A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF ÇANKIRI KARATEKİN UNIVERSITY

ASSESSMENT OF THYROID FUNCTION, VITAMIN D AND SOME BIOCHEMICAL VARIABLES IN HEMODIALYSIS PATIENTS

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
CHEMISTRY

BY

AHMED QASIM SHAKIR SHAKIR

ÇANKIRI

2022

ASSESSMENT OF THYROID FUNCTION, VITAMIN D AND SOME BIOCHEMICAL VARIABLES IN HEMODIALYSIS PATIENTS

By Ahmed Qasim Shakir SHAKIR

June 2022

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ABSTRACT

ASSESSMENT OF THYROID FUNCTION, VITAMIN D AND SOME BIOCHEMICAL VARIABLES IN HEMODIALYSIS PATIENTS

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Master of Science in Chemistry

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Vitamin D binds to the intracellular nuclear receptor VDR, which is expressed in macrophages, monocytes, dendritic cells, T and B lymphocytes, and others. These cells produce the enzymes needed to metabolise vitamin D. Vitamin D receptors interact with 1,25(OH)2D3, generated by 25(OH)D-1-hydroxylase. Vitamin D3 has extensive impacts on numerous lines of defence cells, indicating its importance in immune-mediated changes and autoimmunity. It controls CD4+ T cell differentiation and activation. It suppresses the activation of TCD4+ Th1 cells, lowering the production of IFN-γ, IL-2, and TNF-α, resulting in enhanced CD4+ Th2 T cell proliferation and production of IL-4, IL-5, and IL-10. Multiple sclerosis symptoms worsen in winter and spring when vitamin D levels are low, according to research. Vitamin D pills reduce these symptoms in women, according to studies. Clinical investigations using vitamin D analogues in individuals with DM1 showed that the condition was managed, with a drop in glycated haemoglobin and an increase in plasma C-peptide levels, indicating the prospective relevance of this therapy. Further research is needed.

2022, 62 pages

Keywords: Thyroid function, Vitamin D, Chronic haemodialysis (CDH), hematology and inflammatory status

ÖZET

HEMODİALİZ HASTALARINDA TİROİD FONKSİYONU, D VİTAMİNİ VE BAZI BİYOKİMYASAL DEĞİŞKENLERİN DEĞERLENDİRİLMESİ

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Haziran 2022

Bu çalışmanın amacı, D vitamini, makrofajlar, monositler, dendritik hücreler, T ve B lenfositleri ve diğerlerinde ifade edilen hücre içi nükleer reseptör VDR'ye bağlanır. Bu hücreler, D vitaminini metabolize etmek için gerekli enzimleri üretir. D vitamini reseptörleri, 25(OH)D-1-hidroksilaz tarafından üretilen 1,25(OH)2D3 ile etkileşime girer. D3 Vitamini, bağışıklık aracılı değişiklikler ve otoimmünitedeki önemini gösteren çok sayıda savunma hücresi hattı üzerinde geniş etkilere sahiptir. CD4+ T hücre farklılaşmasını ve aktivasyonunu kontrol eder. TCD4+ Th1 hücrelerinin aktivasyonunu baskılayarak IFN-y, IL-2 ve TNF-a üretimini azaltır, bu da gelişmiş CD4+ Th2 T hücre proliferasyonu ve IL-4, IL-5 ve IL-10 üretimi ile sonuçlanır. Araştırmaya göre, D vitamini seviyelerinin düşük olduğu kış ve ilkbaharda multipl skleroz semptomları daha da kötüleşiyor. Yapılan araştırmalara göre D vitamini hapları kadınlarda bu semptomları azaltıyor. DM1'li bireylerde D vitamini analoglarını kullanan klinik araştırmalar, durumun glise edilmiş hemoglobinde bir düşüş ve plazma C-peptid seviyelerinde bir artışla yönetildiğini gösterdi, bu da bu tedavinin ileriye dönük uygunluğunu gösterir. Daha fazla araştırmaya ihtiyaç var.

2022, 62 sayfa

Anahtar Kelimeler: Tiroid fonksiyonu, D vitamini, Kronik hemodiyaliz (CDH), Hematoloji ve inflamatuar durum

PREFACE AND ACKNOWLEDGEMENTS

I would like to thank my thesis advisor, Assoc. Prof. Dr. Şevki ADEM, for his patience, guidance and understanding. And I thank my parents, my wife and all my family for their support

Ahmed Qasim Shakir SHAKIR
Çankırı-2022

CONTENTS

LIST OF SYMBOLS

%	Percentage
±	Plus-minus
°C	Degree Celsius
g	Gram
IU	International units
mg	Milligram
mL	Milliliters
ng	Nanogram
nm	Nanometer
Pg	Picogram
µg	Microgram
µL	Microliter

LIST OF ABBREVIATIONS

25OHD	25-hydroxy vitamin D
CKD	Chronic kidney disease
CRP	C-reactive protein
CVD	Cardiovascular disease
DCs	Dendritic cells
eGFR	Estimated glomerular filtration rate
FT4	Free thyroxine
HD	Hemodialysis
HDL	High-density lipoprotein
IFN-γ	Interferon gamma
IL-2	Interleukin-2
LDL	Low-density lipoprotein
LPI	Platelet/lymphocyte ratio
NLI	Neutrophil/lymphocyte ratio
OPG	Osteoprotegerin
PTH	Parathyroid hormone
PTH	Parathyroid hormone
RANKL	Receptor activator of NF kappa B ligand
T3	Triiodothyronine
T4	Tetraiodothyronine
TNF-α	Tumour necrosis factor alpha
TSH	Thyroid stimulating hormone
VDBP	Vitamin D-binding protein
VDR	Vitamin D receptor
VEGF	Vascular endothelial growth factor

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1. INTRODUCTION

VitD can also come from foods naturally rich in vitD3 or with added vitamin D2 (vitD2) or vitD3, such as fatty fish (salmon, mackerel), egg yolks, milk and margarine (Canada *et al.* 2012) . VitD2 comes from plant sources (yeasts, fungi and plants), but is only present naturally in very small quantities. Bile acids and pancreatic lipases secreted by the pancreas play a critical role in the absorption of vitamin D2 and D3 in the intestines (Xiao *et al.* 2011). VitD2, vitD3 and the fats thus hydrolyzed will form micelles which will mainly cross the duodenal wall, by diffusion through the enterocytes. From there, vitD2 and vitD3 will almost exclusively be stored in chylomicrons and transported to the systemic circulation via the lymphatic pathway (Xiao *et al.* 2011). To release vitamin D2 and vitD3 from chylomicrons, which are found in adipose tissue and skeletal muscle, tissues that express lipoprotein lipase (VDBP) and vitamin D binding protein (VDP) must be metabolized (VDBP). albumin. VitD2 and vitD3 from dietary or solar sources are believed to be inert, therefore they must be activated by enzymes before they can have a biological effect (Lehmann and Meurer 2010).

The amount of 25-hydroxyvitamin D (25 OH-D) in the blood is used to assess vitamin D levels. Serum 25OHD levels and bone health are often debated. Several investigations on vitamin D supplementation have shown that the active form of vitamin D, 25OHD, has to be kept between 20 and 40 nanograms (nmol/L). This means that levels less than this would be considered inadequate, and levels less than 10 ng/mL would be considered deficient, according to the findings of the research. Some specialists, however, believe that levels below 30 ng/mL are insufficient, particularly for the elderly. A level of 30 to 40 ng/mL (75 to 100 nmol/L) is commonly considered appropriate as a result of this phenomenon. The majority of specialists believe that low concentrations of 20 ng/mL or below are unhelpful for bone health maintenance purposes. It has not yet been determined what the optimal serum 25OHD levels are for bone health (Spiro and Buttriss 2014).

Several experts feel that in elderly people, a level of 30ng/mL (75 nmol/L) is necessary to prevent falls and fractures. While low levels of vitamin D indicate deficiency, insufficiency indicates a lack of vitamin D intake. Vitamin D levels in the blood are affected by sun exposure, season, latitude, skin colour, age, BMI, PAM, dietary consumption (DHA), and medication use. Vitamin D concentrations in equatorial locations tend to fluctuate somewhat throughout the year, although the definition of these concentrations is influenced by a variety of circumstances. Vitamin D concentrations have been observed to vary seasonally, with some studies showing no correlation between the two, while others clearly demonstrate a correlation, with greater amounts present throughout the spring and summer months (Eloi *et al.* 2016).

1.1 Functions of Vitamin D

Vitamin D is essential for the interaction of the kidney, the bones, the parathyroid glands and the intestine. It allows maintenance of extracellular calcium levels in a tightly regulated manner, which is vital for skeletal integrity and cell physiology. Vitamin D increases the absorption of calcium and phosphorus in the proximal duodenum, promoting their passage through the brush border into the cell and their exit through the basolateral membrane. The mechanism of action to promote this transport of calcium in the intestine involves the synthesis of calcium binding protein (CaBP) (Bronner 2003). The synthesis of this protein is dependent on 1,25(OH)2D3 and is regulated both at the transcriptional and post-transcriptional levels.

The skeleton need vitamin D to be healthy and grow properly. Vitamin D deficiency may cause rickets in children and osteomalacia in adults, both of which can lead to bone loss. Osteocalcin, RANKL, and osteoprotegerin (OPG) are all genes that play a role in bone metabolism, and vitamin D works as a transcriptional regulator of these genes. Vitamin D has been shown to increase the growth of osteoclasts and, as a consequence, the release of calcium and phosphate in both vivo and vitro (D'Amore et al. 2006).

Vitamin D's endocrine system affects parathyroid function significantly. Hyperplasia of the thyroid gland and an increase in PTH production and secretion are side effects of

vitamin D insufficiency. 1,25(OH)2D3 is utilized in the treatment of secondary hyperparathyroidism because it suppresses PTH production and cell development, as previously mentioned. In the kidney, 1,25(OH)2D3 has a primary role in regulating its own production and breakdown by blocking 1-hydroxylase and encouraging the expression of 24-hydroxylase, as well as activating megalin expression in the proximal tubules.

The skin, pancreas, liver, and other organs have also been shown to benefit from 1,25(OH)2D3. Known as "non-classical" activities of vitamin D, they occur when VDR is present in a variety of cell types. A few examples of these non-classical activities include cell proliferation and differentiation, as well as hormone and immune function control. In addition to expressing VDR, macrophages and lymphocytes also express 1 hydroxylase, which allows them to manufacture 1,25(OH)2D3 locally under specific conditions. As a result of epidemiological research, 1,25(OH)2D3's in vitro effects on colon, breast, and prostate cancer have been studied. 1,25(OH)2D3 and 1,25(OH)2D3 have been shown in cancer cell studies to reduce proliferation and increase cell differentiation in these cells. 1. (Leyssens et al. 2013). Endometrial cells62, chondrocytes168, and Vascular Smooth Muscle Cells (VSMC) are all examples of cell types that benefit from 1,25(OH)2D3. Vitamin D has several impacts, and more research is needed to understand them all. Although 1,25(OH)2D3's significance in the arterial wall has been established, the presence of VDR in endothelial and vascular smooth muscle cells has raised doubts about these roles.

Despite its original description as a vitamin and its involvement in calcium-phosphorus homeostasis control, vitamin D is today regarded a hormone. Anti-immune illnesses, such as Hashimoto's thyroiditis (HT), such as vitamin D's immunomodulatory activities are now well understood to play a significant part in the development of autoimmune diseases (Niedziela *et al.* 2000).

In autoimmune illness, vitamin D's immunomodulatory effects arise from its direct action on the T cell VDR receptor, resulting in the protection of target organs such as thyroid cells. T-cell-induced apoptosis and suppression of post-activation B cell

proliferation also contribute to the decrease of thyroid antigen-specific antibodies in the presence of this compound. Vitamin D aids in a variety of important bodily functions. When exposed to UVB light, keratinocytes convert 7-dehydrocholesterol into pre-vitamin D3 and transfer this vitamin D3 to the bloodstream as vitamin D3. Extrinsic factors (such as sunblock and garments) may alter a person's capacity to absorb vitamin D from the sun. Foods containing ergocalciferol or vitamin D2 produced from plants and cholecalciferol or vitamin D3 derived from animals include smaller amounts, with intestinal absorption leading to liver storage. The current lifestyle of people with reduced outdoor activities and a diet low in vitamin D has been responsible for the decrease in their levels (Lips *et al.* 2014).

1-25(OH)2D3 (vitamin D3) is generated after two hydroxylations of vitamin D. The liver's D-25-hydroxylase catalyzes the first hydroxylation, while the kidneys' 1-hydroxylase-25(OH)D completes the second. As a result, it regulates calcium and phosphate metabolism, maintains adequate serum levels, promotes intestinal absorption and renal calcium reabsorption, as well as suppressing parathyroid hormone (PTH) secretion, thus preserving bone integrity and acting directly on osteoclastogenesis, effect on osteoblasts, and bone matrix mineralization. As a result of its involvement in regulating the immunological response, vitamin D and its nuclear receptor I have been extensively researched. The steroid receptor Vitamin D Receptor (VDR) (Dowd and MacDonald 2010).

