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STUDY THE RELATION OF SOME BIOCHEMICAL PARAMETERS WITH A VITAMIN D DEFICIENCY IN CARDIOVASCULAR DISEASE PATIENT IN KIRKUK CITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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ZAINAB YALMAS SHAWKAT SHAWKAT

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By Zainab Yalmas Shawkat SHAWKAT

Haziran 2022

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

STUDY THE RELATION OF SOME BIOCHEMICAL PARAMETERS WITH A VITAMIN D DEFICIENCY IN CARDIOVASCULAR DISEASE PATIENT IN KIRKUK CITY

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Haziran 2022

As in any research work, this work also identified some limitations. One of the limitations was the fact that the groups were not homogeneous in terms of the proportion of men and women. The results were not treated according to gender for all variables because, for most of them, we would be working with a very small sample. If we had more women, we could have treated all the data according to gender and verified whether the variables studied had different behaviours between men and women. The differences between controls and patients could have been even greater. However, this is what has been done in most studies published to date. Perhaps this is a gap and studies should be developed in patients monitored over several years to, in a way, assess the role of vitamin D in the evolution and prognosis of these individuals. In the patient group, there were only 16 individuals with vitamin D sufficiency. If the sample had been larger, it is possible that we would have had a greater number of individuals with vitamin D sufficiency in this group and it would have been possible to make comparisons with a greater degree of reliability.

2022, 69 pages

Keywords: Vitamin D deficiency, Cardiovascular disease, Biochemical parameters

ÖZET

KIRKÜK ŞEHRİNDEKİ KARDİYOVASKÜLER HASTALIK HASTALARINDA BAZI BİYOKİMYASAL PARAMETRELERİN VİTAMİN D EKSİKLİĞİ İLE İLİŞKİSİNİN İNCELENMESİ

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Tez Danışmanı: Doç. Dr. Şevki ADEM
Haziran 2022

Herhangi bir araştırma çalışmasında olduğu gibi, bu çalışma da bazı sınırlamalar belirledi. Sınırlılıklardan biri, grupların kadın ve erkek oranı açısından homojen olmamasıydı. Sonuçlar tüm değişkenler için cinsiyete göre ele alınmadı çünkü çoğu için çok küçük bir örneklemle çalışıyor olacağız. Daha fazla kadınımız olsaydı, tüm verileri cinsiyete göre ele alabilir ve incelenen değişkenlerin kadın ve erkek arasında farklı davranışlara sahip olup olmadığını doğrulayabilirdik. Bu ilginç olurdu çünkü 25(OH)D3 gibi bazı değişkenlerde cinsiyetin bir etkisi var gibi görünüyor. Bir diğer olası kısıtlılık, hasta grubunun işe alımdan yaklaşık 2 veya 3 yıl önce kateterizasyon uygulanan koroner arter hastalığı tanısı almış bireylerden oluşmasıydı. Muhtemelen, işe alım kardiyovasküler olaydan birkaç hafta veya ay sonra gerçekleştirilmiş olsaydı, sonuçlar farklı olurdu. Kontroller ve hastalar arasındaki farklar daha da büyük olabilirdi. Ancak, bugüne kadar yayınlanan çoğu çalışmada yapılan budur. Belki de bu bir boşluktur ve birkaç yıl boyunca izlenen hastalarda D vitamininin bu bireylerin evrimi ve prognozunda rolünü değerlendirmek için çalışmalar geliştirilmelidir. Hasta grubunda D vitamini yeterliliği olan sadece 16 kişi vardı. Örneklem daha büyük olsaydı, bu grupta D vitamini yeterliliği olan daha fazla sayıda birey olması ve daha güvenilir karşılaştırmalar yapmak mümkün olabilirdi.

2022, 69 sayfa

Anahtar Kelimeler: Vitamin D eksikliği, Kardiyovasküler hastalığı, Biyokimyasal parametrelerin

PREFACE AND ACKNOWLEDGEMENTS

I extend my sincere thanks and gratitude to my thesis advisor Assoc. Prof. Dr. Şevki ADEM for your patience, guidance and support for me. I also extend my sincere thanks, and appreciation to my beautiful family for their support.

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Çankırı-2022

CONTENTS

LIST OF SYMBOLS

%	Percentage
dL	Deciliter
g	Gram
Kg	Kilogram
m	Meter
mg	Miligram
mL	Milliliter
ng	Nanogram
nmol	Nanomol
µg	Mikrogram

LIST OF ABBREVIATIONS

25-(OH)D	25-hydroxyvitamin D
25-OHase	25-hydroxylase
AMI	Acute myocardial infarction
AST	Aspartate amino transferase
BMI	Body mass idex
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DM	Diabetes mellitus
GGT	Gama glutamyl transferase
HbA1c	Hemoglobin A1c
LDH	Lactate dehydrogenase
LDL	Low density lipoprotein
RAAS	Renin angiotensin aldosterone system
SBP	Systolic blood pressure

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1. INTRODUCTION

1.1 Vitamin D

As a biologically inactive compound, vitamin D needs to be metabolized to become active and perform various functions in the body, regardless of how it is obtained from food or the skin.

No matter where it comes from, adipose tissue will store any additional vitamin D. The buildup of vitamin D2 in adipose tissue makes its half-life lower than that of vitamin D3 (Bartoszewicz *et al.* 2007).

To become biologically active, vitamin D undergoes two hydroxylations that are apparently dependent on cytochrome CYP450 enzymes. The liver performs the first hydroxylation, while the kidneys perform the second. (Lehmann and Meurer 2010).

25-hydroxyvitamin D is produced by converting vitamin D into its hydroxylated form (also known as Calcifediol), an enzyme called 25-OHase in the liver (Christakos *et al.* 2013).

Approximately 15 days are required for the major metabolite of vitamin D, 25(OH)D. This metabolite binds to DBP which transports it to the kidneys, where the second hydroxylation takes place. The complex formed by Vit-Dand DBP binds to megalin. A transmembrane protein, A member of the low-density lipoprotein receptor family, Megalin binds to DBP in renal tubule cells. Megalin is responsible for cellular uptake of the complex formed by Vit-Dand DBP. Once inside the cell, Vit-Dis released and transformed into Due to the enzyme's action, 1,25-dihydroxyvitamin D [Calcitriol or 1,25(OH)2D] the enzyme 1-OHase (Holick 2006).

1.2 Action of Vitamin D on Blood Pressure

Consistently high blood pressure is a sign of chronic hypertension. There are many crucial organs that depend on blood pressure being maintained at a healthy level, such as the heart, brain and kidneys. Blood pressure over 140 or 90 millimeters of mercury (MPH) is considered to be hypertension by the World Health Organization, which defines it as having an SBP more than 140 or a DBP greater than 90 millimeters of mercury (mmHg) (Tomaschitz *et al.* 2010). Hypertension can be caused by endocrine conditions such as hyperparathyroidism or by an increased activity of the RAAS and, in addition, some habits related to an unhealthy lifestyle such as a sedentary lifestyle, tobacco consumption, excessive alcohol and excessive consumption. Hypertension can be exacerbated by a diet high in sodium.

Hypertension is one of the main things that puts people at risk for CVD, and as a chronic disease, it needs to be treated well and kept an eye on all the time.One way that vitamin D and CVD are related is through the effect it has on blood pressure. Vitamin D helps lower blood pressure by stopping the RAAS from working, stopping secondary hyperparathyroidism from happening, protecting the kidneys, and working on the cells of the blood vessels. As previously mentioned, 1-OHase is present in cells of the endothelium and the smooth muscle of the blood vessels. One theory holds that cells of both types are to blame for elevated blood pressure (Vaidya *et al.* 2012).

RAAS suppression appears to be the main antihypertensive mechanism of vitamin D. There is more and more evidence that Calcitriol controls blood pressure by regulating the RAAS. This is a chain of systems that work together to keep blood pressure, electrolyte balance, and intravascular volume stable. (Harinarayan 2014).

This chain reaction begins with renin. In the kidney, the angiotensin-converting enzyme (ACE) converts angiotensinogen to angiotensin I, which in turn is converted to angiotensin II by renin (ACE). Aldosterone production is boosted by angiotensin II receptor interaction when angiotensin II (RAAS) is activated in the brain, heart, kidney,

and adrenal cortex. This results in vasoconstriction and retention of water and salts, thus leading to increased blood pressure (Harinarayan 2014).

Inadequate RAAS stimulation has been linked to hypertension, heart attack and stroke. An example of Calcitriol's ability to influence the RAS was proven in 2002 when mice lacking the VDR were studied and found to have excessive renin concentrations, which resulted in high blood pressure and heart disease (Li *et al.* 2004).

Furthermore, human studies have connected a rise in RAAS activity to a vitamin D shortage. A mechanism of action on angiotensin II that is similar to that of ACE inhibitors is found in calcitriol, as well (Pai *et al.* 2013).

Calcitriol's effect on the RAAS is not the only thing to consider, secondary hyperparathyroidism and hypocalcemia also appear to be related to hypertension. Even though we don't know exactly how PTH affects endothelial cells, it's widely believed that it causes arterial stiffness and atherosclerotic damage. However, more research is required to fully comprehend the role of PTH in the cardiovascular system and the impact it has on blood pressure (Kolb and Mandrup-Poulsen 2005).

1.3 Action of Vitamin D in Diabetes Mellitus

Insulin deficiency or inactivity causes diabetes Mellitus, a metabolic disorder that results in hyperglycemia (DM).DM type 1 and DM type 2 are the two most common kinds of DM. There are several mechanisms involved in the pathogenesis of DM. The autoimmune destruction of the islets of Langerhans in type 1 DM is a multifactorial disease, in type 2 DM there are several changes that result in resistance to insulin action in target cells and in pancreatic β-cell dysfunction (Scragg *et al.* 2004).

Long-term effects of diabetes mellitus (DM) include damage to different organs, particularly the eyes, kidneys, heart, and blood vessels, as well as organ malfunction or

failure. A higher risk of coronary heart disease is associated with diabetes mellitus (DM) (CVD).

The monitoring of DM can be done through the determination of glycated hemoglobin (HA1C). This determination makes it possible to evaluate the average glucose concentration during approximately the last 6 to 8 weeks and presents a closer relationship with the risk of developing complications than the point determinations of blood glucose. It has already been shown that HA1C is related to the risk of developing CVD, even among apparently healthy and non-diabetic individuals, and may constitute an important early marker of CVD (Forman *et al.* 2010).

In the case of type 2 DM, Calcitriol is thought to have a direct action on pancreatic β-cellsincreasing insulin receptor expression in target tissues to improve insulin sensitivity and promoting insulin synthesis by activating the insulin gene. It also appears to have an indirect effect, through calcium homeostasis and calcium influx into cells, a mechanism that is important in insulin synthesis and secretion (Holick *et al.* 2011).

Some research has been done to find out what role vitamin D plays in insulin resistance. One of the first studies carried out in this field was published in 1980, (Norman *et al.* 1980). In vitamin D deficient rats, a study found that supplementing with vitamin D restored insulin production to normal (Li *et al.* 2004).

1.4 Objectives

- The primary goals of this study were to investigate the link between low levels of vitamin D and cardiovascular disease and to conduct a preliminary examination to determine if residents of Kirkuk City were deficient in vitamin D.

- The study's primary focus was on cardiovascular disease and vitamin D deficiency. This research included looking at two distinct groups of people. In this

research, participants with and without cardiovascular disease (controls) were compared to each other (patients).

- Blood count, liver and kidney function evaluation, as well as lipid profile, HA1C, glucose, insulin, fibrinogen, hsCRP, and homocysteine concentrations were measured in the laboratory for each of the study groups.

- Ascertaining the blood's vitamin D levels. In addition, a variety of anthropometric and lifestyle data were gathered on each participant.

- To study the Laboratory parameters and anthropometric and lifestyle data from both study groups (controls and patients) were statistically analyzed and compared. These parameters were related to the concentrations of Calcifediol.

- Finally, the differences between two approaches to determining serum Vit-D concentrations were examined.

2. LITERATURE REVIEW

2.1 Vitamin D as a Direct Factor in Cardiac and Vascular Tissue

Vitamin D has been studied in vivo and in vitro for its effects on damaged heart and vascular tissue. As a result of the inhibition of VDR receptors, (Rahman *et al.* 2007) discovered that matrix metalloproteinases were overstimulated, which have a role in the generation of aberrant cardiomyocytes during remodelling in response to injury and atherosclerosis (Martins *et al.* 2007). Deficiency in cardiac contractility (McCullough *et al.* 2010) and left ventricular hypertrophy (Wanner *et al.* 2015) are two possible consequences of VDR receptor inhibition.

Vitamin D insufficiency has been associated to coronary artery calcification,71 according to one research. With 52 persons with end-stage renal disease, researchers discovered that vitamin D concentrations correlated positively with arterial flexibility (as evaluated by dilation of the Brachial Aorta) and negatively with pulse wave speed. Both results point to a decrease in the vascular wall's flexibility. Vitamin D in the 1,25-dihydroxyvitamin D form has been shown in vitro to reduce profibrotic signals in multipotential mesenchymal cells. This suggests the possibility that injury-induced changes in vascular tissue could be influenced by vitamin D in this way85. Endothelial function, as measured by carotid artery dilation and blood pressure reduction, improved significantly in patients with diabetes who received a vitamin D dose of 100,000 international units (Santiago *et al.* 2012).

