis therapy, guarantee greater preservation of renal function, as well as greater safety for treatments of disorders resulting from chronic kidney disease.

From the results obtained, it can be concluded that the oxidative stress assessed in CKD patients who undergo HD is significantly higher than that observed in healthy individuals, evidenced by the significant increase in the levels of lipoperoxidation and protein oxidation, significantly reducing the activity of antioxidant enzymes SOD and CAT and increased levels of nitrites. A single session of HD did not modify the levels of systemic oxidative stress presented by these patients.

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