To determine vitamin D levels, the concentration of 25-hydroxyvitamin D is measured (25OHD). Serum 25OHD levels for bone health are debatable. According to vitamin D supplementation studies, a range of 20 to 40 ng/mL (50 to 100 nmol/L) is regarded adequate, while levels below 10 ng/mL would be deemed deficient. However, it is recommended that in the elderly, values below 30 ng/mL would be considered inadequate. As a result, a level of 30 to 40 ng/mL (75 to 100 nmol/L) is often regarded acceptable. Low values of 20 ng/mL or below are considered ineffective for bone health maintenance by the majority of experts. In terms of bone health, we don't yet know what level of serum 25OHD is optimal. A minimum value of 30 ng/mL (75 nmol/L) has been recommended for older persons to reduce the incidence of falls and fractures (Bischoff-

Ferrari et al. 2006). While low levels of vitamin D indicate deficiency, insufficiency indicates a lack of vitamin D intake. The levels of vitamin D in the blood are influenced by a variety of factors, including time of year, latitude, skin pigmentation, age, BMI, degree of physical activity, dietary fat consumption, and prescription medication use. The levels of vitamin D in equatorial regions change significantly throughout the year, although the definition of these levels is affected by a number of variables. Vitamin D concentrations have been observed to vary seasonally, with some studies showing no correlation between the two, while others clearly demonstrate a correlation, with greater amounts present throughout the spring and summer months.

Center for Meteorological and Climatic Research Applied to Agriculture at State University of Campinas says ultraviolet radiation has grown considerably in recent years, especially in the Campinas region (Dias and Sentelhas 2021). Following the recommended daily sun exposure of roughly 20 minutes, this quantity would be sufficient to maintain appropriate vitamin D synthesis levels. Low sun exposure is considered when it occurs less than 3 times a week for a period of less than 15 minutes a day of exposure only to the face and upper limbs. It is considered high when sunbathing with exposure of the face, upper limbs and also the chest occurs at least 5 times a week for more than 30 minutes at a time.

Almost all studies in the future show a strong inverse relationship between 25OHD concentrations and cardiovascular disease, serum lipid concentrations, inflammation, glucose metabolism disorders and weight gain as well as various infectious diseases, cognitive disorders and malignancies (Pilz *et al.* 2011). Anti-inflammatory effects of vitamin D in its active form, 1α-25(OH)2D3, include slowing cell differentiation, as well as the proliferation and death of defense cells.

Rheumatoid arthritis, another inflammatory disorder, has been related to a lack in vitamin D. Studies have shown that those with low levels of vitamin D are more susceptible to sickness. Psoriasis, Sjögren's disease, and Systemic Lupus Erythematosus have all been related to vitamin D insufficiency (Borges *et al.* 2010). Anti-thyroid antibodies and changes in thyroid function are connected to low vitamin D levels,

suggesting that vitamin D is implicated in the pathogenesis of ATDs. The anti-inflammatory and immunomodulatory actions of vitamin D have been shown in animal studies. A positive therapeutic outcome associated with manageable dosages and probable side effects is still uncertain in human autoimmune disorders, and additional clinical research are required to clarify whether vitamin D therapy really works. Vitamin D insufficiency has been linked to musculoskeletal health and probably also to cardiovascular and immune system health, thus it is crucial to identify and treat this deficit (Kamen and Aranow 2008).

1.2 Role of Vitamin D in the Immune System

A natural immunomodulator and regulator of a variety of immune-mediated processes, vitamin D is widely accessible and inexpensive. Vitamin D's anti-inflammatory and immunomodulatory properties may be to blame for this capacity to diminish autoimmune disease. According to recent research, vitamin D insufficiency has long been associated to the development of autoimmune illnesses. Type 1 diabetes, rheumatologic illnesses like systemic lupus, rheumatoid arthritis, multiple sclerosis, Crohn's disease, and ulcerative colitis, as well as other autoimmune disorders, are all associated with an increased risk of developing autoimmune thyroid disease, such as Graves' and Hashimoto's disease (Pludowski *et al.* 2013).

It has been shown that 1,25(OH)2D3 has an inhibitory impact on the proliferation and production of T cell proteins and IL-2 and IFN- mRNA, in vitro investigations show. Vitamin D has also been proven to improve the activity of regulatory T cells, according to some research (Treg) (Aranow 2011).

In the immune response, dendritic cells produced from bone marrow act as antigen-presenting cells. For example, T lymphocytes and dendritic cells (DCs) produce IL-12 during antigenic maturation, which then displays antigen-processed antigen on their surface in association with an MHC class II receptor on their surface. For the growth of dendritic cells, vitamin D or its equivalents have been demonstrated to stimulate interleukin-10 and suppress costimulatory molecules, as well as to prevent pro-

inflammatory interleukin IL-12 production. Each tolerant dendritic cell promotes anti-inflammatory responses and the formation of regulatory T cells. To our knowledge, these two enzymes have never been discovered in dendritic cells or macrophages before. 1,25(OH)2D3 generated at this site may have hormone-regulating characteristics, according to researchers. T cells are regulated by vitamin D, which reduces cytokine production and activation and promotes interferon production. Active vitamin D limits the proliferation of Th1 cells while increasing the number of Th2 cells, allowing for direct control of T lymphocyte activity. During activation, latent T cells express low levels of VDR, which grow to five times larger concentrations when they are inactive. It has been hypothesised that 1,25(OH)2D3 can diminish autoimmunity in both animals and humans by increasing the number of regulatory T cells in both *in vitro* and *in vivo.*

An increase in the incidence of autoimmune thyroid illnesses has been linked to VDR gene variants, according to recent research. Numerous polymorphisms in the VDR gene, such as those affecting its exon 2 and intron 8 and those affecting its exon 9 have an effect on where the VDR gene loci are located (Jeffery *et al.* 2009).

The vitamin D binding protein transports vitamin D to the systemic circulation in order for the active form of vitamin D to enter cells (DBP). Serum 1,25(OH)2D3 and DBP levels are directly linked. It's been reported that the DBP gene has many polymorphisms, including an intron 8 (TAAA) repeat, an exon 11 (TG), and an exon 420 (CG) alteration. Vitamin D binding to DBP is affected by polymorphisms in these genes, which are linked to the occurrence of ATDs (Fronczek *et al.* 2021).

The development of autoimmunity is strongly linked to environmental and genetic variables. Vitamin D's involvement as an immunomodulator in the development of autoimmunity is becoming more prominent in this setting. Vitamin D insufficiency is most closely linked to a decrease in sun exposure time among environmental variables (Kurylowicz *et al.* 2006).

1.3 Association Between Vitamin D Serum Levels and Inflammatory Markers in Patients on Hemodialysis

In the general population, vitamin D deficiency is frequent; study reveals that 76% to 92% of those with chronic renal illness have it. 1 Hemodialysis patients with low vitamin D levels are more likely to have low bone mineral density, hyperparathyroidism, and rapid bone turnover. Two and three studies have shown that vitamin D levels under 30 ng/mL increase muscle strength. Studies have revealed, for example, that a rise in the risk of falling has been connected to a decrease in 25-OH-D levels. 2 4 The risk of metabolic syndrome, obesity, and cognitive impairment in HD patients may be increased if vitamin D levels are low (Omar *et al.* 2018).

People with CKD, particularly those on HD and peritoneal dialysis, had lower levels of 25-OH-D and all-cause mortality and cardiovascular mortality, according to various research published in the last few years. Interleukin-6, tumor necrosis factor alpha, procalcitonin, albumin, ferritin, and cholesterol may all be used to evaluate inflammation in HD patients, as can C-reactive protein (CRP), usCRP (ultrasensitive CRP), and usCRP. Low cost and wide availability make hsCRP quantitation the gold standard for these markers. The neutrophil/lymphocyte ratio (NLI) and the platelet/lymphocyte ratio (LPI) have been advocated by researchers for a range of illnesses, including HD, as patient proxies for inflammation. It's still unclear which how vitamin D levels and inflammatory biomarkers are linked. Increasing vitamin D levels may decrease inflammation, according to some research, but inflammation itself may lower vitamin D levels. HD patients often have low 25-OH-D levels, although the link between vitamin D and inflammation in HD patients has received very little research attention.

Uremic syndrome: The uremic syndrome represents a panoply of metabolic effects and symptoms caused by uremia, itself presumably defined by the retention of organic waste normally excreted by the kidneys. The clinical manifestations of uremia are diverse and affect the quality of life, neurological, cellular and physical functions of ESRD patients. These include insulin resistance, fatigue, sleep disorders, peripheral neuropathies, loss

of appetite, nausea, cramps, etc, (Kim 2017). High blood levels of creatinine and urea are also present. A replacement treatment (kidney transplant, Hemodialysis, peritoneal dialysis) must be initiated to allow the survival of the individual. More than 80 uremic substances have been identified, however the toxicity and their relationship in the so-called uremic clinical manifestations would be difficult to quantify. Some uremic toxins, due to their size or their ability to bind to proteins (e.g. albumin), are only slightly removed from the body during dialysis.

1.4 Hemodialysis

According to the (Foley and Collins 2013), almost 93% of CKD patients requiring dialysis treatments were on Hemodialysis (HD), of which approximately 45% of them were aged 65 and over. Even though survival rates have increased in recent decades, a 60-year-old patient commencing hemodialysis should expect to live for about 4 to 5 years at most. Cardiovascular disease, especially in diabetics, accounts for more than half of HD fatalities. This high mortality results from the coexistence of various comorbidities, age, low hemoglobin levels, malnutrition, inflammation and disorders of bone mineral metabolism.

1.5 General Principles of Hemodialysis

Hemodialysis removes solutes from the blood (potassium, urea, small uremic toxins, etc.) as well as excess fluid mainly by diffusion and convection (ultrafiltration) through a semi-permeable synthetic membrane. The action forces of diffusion and ultrafiltration are respectively the transmembrane concentration gradient and the transmembrane hydrostatic pressure. The semi-permeability of the membrane refers to its ability to diffuse small solutes towards the dialysate and to "block" the passage of large molecules, including red blood cells and albumin, to return them to the bloodstream. Dialysate is a liquid whose concentration of its components (sodium, potassium, chloride, bicarbonate, acetate, calcium, magnesium, dextrose, inorganic acids) is optimized to limit losses and control the acidity of the blood of HD patients. Ultrafiltration aims to reduce fluid volume until a physiological extracellular volume, or

dry weight, is reached. The optimal weight and extracellular volume are difficult to identify clinically and must be regularly redetermined based on the patient's symptoms. Hypovolemia is associated with hypotension (inter and intradialytic) and severe symptoms of ischemia, while hypervolemia is associated with hypertension, cardiovascular events and pulmonary or peripheral oedema. Interdialytic volume gains, hypervolemia and hypovolemia are thus associated with greater morbidity and mortality. HD treatments are usually repeated three times a week, involving a two to three day retention of solutes, toxins and fluid. The rate of interdialytic accumulation differs for each patient depending on their metabolic and nutritional status as well as their adherence to numerous dietary restrictions.

1.6 Objectives

- Patients with hypothyroidism who visit the Hemodialysis Center at Baghdad Teaching Hospital will be studied for the incidence of vitamin D deficiency and the association between vitamin D and thyroid hormone levels throughout the 2019-2020 period.
- To assess serum concentrations of vitamin D (25OHD) and the prevalence of vitamin D deficiency in patients with hypothyroidism compared to subjects without thyroid disease in a control group.
- Relate serum 25OHD concentrations to thyroid hormone status and demographic characteristics in patients with hypothyroidism and in control subjects.
- In individuals with thyroiditis, correlate blood 25OHD concentrations with serum indicators of thyroid autoimmunity, AcTPO, AcTG, and TRAb.
- Relate ultrasound estimated thyroid volume in patients with hypothyroidism to demographic parameters and characteristics of thyroid hormone status, serum 25OHD concentrations.
- To determine the incidence of Hypothyroidism in patients with Chronic Hemodialysis in the Baghdad teaching hospital.
- Identify the sex and age with the highest incidence of hypothyroidism.

- Know the risk factors additional to Renal Insufficiency and Chronic Hemodialysis for the development of Hypothyroidism.
- To evaluate the changes in renal function of patients with chronic kidney disease and subclinical hypothyroidism.

2. LITERATURE REVIEW

Based on the guiding question, "What is the relationship established in the literature between vitamin D supplementation in individuals with chronic kidney disease and thyroid dysfunction?" In accordance with (Bellucci Júnior and Matsuda 2012) proposal, this is an integrated review (Rydzewska *et al.* 2018). The bibliographic survey was carried out in February 2022 in the databases Pubmed, Lilacs, SciELO, and MedLine, among others. Research that is publicly available online, in full, in the form of an article, and that was first published at least 20 years ago is eligible for consideration. Excluded from consideration are articles that are not related to the specified topic as well as studies of methodological design that do not define the desired purpose.

All-cause mortality and cardiovascular mortality in patients with CKD have been shown to be strongly linked to decreased 25-OH-D levels, both in those newly diagnosed and in those already unwell. Dialysis and hypothyroidism (HD), when inflammation is a major factor. In HD patients, 7-9 C-reactive protein (CRP), ultrasensitive CRP (usCRP), interleukin-6, tumor necrosis factor alpha, procalcitonin, albumin, ferritin, and cholesterol may all be used to measure inflammation. hsCRP quantification is the gold standard since it is so cheap and readily accessible. The neutrophil/lymphocyte ratio (NLI) and the platelet/lymphocyte ratio (LPI) have been advocated by researchers for a range of disorders, including HD. Research on the relationship between vitamin D status and inflammatory indicators is currently ongoing. Inflammation has been demonstrated to diminish vitamin D levels, but it has also been shown to increase vitamin D levels. HD patients often have low 25-OH-D levels, despite the fact that there has been minimal investigation into the relationship between vitamin D and inflammation in HD patients. The prevalence of vitamin D deficiency in HD patients, as well as the link between 25-OH-D levels and inflammatory indicators, were thus critical research goals in this study.