Research on vitamin D and SLE-related cardiovascular risk factors has been sparse in comparison to other studies. Since they are more likely to suffer from vitamin D deficiency, they are more likely to suffer from cardiovascular disease. A blood vitamin D level that is less than or equal to 75 nanomol/L (30 nanograms per millilitre) LDL cholesterol, lipoprotein A, fibrinogen, and diabetes were all linked to elevated diastolic blood pressure (hypertension) (Wu *et al.* 2009). An American Journal of Clinical Nutrition study found that vitamin D deficiency may increase the risk of type 2 diabetes, cardiovascular disease, and other health problems. a greater BMI (body mass index),

higher fasting insulin levels (fasting insulin resistance), according to vitamin D deficiency are all associated with an increased risk of diastolic hypertension (Reynolds *et al.* 2016). Cardiovascular disease and high levels of triglycerides were linked to low vitamin D levels in a study published in 2012 (Alves *et al.* 2013)

2.2 Vitamin D and Cardiovascular Risk

Many chronic conditions that raise the risk of cardiovascular disease have been linked to a lack of vitamin D. Chronic renal disease, hardening of the coronary arteries, atherosclerosis, and peripheral arterial disease are just a few examples of the disorders that fall under this category (National Institute of Statistics 2014). Low levels of vitamin D have been found to be associated with high blood pressure in Caucasians, Hispanics, and African Americans, according to research conducted through observation (Chen 2010). Vitamin D deficiency increases the risk of heart attack in men. The risk of heart failure or sudden death is three to five times greater in people with severe vitamin D deficiency (Vit-D 75 nmol/L) than in those with adequate levels of vitamin D (Chan and Watts 2006).

A growing body of studies suggests that vitamin D administration may improve cardiovascular health while also lowering proteinuria. A study found a link between vitamin D deficiency and obesity, insulin resistance, and metabolic syndrome (Chen 2010).

A combination of vitamin D and calcium supplementation may help prevent type 2 diabetes in high-risk groups, according to randomized controlled trials (glucose intolerance) (WHO 2014). There may be a link between improved insulin sensitivity in people with normoglycemia and increased osteocalcin-aldosterone system (RAAS) and decreased Renin-Angiotensin-Aldosterone system (RAAS) modulation. adiponectin. However, the underlying mechanism for vitamin D deficiency-induced RAAS hyperactivity is still a mystery. (Chan and Watts 2006).

Metabolic processes such as insulin release and glucose tolerance have been linked to a number of vitamin D polymorphisms. After supplementation with Calcitriol3, there is a decrease in glucose and insulin levels.

High systolic blood pressure and plasma glucose concentrations, two markers of cardiovascular and metabolic disease in adolescents, were shown to be negatively correlated with vitamin D deficiency (Holick 2008). Both normal and hypertensive individuals were shown to have a negative correlation between serum calcitriol and blood pressure. In the treatment of hypertension, vitamin D analogues and supplements have been found to be effective (Kienreich *et al.* 2013). A1 cyclins C and E and Cdk2 and Cdk4 cyclin dependent kinases Cdk2 and Cdk4 have been shown to lower cardiac myocyte proliferation and increase their number, protect them from apoptosis, resting phases of the cell cycle by supplementing with active vitamin D

It has been demonstrated that vitamin D helps to prevent left ventricular dysfunction (LVD), according to a new study (Li *et al.* 2002). Vitamin D analogues reduce the production of proinflammatory Th1 cytokines, such as IL-2 and IFN-, which stimulate Th2 lymphocytes, resulting in decreased platelet formation. These drugs are effective in preventing heart disease because they stop the growth of certain lymphocytes and cytokines that have been linked to coronary artery disease (Tishkoff *et al.* 2008)

Protective doses of calcitriol suppress the gene expression of aortic osteoblasts that is normally increased in cardiovascular disease, suggesting a mechanism for this inhibition of aortic calcification.

Hypovitaminosis D, an independent risk factor for chronic kidney disease, has a negative correlation with the estimated glomerular filtration rate in stage 3 of chronic renal failure (Ramjee *et al.* 2011).

Observational studies have shown that in ESRD, patients prescribed active vitamin D compounds appear to experience a 26% reduction in mortality, irrespective of other cardiovascular risks, PTH, and bone mineral biomarkers.

It's been shown in a slew of large cohort and prospective observational research studies that low vitamin D status is associated with a 50% lower mortality and morbidity risk than high vitamin D status (WHO 2014).

3. MATERIALS AND METHODS

3.1 Recruitment and Selection of Participants

This study's participants were recruited from outpatient clinic of Kirkuk General Hospital, the oldest hospital in Kirkuk, Iraq, when they went to that unit for follow-up consultations of the current research project, which is taking place there.

The recruitment of participants took place between January 2021 to January 2022.

In accordance with Kirkuk General Hospital's Ethics Committee, the study's protocols have been approved by all participants, a total of 412 individuals, signed the Informed Consent document (Annex 3) after having been given the Information to the Subject of Investigation document (Annex 1 and 2) and having been provided with all clarifications they deemed relevant.

For the study's purposes, participants were split into two groups.

A. **A group** of subjects with a previous history of AMI (acute myocardial infarction) or coronary disease confirmed by coronary angiography with at least 75% obstruction of one of the coronary vessels and
B. **Another** group of healthy subjects (control) without heart disease, hypertension, DM or any other chronic disease. Exclusion criteria for both groups were liver failure, renal failure, intestinal malabsorption syndromes, cancer, autoimmune disease, neurodegenerative disease, and intake of supplements of vitamin D, β-carotene, vitamin A, vitamin E and treatment with anticoagulants.

After applying the exclusion criteria, 194 individuals remained eligible. Of the 194 selected subjects, The cardiovascular patients comprised 94 of the participants, while the control group consisted of 100 subjects. A protocol was applied to all individuals

who participated in the study, which involved physical assessment, filling out a questionnaire and laboratory tests.

3.2 Data Collection

3.2.1 Questionnaires

Personal characteristics, such as smoking, drinking, exercise, and sun exposure, as well as a history of chronic disease are all taken into consideration (heart disease; hypertension; DM, cancer, autoimmune, neurodegenerative, and liver or kidney disease), type of diet and vitamin supplementation; and their level of sun exposure were gathered using a short questionnaire (Appendix 4).

Smoking habits and alcohol consumption: Individuals who had never smoked or who had stopped smoking for more than five years were considered non-smokers, and alcohol consumption was considered to be present when it occurs with a frequency equal to or greater than once a week.

Physical activity: Subjects were asked what kind of physical activity they do most often (moderate and/or intense) and its duration.

Data regarding the amount of time spent participating in moderate or strenuous physical activity on a particular workday was collected through the use of a modified version of the International Physical Activity Questionnaire that was developed by the WHO.

Food: Subjects indicated how often they consumed foods rich in vitamin D (salmon, sardines, tuna, eggs and beef liver).

Consumption of foods rich in vitamin D was considered very frequent when it occurred four times a week or more, frequent when it happened with a regularity of one to three times a week and rare when it happened less than four times a month. All individuals

who reported taking or having taken vitamin supplements in the last 12 months were excluded.

Sun Exposure: Vitamin D levels can be maintained by exposing one's arms and legs to sunlight between the hours of 10:00 and 15:00, twice a week, for a total of five to thirty minutes (Lehmann and Meurer 2010). However, as it was not possible to know exactly if the individuals always maintained an exposure of arms and legs, sun exposure was considered high when individuals reported being exposed to sunlight for more than one hour a day, moderate when they claimed to maintain it. Sun exposure lasting between 30 and 60 minutes a day and low when they said that they were only exposed to the sun for a total of less than half an hour each day.

3.2.2 Anthropometric tests

The measurement of blood pressure as well as the rate of the heart: An automatic sphygmomanometer was used to take readings of the patient's blood pressure and heart rate from the right arm (OMROM M6 Comfort). Following a period of rest during which the individual sat for ten minutes, three measurements were performed, The arithmetic mean was calculated after each recording was separated by two minutes.

A blood pressure reading of 140 mmHg or higher was considered to be hypertension by the World Health Organization, which suggests that those taking antihypertensive medication or experiencing symptoms of hypertension should seek medical attention.. A few examples include: The European Association of Cardiology and the European Society of Hypertension (Pilz *et al.* 2009).

Registration of body mass, height, waist circumference and BMI: Body mass was determined with the individual barefoot and wearing light clothes, using a scale (Krups). This measurement was determined with an error of 50g. Height was recorded according to the information described in the individual's medical record. Body mass index (BMI) was calculated using the following formula:

$$BMI = body\ mass\ (Kg) / height\ (m)^2 \quad 3.1$$

After the individual had expelled all of their pulmonary air, an anthropometric tape was used to measure the individual's abdominal circumference while the individual was in the vertical position. The midpoint between the iliac crest and the bottom border of the last rib was marked with a piece of tape. A body mass index (BMI) greater than 30 kg/m2 and a waist circumference greater than 102 cm in men or 88 cm in women are considered obese by the World Health Organization (WHO).

3.3 Laboratory Procedures

3.3.1 Blood collection

Every individual who took part in the research project was asked to complete a series of predetermined laboratory examinations. The Clinical Pathology Service of Kirkuk General Hospital was the site of the blood collection, by duly qualified professionals, following a 12-hour fast, as confirmed verbally by each individual. Blood samples were obtained by venipuncture using the Vacuette® vacuum system (Greiner Bio-One, Austria). Blood was collected in properly labelled tubes. All participants received a 3 mL tube containing ethylenediaminetetraacetic acid (EDTA), a 3 mL tube containing sodium citrate and a 5 mL dry tube.

The EDTA tube was then processed for the determination of hemogram and HA1C. The sodium citrate tube was centrifuged in the Remi Shaker 2L (Remi, India) at 3500 rpm for 15 minutes to obtain plasma for fibrinogen assay. The dry tube was allowed to stand at room temperature in an upright position for half an hour to facilitate clot retraction. After this period of time had elapsed, the empty tube was centrifuged at a speed of 4200 rpm for a duration of 8 minutes in order to obtain serum.

The serum isolated from each sample was transferred to properly identified microtubes for the determination of Calcifediol, insulin, APO B, Lp(a), hsCRP and homocysteine.

Two aliquots with 500 μL of serum were taken from each sample, which were stored at -20ºC until processing. The remaining serum was processed on the same day for the determination of several routine biochemical parameters, which served to complement the clinical information of the individuals parameters (Holick 2008) .

3.3.2 Laboratory tests

Blood count: The blood count was performed on the Coulter LH780 automatic counter (Beckman Coulter Inc, USA). The operation of this equipment is based on the Coulter Principle, which uses electrical impedance to measure the volume of particles.

In this equipment, a current is passed through a small hole of a predetermined size while a suspension of blood cells does the same thing. The passage of individual cells through the hole causes an impedance change proportional to the cell's size. This enables the system to count the individual cells and provide a distribution of cells according to size.

HA1C: HA1C was determined on the VARIANT II TURBO analyzer (Bio-Rad company, USA), a fully automated instrument that uses the principle of high performance liquid chromatography (HPLC). The HA1c 2.0 kit, was used.

The VARIANT TURBO II consists of two modules: a chromatography station and a sample pipetting module. Automatic injection of samples into the analytical column dilutes them in the pipetting module. At the chromatography station, two pumps supply the analytical column with buffer solution and the hemoglobins are separated according to their ionic strength. After separation, the hemoglobins pass through a photometer, where the absorbance is measured.

The data obtained are transmitted to the equipment software and the HA1C value is calculated based on the calibration curve generated for this test (Figure 3.1).

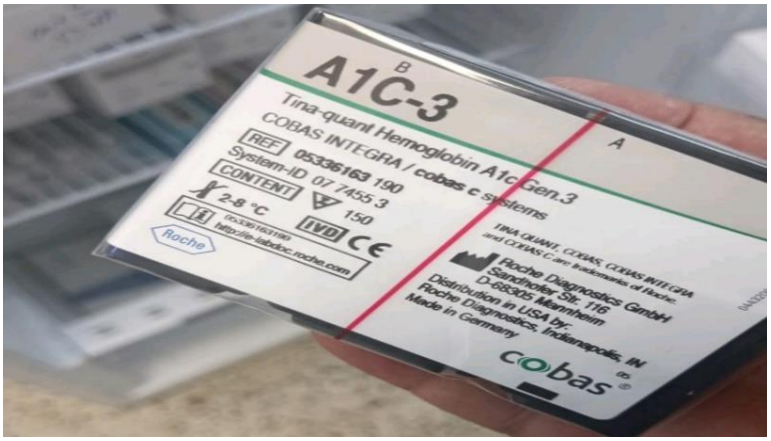

Figure 3.1 A1C-3 kit (Germany)

Fibrinogen: Fibrinogen was determined using the Fibrinogen C kit, (HemosIL® Fibrinogen-C - 0020301100), on the ACL TOP 700 CTS (USA), based on the Clauss method. This method assesses the rate of conversion of fibrinogen to fibrin using an excess of thrombin in diluted plasma. When fibrinogen concentration is inversely proportional to excess thrombin concentration in diluted plasma, clotting time occurs. Next, we compare our results with a standard fibrinogen preparation and figure out how much fibrinogen is in our sample.

25(OH)D: Vitamin D levels in the bloodstream was determined by electrochemiluminescence on the Cobas e411 autoanalyzer (Roche Diagnostics GmbH, Germany) (Figure 3.2), by an immunocompetition principle. The Vitamin D Total kit, Ref.05894913 190 (Roche Diagnostics GmbH, Germany) was used.

Figure 3.2 Cobas e411 (Roche Diagnostics GmbH, Germany)

The sample is initially treated with a pre-treatment reagent, which releases the bound Vit-D derived from the protein that binds vitamin D. Vitamin D and the protein that binds vitamin D were rutenylated were added to the sample for a second incubation, which resulted in a compound between the two (Figure 3.3)

Figure 3.3 Vitamin D total kit

Third incubation is supplemented with biotin-labeled vitamin D streptovidin-coated microparticles. Because of this, ruthenium-labeled vitamin D binding proteins previously inactive may now bind vitamin D. In this case, a combination of rutenylated

The biotin-strepavidin interaction is responsible for the binding of vitamin D-binding protein and biotinylated vitamin D to the solid phase (Figure 3.4).

Into the reading cell is sucked the reaction mixture, where microparticles are attracted to the electrode surface by magnetic attraction and unbound elements are removed. A photomultiplier measures a chemiluminescent emission induced by the application of an electric current to the electrode.