(Dekker *et al.* 2017) reported, after studying the survival of Hemodialysis patients, that survivors ingested an average of 27.4 cal/kg/day, while non-survivors ingested an average of 23.5 cal/kg/day. This same study revealed that the amount of protein made a

difference too. Survivors ingested an average of 1.01 g/kg/day, while non-survivors ingested 0.92 g/kg/day2. Recent research also indicates an increase in the survival of individuals with obesity. (Elbaz-Greener *et al.* 2021) observed in their study that individuals with a BMI equal to or greater than 27.5 had lower mortality and shorter hospitalization time. This demonstrates that, although the opposite of the epidemiological curve may exist for the healthy population in general, the protective effect of obese chronic renal patients is being taken into account and the importance of a specialized diet, whether in case of malnourished or obese.

According to (Levey *et al.* 2017) There is a worldwide epidemic of chronic kidney disease (CKD). Chronic renal failure is the most severe symptom of kidney disease and requires renal replacement treatment such as dialysis or a kidney transplant (Elrefaey *et al.*2018). There is evidence that early treatment of kidney disease can prevent or delay progression to more serious stages, its complications, as well as reduce the risk associated with cardiovascular disease (CVD). Some authors state that the abnormalities that occur in patients with CKD and thyrotropin (TSH), in addition to representing a risk factor for CVD, could be involved in the progression of kidney disease.7, 9 Other publications deal with specific clinical cases in which the patient has CKD and TSH and an improvement in kidney function is shown after timely treatment with levothyroxine. 3, 4, 10 However, there are currently no recommendations on the treatment of TSH in patients with CKD. The pathophysiogenic mechanism attributed to the low glomerular filtration rate associated with TSH is due to the occurrence of a systemic or local hemodynamic compromise and the action of thyroid hormones at the level of the renal tubules. Despite the above, few studies have evaluated the relationship between mild TSH elevations in patients with kidney disease and the possibility of treating them with thyroid hormone replacement.

Other publications (Kumari *et al.* 2021) deal with specific clinical cases, in which the patient has CKD and TSH and an improvement in renal function is shown after timely treatment with levothyroxine. Likewise, in another study carried out in Argentina, the pre- and post-treatment impact with levothyroxine is observed, expressed as an improvement in the mean estimated glomerular filtration rate.

(El Ters *et al.* 2014) Patients with renal illness who have modest TSH rises may benefit from thyroid hormone replacement therapy, according to a number of studies. Even if the cause of renal failure cannot be determined, it is advised that thyroid function be studied as a probable cause of reduced renal function, whether asymptomatic or evident. Free thyroxine (FT4) may be aberrant in the early stages of thyroid dysfunction because TSH reacts exponentially to changes in FT4 that are still within the reference range of the population.

In times of unstable thyroid function, TSH readings might be diagnostically deceptive, such as when adjusting levothyroxine dose or beginning therapy for hyperthyroidism or hypothyroidism. A rebalancing of the pituitary TSH in accordance with the new condition of thyroid hormones takes six to twelve weeks. After thyroiditis, such as postpartum thyroiditis, these periods of unstable thyroid function may occur, in which the TSH and FT4 levels are inconsistent (Tomasello 2008).

At the time of diagnosis, it is necessary to differentiate CAH from other situations that also present with elevated TSH levels, not secondary to thyroid hormone deficiency (recent adjustment in the dose of levothyroxine, or in those who do not take the medication correctly, recovery phase of severe non-thyroid disease, untreated Primary Adrenal Insufficiency, injections of recombinant TSH and heterophile antibodies) (Haussler *et al.* 2012).

For this reason, it is crucial to keep in mind that TSH may be affected by non-thyroidal pituitary factors (glucocorticoids, Somatostatin, Dopamine, etc.) that might modify the TSH/FT4 ratio in the presence of normal FT4 levels (Villafuerte-Ledesma *et al.* 2021).

The pathophysiogenic mechanism attributed to the low glomerular filtration rate associated with TSH is due to the occurrence of a systemic or local hemodynamic compromise and the action of thyroid hormones at the level of the renal tubules (Kara and Soylu 2019).

(Melamed *et al.* 2006) aimed to analyze changes in phosphorus, calcium and PTH metabolism, seen for the first time at the Uremia outpatient clinic of the Nephrology Service. Of all the patients seen, 147 patients showed chronic renal failure, and patients with more severe CKD had low levels of calcium, increased levels of phosphorus and calcium x phosphate product 10. This study confirmed what is reported in the literature. Vitamin D influences bone metabolism and intestinal calcium absorption. In CKD, it inhibits PTH production, whereas low phosphorus levels seem to inhibit PTH secretion. On the other hand, PTH acts by stimulating the removal of calcium from the bones into the blood. Thyroid hormone levels are significantly associated with those of angiotensinogen, angiotensin II (AT II), and aldosterone in the blood. It is the renin-angiotensin-aldosterone system (RAAS) that is important for controlling the renal and cardiovascular changes that occur in people with thyroid disease. Thyroid hormones have the potential to regulate the RAAS despite the fact that renin production is mostly located in the kidney. Presodium shortage and water reabsorption in proximal tubule epithelial cells are thought to cause preglomerular vasoconstriction in hypothyroid animals as an adaptive mechanism to filtrate overload. Hypothyroidism has previously been linked to a variety of glomerular vasodilators, including insulin-like growth factor 1 (IGF-1) and vascular endothelial growth factor (VEGF) (VEGF).

In CKD, the most frequent laboratory finding is low T3, due to decreased action of deiodinases due to the effect of chronic metabolic acidosis and chronic protein malnutrition that many patients present (2). The most common thyroid disorder is TSH (7-18%). By decreasing the excretion of inorganic iodine, its excess causes hypertrophic effects on the thyroid gland and its dysfunction due to the Wolff-Chaikoff effect.

In people with chronic kidney disease (CKD), the cause of thyroid abnormalities has yet to be discovered in its entirety. Triiodothyronine (T3) is synthesised locally in the kidney from thyroxine (T4) by the enzyme 5'deiodinase (D1) (Foley and Collins 2013). T3 levels in the blood and tissues are low in individuals with advanced CKD due to diminished D1 enzyme activity. In addition to malnutrition and metabolic acidosis, other factors that may contribute to poor T3 deiodination include: Inorganic iodine excretion decreases in the latter stages of chronic kidney disease, resulting in decreased

thyroid hormone production (CKD). The aberrant TSH response to thyrotropin-releasing hormone causes uremia-induced thyroid dysfunction. An alternative to this is to use many types of uremic poisons at the same time.

(Liu *et al.* 2013), Chronic kidney disease patients may have a larger proportion of 25(OH)D utilized for the formation of 1,25(OH)2D, which works through paracrine and autocrine pathways, according to a review paper on the pleiotropic effects of vitamin D on chronic kidney disease. In patients with CKD stages 3 and 4, extra-renal synthesis of 1,25(OH)2D has been demonstrated to be reliant on 25(OH)D levels more than 100-250 ng/mL. (26). CKD patients might benefit from 25(OH)D levels over 30 ng/mL, according to the VDR Expert Centers group. Since this group's metabolism and the many effects of vitamin D on the body are so distinct, further study is required to establish reference values for 25(OH)D levels in this population. These values may or may not be associated to GFR.

3. MATERIALS AND METHODS

3.1 Theoretical Definitions of Variables

In this study, two variables were considered, which are defined below:

- Subclinical hypothyroidism is defined when there are subtle changes in thyroid function without specific clinical manifestations, and it manifests itself when elevated values of TSH with normal values of thyroid hormones, having ruled out other causes of elevated TSH.
- In order to qualify as having chronic kidney disease, a patient must have had damage to the kidneys for more than three months and evidence of renal function changes, such as an eGFR (estimated glomerular filtration rate) or measured less than 60 milliliters per minute per 1.73 square meters, or the presence of kidney damage markers (abnormalities in the urinary sediment or structural abnormalities detected by means of imaging studies or biopsy documenting abnormalities).

3.2 Type of Study and Design

Prospective –Analytical, descriptive, observational study in a first stage and analytical, experimental in the last one.

Sample of the study: At Baghdad Teaching Hospital throughout the research period, there were 293 hemodialysis patients. Of them, 145 were eligible and freely accepted to participate, while the other 148 declined to participate. Between January 2021 and January 2022, 95 of them completed the one-year follow-up period.

All patients on Chronic Hemodialysis at the Baghdad Teaching Hospital, Baghdad, Iraq.

Patients of both sexes, over 18 years of age who receive treatment with Chronic Hemodialysis at the Baghdad Teaching Hospital, Baghdad, Iraq regardless of their indication.

The eligibility criteria for the study required Arabic speaking status, being of legal age (≥ 18 years old), chronically treated with Hemodialysis (≥ 3 months) and with good treatment compliance (3 times/week). Patients were excluded for the following reasons: amputation or any other condition severely limiting bipodal posture and mobility, significant cognitive impairment limiting the ability to follow instructions, medical contraindications to resistance exercise, and inability to give informed consent. Researchers from Iraq's Baghdad Teaching Hospital (BTH) received ethics committee approval for this study. The data concerning the medical history, the demographic data, the history of falls for the last year, all the pharmacological treatments, the dosage of calcidiol (Vitamin D) as well as the muscular and functional physical parameters were collected during the first meeting, i.e. the one scheduled. for the assessment of physical abilities.

Limits

- Patients with a diagnosis of Hypothyroidism
- Patients admitted by emergency to the bed-ridden services and who have already been included in the study.

Variables

- Age
- Sex
- Risk factors for Hypothyroidism
- Etiology of Chronic Renal Failure

- Thyroid Hormones
- Time on Hemodialysis
- Dialytic Efficiency

3.3 Diagnostic Criteria for Hypothyroidism and Changes in Vitamin D Concentrations

TSH levels above the guideline and free T4 levels below the reference level were regarded to have a prior diagnosis of primary hypothyroidism before starting a specific medication if they had previously been diagnosed with primary hypothyroidism. All of the patients were having levothyroxine medication at the time. Only patients with high levels of AcTPO and/or AcTG were included in the study, confirming that hypothyroidism is caused by an autoimmune process. Participants in the control group were found to be euthyroid and to have undetectable levels of AcTPO, AcTG, and TRAb.

3.4 Blood Sample Collection

A single collection of 20 mL of peripheral blood was performed from patients and individuals in the control group for laboratory measurements on the same day as the clinical evaluation. The blood was collected by puncture of the antecubital vein, and then immediately centrifuged and stored at -20°C for later measurement.

3.5 Biochemical Analysis

The plasma concentration of total calcidiol (25OHD) was obtained initially for each of the participants between the months of May and July by electrochemiluminescence (Elecsys® Total Vitamin D, Roche Diagnostics). This derivative is the best indicator of vitamin D reserves in addition to being the metabolite of vitamin D most correlated with various pathologies.

5 mL of venous blood sample was collected in a tube with a red cap serum separator gel. These were placed in a Styrofoam and later taken to a clinical analysis laboratory, where free T4 (thyroxine) and TSH (thyrostimulating hormone) were measured. Soon after collection and transport, the samples were read by chemiluminescence methods. TPOAb, TgAb, and TRAb were among the many tests we ran on the patients. We also tested for 25OH vitamin D in the blood (Garland *et al.* 2014).

To determine the serum concentrations of thyrotropin mIU/L (TSH), triiodothyronine ng/mL (T3), thyroxine µg/dL (T4) and free thyroxine (FT4) ng/dL, a Chemiluminescence assay (ACCES Autoanalyzer) was used. (Immunoassay System-Beckman). For the biochemical parameters urea g/L, creatinine mg/L, albumin g/dL and total protein g/dL, a SYNCHRON CX4-Clinical System-BECKMAN Autoanalyzer and reagents: Wiener Lab were used.

With the second version of the Daugirdas equation (2.1), we attempted to estimate Kt/v (Broers 2017)

$$Kt/V = -Ln[(C2/C1) - (0.008 \times T)] + [4 - 3.5 \times (C2/C1)] \times FU/weight \qquad (2.1)$$

Where;

C1, C2 = initial and final urea. T = time in hours.

FU = pre-post dialysis weight change in kg.The Kidney Disease Outcomes and Quality Initiative (KDOQI) will use the blood level of 25-OH-D to determine the vitamin D status of HD patients:

- Deficiency, < 20 ng/mL.
- Insufficiency, from 21 to 30 ng/mL.
- Sufficiency, > 30 ng/mL.

One group had acute vitamin D deficiency when their blood vitamin D level was less than 10 ng/mL. By dividing the total number of lymphocytes in peripheral blood, we were able to determine the amount of neutrophils and platelets in each sample. We were able to figure out how much erythropoietin our bodies need by using the erythropoietin resistance index (erythropoietin units per week/weight in kg/hemoglobin grams per 100 mL). Statistical analysis was performed using SPSS 21.0 for Windows. Descriptive statistics were used for all variables. We were able to establish quantitative variables using the median and interquartile range of asymmetric distributions. Groups differed significantly in the Mann-Whitney U and Pearson's 2 tests for categorical variables (for continuous variables without normal distribution). Analysis of correlations between variables was done using Spearman's Rho (for data that are not normally distributed). A p value less than 0.05 was considered statistically significant. Twenty to twenty ng/mL was the cut-off point for the two groups of patients to be analyzed. It was necessary to follow established protocols in order to get clinical data, such as 25-OH-D and hemocrit levels and counts of white blood cells and albumin. Ferritin and PTH levels were also measured as well as calcium and phosphorus levels. HDL and LDL levels of cholesterol and triglycerides were also measured.

3.6 Hormonal, Biochemical and Thyroid Autoimmunity Markers Serum Levels

TSH receptor TRAb was detected using an Electrochemiluminescence Immunoassay with reference values as high as 1.22 IU/L and a detection range of 0.33-40.0, 0.3% CV, and a functional detection limit of 0.9% using Elecsys Trab TSH receptors. Elecsys chemiluminescent immunometric assay was used to quantify AcTPO and AcTG with reference concentrations as low as 34 IU/mL and as high as 115 IU/mL, respectively. Between 5 and 600.0 IU/ml, the AcTPO measurement range was employed; the CV was 5%, the analytical sensitivity was 5 and the functional 34 IU/mL. Analytical sensitivity is 10 IU/mL, while functional sensitivity is 34 IU/mL for AcTG measurements between 10.0 and 4000.0 IU/mL.

The amount of total vitamin D (25OHD) was determined using an immunometric chemiluminescent assay with a reference range of 30 to 100 ng/mL and a sample

collected in dark-protected test tubes. After ten minutes of centrifugation at 3500 rpm, the sample was analyzed. Storage at -20°C of aliquots of the centrifuged serum supernatant was done for subsequent dosing. Those with values below 30 ng/mL were considered insufficient; those with concentrations below 10 ng/mL were considered deficient. Detection sensitivity ranges from 3.0 to 70.0 ng/ml, with intra-assay accuracy of 5%, an analytical detection limit of 5.0 ng/mL, and a functional detection limit of 4.01.

Parathyroid hormone (PTH) concentrations were determined by electrochemiluminescence with reference values between 15 and 65 pg/mL, and the samples were collected in an ice bath with a measurement range between 1.2 and 5000 pg/mL, intra-assay precision (CV) of 5%, analytical sensitivity 1.2 pg/mL, functional sensitivity 6.0 pg/mL and coefficient of variation 20%. Phosphorus was measured by UV photometry with reference values between 2.7 and 4.5 mg/dL for adults. Calcium was measured by colorimetry with reference values between 8.8 and 10.2 mg/dL for the age group from 21 to 50 years and between 8.4 and 9.7 mg/dL for the age group over 50 years.

3.7 Statistical Analysis

The data was presented as the mean standard deviation (X SD). Non-parametric t-test was used to compare patients with CKD on Hemodialysis (HD) vs. control and Analysis of Variance (ANOVA) for repeated samples in the studies of one year of evolution. For the comparison between the different groups, Tukey's test was used. The correlations between the different variables were evaluated by a non-parametric test (Spearman).

3.8 Ethical Approval

With the proper authorization of the professional responsible for the Include Health program, the data and medical records of this group of patients were used. In turn, the approval and support of the executive management of BTH was obtained for the

collection of samples and their processing in said hospital. Subsequently, the doctor responsible for the endocrinology service was in charge of consulting patients at the BTH, Baghdad, with the corresponding consent from this hospital (Elbaz-Greener *et al.* 2021).

4. RESULTS AND DISCUSSION

4.1 Results

A breakdown of the patients' demographic and clinical data is provided in Table 4.1.

Table 4.1 Characteristics of patients who completed physical assessments

Physical assessments completed N=145	
Weight (kg)	78.4±19.8
BMI (kg/m^2)	28.9±7.0
Years in HD	4.0 ± 4.3
Hypertension	137 (94.5)
Diabetes	72 (49.7)
25-OH D (nmol/L)	58.0 (14.0-134.0)
Medication (#)	12.3±3.8
Alphacalcidol	99 (68.3)
Alphacalcidol dose (µg/week)	1.9 ± 2.3
CNS	52 (35.9)
The results are mean ± standard deviation, Nb (%) and median (min-max) HD: Hemodialysis, CNS: drugs acting on the central nervous system (anti-manic, anti-depressant, anti-convulsant, anti-epileptic, anti-psychotic)	

Of the 145 patients who completed the initial assessment, 77% were insufficient in 25OHD (<75 nmol/L), including 11% with severe deficiency (< 25 nmol/L) (Figure 4.1). Active vitD was widely prescribed, mainly with alphacalcidol, to 68% of patients. The main etiologies of end-stage renal disease were diabetic nephropathy (n=41), glomerulonephritis (n=27), nephroangiosclerosis (n=19), polycystic disease (n=12) and others (n=46). (Table 4.2)

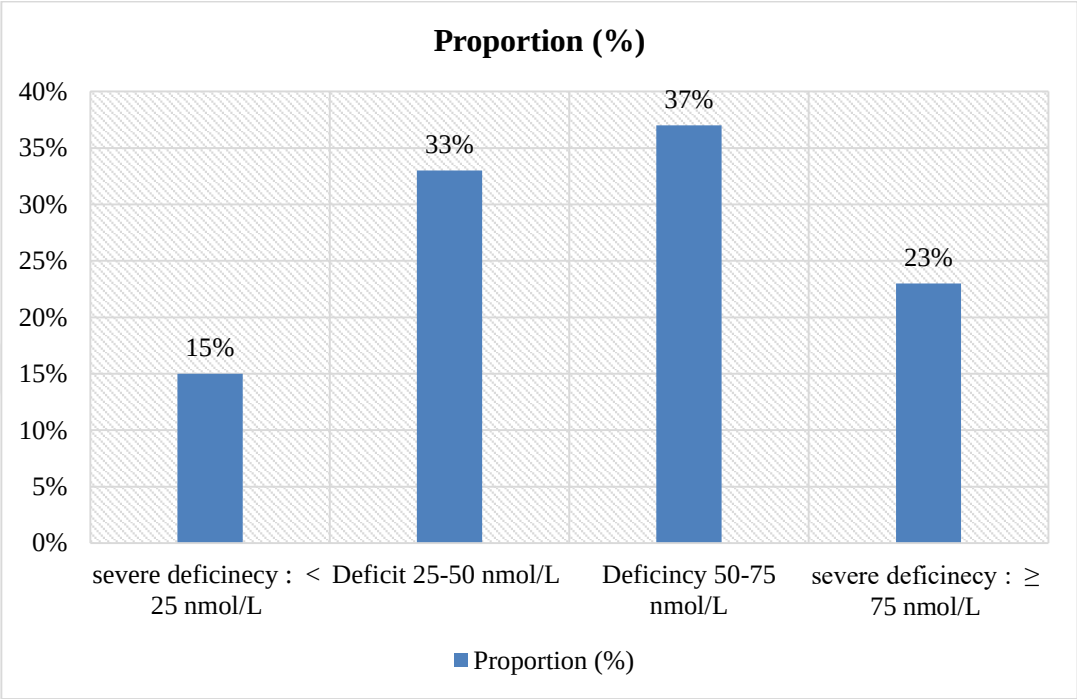

Figure 4.1 Proportion of patients according to 25OHD status, n=145

Table 4.2 Results of the operational variables

		Frequency	Percentage
Age	**18 to 29**	12	8.5%
	30 to 39	52	36.1%
	40 to 49	59	40.5%
	50 to 59	12	8.5%
	60 to 69	10	7%
	Male	71	48.9%
	Female	74	51.1%
DOQI	**Stage III (30 to 59)**	3	2.1%
	Stage IV (15 to 29)	6	4.2%
	Stage V (<15)	136	93.7%
RISK FACTOR'S	**CNS + Chronic Malnutrition**	3	2.1%
	Chronic liver disease	6	4.2%
	Chronic malnutrition	16	10.7%
	No Additional Risk Factors	120	83%
ETIOLOGY OF CHRONIC KIDNEY FAILURE	**diabetic nephropathy**	56	38.3%
	Hypertensive Nephropathy	43	29.8%
	Diabetic and Hypertensive Nephropathy	25	17%
	Lupus Nephropathy	3	2.1%
	Rhabdomyolysis	3	2.1%
TIME ON HEMODIALYSIS	**6-12 months**	16	34%
	>12 months	31	66%
EFFICIENT DIALYTIC		**Value**	**Frequency**
	Kt/V	<1.2	139(95.8%)
		>1.2	6(4.2%)
	URR	<65%	139(95.8%)
		>65%	6(4.2%)
CLASSIFICATION OF HYPOTHYROIDISM		**FREQUENCY**	**Percentage**
	SUBCLINICAL	97	67%
	PRIMARY	48	33%

A Descriptive Prospective study was carried out in which 145 patients who are in a Chronic Hemodialysis plan were included in the Baghdad Teaching Hospital, with the main objective of knowing the incidence of Hypothyroidism during the year 2019 to 2020 in the absence of local records regarding the mentioned pathology that allow us to know these data.

The largest number of those evaluated go to the outpatient Hemodialysis unit and only a minority were included in the research process as they were hospitalized in said care

center, they received sessions on average with a weekly frequency of 3 and a duration of 3 hours to each.

The group of patients is made up of an almost equivalent percentage for both sexes, however the highest rate of hypothyroidism was detected in women, this result being similar to that described in the literature and reviews made on the subject, where the trend of sex stands out. Female to present greater Dysthyroidism. Currently, 67% of hypothyroidism cases were detected in this group.

It was determined that the age range most likely to suffer from hypothyroidism is between 40 and 49 years, it is important to note that despite being this same group the most frequent, its incidence is higher compared to other age ranges, being 67%.

We discovered that 94% of long-term hemodialysis patients are in Stage V of the KDOQI classification for creatinine clearance, which is more frequent among patients with chronic kidney disease and the need for renal replacement therapy.

Of the investigated risk factors, Alcoholic Liver Disease and Adult Protein Calorie Malnutrition were documented as conditions that, added to chronic nephropathy and Hemodialysis, increase the risk of hypothyroidism. It is noteworthy that 83% had no identified associated factors (El Ters *et al.* 2014).

The main causes of chronic kidney disease detected are those already known in the literature reviews; Diabetic, Hypertensive Nephropathy and the combination of both, 66% have been on Hemodialysis for more than 1 year and 96% do not have adequate dialytic coefficients.

With respect to the patients in whom the problem under study was found, it is mentioned that; the incidence of hypothyroidism is 13%, of which 67% are cases of subclinical hypothyroidism and the remaining 33% correspond to primary hypothyroidism.

All of them had TSH levels below 10 mIU/L with which the exogenous supply of thyroid hormone is not indicated, so the therapeutic plan undertaken was conservative, in addition to timely monitoring of the functional evolution of the thyroid and other biochemical parameters, according to the recommendations described for these patients.

The incidence data obtained are similar to those obtained in the (Lee *et al.* 2018) study (Lo *et al.* 2005) and the trend towards a greater presentation of Subclinical Hypothyroidism compared to Primary Hypothyroidism is also coincident.

According to the incidence of hypothyroidism in the population studied, it is important to have a high index of suspicion, mainly due to its masking with symptoms attributed to chronic kidney disease, and comprehensively approach the patient to thereby improve their quality and life expectancy. The importance of monitoring the thyroid function of these patients for early diagnosis and timely follow-up.

During the study, the most important limitation was the reduced population that we were able to evaluate, given the scarce incorporation of new patients to the program, therefore the most important suggestions after what was done are; the continuity of the research process to improve the validity of this research, timely monitoring of hypothyroidism in whom this diagnosis was established and making improvements in all those factors that contribute to favorably modify dialytic coefficients, which, as is known, have an important impact in relation to morbidity and mortality rates.

4.2 Thyroid Hormone Levels in Chronic Kidney Patients on Hemodialysis

Evaluation of the Hypo Thyroid Disease (HHT) axis in CKD patients on Hemodialysis

TSH, T3, T4, and FT4 were all measured before the dialyzer was connected to each patient on the day of sample collection, and the findings were compared to those obtained in the control group (CG). Figure 4.2 shows the distribution of 0.52-3.36 vs. 0.59-2.93 mIU/L).

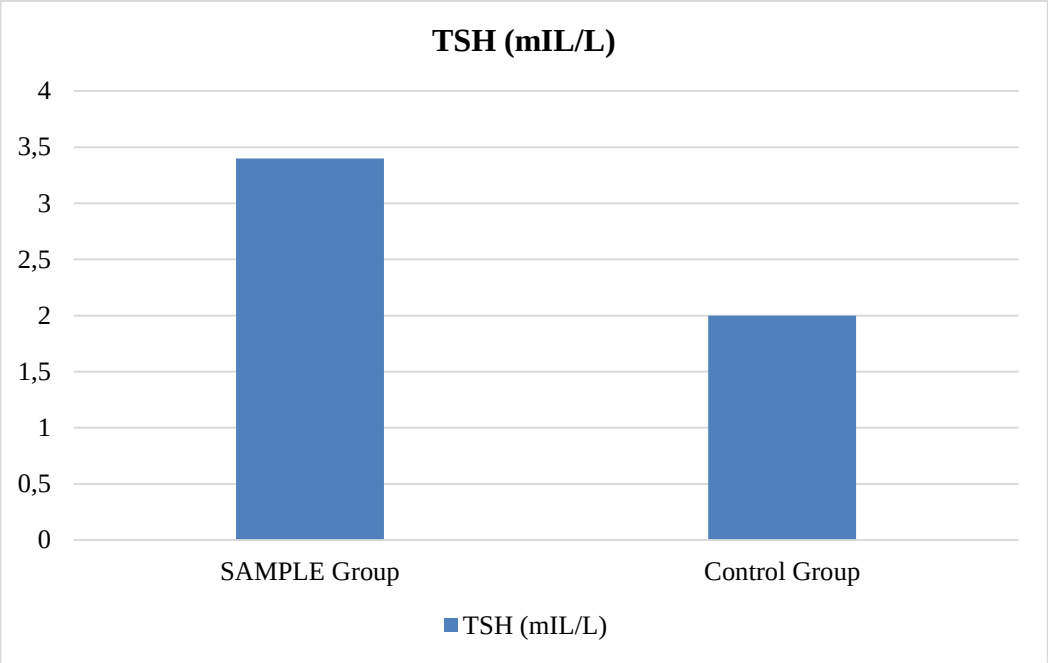

Figure 4.2 Serum TSH values in CKD patients on Hemodialysis (HD) (pre-dialysis) n=145 vs. control group (CG) n=25

TSH levels in individuals with chronic kidney disease (CKD) were found to be significantly higher than those in the control group (X ± SD; HD: 3.36 ± 0.036 vs. CG: 2.01 ± 0.025 mIU/L, $p<0.05$), without However, within the reference values (0.40 - 4.00 mIU/L). Figure 4.3 shows the results obtained for total T3 in Hemodialysis patients (range: 0.50 - 1.10 ng/mL) with very little overlap with the control group (range: 0.98 - 1.54 ng/mL). On average, CKD patients showed a 42% decrease in T3 concentration (HD: 0.74 ± 0.019 vs. CG: 1.06 ± 0.026 ng/mL, $p<0.05$).