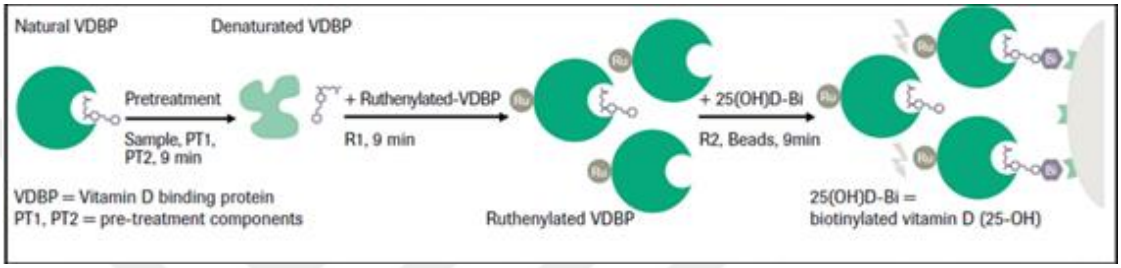

Figure 3.4 Principle of immunocompetition for the determination of Calcifediol

In order to get vitamin D concentration values in nanograms per milliliter (ng/mL). The reagent barcode serves as a master curve for an analyzer, which is calibrated with two points. There is a range of 3 to 70 ng/mL in the test's detection range.

Vit-D serum levels were termed deficient if they were less than 20 ng/mL, they wouldn't be enough if they were between 21 and 29 ng/mL, and sufficient if they were greater than 30 ng/mL (Holick 2008).

Insulin: Insulin was determined by chemiluminescence on the Unicel DXI 800 autoanalyzer (USA) by the sandwich principle. A conjugate and paramagnetic particles coated with anti-insulin monoclonal antibodies are added to the sample. On the solid phase, serum insulin binds to the antibody, while the conjugate reacts with another location on the insulin molecule. The material bonded to the solid phase is kept in a magnetic field after incubation, while the unbound material is washed and discarded. The light created in the reaction is then monitored with a luminometer after the addition

of a chemiluminescent substrate. The intensity of the light is related to the amount of insulin in the sample.

The Homeostatic Model Assessment (HOMA) and the accompanying formula were used to quantify insulin resistance, which was based on the measurement of blood insulin concentration:

$$HOMA = \frac{Glucose\left(\frac{mg}{dl}\right) \times Insulin\frac{\mu U}{ml}}{405} \qquad 3.2$$

HOMA values were considered normal when lower than 2.33

Routine biochemical parameters

The remaining biochemical parameters were determined on the AU5400 autoanalyzer (Beckman Coulter Inc, USA). This fully automated analyzer makes the determination of several biochemical tests by a spectrophotometric principle, through colorimetric, immunoturbidimetric, enzymatic and kinetic reactions.

The reagents used were purchased from Beckman Coulter and do not require preparation. After the calibration of the reagent lots in use, the concentration values were automatically obtained for each analyte, using the respective calibration curve that is stored in the equipment's database.

Kinetic reactions

Urea: The UREA kit was used to determine urea levels. The urease method was used to determine urea. This method produces ammonia and carbon dioxide by hydrolyzing urea in the presence of water and urease. In the presence of glutamate dehydrogenase, the ammonia produced in this reaction reacts with 2-oxoglutarate and nicotinamide adenine dinucleotide in its reduced form (NADH) to produce glutamate and

nicotinamide adenine dinucleotide in its oxidised form (NAD+). As urea concentration increases, so does the reduction in NADH absorbance with time (Figure 3.5).

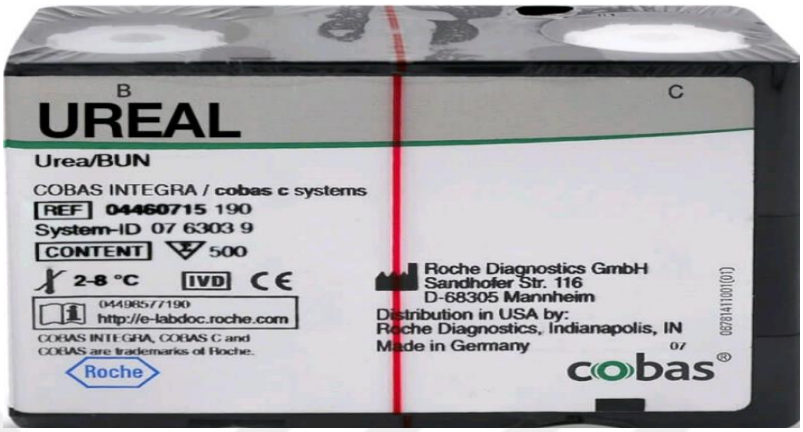

Figure 3.5 Kit of urea

The CREATININE kit was used to determine creatinine using the Jaffé method (Beckman Coulter Inc, USA). Creatinine interacts with picric acid in an alkaline media to generate an orange complex in this approach. The sample's creatinine concentration is proportional to absorbance change at the wavelengths of 520 and 800 nm (Figure 3.6).

Figure 3.6 Kit of Creatinine

Alkaline phosphatase: The ALP kit was used to determine alkaline phosphatase activity (Beckman Coulter Inc, USA). Rate of p-nitrophenylphosphate to p-nitrophenol

conversion in presence of magnesium and zinc ions was utilised to determine alkaline phosphatase activity. The rate of change in absorbance owing to p-nitrophenol production is measured bichromatically at 410 and 480 nm and it's based on the alkaline phosphatase activity of the sample.

Alanine aminotransferase: The method for determining alanine aminotransferase (ALT) is based on the International Federation of Clinical Chemistry's recommendations. It was decided to use the ALT kit. In this experiment, the amino group from alanine is transferred to 2-oxoglutarate by ALT, resulting in pyruvate and glutamate. There is an increased catalytic activity when the reaction mixture is supplemented with Pyridoxal Phosphate. Lactate dehydrogenase (LDH) catalyzes a reaction involving pyruvate and NADH to produce lactate and NAD+. The sample's ALT activity is directly correlated to the decrease in absorbance caused by NADH consumption, which is measured at 340 nm. Endogenous pyruvate is excreted during the incubation period.

Aspartate aminotransferase: Aspartate aminotransferase was measured using an AST kit (Beckman Coulter Inc, USA) (AST). AST catalyzes the transamination of aspartate and 2-oxoglutarate, resulting in L-glutamate and oxaloacetate, as recommended by the International Federation of Clinical Chemistry. The AST enzyme's catalytic activity is enhanced by adding pyridoxal phosphate to the reaction mixture. Malate dehydrogenase (MDH) converts oxaloacetate to L-malate, while NADH is transformed to NAD+ at the same time. Measurement of the reduction in absorbance due to the consumption of NADH may be done at 340 nm and the amount of activity in the sample. The LDH process eliminates endogenous pyruvate throughout the incubation phase.

γ-glutamyltransferase

γ Glutamyltransferase (GGT) was measured using the GGT kit, Ref.OSR6120, according to the International Federation of Clinical Chemistry's standards (Beckman Coulter Inc, USA). Gamma-glutamyl groups in the substrate of the reaction are transported to GGT, where they are transformed into 5-amino-2-nitrobenzoate by the

GGT catalyst. GGT activity is closely correlated with the quantity of 5-amino-2 nitrobenzoate generated in a sample.

Homocysteine: The Homocysteine Liquid kit was used to determine homocysteine levels (Sentinel Diagnostics, Italy). Dimerized and oxidised homocysteine is reduced to free homocysteine, which then combines with serine to generate cystathionine, catalysed by the enzyme cystathionine beta synthase. By using cystathionine beta-lyase cystathionine is converted into homocysteine, pyruvate, and ammonia. LDH uses NADH as a cofactor to convert pyruvate to lactate. The rate at which NADH is converted to NAD+ is proportional to the amount of homocysteine present.

Colorimetric reactions:

Total proteins: The TOTAL PROTEIN kit was used to determine total protein concentration using the biuret technique (Beckman Coulter Inc, USA) (Beckman Coulter Inc, USA). In this method, copper ions react with the sample's proteins in an alkaline solution to produce a violet complex where the ratio between absorbance and total protein concentration is known.

Albumin: ALBUMIN kit (Beckman Coulter Inc, USA) was used to measure albumin using the bromocresol green method. Because of the reaction between bromocresol green, which is in the reagent, and albumin, a colourful complex forms. The complex's absorbance increases as the amount of albumin in the sample increases.

Bilirubin: The diazo reaction was used to determine bilirubin using a colorimetric approach. Conjugated bilirubin and unconjugated bilirubin react with a diazonium salt in the presence of a catalyst to create azobilirubin in the TOTAL BILIRUBIN kit (Beckman Coulter Inc, USA). When total bilirubin concentrations are tested using absorbance at 540 nanometersThe outcome is influenced by the sample's bilirubin content.

Enzymatic reactions

Glucose: Glucose was evaluated using the GLUCOSE kit and the hexokinase technique (Beckman Coulter Inc, USA) (Beckman Coulter Inc, USA). In this process, glucose is phosphorylated to produce glucose-6-phosphate and adenosine diphosphate, which are both produced by the enzyme hexokinase and magnesium ions. Glucose-6-phosphate dehydrogenase reduces NAD+ to NADH by oxidising glucose-6-phosphate to gluconate-6-phosphate. The amount of NADH produced is directly proportional to the absorbance at 340 nm, which measures the glucose concentration in the sample. Having a fasting blood glucose level greater than 126 mg/dL or being treated with oral antidiabetic medications or insulin was defined as having diabetes for this study's purposes.

Total cholesterol: It was determined using the CHOLESTEROL kit (Beckman Coulter Inc, USA), an enzymatic method. Cholesteryl esterase hydrolyzes the sample's cholesteryl esters in this procedure. Spectroscopy can measure quinoneimine red, which is formed when hydrogen peroxide reacts with 4-aminoantipyrine and phenol to oxidize free cholesterol. As the sample's total cholesterol concentration rises, so does its absorption.

Triglycerides: Triglyceride concentrations in samples are measured using an enzyme reaction-based approach. The TRIGLYCERIDE kit (Beckman Coulter Inc, USA) was utilised to make this determination. The triglycerides in the sample are hydrolyzed by a combination of microbial lipases, resulting in glycerol and fatty acids. Glycerol kinase catalyzes glycerol's phosphorylation, which results in 3-phosphoglycerol. In the presence of the enzyme glycerol phosphate oxidase, hydrogen peroxide and dihydroxyacetone phosphate are generated during the oxidation of glycerol-3-phosphate. At the conclusion of the process, a chromophore is formed and may be measured at 660 and 800 nm. The greater the sample's absorbance, the higher the concentration of triglycerides.

HDL cholesterol: HDL-CHOLESTEROL kit (Beckman Coulter Inc, USA) was used to measure HDL cholesterol using an enzymatic approach that involved cholesterol esterase, cholesterol oxidase, and peroxidase. Anti-human lipoprotein antibodies, which bind to all lipoproteins except HDL, are introduced in the initial step of the procedure. The lipoproteins that are bound will be prevented from undergoing the enzymatic action that occurs in the second portion of the reaction. These enzymes are able to target exclusively HDLs because of this. To quantify the HDL concentration in the sample, a chromogen is generated at the end of the procedure, and a change in absorbance can be used to measure it.

LDL cholesterol: For triglyceride readings below 400 mg/dL, the Friedewald formula was used to determine LDL cholesterol. The LDL-CHOLESTEROL kit was used to measure LDL-Cholesterol in those with triglyceride levels more than 400 mg/dL. LDL and all other lipoproteins are destroyed in this reaction because a protective agent is introduced to the sample. An enzyme known as cholesterol esterase is used to break down LDL, which has been freed from its protective agent. Cholesterol oxidase oxidises the free cholesterol generated. The amount of LDL cholesterol in the sample can be determined by measuring the absorbance of the solution once the reaction is complete. The term "dyslipidemia" is used to describe a condition in which a person's serum triglyceride level is greater than 150 milligrams per deciliter, or if triglyceride levels are greater than 150 milligrams per deciliter in the blood.

Immunoturbidimetric reactions

A C-reactive protein that is highly sensitive: In order to measure high sensitivity C-Reactive Protein (hsCRP), an immunoturbidimetric test was carried out using a CRP Latex kit (Beckman Coulter Inc, USA). PCR binds to anti-PCR antibodies on latex particles, generating insoluble aggregates, and then the sample is combined with a buffer and the latex particle suspension. An increase in the PCR concentration in the sample causes these aggregates to absorb more radiation. This method's ultra-sensitive application allows the detection of CRP concentrations as low as 0.08 mg/L. The CRP Calibrator Highly Sensitive Set was used to plot the calibration curve

APO B: The APO B kit was used to perform an immunoturbidimetric test to evaluate the presence of APO B. (Beckman Coulter Inc, USA). A buffer solution and an anti-Apo B antibody solution are added to the sample. The absorbance of the insoluble aggregates that are formed as a result of APO B binding to these antibodies is found to be proportional to the amount of APO B that is present in the sample.

Lp(a): The Lp(a) Ultra kit was used to perform an immunoturbidimetric test to determine Lp(a) (Sentinel Diagnostics, Italy). To induce agglutination, the sample is incubated in an anti-Lp(a) antibody-coated latex particle solution. As a result of this agglutination, the sample's absorbance changes in a way that is proportional to the amount of Lp(a) present.

3.4 Statistical Analysis

For this project's statistical analysis, SPSS 23.0 for Windows was used (IBM SPSS statistics 23.0, Chicado, Inc).In descriptive statistics, a measure of central tendency or dispersion may be used to characterize continuous data (mean standard deviation). ANOVA, Mann-Whitney, Kruskal-Wallis, and the Chi-square test of homogeneity were all used in the research between groups. Testing for normality and consistency of the variances using Shapiro-Wilk and Levene tests was carried out. The category variables were examined using the chi-square test of homogeneity. Methods such as one-way ANOVAs, Mann-Whitney tests, in addition to using Kruskal-Wallis tests analyze continuous variables. As an option, non-normal distributions were examined. The research examined the variance in both directions using an ANOVA test and a logistic regression model. It's necessary to use P values lower than 0.05 to consider something statistically significant..

4. RESULTS AND DISCUSSION

4.1 Characterization of the Population

A total sample of 194 individuals distributed into two groups was evaluated. A group of 100 healthy individuals (controls), of which 89 men (89.0%) and 11 women (11.0%) and a group of 94 individuals with coronary heart disease (patients), of which 64 men (68.1 %) and 30 women (31.9%). The population is broken down into its various subgroups based on gender and age, as shown in Figure 4.1.

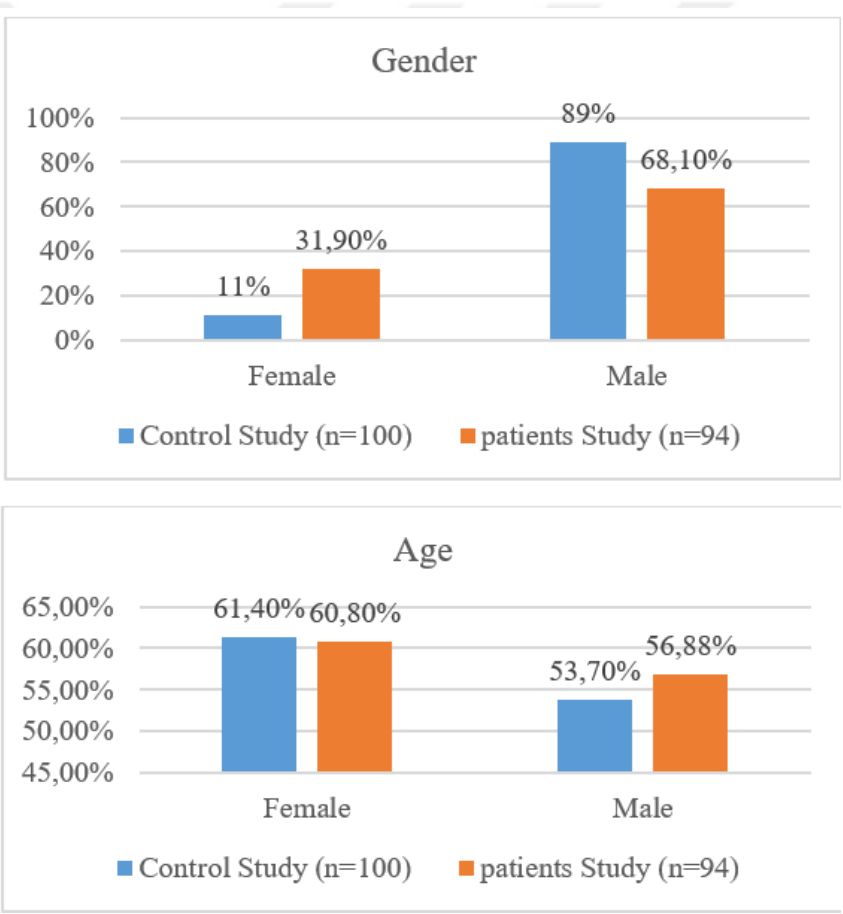

Figure 4.1 Characterization of the population in terms of gender and age

The values presented at the top of the columns refer to the mean value ± standard deviation. n refers to the sample size. The P values shown in the image were obtained by applying either the Student's t test (with n fewer than 30) or the Mann-Whitney test

(with n fewer than 30). (The significance level for all values was set at less than or equal to 0.05).

• The values presented at the top of the columns refer to the mean value ± standard deviation. n refers to the sample size.

The results of the Student's t test (n fewer than 30) or the Mann-Whitney test (n fewer than 30) were used to calculate the P values that are displayed in the image (The significance level for all values was set at less than 0.05). When it comes to gender distribution, both the control group and the sick group had a higher percentage of men. In both groups, the average male age was found to be considerably lower than the average female age (Figure 9). The average age of the women in both groups was not significantly different. When it came to men in the control group, they had a few years of age advantage over those who were in the ill group in terms of statistical significance (P = 0.004).

4.1.1 Biometric characteristics and lifestyles

The general characteristics of the individuals who made up the groups of patients and controls are outlined Table 4.1 shows.

Table 4.1 Biometric characteristics of the population

	Controls (n=100)	**Patients (n=94)**	***P***
BMI	26.3±4.6	28.3±4.5	0
Abdominal perimeter (with)	94.8±10.7	101.1±9.9	0
Systolic blood pressure (mmHg)	126.9±7.7	136.4±18.8	0
Diastolic blood pressure (DBP) (mmHg)	76.8±5.1	78.7±9.1	0.094
Heart rate (bt/min)	63.8±11.1	65.1±9.9	0.353

The results presented refer to the mean ± standard deviation values.

It was possible to calculate the P-value by using Student's t test (P values must be less than 0.05 to be considered statistically significant).

After determining the BMI, Individuals in the control group scored significantly lower than those in the experimental group (26.3±4.6) than individuals in the patient group (28.3±4.6) (P = 0.000), verifying the same condition in the value of the abdominal perimeter (P= 0.000). Control group blood pressure was lower than patient group SBP and DBP, respectively. The mean SBP was 126.9±7.7 mmHg in the control group, 136.4±18.8 mmHg in the patient group, and the mean DBP was 76.8±5.1 mmHg in the controls and 78.7±9.1 mmHg in the patient group. A statistically significant difference (P=0.000) was found between the two groups in SBP, but the difference in DBP (P=0.094) was not statistically significant.

Both groups had similar resting heart rates (P=0.353).

Comorbidities

Figure 10 shows the characteristics of the population with regard to comorbidities. Considering the BMI values, according to the WHO norms, 12 individuals (12.0%) were considered obese in the control group and 29 individuals (30.9%) in the patient group. Hypertensive and/or diabetic individuals were not admitted to the control group because hypertension and DM are factors changes in vitamin D concentration may be to blame. In contrast, the World Health Organization has established criteria for determining whether or not a substance is safe to consume, 94.4% of the patients in the patient group were categorized as having hypertension, and 29.8% of the patients were categorized as having diabetes. Although there were no diabetic subjects in the control group, 16.0% had insulin resistance, according to HOMA values. The percentage was higher in the patient group, in which 37 subjects (39.4%) were insulin resistant.

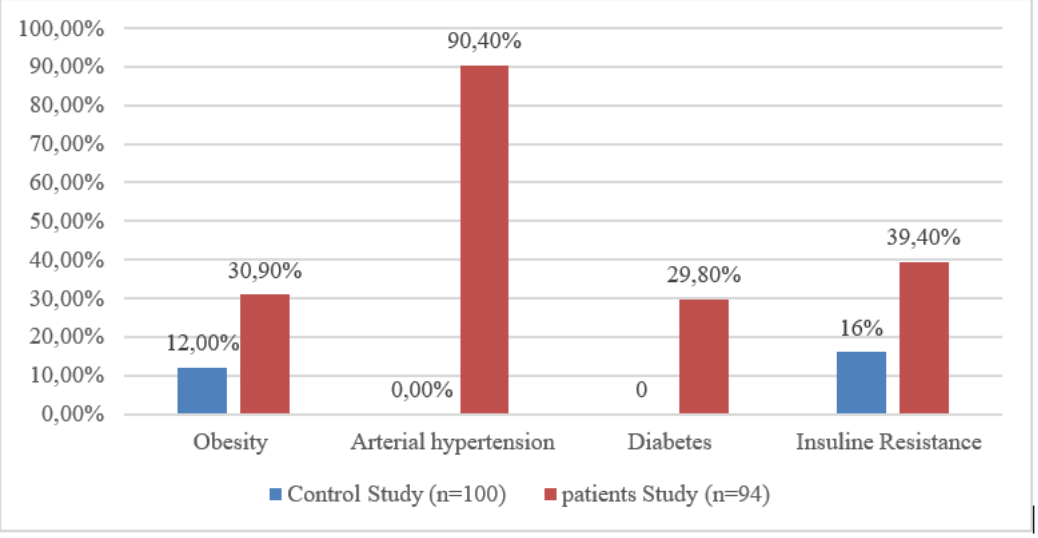

Figure 4.2 Comorbidities population

1. Obesity is defined as a BMI of 30 kg/m2 or higher (Pittas *et al.* 2010).

2. High blood pressure (SBP) greater than 140 mmHg and/or diastolic blood pressure greater than 90 mmHg, as well as the use of antihypertensive medication, were referred to as hypertensive conditions (Zittermann and Koerfer 2008).

3. In order to be diagnosed with diabetes, oral antidiabetic medication such as insulin had to be used if one's fasting glucose level was greater than 126 mg/dL

4. Insulin resistance was considered for HOMA values equal to or greater than 2.33. n-refers to the sample size.

Population lifestyle: The two groups (patients and controls) were characterized in terms of the individuals' lifestyles. Habits such as smoking, consumption of alcoholic beverages, and consumption of foods rich in vitamin DThe degree of sun exposure and level of physical activity were both taken into consideration. Additionally, the skin phototype of the individuals was evaluated. The parameters evaluated are shown in Table 4.2.

Table 4.2 Lifestyle of the population

	Controls		Patients		
Smoking	**n**	**%**	**N**	**%**	**P**
Yes	16	16	8	8.5	
Ex-smoker	3	3	28	29.8	--
No	81	81	58	61.7	
Consumption of alcoholic beverages					
Yes	76	76	37	39.4	--
No	24	24	57	60.6	
Consumption of foods rich in vitamin D					
Very frequent	4	4	4	4.3	
Frequent	67	67	49	52.1	0.097
Rarely	29	29	41	43.6	
Physical activity level					
Very active	6	6	--	--	
Active	64	64	70	74.5	0.024
Irregularly active	18	18	9	9.6	
Sedentary	12	12	15	15.9	
Sun exposure					
Low	18	18	25	26.6	
Moderate	29	29	28	29.8	0.286
High	53	53	41	43.6	
Skin phototype					
1	4	4	8	8.5	
2	23	23	19	20.2	
3	44	44	30	31.9	0.207
4	20	20	29	30.9	
5	9	9	8	8.5	
statins					
Take	8	8	66	70.2	--
Do not take	92	92	28	29.8	

Does not take 92-92.0-2- 29.8

The P value was calculated using the significance threshold of the chi-square test of homogeneity (Statistics were deemed significant for P values less than 0.05).

a) Never smoked or quit smoking for more than 5 years

b) Yes – at least once a week; No – less than once a week

c) Very frequent – 4 times a week or more; frequent - 1 to 3 times a week; rarely – less than 4 times a month.

d) High-intensity physical activity is defined as five or more days of intense activity per week, as well as an additional five days per week of moderate exercise. Regular physical activity is defined as having at least three days per month of moderate or vigorous exercise. The term "infrequently active" refers to someone who participates in only a few activities each week. A person who is sedentary does not do anything.

e) Low is defined as less than thirty minutes per day, moderate as thirty to sixty minutes per day, and high as sixty minutes or more per day.

f) Skin phototype: 1 – Very Light. It burns frequently. Never tans; 2 – Clara. It burns frequently. Sometimes tans; 3 – Medium light. Sometimes it burns. Often tans; 4 – Medium dark. It rarely burns. Often tans; 5 – Dark. It rarely burns. It tans a lot.

Smoking and consumption of alcoholic beverages: In the analysis of tobacco consumption (Table 4.2), it was found that 81 individuals (81.0%) in the control group and 58 individuals (61.7%) in the patient group were non-smokers, thus having a predominance of individuals non-smokers in both groups.

The main difference was observed in the percentage of ex-smokers. While only 3 individuals (3.0%) There were more ex-smokers in the patient group than there were in the control group, with 28 individuals (29.8%) ex-smokers. The consumption of alcoholic beverages It was discovered that in the control group (76.0 percent) than in the patient group, was a more common habit (39.4 percent) (Table 4.2).

Food: In the assessment of eating habits and, with regard to the consumption of foods rich in vitamin D, it was found that it found in the control group compared to those who received treatment (Table 4.2 and Figure 4.3). In any of the groups, few individuals (only 4 in each group) reported consuming foods rich in vitamin D more than 3 times a week. However, it was possible to verify that, in the control group, a greater number of individuals (67.0%) reported consuming foods rich in vitamin D between 1 and 3 times a week, than in the patients group (52, 1%). Finally, while in the group of controls only

29 individuals (29.0%) reported consuming Vitamin D-rich foods are extremely rare. This higher proportion of patients was discovered to exist (41 individuals; 43.6%). In the homogeneity chi-square test, there was no statistically significant difference between the two groups in terms of eating patterns (P=0.097).

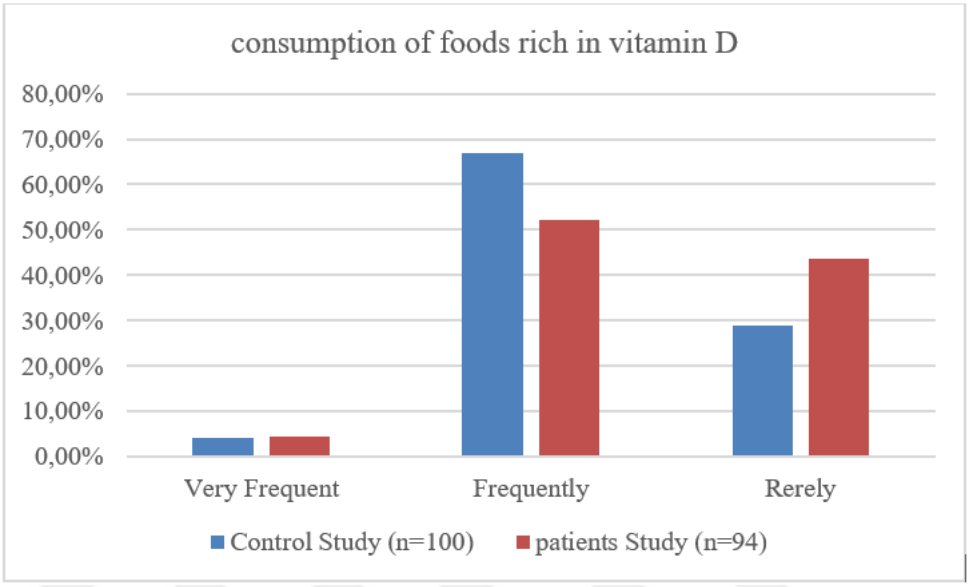

Figure 4.3 Lifestyle of the population: consumption of foods rich in vitamin D

1Very frequent – 4 times a week or more; frequent - 1 to 3 times a week; rarely – less than 4 times a month. The chi-square test of homogeneity was used to calculate the P value (We defined statistical significance as being less than 0.05 as the smallest p value). n refers to sample size.