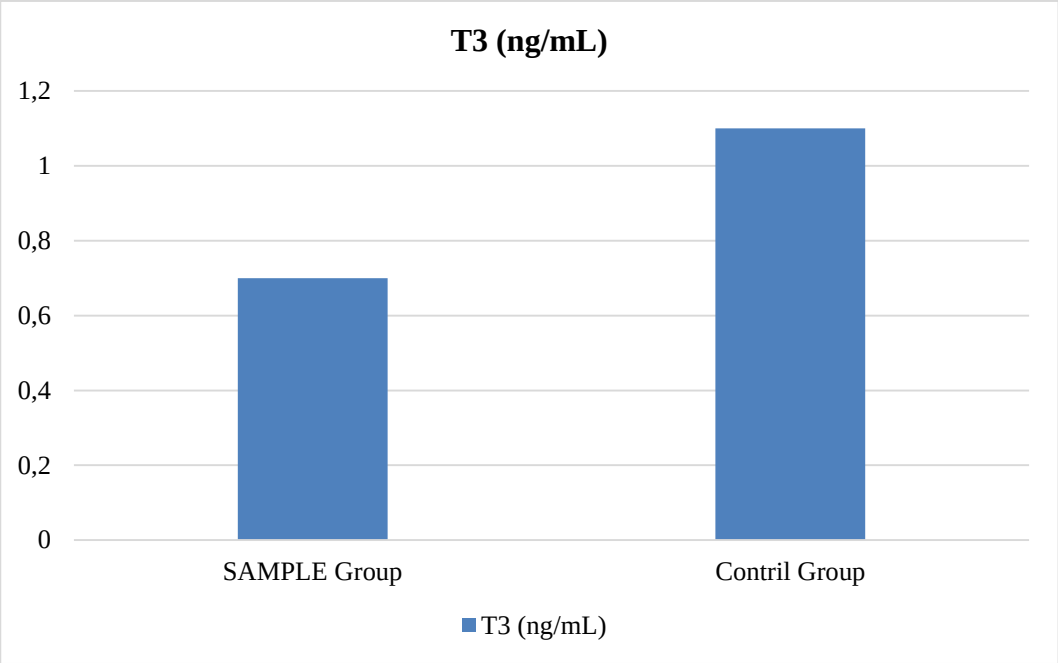

Figure 4.3 Serum T3 values in patients with CKD on Hemodialysis (HD) (pre-dialysis) n=145 vs. control group (CG) n=25

Likewise, a 19% decrease in the mean total T4 concentration was observed in Hemodialysis patients compared to that observed in control individuals (HD: 9.014 ± 0.09 vs. CG: 7.02 ± 0.033 µg/dL, $p<0.05$). The range of values for total T4 observed in IRC (5.00 - 14.00 µg/dL) was somewhat wider than in controls (7.90 - 13.10 µg/dL) (Figure 4.4).

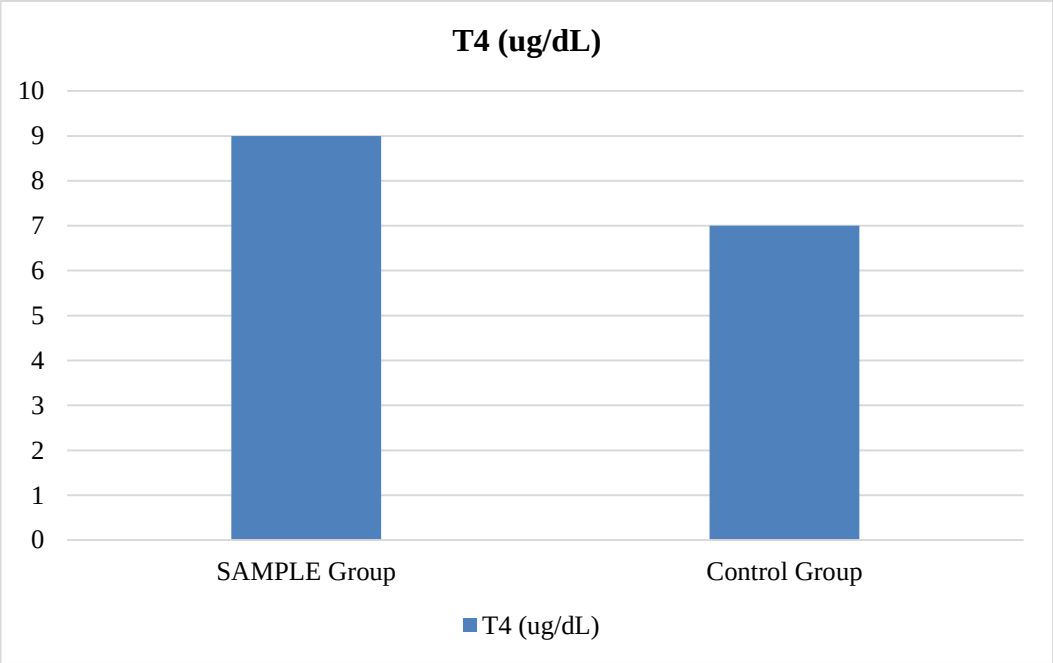

Figure 4.4 Serum T4 values in patients with CKD on Hemodialysis (HD) (pre-dialysis) n=145 vs. control group (CG) n=25

Similarly, Figure 4.5 shows a decrease in mean FT4 values in CRI compared to CG (X ± SD; HD: 0.87 ± 0.035 vs. CG: 0.72 ± 0.027 ng/dL, $p<0.05$). In this case, the individual values show a range shift towards lower values (0.51 - 1.4 vs. 0.9 - 1.5 ng/dL).

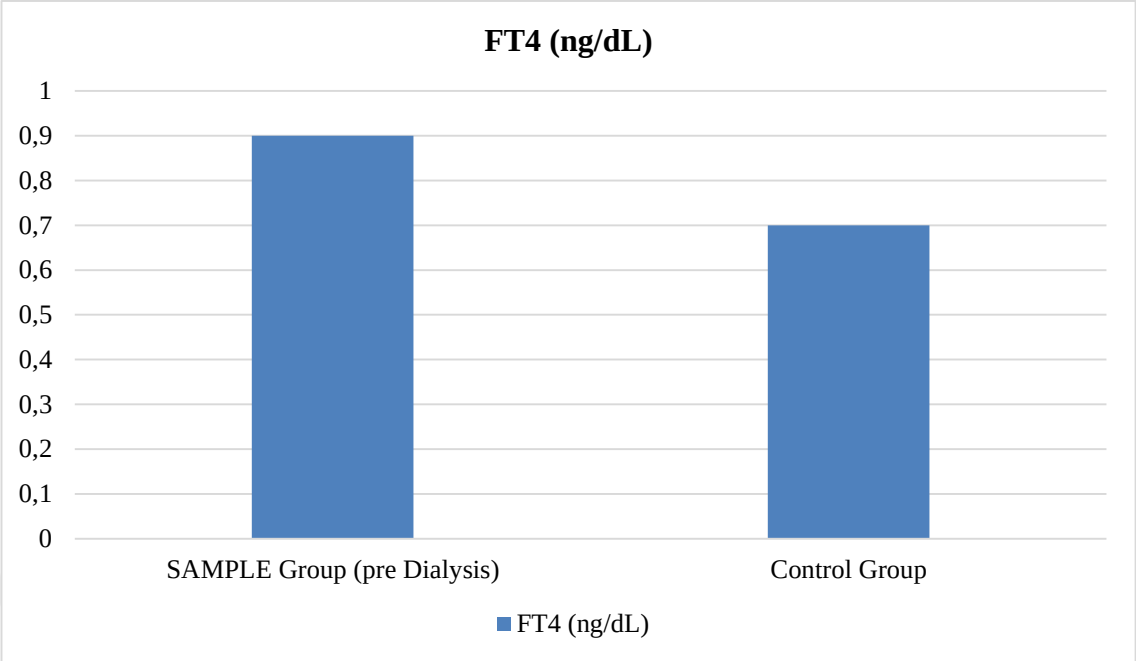

Figure 4.5 Serum FT4 values in patients with CKD on Hemodialysis (HD) (pre-dialysis) n=145 vs. control (CG) n=25

4.3 Effect of HD Therapy on Thyroid Axis Hormones

In order to analyze the changes experienced by the hormones of the hypothyroid axis during Hemodialysis therapy, in these patients, samples were taken immediately after its disconnection (post-dialysis sample). As our results show, it is clear that the Hemodialysis process affects thyroid hormones, both total and free, and not TSH. No significant changes were observed in mean TSH levels (Pre-dialysis: 3.36 ± 0.036 vs. Post-dialysis: 3.02 ± 0.26 mIU/L). The increase in T3 was statistically significant in post-dialysis determinations compared to pre-dialysis (Post-dialysis: 0.92± 0.032 vs. Pre-dialysis: 0.74 ± 0.019 ng/mL; $p<0.001$). The ranges found were 0.50 - 1.10 and 0.60 - 1.40 ng/mL, respectively. As well,

Similarly, the increase observed in post-dialysis FT4 is shown with respect to predialysis samples (Post-dialysis: 9.44 ± 0.05 vs. Pre-dialysis: 9.01 ± 0.03 ng/dL;

p<0.001), and in this case with ranges 0.52 - 1.40 ng/dL and 0.59 - 1.85 ng/dL respectively. Figure 4.6 shows the hormone levels in CKD before and after dialysis compared to the CG group.

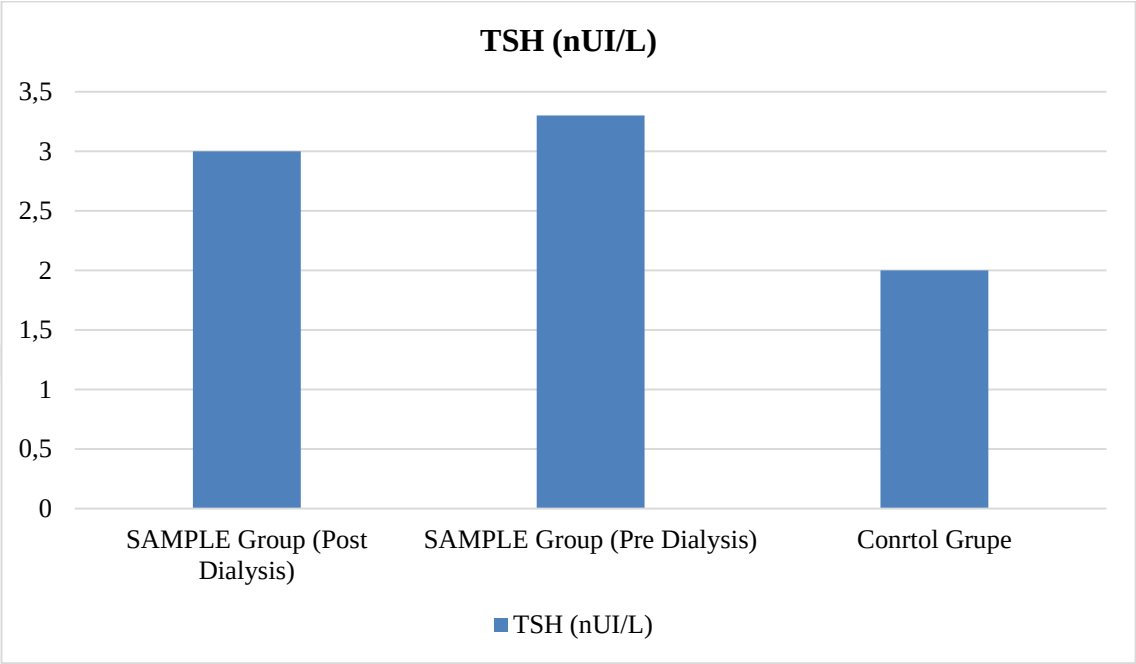

Figure 4.6 Serum TSH levels in CKD. Pre-dialysis: before and Post-dialysis: after Hemodialysis treatment (n=145); CG: control (n=25), ns: not significant; Pre-dialysis vs CG, p<0.05; Pre-dialysis vs Post-dialysis and Post-dialysis vs CG, ns; Tukey's test. The bars express the mean ± SD

TSH Post-dialysis and CG mean readings are not significantly different after the treatment (Figure 4.6). Patients with CKD had lower post-dialysis T3 levels than those in the control group (Figure 4.7, Figure 4.8 and Figure 4.9). The average post-dialysis FT4 value is likewise somewhat higher than the control after replacement treatment (Figures 2D).