Physical activity: The amount of difference between the patient and control groups of time spent engaging in physical activity did not reveal any significant differences (Table 4.2). It was believed that the majority of the subjects participated in both of the groups (controls 64.0 percent ; patients 74.5 percent). The percentage of people leading sedentary lifestyles did not significantly differ between the groups either. 12.0 percent of the subjects in the control group could be classified as sedentary, while 15.9 percent of the subjects in the patient group fit that description. It is important to point out that none of the patients in the sample could be described as being particularly active.

Sun exposure and skin phototype: The evaluation of the amount of time spent in the sun revealed that the two groups were comparable in many ways (Table 4.2 and Figure 4.4). After verifying, using the homogeneity test of the Chi-Square, When it comes to sun exposure, there were no statistically significant differences (P=0.286) between the two groups, the majority of the individuals in both the control group (53.0 percent) and the patient group (43.6 percent) claimed to be exposed to the sun for more than one hour a day. Patients and controls alike were found to have the same results. As a result of this study, approximately 26.6% of all patients spent less than 30 minutes a day outside of their homes, compared to 16.4 percent of those in the control group (18.0 percent). In both groups, the majority of people had skin phototypes 2, 3 and 4, with a small number of people having skin types 1 and 5. (Table 4.2 and Fig. 4.4).

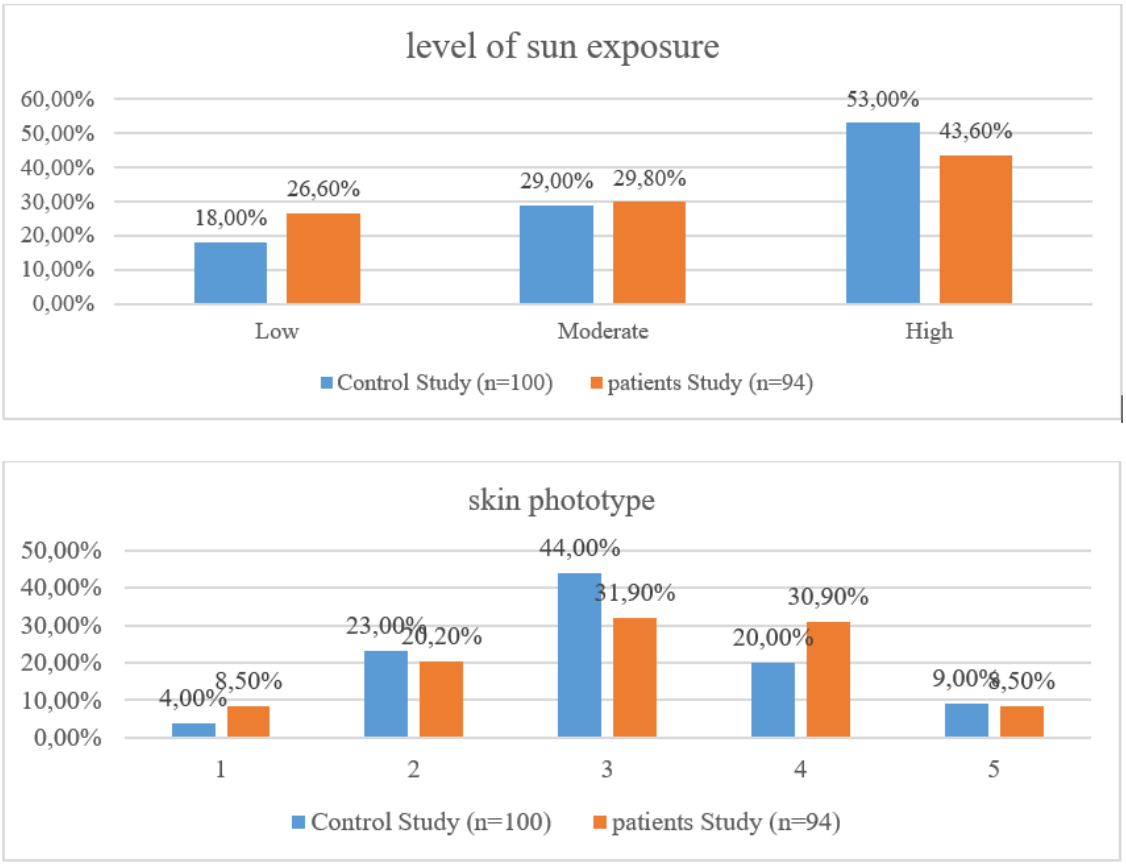

Figure 4.4 Lifestyle of the population: level of sun exposure and skin phototype

1) A person should limit their time spent in the sun to 30 minutes or less, 30 to 60 minutes, or more than 60 minutes each day.

2) Skin phototype: 1 – Very Light. It burns frequently. Never tans; 2 – Clara. It burns frequently. Sometimes tans; 3 – Medium light. Sometimes it burns. Often tans; 4 – Medium dark. It rarely burns. Often tans; 5 – Dark. It rarely burns. It tans a lot. The P value was obtained by applying the chi-square test of homogeneity (There was no statistical significance for any value lower than 0.05) n refers to the sample size.

Taking statins: Finally, another of the characteristics studied was taking medication (Table 4.4). It was found that in the group of patients, the use of statins was much more frequent (70.2%) than in the group of controls (8.0%).

4.1.2. Biochemical characteristics

All subjects underwent routine laboratory tests that were supplemented with specific laboratory tests. Initially, all individuals underwent a blood count and, since renal and hepatic insufficiency were considered as exclusion criteria, biochemical tests were performed that allowed the evaluation of renal function and liver functionFigures 4.5, 4.6, 4.7, and 4.8 show the results of these laboratory tests. There were no patients with hepatic or renal impairment in the study participants.

Cardiovascular risk assessment

In order to assess the cardiovascular risk of individuals, several biochemical parameters were determined, These findings are shown in Figures 4.5, 4.6, 4.7, and 4.8, respectively. The lipid profile was analyzed and the fasting serum glucose and insulin concentrations, the percentage of HA1C and the HOMA value for insulin resistance were determined. In addition, some laboratory parameters that are related to increased cardiovascular risk such as fibrinogen, homocysteine and hsCRP were also determined. The Mann-Whitney test ($n<30$) or the Student's t test ($n\geq30$) to determine the significance of the P value (Statistical significance was defined as a P value of 0.05 or less).

Lipid profile: Total cholesterol levels in the bloodstream (208.3±39.5 mg/dL), High density lipoprotein (HDL) (52.7±12.6 mg/dL), and LDL cholesterol (139.4±34, 1 mg/dL) controls had a higher incidence of disease than patients (188.4±48 mg/dL; 46.2±12 mg/dL and 113.7±39.1 mg/dL respectively). On the other hand, the group of patients had mean concentrations of triglycerides and Lp(a) in contrast to the group of controls. However, these differences only showed statistical significance (P = 0.022) for the concentration of Lp(a) (Figure 4.5).

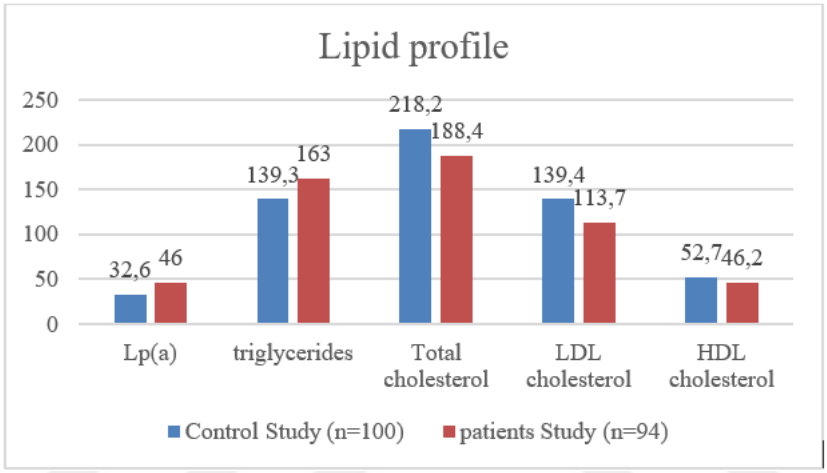

Figure 4.5 Lipid profile

The numbers shown with the bars correspond to the average minus the standard deviation of the results.

Applying the Student's t-test allowed for the calculation of the P values that are displayed in the image (All P values with a value that is less than 0.05 will be regarded as statistically significant).

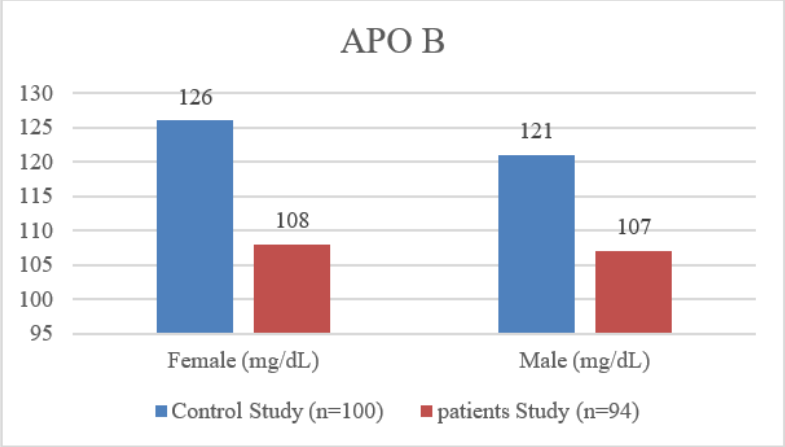

Figure 4.6 Serum concentration of APO B

The values presented at the top of the columns refer to the mean value ± standard deviation. n refers to the sample size. The P values presented in the figure were obtained using either a Student's t test ($n \geq 30$) or a Mann-Whitney test ($n < 30$) (It was determined that any P value that was less than 0.05 was statistically significant).

Because the APO B reference values for men and women are different, statistical analysis was conducted for this parameter based on gender (Figure 14). Among both the healthy controls and the patients, females had higher serum concentrations of APO B than did males. Patients in the patient group had lower APO B concentrations (109±28.1 mg/dL in women, and 108±25.6 and 107.2±27.6 mg/dL in women and men) than those in the control group (126.1±27.1 mg/dL in women, and 122±30.1 mg/dL in men). Student's t test was used to see if statistically significant differences existed between the two groups. Males and females differed statistically (P=0.001), There was, however, a statistically insignificant difference in results. A statistically insignificant difference was found between males and females (P=0.102).

Glucose, HA1C and Insulin: Serum glucose and insulin concentrations were determined in the fasting state and the percentage of HA1C was evaluated for all subjects. The results are shown in Figure 15.

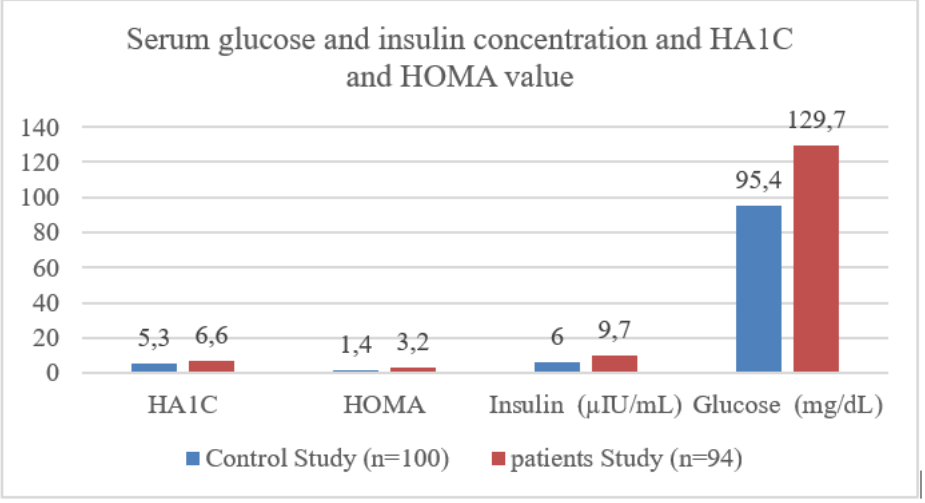

Figure 4.7 Serum glucose and insulin concentration and HA1C and HOMA values

As you can see from these numbers, they're averages rounded up to their nearest standard deviation. The P values depicted in the image were obtained using the Student's t test (Statistical significance was considered to exist for all P values less than 0.05). Study participants with diabetes had lower HA1C percentages, lower blood glucose and insulin concentrations, and lower HOMA values than those without diabetes.

Fibrinogen, hsCRP and homocysteine: Fibrinogen, hsCRP, and homocysteine levels in both groups' blood were measured, and the results are shown in Figure 4.8.