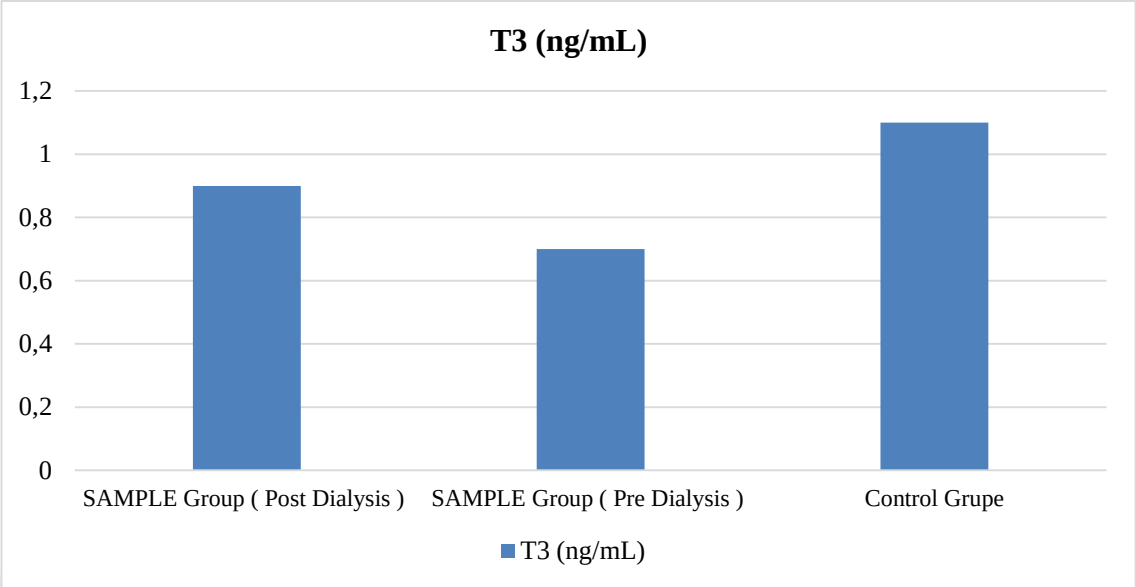

Figure 4.7 Serum T3 levels in CKD. Pre-dialysis. Post-dialysis and CG: Fig. 2A. Pre-dialysis and Post-dialysis vs CG and Pre-dialysis vs Post-dialysis, p<0.001; Tukey's test. The bars express the mean ± SD.

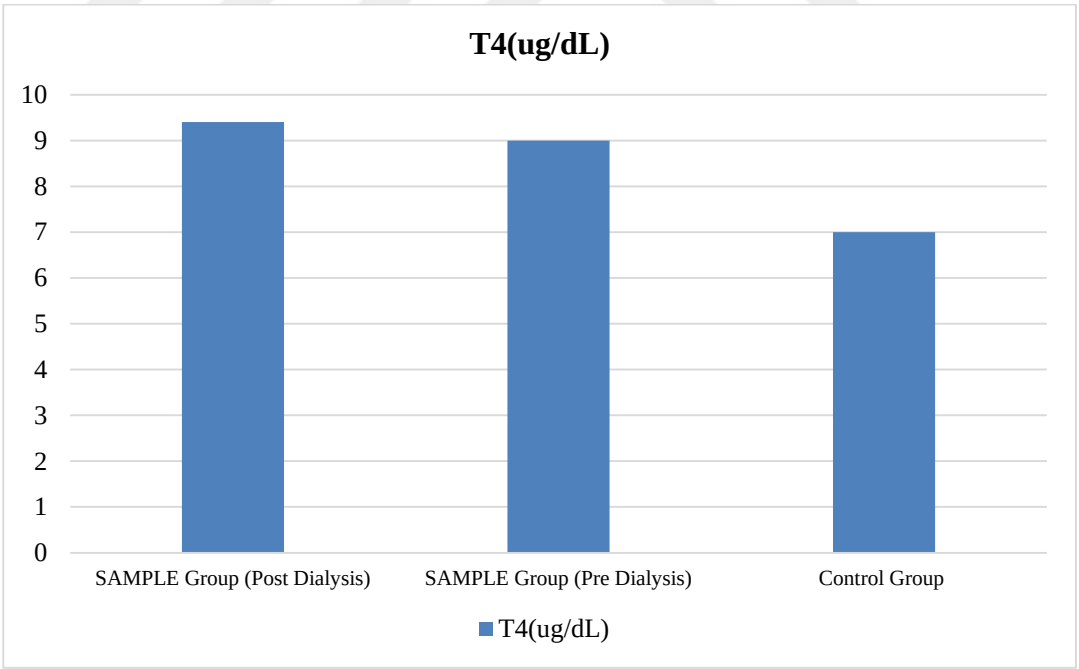

Figure 4.8 Serum T4 levels in CKD. Pre-dialysis Post-dialysis and CG: Idem Fig. 2A . ns: not significant: Pre-dialysis vs CG, p<0.001; Pre-dialysis vs Post-dialysis, P<0.01; Post-dialysis vs CG, ns; Tukey's test. The bars express the mean ± SD

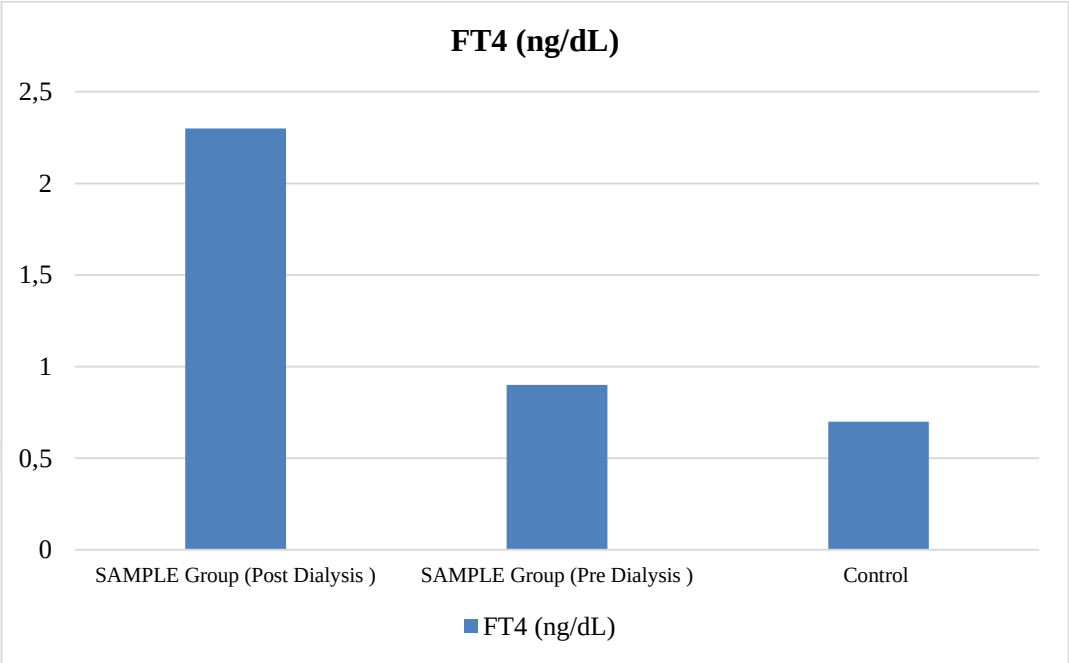

Figure 4.9 Serum FT4 levels in CKD. Pre-dialysis Post-dialysis and CG: Idem Fig. 2A. ns: not significant: Pre-dialysis vs CG, $p<0.001$; Pre-dialysis vs Post-dialysis and Post-dialysis vs CG, ns; Tukey's test. The bars express the mean ± SD

4.4 Analysis of the HTD Axis During One Year of Disease Evolution

In order to evaluate possible endocrine changes over time, a follow-up was carried out during one year of renal replacement therapy. Twelve samples were taken from each patient, six pre-dialysis and six post-dialysis, in different time intervals. An ANOVA was applied for repeated samples, it was found that the values of TSH and thyroid hormones T3, T4 and FT4 do not change significantly in these patients throughout the year of study. We only observed a downward trend in the average levels of triiodothyronine (Table 4.3 and Table 4.4)

Table 4.3 Average levels of TSH and thyroid hormones in Hemodialysis chronic renal patients predialysis during one year of disease evolution. Values are expressed as mean ± SD

Month	TSH (mIU/ L)	T3 (ng/ mL)	T4 (µg/ dL)	FT4 (ng/ dL)
January	3.31 ± 0.136	0.81 ± 0.013	9.09 ± 0.110	0.86 ± 0.015
March	3.38 ± 0.130	0.81 ± 0.019	8.98 ± 0.111	0.86 ± 0.015
May	3.39 ± 0.133	0.80 ± 0.011	8.91 ± 0.115	0.84 ± 0.021
July	3.33 ± 0.117	0.69 ± 0.018	9.08 ± 0.119	0.88 ± 0.017
September	3.36 ± 0.133	0.68 ± 0.017	8.91 ± 0.115	0.88 ± 0.020
November	3.37 ± 0.119	0.66 ± 0.011	9.09 ± 0.193	0.87 ± 0.020

Table 4.4 Mean levels of TSH and thyroid hormones in post-dialysis Hemodialysis chronic kidney patients during one year of disease evolution. Values are expressed as mean ± SD.

Month	TSH (mIU/ L)	T3 (ng/ mL)	T4 (µg/ dL)	FT4 (ng/ dL)
January	2.97± 0.247	0.99 ± 0.024	9.29 ± 0.244	2.20 ± 0.035
March	3.03 ± 0.244	0.94 ± 0.046	9.94 ± 0.234	2.29 ± 0.037
May	3.04 ± 0.244	0.92 ± 0.044	9.94 ± 0.249	2.27 ± 0.043
July	3.03 ± 0.242	0.90 ± 0.042	9.24 ± 0.239	2.24 ± 0.034
September	3.06 ± 0.243	0.89 ± 0.044	9.02 ± 0.244	2.22 ± 0.035
November	2.93 ± 0.243	0.89 ± 0.044	9.20 ± 0.242	2.27 ± 0.037

4.5 Evaluation of the Thyroid Axis in CKD Patients with Different Times on HD

When evaluating whether the time spent under Hemodialysis therapy influenced the values of TSH, T3, T4 and FT4, the patients were grouped into G1: with a time less than 2 years, G2: between 2 and 5 years and G3: over 5 years in replacement therapy (with the same frequency). The number of patients, the distribution and the average values of the ages (X ± SD; G1: 61 ± 3.41; G2: 59.27 ± 2.67; G3: 54.9 ± 2.69 years) were similar in the three groups, since it is appropriate to analyze the effect of treatment time independently.

Results shows the mean TSH levels in each group studied and it is observed that the group of patients with the longest stay on Hemodialysis (G3) have a significantly higher mean TSH value (G3: 3.12 ± 0.231 vs. G2: 3.02 ± 0.271 and G1: 1.69 ± 0.512 mIU/L, $p<0.05$). Results shows the mean values of serum T3 in the patients of each particular

group and shows significant changes in groups G2 and G3 with respect to G1 (G2: 0.56 ± 0.042 and G3: 0.26 ± 0.012 vs. G1: 0.27 ±0.051, ng/mL, $p<0.05$). However, no statistically significant variations were observed between the different groups in the levels of total T4 (G1: 8.02 ± 0.161; G2: 8.025 ± 0.17; G3: 7.61 ± 0.13 μg/dL) and free T4 (G1: 0.81 ± 0.03; G2: 0.89 ± 0.05; G3: 0.91 ± 0.05 ng/dL) (Tukey's test). However, there is a tendency for both hormones to decrease as the time spent on replacement therapy increases.

4.6 Evaluation of Biochemical Parameters in Kidney Patients on HD

In order to evaluate kidney patients on Hemodialysis, serum biochemical parameters sensitive to the patient's critical condition were studied: pre- and post-dialysis urea, creatinine, albumin, and total protein. The CKD patients studied have average predialysis urea values approximately 3.5 times higher than control individuals (Pre-dialysis: 1.12 ± 0.05 vs CG: 0.31 ± 0.013 g/L; $p< 0.001$). In addition, it was found that the average value of urea after the Hemodialysis procedure is similar to the value found in the control group (Post-dialysis: 0.43 ± 0.012 vs. CG: 0.23 ± 0.011 g/L). Results obtained in Pre-dialysis and Post-dialysis kidney patients are compared with the control group. We analyzed whether, in chronic kidney patients, the time spent on Hemodialysis influenced the observed values of serum urea Pre-dialysis and Post-dialysis. The results obtained show that no statistically significant differences are observed between the different groups both before substitutive therapy (G1: 1.09 ± 0.068; G2: 1.41 ± 0.065 and G3: 1.61 ± 0.181 g/L), and after from disconnection to the dialyzer (G1: 0.43 ± 0.013; G2: 0.73 ± 0.012 and G3: 0.26 ± 0.043 g/L). However, we were able to verify that the G2 group, both pre-dialysis and post-dialysis, had slightly higher serum urea values than the other two groups. Chronic kidney patients have an average creatinine value ten times higher than control individuals (HD: 93.2 ± 3.45 vs. CG: 9.1 ± 0.10 mg/dL; $p< 0.001$). Results shows that the mean level of serum albumin in control individuals is higher (15%) than that seen in kidney patients (CG: 4.2 ± 0.03 g/dL vs. HD: 3.3 ± 0.04; $p <0.001$).

Since hypoalbuminemia has a high prognostic value for morbidity and mortality in dialysis patients, changes in its levels were analyzed at different lengths of stay under HD. Results shows a progressive decrease in mean serum albumin values in patients with longer time under therapy (G3: 2.9 ± 0.06 vs. G1: 3.69 ± 0.04 and G2: 3.3 ± 0.04 g/dL; $p < 0.05$). A 6% decrease in mean total protein values was observed in dialyzed individuals compared to healthy subjects (HD: 6.7 ± 0.04 vs. CG: 7.1 ± 0.03 g/dL; $p < 0.01$).

4.7 Correlation Studies Between Thyroid Axis Hormones and Biochemical Parameters

Based on the results obtained, correlation studies were carried out between the levels of thyroid axis hormones and parameters sensitive to the critical condition of renal patients. Indeed, we were able to verify some correlations between the hormones of the hypothyroid axis and urea or albumin in CKD patients on Hemodialysis therapy. Findings reveal that in renal patients, TSH is linked to urea directly, and that T3 is linked to urea in an anti-clockwise direction.