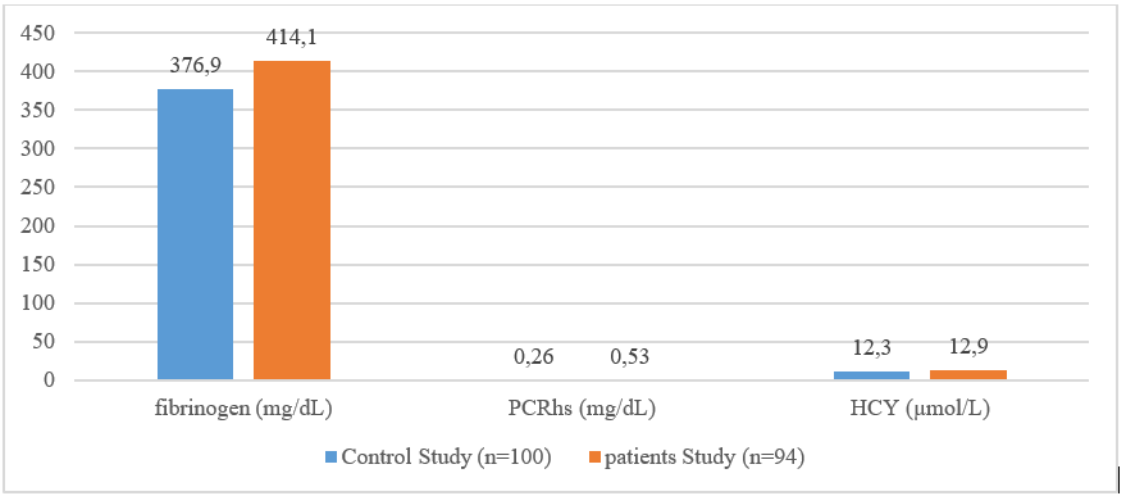

Figure 4.8 Serum concentration of fibrinogen, hsCRP and homocysteine

As you can see from these numbers, they're averages rounded up to their nearest standard deviation. To arrive at the P values displayed in the image, The Student's t test was used (Any P-value less than 0.05 was considered statistically significant).

A significant difference in fibrinogen levels was found between patients and controls (P=0.003). When it came to hsCRP, the sick group had a higher concentration than the control group, which was statistically significant (P=0.027).

The differences in homocysteine levels between the patient and control groups were insignificant (P=0.311), despite a slightly greater concentration in the patient group.

4.2 Vitamin D [25(OH)D]

4.2.1 Determination of the serum concentration of 25(OH) D

Figure 4.9 depicts blood samples from the two study groups were compared for 25(OH)D serum levels.

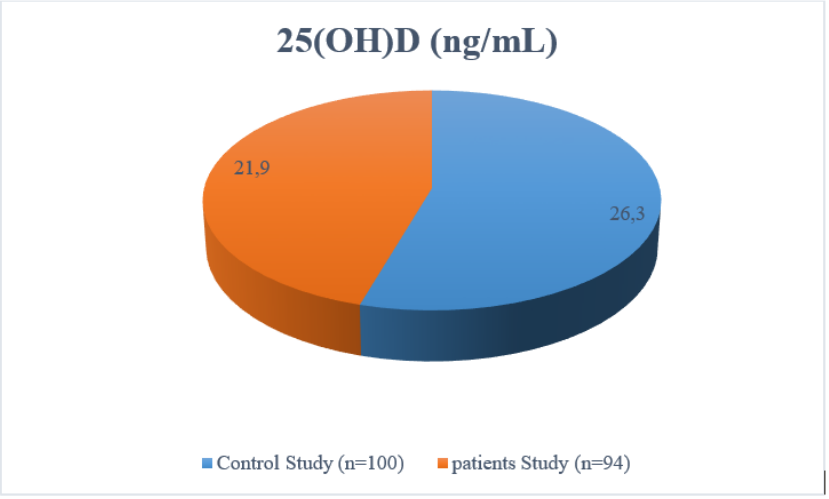

Figure 4.9 Serum concentration of calcifediol

Top-column figures relate to mean values minus standard deviations.

To get this P value, we used a method known as the Student's t test: Statistical significance was assumed to exist for any P values less than 0.05.

Serum Vit-D in both the control and the treatment group were found to be higher in the former than in the latter, with the patient group showing a mean value lower. A statistically significant (P= 0.001) difference was found between the two groups using the Student's t test. However, despite the fact that the scientific literature does not differentiate between male and female Vit-D concentrations, significant variations were identified in the group of patients (P=0.021). Both men and women in the control group (P=0.067) did not show a significant difference between the two sexes (Figure 4.10).

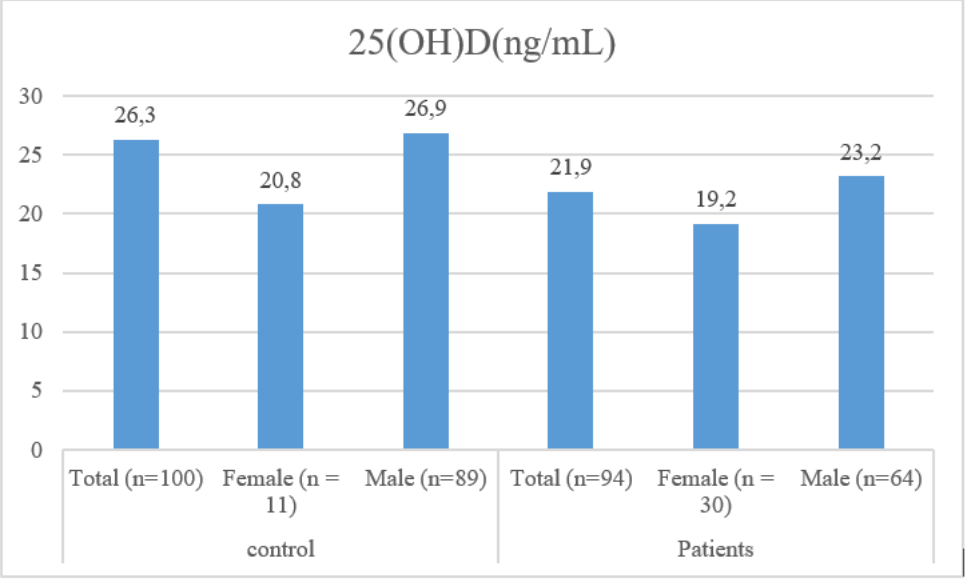

Figure 4.10 Serum concentration of Vit-D according to gender {P: 0.067 for control group and 0.021for patient group}

Statistically substantial disparities in the mean serum concentrations of men and women in both research groups led to the decision to compare them. This difference was statistically significant (P=0.009) between males and females (Table 4.4). Chi-square tests of homogenity were utilized after determining that the proportions of men and

women in patients and controls differed statistically significantly, taking into consideration the prior results. (P=0.000).

4.2.2 Assessment of vitamin D status

The serum concentration of Calcifediol is determined by the value, the vitamin D status of each individual was determined, that is, it was verified whether this value corresponded to one of the following categories: sufficient, insufficient or deficient in vitamin D For each of the groups studied, the percentage of individuals in each of the vitamin D categories was determined.

Only a small percentage of the individuals in both groups were able to meet the recommended levels of vitamin D, as shown in Figure 4.11.

46% of patients evaluated had vitamin D insufficiency compared to a lower number of control subjects (28.0 percent). Only a small fraction of the control group had vitamin D deficiency. However, no discernible difference could be found between the two groups. In the control group, 31.0 percent of the participants were found to have appropriate vitamin D blood concentrations, but only 17.0 percent of the subjects in the sick group reached this criteria.

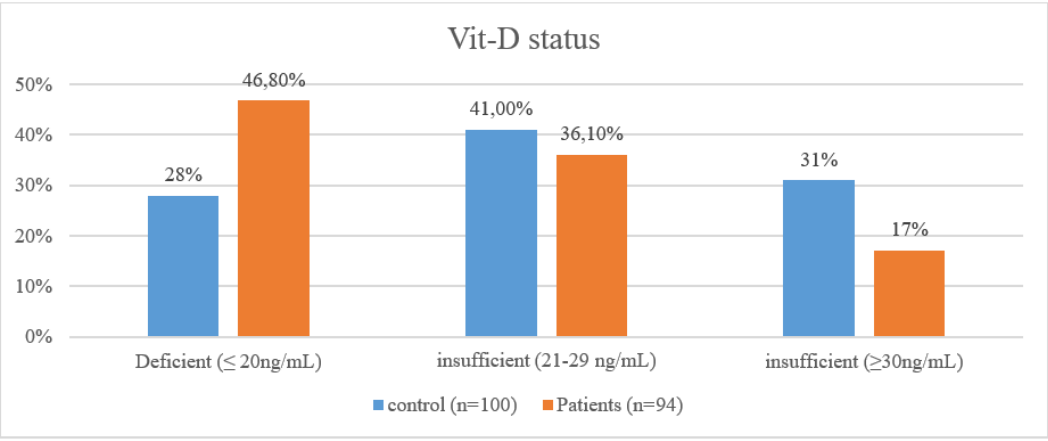

Figure 4.11 Vitamin D status of the population, according to the individuals' serum Vit-D concentration.

4.2.3 Serum concentration of Vit-D as a function of population comorbidities

Vitamin D levels in the blood were linked to obesity, insulin resistance, hypertension, and type 2 diabetes, according to this study. This image displays the results of our research (Figure 20). Vitamin D levels are lower in obese people than in non-obese people. Both the control (P=0.203) and patient (P=0.174) groups showed no statistically significant difference in this measure. Obese people in the sick group were found to have a lower concentration of vitamin D than non-obese controls, with a significant difference between the two groups. a difference between two groups of patients that is statistically significant

Both insulin-resistant and non-insulin-resistant groups had reduced blood levels of vitamin D, despite the fact that it was insignificant.

A significant difference (P=0.003) was seen in blood Vit-D levels between those of people without insulin resistance and those of patients with the same disease.

Because there were no diabetics or hypertensives in the control group, it was difficult to relate vitamin D's value to these illnesses. In this research, In this study, diabetic and non-diabetic participants had different levels of vitamin D (P=0.005).

Hypertension patients showed greater Vit-D concentrations than healthy controls, The difference was statistically insignificant (P=0.542), despite this fact However, there was no statistically significant difference in the Vit-D concentrations between controls and patients without HTA (P=0.089).

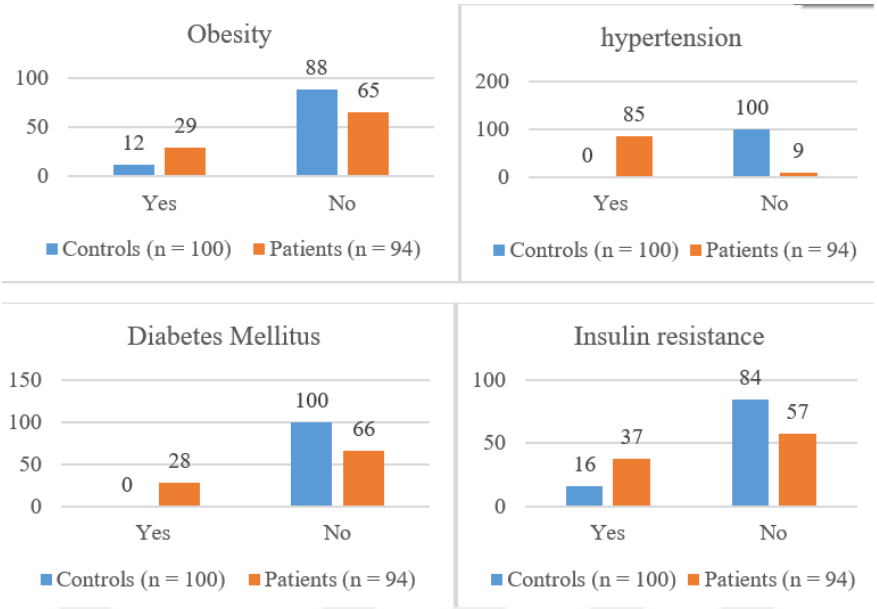

Figure 4.12 Serum concentration of Vit-D as a function of population comorbidities

The values presented at the top of the columns refer to the mean value ± standard deviation.

The P values depicted in the figure were computed using either the Mann-Whitney test ($n<30$) or the Student's t test ($n\geq30$) (Statistical significance was defined as a P value of 0.05 or lower). Size of the sample is expressed in terms of n.

a) Obesity is defined as a BMI of 30 kg/m2 or higher.

b) Those who had SBPs of 140 mmHg or higher and/or DBPs of 90 mmHg or higher were considered to have hypertension.

c) In order to be diagnosed with diabetes, one must have a fasting glucose level of at least 126mg/dL and/or be taking insulin or an oral antidiabetic medication.

d) Insulin resistance was considered for HOMA values equal to or greater than 2.33.

4.2.4 Serum concentration of Vit-D as a function of the population's lifestyle

In Table 4.3, you can see how Vit-D levels differ between the two groups of people studied (controls and patients).

Table 4.3 Serum concentration of Vit-D as a function of the population's lifestyle

	Controls (n = 100)	**Patients (n = 94)**	***P***
Smoking[1]	Vit-D (ng/mL)	Vit-D (ng/mL)	
yes	24.1 ± 7.2	18.7 ± 5.9	0.106
Former smoker	27.1 ± 0.98	23.7 ± 8.6	0.59
None	25.92 ± 8.9	21.5 ± 9.0	0.002
Consumption of alcoholic beverages[2]			
yes	26.6 ± 9.0	23.8 ± 7.5	0.107
None	25.2 ± 10.7	20.7 ± 9.3	0.075
Consumption of foods rich in vitamin D[3]			
Very Frequent	33.1 ± 14.6	22.0 ± 3.0	0.343
Frequent	25.8 ± 8.7	22.9 ± 8.6	0.072
Rarely	26.4 ± 10.1	20.8 ± 9.3	0.032
Physical activity level[4]			
very active	34.6 ± 7.5	--	--
Active	26.9 ± 9.1	22.2 ± 8.6	0.002
irregularly active	24.0 ± 9.1	22.5 ± 9.3	0.668
Sedentary	22.2 ± 9.4	20.5 ± 9.3	0.614

The P value was calculated using either the Mann-Whitney or the Student's t test (n=30) (Statistical significance was defined as a P value of 0.05 or lower).

1 - Never smoked or quit smoking for more than 5 years

2 - Yes – at least once a week; No – less than once a week

3 - Very frequent – 4 times a week or more; Frequent - 1 to 3 times a week; Rarely – less than 4 times a month.

4 - a high level of physical activity, defined as at least five days per week of vigorous activity, or three days per week of vigorous activity combined with five days per week

of moderate activity. At least three times a week, or five times a week, you should engage in some form of physical activity. Irregularly active - less than three times a week; moderate activity To be sedentary means to be inactive.

Serum concentration of Vit-D as a function of smoking and alcohol consumption

The concentration of Vit-D was evaluated as a function of the consumption of tobacco and alcoholic beverages. Table 4.3 shows the findings. Nonsmokers had higher levels of vitamin D than smokers, despite the fact that this difference was statistically insignificant (P=0.848 in the control group; P=0.301 in the sick group) in both groups.

Serum Smokers, ex-smokers, and non-smokers. Patients had significantly lower vitamin D concentrations than control subjects. However, comparing the two groups studied, by category, this difference was only considered significant for the non-smoking individuals (P= 0.002).

When compared to those who never drink alcohol, Vitamin D levels in the blood of regular drinkers were higher than those of non-drinkers. Patients who drank alcohol and those in the control group had lower levels of vitamin D, as was the case with those who smoked. All of these differences, however, were negligible.

Serum concentration of Vit-D as a function of food

According to the results of an investigation into the relationship between vitamin D intake and levels, Table 4.3 shows the findings. Vitamin D levels were greater in the control group (33,1 14,6 ng/mL) than in the group that did not consume foods high in vitamin D. Vitamin D concentrations in the same group did not differ statistically significantly between those who consumed vitamin D-rich foods frequently and those who did not.

On the other hand, in the group of patients, the concentrations of Vit-D were very similar in individuals who consumed foods rich in vitamin D very often, frequently or who consumed them rarely. P=0.323 and P=0.591, respectively, were statistically insignificant differences in comparison to the control group.

Although it was found that regardless of how often they ate foods rich in vitamin D, As a result, patients' vitamin D concentrations were lower than those in the control group, this difference was significant in those who rarely ate foods rich in vitamin D (P=0.032).

Serum concentration of Vit-D as a function of physical activity

Table 4.3 displays the relationship between Vit-D concentration and physical activity level in individuals.

Calcifediol concentrations increased with increasing levels of physical activity in the control group (P=0.035), and the statistical significance of these differences was clear.

Sedentary people had lower levels of Calcifediol than active people, despite the fact that the difference was insignificant statistically.

When comparing the same amount of physical activity between the patient and control groups, researchers discovered that the Vit-D concentrations of the patients were lower. This difference proved to be statistically significant in active individuals (P=0.002).

Serum Vit-D concentration as a function of time of year, sun exposure and skin type

Serum vitamin D levels are affected by the season. Individuals recruited in the summer months and those recruited in the winter months were both studied for the mean serum concentration of vitamin D. Table 4.4 displays the findings The months of May to

October were considered as summer months, as they are the months with the highest sun exposure and milder temperatures, and the remaining months (November to April) were considered summer months. Winter.

In addition, the amount of sun exposure that individuals in both groups received as well as their skin type were taken into consideration when calculating the mean serum concentration of vitamin D.

Table 4.4 Serum concentration of Vit-D according to time of year, sun exposure and skin phototype

	Controls (n = 100)	**Patients (n = 94)**	
Time of year[1]	Vit-D (ng/mL)	Vit-D (ng/mL)	***P***
Summer months	27.8.7 ± 8.4	23.5 ± 7.7	0.011
Winter months	20.7 ± 6.6	18.9 ± 9.0	0.143
Sun exposure[2]			
Low	21.2 ± 6.1	16.9 ± 9.6	0.195
Moderate	25.7 ± 7.6	18.7 ± 6.2	0.016
High	28.2 ± 8.3	25.9 ± 8.1	0.081
Skin phototype[3]			
1	21.1 ± 7.4	20.9 ± 10.1	1.000
2	21.9 ± 7.3	18.1 ± 8.2	0.026
3	27.4 ± 8.5	24.02 ± 9.1	0.042
4	27.5 ± 7.2	22.2 ± 7.7	0.057
5	20.5 ± 9.98	20.9 ± 6.7	0.961

Serum Vit-D concentration is shown as mean ± standard deviation.

The Mann-Whitney test ($n<30$) or the Student's t test ($n\geq30$) were used to determine the P value (Statistical significance was defined as a P value of 0.05 or less).

1. Summer (May to October) and Winter (November to April)

2. Low is defined as less than thirty minutes per day, moderate as thirty to sixty minutes per day, and high as sixty minutes or more per day.

3. Skin phototype: 1 - Very Light. It burns frequently. Never tans; 2 – Clara. It burns frequently. Sometimes tans; 3 – Medium light. Sometimes it burns. Often tans; 4 – Medium dark. It rarely burns. Often tans; 5 – Dark. It rarely burns. It tans a lot.

For both controls and patients, A notable distinction existed between the two teams (P=0.000; P=0.001) when subjects were recruited in summer as compared to winter. Participants in the study had lower levels of vitamin D than the control group, the researchers discovered, in the summer recruiting group, however, this difference was statistically significant (P=0.011).

The control group's Calcifediol serum concentrations were found to be higher (P= 0.001) and in the sick group (P=0.000) when the amount of time spent outside was longer. This difference was significant in both groups. Regardless of the amount of sun exposure, none of the groups had adequate levels of Calcifediol. Between the control group and the patients who had been exposed to moderate amounts of sunlight, there was a significant difference (P=0.016) between Vit-D concentrations in the patient group and the control group.

Serum Vit-D concentrations were higher in people with skin types 3 and 4 than in controls with the same skin type, however, patients' levels of serum vitamin D were lower. Patients with skin type 2 (P=0.026) and 3 (P=0.042) differed significantly from controls (P=0.026).

4.2.5 Biochemical tests as a function of vitamin D status

Lipid profile: Vitamin D deficiency, normal levels of vitamin D, healthy levels of vitamin D were the four categories of participants in the study. Table 4.5 shows the outcomes.

When vitamin D sufficiency was present in the control group, total cholesterol levels were lower than when vitamin D deficiency was present. There was no statistically significant change in any of the cases.

However, the difference between the deficient and healthy control groups was not statistically significant in terms of HDL cholesterol concentrations. Because of this, the healthy and ill groups were statistically distinct.

There was a significant difference in LDL cholesterol levels between those in the Vit-D-sufficient group and those in the control group, even if statistical significance was lacking. Even yet, the statistical significance of the two results was so minute as to be meaningless in this case.

Vitamin D deficiency has been linked to an increased risk of cardiovascular disease, however a statistically insignificant difference was detected between the two groups of individuals in this study.

For women with vitamin D deficiency, the APO B levels were higher than for those with normal Calcifediol values, even though statistically no difference was found between the two groups. Vitamin D deficiency has been linked to a higher level of APO B in men compared in those with adequate levels of vitamin D. The outcomes for the patients varied widely. APO B concentrations were lower in guys who were deficient in vitamin D compared to those who received adequate levels. According to the findings, there were no significant differences among the groups.

Lp(a) concentrations were higher in the vitamin D-sufficient group than in the vitamin D-deficient group in both the control and patient groups.

Insulin, glucose, and HA1C: Glucose, insulin, and HbA1C levels were shown to be correlated with vitamin D status across all participants in this study. Participants with adequate amounts of vitamin D had lower glucose values than those with inadequate

levels of vitamin D. Individuals with vitamin D deficiency had higher HA1C values than those with adequate vitamin D levels.

The blood levels of fibrinogen, hsCRP, and homocysteine were examined and compared to the vitamin D status of the participants as shown in Figure 4.13.

People with adequate levels of vitamin D had lower levels of fibrinogen than those with insufficient levels of vitamin D, in both the healthy and ill groups. Despite the absence of a statistically significant difference, those with low levels of vitamin D had reduced fibrinogen in both groups.

Vitamin D deficiency was connected to higher hsCRP levels than appropriate levels, however this was not statistically significant. There was a correlation between vitamin D insufficiency and decreased circulating levels of CRPhs, however it is crucial to note that individuals who were deficient in vitamin D also had lower levels of CRPhs in the control group than those who had sufficient vitamin D.

Homocysteine levels in the blood were drastically different between the two groups. Homocysteine concentrations were lower in individuals with enough vitamin D in the control group than those with deficiency, but this was reversed in patients. Deficiency patients had lower homocysteine levels than controls, although the control group had higher homocysteine levels. Despite this, the groups did not differ significantly.

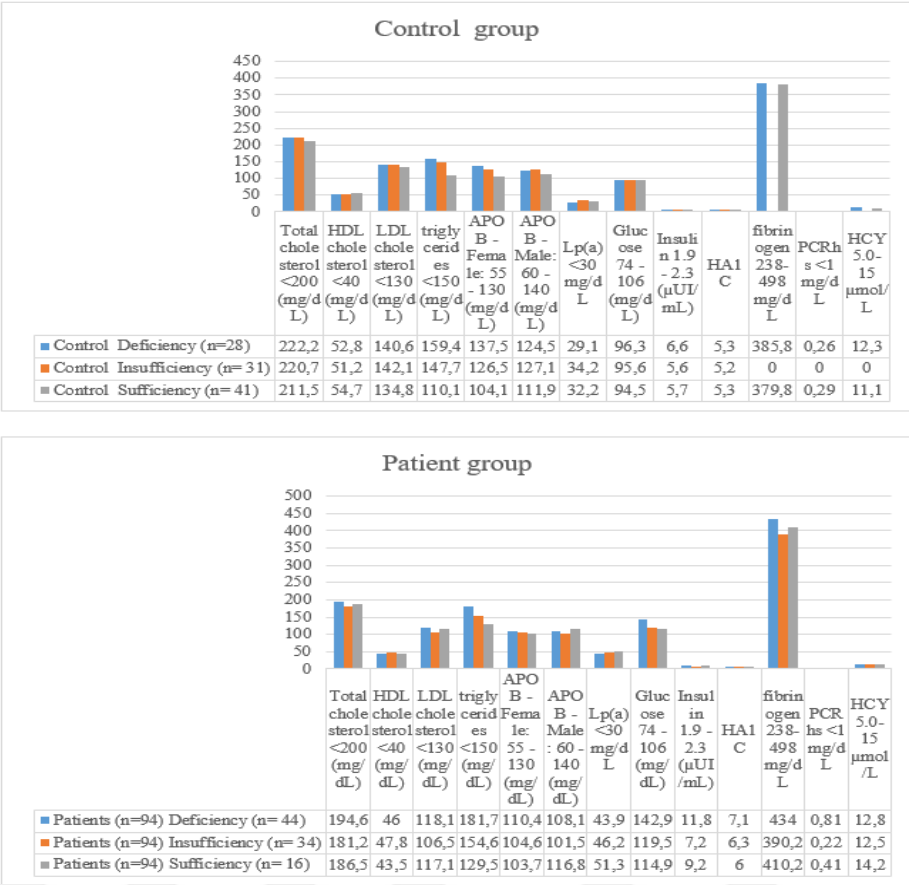

Figure 4.13 Biochemical tests as a function of the vitamin D status of individuals

The mean ± standard deviation are shown in the results. The Kruskal-Wallis test was used to get the P value (Statistical significance was defined as a P value of 0.05 or less). (SD ± 5.0 to 50); p value 0.034 to 0.864 for control group and 0.009 to 0.87for patient group

4.2.6 Serum concentration of Vit-D as a function of all factors studied

The fact of testing each of the independent variables separately, as a function of the response variable, may introduce a bias in the study. After the statistical tests already presented, a Double Analysis of Variance was carried out in which the response variable was vitamin D and the independent variables, Controls, Patients and Gender, in order to verify if there was interaction. It was found that there was no interaction and the sex was significant. From here it was possible to join Controls and Patients in the study. A

simultaneous analysis of all the variables studied was then performed through the application of a Logistic Regression to determine possible risk factors (probabilities, as well as their respective 95% confidence intervals). The variable that most influenced the serum concentration of Vit-D was the height of the individuals (P=0.033).

4.2.7 Vit-D concentration by electrochemiluminescence and by immuno chemiluminescence

In the present work, 70 of the 194 samples were tested by two tests from two different commercial houses, in order to compare a new immunochemiluminescence method with the existing electrochemiluminescence method. These samples were tested by Roche's Vitamin D Total Test, which is an electro chemiluminescence immunoassay, and by Beckman Coulter's Vitamin D Total Test, which is a chemiluminescent immunoassay. The results of this comparison are shown in Figure 21Vit-D levels in these 70 samples were slightly different, but this difference was not considered statistically significant (P=0.099) when analyzing data.

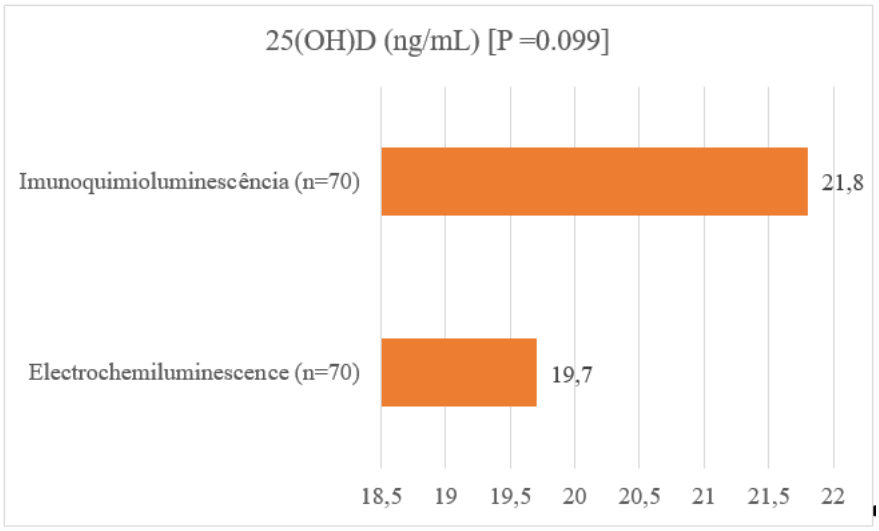

Figure 4.14 Vit-D concentration by electro chemiluminescence and immuno chemiluminescence

Serum Vit-D concentration is shown as mean ± standard deviation.

The P value was determined using the Student's t test (P values lower than 0.05 were considered statistically significant).

4.3 Discussion

The initial objective of this research was to examine whether or not there was an association between vitamin D insufficiency and cardiovascular disease in a sample of Madeira residents who were found to be vitamin D deficient.