TSH and albumin have an inverse relationship, but T3 and albumin have a direct correlation. T3 levels were shown to be statistically associated with albumin levels in patients who had been on HD for less than two years, between two and five years, and for more than five years (G3). The hypothyroid axis hormones and the same biochemical indicators examined in the control people had no statistically significant connection in the control group.

On the other hand, in order to evaluate the effect of the HD procedure at an individual level, the changes (Δ) in the different hormones were analyzed; TSH, T3, T4 and FT4 (post dialysis level minus pre-dialysis). The Δ for each of the individuals were similar except for FT4 and a certain degree of inverse correlation was found between ΔFT4 and the urea concentration reached in these patients (Table 4.5).

Table 4.5 Serum levels of 25(OH)D in relation to sex, age and duration of Hemodialysis performed by the patients in the sample

Sex	**Serum value 25(OH)D Mean ± SD, r, rs, Median (Min – Max)**	**p**
Male	24.08 ± 11.24	0.388*
Female	20.31 ± 9.52	
Age	0.112	0.586^{r}
HD time	1,000	0.333^{rs}
* The t test for students. correlation coefficient r: Pearson's The Spearman correlation coefficient (rs) is a measure of how well two variables are related. It is 25-hydroxyvitamin D. Hemodialysis (HD)		

Table 4.5 presents the correlation calculations between serum 25(OH)D levels with sex, age and duration of Hemodialysis. However, there was no statistical significance between the variables analyzed, obtaining only a weak correlation between these levels with age and a strong positive correlation with the duration of Hemodialysis

This study showed vitamin D levels in female patients of 20.31 ± 9.52 ng/mL ng/mL versus 24.08 ± 11.24 ng/mL in men. (Nalbant *et al.* 2017) showed a negative correlation between these levels and women (β = -0.332, $p<0.0001$), as did (Ahmad *et al.* 2020), who showed severe deficiency of this hormone in females ($p = 0.009$). It is known that there is a natural deficit of it in this gender, accentuated after menopause and justified by changes in bone mineral composition, however, our study did not obtain statistical significance for this association. Regarding the comparison between age and vitamin D level, this study showed a weak correlation and also did not obtain statistical significance ($r = 0.112$, $p = 0.586$). (Table 4.6)

It has been shown that patients with renal illness who had greater levels of 25(OH)D had better outcomes if they were on hemodialysis for a longer duration (rs = 1.000), which is consistent with results from a comprehensive review and meta-analysis of prospective observational studies (35)

Table 4.6 Thyroid Patients' Pre-Hemodialysis Clinical and Laboratory Characteristics

Parameters	Sample Group (n = 145)	Control (n = 25)	
	Average Value	Average Value	P value
atrioventricular fistula	49.00	0.00	0.516
jugular catheter	91.00	0.00	0.848
femoral catheter	5.00	0.00	0.207
Diabetes	62.00	2.00	0.426
Charlson index	6.01	1.56	0.067
Hemoglobin (g/dL)	11.80	12.01	0.848
Leukocytes (×103/mm3)	6.40	9.25	0.984
Albumin (g/dL)	3.40	4.10	0.047
hsCRP (mg/dL) (ultrasensitive C-reactive protein)	0.80	1.89	0.039
INL (neutrophil/lymphocyte ratio)	2.60	0.86	0.042
IPL (platelet/lymphocyte ratio)	130.20	37.95	< 0.01
Ferritin (ng/mL)	594.40	290.00	0.984
i-PTH (pg/mL)	256.20	61.00	0.13
Calcium (mg/dL)	8.30	9.10	0.669
Phosphorus (mg/dL)	4.60	3.20	0.252
LDL-C (mg/dL)	78.20	120.00	0.552
HDL-C (mg/dL)	36.00	52.00	0.869
Triglycerides (mg/dL)	115.60	142.00	
erythropoietin (mU/mL)	76.00	17.10	0.218
Antihypertensives	56.00	NA (Not Available)	
Vitamin D analogs	61.00	NA	
EPO dose (U/week)	8510.00	NA	
Iron dose (mg/week)	32.20	NA	
ERI (erythropoietin resistance index)	9.50	NA	
TPOAb (UI/ml)	267 (132-476)	0	NR
TGAb (UI/ml)	426.9 (97-704)	0	NR
TRAb (UI/L)	0	0	NR

The research involved 145 patients in total. A sampling group and a control group were created for the patients. It was found that the median 25-OH-D concentration was 25.10 ng/mL in the group with deficit, ranging from 9.8 to 14.5, and 39.20 ng/mL in the group with normal concentrations. We found statistically significant variations in hsCRP, INL, and IPL between the two groups. Analyzing the relationship between 25-OH-D and other variables, bivariate correlation analysis was performed (Table 4.7). Kt/v ($r = 0.402$, $p = 0.034$) and INL ($r = 0.540$, $p = 0.013$) had a negative association with 25-OH-D, whereas hsCRP, INL, and IPL had a positive connection with 25-OH-D. $R = 0.002$, $p = 0.98$; there was no correlation between albumin and vitamin D levels. Neither calcium, phosphorus nor parathyroid hormone were shown to have a significant

correlation with 25-OH-D, despite the fact that the correlation was 0.189, which was statistically insignificant (p = 0.079). No correlation was detected between vitamin D levels and erythropoietin needs (r = -0.037, p = 0.694).

Table 4.7 Bivariate correlation between levels of 25-OH-vitamin D and other associated parameters (N = 145) in Sample group

Parameter	Spearman's correlation coefficient (ro)	p
Kt/v	0.402	0.035
Hemoglobin (g/dL)	0.069	0.540
Leukocytes (× 103/mm3)	0.079	0.491
Albumin (g/dL)	0.002	0.990
hsCRP (mg/dL)	−0.296	0.027
INL	−0.540	0.013
IPL	−0.460	0.022
Ferritin (ng/mL)	0.049	0.657
i-PTH (pg/mL)	−0.089	0.419
Calcium (mg/dL)	0.189	0.082
Phosphorus (mg/dL)	−0.059	0.591
LDL-C (mg/dL)	−0.039	0.675
HDL-C (mg/dL)	−0.067	0.536
Triglycerides (mg/dL)	0.084	0.403
EPO dose (U/week)	−0.037	0.708
Iron dose (mg/week)	−0.068	0.537
IRE	−0.004	0.985

In terms of thyroid autoimmune indicators, high levels of TPOAb and TgAb were observed in 96.2 percent of hypothyroid patients and in 76.5 percent of those with hyperthyroidism. Seventy-three percent of those tested exhibited elevated levels of both biomarkers. There was an increase in TRAb in 13% of those studied. All members of the control group had undetectable levels of TPOAb, TGAb, and TRAb in their blood.

Patients had an average of 2.4 patients per woman, while the control group had an average of 1.6 patients per woman. With an average thyroid volume (RV) between 6 and 15 millilitres in the hypothyroid group, goitre was found in about a third of the patients (36 percent), whereas 65 percent of those with hypothyroidism had normal or decreasing thyroid volumes. More than 60% of patients without goitre had vitamin D deficiency. Patients with goitre were 85 percent likely to experience this. When patients

with adequate amounts of vitamin D were compared to those with low levels, there was no change in volume.

Vitamin D levels, demographic characteristics, calcium metabolism parameters, thyroid hormone status, and serum indicators of thyroid autoimmunity were compared between patients with Hashimoto's thyroiditis and a control group:

Vitamin D levels were not associated with any of the following: age, BMI, parity, thyroid volume, blood calcium and phosphorus levels, parathyroid hormone (PTH), thyroid-stimulating hormone (TSH) or free T4 levels. Study No. 2 revealed no correlation between thyroid volume and parameters such as age, BMI, Calcium (Phosphorus) PTH (TRAb), TGAb, and free T4. P=0.01 for a positive correlation between thyroid volume and TPOAb; both groups had the same quantities of calcium and vitamin D circulating in their bloodstreams. There was a correlation between vitamin D and phosphorus and parity. None of the factors examined were associated with the individuals' levels of vitamin D, who acted as controls (Data not shown).

In hypothyroid patients, we used a single-variable linear regression model to predict vitamin D levels in the blood.

Vitamin D had no effect on any of the thyroid hormones measured in the univariate regression analysis: TSH, free T4, TPOAb, TGAb, TRAb, and thyroid volume (Data not shown).

The multivariate analytic model comprised gender, age, TSH, free T4, TPOAb, TGAb, TRAb, and thyroid volume. We discovered that B=0.013; IC 95%:0.019; -0.008; p0.0001 was independently predictive of free T4 levels. It has been shown that -TPOAb may independently predict thyroid volume at B=0.006, IC 95 percent =0.001, 0.01 and p=0.002 (Table 4.8).

In individuals with hypothyroidism, there was no correlation between thyroid hormone levels or serum autoantibody indicators and the prevalence of vitamin D insufficiency. Higher TPOAb serum concentrations were associated with larger thyroid volumes, suggesting an increased thyroid autoimmune inflammatory activity.

Table 4.8 Analysis of the research factors in relation to each other in the group of patients rho) for the correlations between vitamin D levels, thyroid volume, thyroid autoimmune indicators, and thyroid function measures

	TPOAb	**TGAb**	**TRAb**	**Vitamin -D**	**Thyroid Volume**
Vitamin D r	-0.088	0.174	0.145	1.000.	-0.232
p	0.525	0.805	0.209	.	0.092
Thyroid Volume r	0.319	0.06	-0.123	-0.232	1
p	0.019	0.665	0.374	0.092	.

4.8 Discussion

In pre-dialysis samples from CKD patients, the mean TSH value was considerably higher than in controls, although it remained within the reference range. In severe non-thyroid pathologies, T3 is the first hormone to decrease (5). In these patients it decreased by approximately 40%. Most of the T3 values (75%) were below the reference range. The levels of T4 and FT4 were also lower than the control group, although within the reference range. TSH levels and the marked decrease in T3 are similar to those previously observed by other authors in chronic kidney patients; although the decrease in T4 described by some groups was of lesser magnitude (Nalbant *et al.* 2017).

Because of the negative feedback from thyroid hormones on pituitary TSH release, there is an inverse logarithmic/linear connection between serum TSH and FT4 when the Hypothyroid axis is functioning normally (Lim *et al.* 2005) . Loss of rhythm and lower amplitude of the TSH pulse, diminished response to stimulation with TSH (15) and alterations in Thyrotropin glycosylation (20) have been described in critically ill patients. Although the "in vitro" bioactivity of TSH correlates with immunoreactivity in normal subjects as well as in Hemodialysis patients, the same does not occur in "in vivo" studies. The bioactivity of TSH in CKD decreases in relation to normal subjects. Apparently the lack of increase in TSH does not correspond to a decrease in stimulation by TSH (Ahmad *et al*. 2020).

In the present work we did not evaluate TSH levels, however (Duntas *et al.* 1992) show that chronic renal failure reduces its degradation and clearance. These results suggest that low levels of circulating thyroid hormones could be attributed to decreased sensitivity of thyrotropes to TSH. In contrast, (Fliers *et al.* 1997) observed a positive correlation between the expression of the messenger RNA for TRH and the levels of circulating T3, suggesting that the drop in this hormone was mainly due to an inappropriate stimulation of the thyrotrope.

On the other hand, a possible explanation for the reduction in circulating total T4 in these patients could be the presence of serum inhibitors that reduce the binding of the hormone to its transport proteins. Inhibitors of T4 binding to its carrier protein include compounds that accumulate in the circulation in uremic patients, such as 3-carboxy-4-methyl-5-propyl-2-furanopropanoic acid, indoxyl sulfate, and hippuric acid (Lee *et al.* 2015). Interleukin-1β (IL-1β) and Tumor Necrosis Factor α (TNF-α) (Terpstra *et al.* 2011) and certain commonly used drugs have also been proposed as binding inhibitors. On the contrary, an in vitro study suggests that the accumulated substances could inhibit the uptake of T4 by the tissues (Lo *et al.* 2005). The decrease in total T4 could also be due to the decrease in protein concentration due to nutritional deterioration in CKD.

The greatest discrepancy between our results and those of other groups corresponds to FT4 levels, which largely depend on the methodology used in renal patient monitoring.

Earlier studies by (Faber *et al.* 1983) described elevated FT4 levels in the presence of total T4 below the reference range, which could be linked to a highly deteriorated nutritional status or the effect of a concurrent disease that worsens the patient's general condition.

Subsequently, (Kaptein *et al.* 1988) observed normal or slightly increased FT4 values in kidney patients with normal concentrations of TBG and transthyretin and low albumin. In agreement with (Lim 2001) we also detected low levels of FT4. The finding of decreased values of T4 and FT4 in the same proportion could be due to the relatively satisfactory nutritional status of our patients (measured as the Body Mass Index and the individual's weight).

The decreased serum levels of T3 in CKD patients would be linked to a lower tissue conversion of T4 to T3. It is clear that the main source of circulating T3 comes from the conversion of peripheral T4 (≈80%). This altered step would be the fundamental cause of the decrease in T3 in the presence of a normal supply of T3 by the thyroid gland and low clearance, a consequence of kidney disease. In this regard, (Peeters *et al.* 2003) describe changes in tissue deiodinases in kidney patients as in other critical patients. Likewise, they observed that liver deiodinase D1, responsible for the conversion of T4 to T3, is down regulated and liver and skeletal muscle deiodinase D3, involved in the inactivation of T4 and T3, is induced (D3 is not expressed in these tissues) in healthy individuals). Consequently, T3 levels are markedly decreased and T4 levels to a lesser extent.

In addition to the changes observed in the Hypothyroid axis, characteristic of critically ill patients, hemodialyzed individuals exhibited significant changes in circulating hormones due to the dialysis procedure. Accumulated organic acids in IRC can exert an effect in vivoconditioning binding to albumin and carrier proteins. There is also an interference in the in vitro FT4 technique since the recognition of the epitope by the antibody is altered. To date, we do not find in the literature comparative studies between pre- and post-dialysis thyroid hormones in the same patient. In this case-control study, we did not observe changes in TSH, but we did find fluctuating values of thyroid

hormones that resulted in an increase of approximately 11% in T3, T4 and FT4 after dialyzer disconnection. These results confirm that the Hemodialysis procedure transiently elevates serum thyroid hormones in uremic patients. The case-control study was repeated under identical conditions for one year. Both the individual levels and the average values of each hormone before and after dialysis showed a very homogeneous profile in the six samples analyzed. We were only able to detect a downward trend of T3 over the course of the year. Although this change was not statistically significant, it is probably indicating an evolution of the disease, which is clearly manifested when longer periods of treatment elapse. Axis hormones were also analyzed, after grouping CKD patients according to time spent on HD. In this case, an average TSH increase of approximately 50% was observed in individuals under HD therapy for more than 5 years (G3), compared to the other two groups. Also, a decrease in T3 was observed as the time on replacement therapy increased and only a tendency for T4 and FT4 to decrease over time, since the differences between the groups were not statistically significant. The increase in TSH has been considered as a marker in the recovery phase of critical illness. Although we cannot mention this fact as an indicator of patient improvement, it probably reflects the influence of time on the adaptation of the Hypothyroid axis, which tends to normalize. Patients with a shorter stay on HD have a lower thyrotrope response to T3 and FT4 variations, trying to preserve the patient's metabolic status.

Renal function was assessed from circulating urea and creatinine levels. According to (Owen *et al.* 1993) dialysis is successful when urea levels decrease by at least 60% (post vs. pre-dialysis) in each replacement therapy. Post-dialysis urea levels in CKD patients were comparable to those in the control group, which explains the procedure's success. For patients with varied dialysis times, there was no statistically significant variation in pre- and post-dialysis urine levels. However, urea in G2 was moderately higher. It is likely that this slight increase is a consequence of the organic adaptation of the renal patient, induced by changes in dietary protein intake. There is sufficient consensus about protein intake in kidney patients. This must result from a balance between a sufficiently low-protein diet in order to restrict the progression of kidney injury, but with the necessary caloric-protein value to prevent nutritional deterioration.

Serum albumin reflects the degree of nutrition both in the healthy individual, in chronic kidney disease and in other non-thyroid non-renal pathologies. In this study, CKD patients showed a 15% decrease in the mean albumin value compared to controls, suggesting a greater degree of nutritional compromise in the uremic population. A similar decrease was observed in total protein. Albumin concentrations progressively decreased in groups G1, G2, and G3 as HD time increased. Given that albumin concentration has the main prognostic value for mortality in CKD patients on HD found to date, these results suggest a certain progression of kidney disease, which translates into a deterioration of the patient's general condition.

The HD procedure also influences protein metabolism. In this sense, (Lim *et al.* 2005) suggest that the reduction in protein synthesis could be a compensatory adaptation to the loss of amino acids during Hemodialysis. Other studies confirm that the process would be clearly catabolic, caused by a lower protein synthesis, a decrease in amino acids and an additional effect due to the increase in proteolysis in some tissues as a consequence of the associated inflammatory response.

Previously described direct correlations between T3 and albumin in both groups (CRI and control) and between T4 and albumin, only in the controls. In this study, we did not observe any statistically significant correlation in the control group, although we did in CKD patients on HD. Pre-dialysis urea values increase with protein intake and as the number of functioning nephrons decreases. We found a statistically significant association between TSH and urea and an inverse link between T3 and urea in individuals undergoing hemodialysis (pre-dialysis). As a result, lower T3 readings are associated with greater urea levels in renal patients. The rate of urea decrease is generally considered as the mortality rate in CKD. Serum albumin content is the primary predictor of death and morbidity in renal patients on HD. HD patients showed an inverse association between TSH levels and albumin, whereas T3 levels were directly linked to albumin. Probably, the decrease in T3 contributes to the lower catabolism that keeps kidney patients in a low basal metabolism.

When analyzing the CKD patients grouped according to the time spent on HD, we observed a progressive decrease in T3 that correlates with the decrease in albumin, indicating that T3 could be a marker in the evolution of the patients. (Lim *et al.* 2005) observed a negative nitrogen balance with increased leucine flow and protein degradation in Hemodialysis patients after administering small amounts of T3. He then suggested that a tissue hypothyroid state would be necessary to control protein expenditure in patients with restricted protein intake and protein loss during therapy. There are currently no clinical data on tissue T3 content in kidney patients. In this regard, an experimental study demonstrates tissue hypothyroidism in a uremic rat model.

On the other hand, one way of preventing the stimulation of the thyroid gland by the pituitary gland could be due to a dissociation between the tissue generation of T3 and the pituitary in situ, in order to preserve protein reserves.

Finally, we analyzed at the individual level, in the group of dialysis patients, the relationship between the variation (Δ) of thyroid hormones due to the effect of the procedure (post less pre-dialysis) and urea or albumin. The most significant change due to the dialysis procedure corresponded to FT4. We were only able to verify a significant inverse correlation between the values of ΔFT4 and post-dialysis urea. In light of these results, it could be inferred that the difference between pre- and post-dialysis FT4 would also indicate the effectiveness of dialysis therapy.

In summary, we can conclude that the most notable change in the thyroid axis in CKD patients under HD therapy was the marked decrease in T3; furthermore, a fluctuation of hormone levels was observed due to HD therapy. Time under therapy affects hormone concentrations and there are correlations between critical biochemical parameters in these patients and axis hormones (TSH and T3).

In conclusion, T3 is an indicator of morbidity in chronic kidney patients on HD, accompanying serum albumin, while variations in FT4 levels reflect the effectiveness of the procedure.

4.8.1 Comparison between vitamin D levels and biochemical parameters

Few studies have examined the connection between vitamin D and inflammatory markers in the HD population. 25-OH-D, INL, and CRP levels were substantially higher in the group with 25-OH-D 10 ng/mL; a modest inverse association between 25-OH-D and CRP was detected (Mirchi *et al.* 2016). Researchers (Kara and Soylu, 2019) identified a statistically significant negative connection between vitamin D levels and inflammatory markers CRP and INL levels.

It was revealed that 25-OH-D levels were negatively correlated with INL and IPL levels, which supports the findings of earlier studies. Another link was found between Kt/v and the urea distribution volume, where K is the dialyzer urea clearance and t is the dialysis time. Cunningham-Rundles and Nesin (2000). Kt/v has also been proven to be related to vitamin D levels. New research shows an association between increased uremic clearance in HD and metabolic impacts on the formation of vitamin D. According to experimental observations, uremic toxins interfere with the skin's production of vitamin D in a fashion similar to the deposition of melanin in the Malpighian layer, where vitamin D synthesis occurs. Having a Kt/v ratio of less than 1.2 shows that the patient is suffering from an inflammatory disease. 27 Correlations between CRP levels and Kt/v were shown to be negative in individuals with HD (Borazan and colleagues 2004). 28 Peritoneal Dialysis Patients (Herzig *et al.* 2001) found this link (r = –0.30, P0.05).

It is possible to develop secondary hyperparathyroidism in people with normal renal function who are deficient in vitamin D. In clinical studies including dialysis patients, the link between parathyroid hormone and 25-OH-D was less obvious. No correlation was identified between 25-OH-D, calcium, phosphorus, and parathyroid hormone levels in our investigation. Vitamin D levels and bone metabolism indicators were shown to have no association in other studies. It was shown that after three months of therapy with calcifediol in HD patients with 25-OH-D insufficiency, CRP and parathyroid hormone levels decreased (Ojeda López *et al.* 2018).

Patients with vitamin D insufficiency had a higher BMI, although the differences were not statistically significant in the research by (Ahmadi *et al.* 2016). There was a lower albumin level in individuals with 25-OH-D levels 20 ng/mL than in patients with levels 20 ng/mL, but this was not statistically significant. Among HD patients, a slight negative connection was discovered between 25-OH-D levels and their BMI, sex, and albumin in a research by (Bansal *et al.* 2012).

But the changes in hemoglobin and erythropoietic resistance index were not statistically significant in our individuals with vitamin D insufficiency. One possible explanation is that our unit has achieved its treatment objectives for anemia in HD patients. In addition, it has been shown that the inflammatory state is one of the most important variables influencing resistance to erythropoietin, which in our research was larger in the group without vitamin D.

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusion

Patients with chronic kidney disease (CKD) are more susceptible to vitamin D shortage or insufficiency than the general population. Deaths from all causes and cardiovascular disease, as well as concomitant conditions such as cardiovascular disease, infection and renal failure, have been associated to low levels of 25(OH)D. It is also used in a broader sense when discussing chronic renal disease. There is a strong scientific basis for vitamin D's expected non-skeletal effects since VDRs (vitamin D receptors) are expressed in a broad variety of systems and organs. Compounds with RAAS inhibitory properties, endothelial protection, immunological modulation, and anti-inflammatory effect have been identified. Diabetes mellitus, insulin resistance, heart failure, atherosclerosis and thrombosis have all been related to vitamin D deficiency, as has an impaired immune system, increased infection risk and the maintenance of inflammation. Vitamin D deficiency may cause a wide range of health problems. It has been shown that a lack of vitamin D is associated to insulin resistance. If vitamin D supplementation may reduce cardiovascular events and progression in those with chronic renal disease, additional study is required before it can be stated that it is helpful (CKD).

When it comes to providing people with renal disease with a nutritious diet, it's clear that it's not an easy job. Ensuring adequate levels of minerals such as calcium and phosphorus, since a large part of the phosphorus chelator used promotes the precipitation of salts and which in turn is deposited in arteries and veins, is of great importance; control sodium, potassium and magnesium levels, especially in cases of dialysis, whether Hemodialysis or continuous ambulatory peritoneal dialysis. Offering quality proteins in order to guarantee the intake of essential amino acids and promote the reduction of urinary albumin excretion, improve the inflammatory condition, reduce the risk of cardiovascular diseases, without harming kidney function, is something to be taken into account. By offering a nutritional supplement, it is possible to provide quality of life to renal patients, even at a more advanced stage of their pathology, and prevent

differences between caloric and protein intake from making the difference between life and death of these patients.

Patients with chronic renal illness and hypovitaminosis D have only been investigated in a few studies so far. This has resulted in a lack of interest among scientists in this field, which is evident. These studies, taken together, allow us to draw the conclusion that vitamin D administration is beneficial for these individuals.

In this study, the effects of vitamin D supplementation in patients with chronic kidney disease (CKD) were better explored. As a result, it is expected to aid in the care and clinical practise of health professionals, as well as the creation of processes for this particular demographic grouping.

- The incidence of Hypothyroidism in patients with Chronic Hemodialysis in the Baghdad Teaching Hospital during the year 2019 to 2020 was 13%.
- The incidence was higher in females and in the fifth decade of life, with 66% of cases found here.
- Additional risk factors to Chronic Renal Insufficiency and Hemodialysis for the development of Hypothyroidism are; Protein Calorie Malnutrition in Adults and Alcoholic Liver Disease.
- A deficiency in vitamin D was found in 40 percent of the patients studied. It was discovered that the vitamin D deficient group had significantly higher levels of hsCRP, INL, and IPL, whereas the vitamin D-sufficient group had significantly lower levels of Kt/v. We discovered a significant negative relationship between low-cost inflammatory indicators such as hsCRP, INL, and IPL and vitamin D levels in the blood. The findings may hint to an unknown mechanism at work in HD patients, which would explain the apparent inverse relationship between the two conditions. Adding to the corpus of information on this topic, which has so far generated a mixed bag of results, our research makes a substantial contribution.
- In patients, there was no correlation between vitamin D levels and thyroid autoimmunity indicators such as AcTPO, AcTG, and TRAb.

- Vitamin D levels did not correspond with changes in thyroid volume, although increased thyroid volume was linked to higher AcTPO levels.

- Patients with hypothyroidism are more likely to be deficient in vitamin D, according to our data. Thyroid hormone status or thyroid autoimmunity serum indicators did not correlate with vitamin D concentrations, on the other hand. Greater thyroid volume and greater AcTPO concentrations are associated, which may be indicative of an even more severe case of autoimmunity and chronic inflammation.

- The vast majority of patients in this research had hypovitaminosis D, although only a small percentage of them received vitamin D supplements. Increased 25(OH)D levels were shown to be positively correlated with the length of time patients spent on hemodialysis. An intervention research with varying vitamin D objectives is needed to examine the effect of this vitamin on mortality, since this analysis will have a direct influence on the serum target to be sought and the therapeutic supplementing approach to be implemented to these patients.

5.2 Recommendations

- Continuity of the study to improve its internal and external validity.
- Multidisciplinary patient care according to comorbidities associated with Chronic Kidney Disease to reduce morbidity and mortality and improve her quality of life.
- Modify in a timely manner all the factors that intervene in adequate Hemodialysis
- Use of the proposed guidelines for diagnosis and follow-up of patients with Hypothyroidism and Chronic Hemodialysis.

- Understanding the existence of a correlation between CKD and thyroid disorders, even in the long term, it is extremely important that health professionals pay attention to this clinical condition and encourage renal patients to undergo laboratory tests to assess thyroid function. It is also worth noting the scarcity of research similar to this, being necessary to carry out more studies that trace the clinical and epidemiological profile of these diseases with a larger population of individuals, or even the application of tests in animals for a better observation of the evolution and correlation of these diseases.

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