For this, 194 subjects divided into two groups (There were 100 healthy people and 94 people with heart disease) were studied and it was found that healthy subjects had a higher serum concentration of Vit-D (26.3±9, 4 ng/mL) than individuals with cardiovascular disease (21.9±8.7 ng/mL), P = 0.001 indicates that the difference is statistically significant Patients with coronary heart disease (46.8%) were found to be more likely than healthy subjects to be vitamin D deficient (28.0 percent). Several other studies have found similar outcomes, so these aren't entirely unexpected. The Fragmingham Offspring Study (Christakos *et al.* 2013) found a link between low levels of 25 (OH)D and an increased risk of cardiovascular events in general (Schöttker *et al.* 2013) and that vitamin D deficiency was an independent risk factor for cardiovascular disease mortality in populations without a history of cardiovascular disease. ADE was similarly linked to reduced vitamin D levels in the Health Professionals Follow-up research (VanPutte *et al.* 2019). Vitamin D levels in the blood seem to be an additional predictor of problems after an AMI or cardiac surgery (Dusso 2011).

It's also possible that vitamin D deficiency isn't a direct cause of cardiovascular disease, but rather an indirect risk factor that affects inflammatory markers and has potentially fatal consequences.

This means that there has been no proof of a causal link between vitamin D insufficiency and cardiovascular disease (CVD), and some research even suggests that poor lifestyle choices may be to blame for vitamin D deficiency (Van Holten *et al.*

2013). This research demonstrated a statistically significant difference in vitamin D levels between healthy persons and those with cardiovascular disease. In the patient group, the difference in Vit-D concentration between men (23.2±7.9 ng/mL) and women (19.2±9.8 ng/mL) showed statistical significance (P= 0.021). Males (26.9±9.1 ng/mL) and females (20.8±9.7 ng/mL) had different levels of vitamin D in the control group, This difference was statistically insignificant (P=0.067), despite this fact. Even though there aren't many studies on the topic, some research suggests that this distinction does exist and should be researched. Vitamin D insufficiency in women is linked to a more severe type of coronary heart disease than in males, according to a research published in 2015. Because vitamin D concentrations of some sex hormones might be affected, generic variations in 25(OH)D levels are to be anticipated. The proportion of fat mass in women is also larger, as is the amount of subcutaneous fat in women. Women's adipose tissue should have more vitamin D than men's since vitamin D is fat-soluble. However, many studies fail to account for the fact that vitamin D levels in the circulation vary between men and women.

A possible explanation for the difference found in the concentration of Vit-D between controls and patients could have been the different proportion of men and women in the two groups (controls and patients). To verify this possible difference, the chi-square test of homogeneity was applied, which indicated that there was a significant difference in that percentage. A possible explanation for the discrepancy between the concentrations of vitamin D in men and women found in this study is that of age. Taking into account the average age of those who took part in the research, it is possible to see that, in the control group, women were older (61±4.1) than men (53±7.1). Likewise, in the group of patients, women were also older (60±8.0) than men (56±8.8). It is known that age is an important factor in the cutaneous synthesis of vitamin D because, with increasing age, the synthesis of vitamin D tends to decrease for two main reasons, which are the thickening of the skin and the decrease in 7- dehydrocholesterol (Mandarino *et al.* 2015).

In addition to factors such as gender and age, it is necessary to take into account some aspects that could influence the results, such as obesity, smoking and physical inactivity

(Van Holten *et al.* 2013, Gunta *et al.* 2013, Pludowski *et al.* 2013). To avoid these confounding factors, the population was evaluated for the existence of comorbidities and the population's lifestyle was evaluated in terms of smoking, consumption of alcoholic beverages, The amount of physical activity and consumption of vitamin D-rich foods. Researchers also tallied the participants' level of sun exposure and skin type.

4.3.1 Biochemical tests as a function of vitamin D status

Vitamin D and the lipid profile: Interestingly, and contrary to what would be expected, the control group had a higher mean total cholesterol concentration (208.3±39.5 mg/dL) than the patients group (188.4±47.2 mg/dL). However, in the patient group there was a higher percentage of individuals taking statins (70.2%) than in the control group (8.0%), which is the possible explanation for this result.

One concept that supports the significance of vitamin D in cardiovascular disease is the link between vitamin D and lipid profile.Although there were no statistically significant differences, lower total cholesterol values were obtained in individuals with a concentration of Vit-D greater than 30 ng/mL (211.5±35.4 mg/dL in the group of controls and 186.5±36.3 mg/dL in the patient group) than in vitamin D-deficient subjects (222.2±44.0 mg/dL in the control group and 194.6±50.5 mg /dL in the patient group). As for HDL cholesterol, it was found that, in the control group, individuals with vitamin D deficiency had a lower HDL cholesterol concentration (52.8±11.9 mg/dL) than individuals with enough vitamin D (54.7±16.3 mg/dL). However, this was not the case in the patient group (P=0.461), where the contrary was found. Vitamin D deficiency may have contributed to this finding in the group of patients, since just 16 of them had adequate levels. People with a concentration of Vitamin D greater than 30 ng/mL had a lower level. Vitamin D-deficient subjects had higher levels of LDL cholesterol (134.8±29.3 mg/dL versus 117.1±32.1 mg/dL) than those with normal levels (140.6±38.2 mg/dL versus 118.1±46.0 mg/dL). However, there was no statistical significance in any of the groups.

The triglyceride concentration was also higher in subjects with vitamin D deficiency (159.4±120.6 mg/dL in the control group and 181.7±152.6 in the patient group) than in those who were deficient in vitamin D. vitamin D (110.1±35.9 mg/dL in the control group and 129.5±56.3 mg/dL in the patient group), despite the fact that this difference was not statistically significant. People with a vitamin D deficit had considerably higher levels of total cholesterol than those in the control group, according to another research (Sun *et al.* 2014). Greater vitamin D status is also connected with higher HDL cholesterol levels and lower LDL and triglyceride cholesterol levels.

A association between vitamin D and cholesterol may be linked to photometabolism, although the specific mechanism remains a mystery. In the presence of sunlight, squalene present in the skin is converted to 7-dehydrocholesterol and vitamin D, while in the absence of sunlight, it is metabolized to cholesterol (Alves *et al.* 2013). Furthermore, intestinal calcium absorption is thought to be a mechanism for lower triglyceride concentrations in individuals with sufficient Vit-D concentrations. When vitamin D promotes calcium absorption in the intestines, it raises calcium levels in the blood, which lowers triglyceride levels in the bloodstream by decreasing liver triglyceride production and secretion (Alves *et al.* 2013).

Vitamin D levels appear to be linked to APO B serum levels, as well. The concentration of APO B appears to be higher when there is a severe lack of vitamin D than when there is an adequate supply. APO B concentrations were higher in women with vitamin D deficiency, both in the control group (137.5 ±32.5 mg/dL) and in the patient group (110.4 ±29.9 mg/dL), but there was no statistically significant difference in either group. A higher level of APO B was found in people with vitamin D deficiency (124.5 ±35.6 mg/dL) in men in the control group compared to individuals with adequate vitamin D levels. At least (111.9 ±2.4 mg/dL). A different outcome was seen in the patient group. Those men who were deficient in vitamin D had a lower concentration of APO B in their blood (108.1 ±29.1 mg/dL) than did those who had adequate levels of vitamin D (116.8 ±27.6). Despite this, there was no difference that could be considered statistically significant. Again, this patient group's outcome may be influenced by the fact that people with CVD who also have adequate levels of vitamin D are a rare breed.

Despite several studies associating a desirable concentration of Vit-D with a more favorable lipid profile, there are few data linking Vit-D with Lp(a). Lp(a) concentrations were higher in those with adequate vitamin D intake (32.1±37.5 mg/dL versus 50.23±46.5 mg/dL) than in those with deficiency (28.1±21.6 mg/dL versus 42.8±49.1 mg/dL) in both the control and patient groups. Since a better lipid profile is associated with greater levels of Vit-D, one would predict a lower level of Lp(a) in patients with better vitamin status. However, our findings show the opposite. D. Lp(a) concentrations have been shown to rise in studies in which vitamin D consumption was increased, and this has been linked to an improvement in vitamin D status (Saedisomeolia *et al.* 2014). However, only the APO A1, APO B, and Lp(a) concentrations were measured in this investigation, and the concentration of Vit-D was not measured to evaluate the participants' vitamin D status.

According to the literature, this study's results are in agreement with those of other studies. There was a correlation between greater vitamin D levels in the bloodstream and a reduced incidence of metabolic syndrome, as well as improved HDL cholesterol levels and triglyceride levels. Serum triglyceride levels, APO B levels, and the LDL/HDL ratio seem to be negatively associated to vitamin D levels (Jorde and Grimnes 2011). Research shows that people with diabetes who are deficient in vitamin D had higher levels of total cholesterol, triglycerides, and LDL-cholesterol, as well as lower levels of HDL-cholesterol (Kositswat 2010). Treatment for dyslipidemia seems to enhance Vit-D concentration through an as yet unknown mechanism, and vitamin D insufficiency also appears to be connected with familial hyperlipidemia. However, more research is required to understand the underlying mechanism of this connection (Mellenthin *et al.* 2014).

22 cross-sectional research papers and 10 placebo-controlled clinical trials examined the link between Vit-D and lipid profile in a 2011 review article on vitamin D supplements (Mangin *et al.* 2014).

Vit-D was found to be directly linked to HDL cholesterol concentration and negatively linked to triglyceride and cholesterol levels in LDL in all cross-sectional studies

examined. However, the results of the various clinical trials that were examined did not all agree. While some studies show that supplementing with vitamin D improves lipid profiles, others show that it has the opposite effect. In contrast, none of these clinical studies were specifically designed to investigate the role of vitamin D in the lipid profile. It's still unclear how vitamin D supplementation affects a person's lipid profile, and more research is needed to answer this question.

Vitamin D, glucose, insulin and HA1C: The control group's mean glucose concentration was lower (93.5 ±10.6 mg/dL) and the patient group's was higher (113.9 ±39.5 mg/dL) than in those with low levels of vitamin D (95.3 ±10.2 mg/dL and 141.9 ±63.0 mg/dL, respectively). In the group of patients, Serum glucose concentrations differed statistically significantly between the two groups. depending on the different vitamin D status of the individuals (P= 0.05). It was also found, in the two groups studied, that the mean serum insulin concentration was lower in individuals with sufficient levels of vitamin D (5.6±5.3 µUI/mL in the control group and 9.1±4.1 µIU/mL in the patient group) than in vitamin D-deficient subjects (6.6±2.9 µIU/mL in the controls group and 11.7±13.3 µIU/mL in the patient group). In the control group, there was a statistically significant difference between the subjects' serum insulin concentration, depending on their vitamin D status (P=0.034). Similarly, People in the study who had adequate levels of vitamin D had lower HA1C values (6.0±1.5 percent) than subjects with vitamin D deficiency (7.0±1, 8%), with a significant difference depending on the vitamin D status of the individuals (P= 0.009).

Because of previously published findings, these findings are in line with expectations. (Chung *et al.* 2014, Khan *et al.* 2013, Laway *et al.* 2014, Mozos and Marginean 2015, Viadya and Forman 2010), Vitamin D deficiency is linked to insulin resistance, according to research. Vit-D has been linked to HA1C in a study published in 2010 despite a lack of evidence (Mangin *et al.* 2014). analyzed the relationship between Vit-D concentration and HA1C value, dividing subjects into three groups according to age (18-34 years; 35-74 years; 75 years and over). The results of this study identified an inverse relationship between the concentration of Vit-D and HA1C in the group of individuals aged between 35 and 74 years. In the other two groups, this relationship was

not verified, but, according to the researchers, in the group of individuals between 18 and 34, a high incidence of abnormal HA1C values was not observed, which could have made this statistical relationship difficult to detect. On the other hand, the group of individuals aged 75 years or older was the smallest group in which possible treatments could have been influenced.

5. CONCLUSIONS AND RECOMMENDATION

Vitamin D deficiency in humans has become a major public health issue in our day, and it only appears to be growing worse. This discovery sparked a flurry of research into the true consequences of hypovitaminosis, its risk factors, and the best ways to diagnose and treat it.

Bone remodeling and a good phosphorus-calcium balance depend on the skin- and food-derived prohormone 25(OH)D. (Liu *et al.* 2013). A number of studies have shown that people with vitamin D insufficiency are more likely to have bone loss, falls, and low-energy fractures than those with adequate levels of vitamin D.

On top of vitamin D's intra-osseous effects, at least 38 different body tissues have been shown to have the vitamin D receptor.

Vitamin D deficiency is mostly caused by a lack of exposure to the sun. When the skin is exposed to sunlight, vitamin D is formed. This is important to note since it is almost hard to get enough vitamin D through diet alone.

Calcifediol levels may be determined using radioimmunoassays, the gold standard approach. These pricey approaches, on the other hand, do not support widespread screening (Holick 2006).

Calcifediol deficiency may cause rickets and osteomalacia in children and adults, respectively, if the blood calcifediol content is below 20ng/mL.

For the diagnosis of rickets and osteomalacia, osteoporosis, and chronic kidney disease, calcifediol levels are necessary (Holick 2006).

Hypovitaminosis may be diagnosed using x-rays, clinical tests, and laboratory tests when there are bone abnormalities or high chemical indicators for metabolic bone disease (Ke *et al.* 2013)

Supplementing with vitamin D has been shown in several studies to increase muscle performance and reduce the chance of acquiring major illnesses such as cancer, autoimmune disorders, infectious diseases, type 2 diabetes, heart disease, stroke, neurological disorders, and gynecological difficulties. ' Other studies have shown a connection between vitamin D deficiency and all of the major causes of death. Only treatment and prevention of osteometabolic disorders and their consequences have been found to benefit from vitamin D supplementation.

everyday usage of vitamin D supplements, such as those made by the pharmaceutical company Roche, does not pose a risk of adverse effects or financial difficulty (Gunta *et al.* 2013). Studies show that the two methods have the same effect on elevating blood levels of vitamin D, and this is supported by the evidence. After 14 days, vitamin D3-treated patients have reached their maximal concentrations of vitamin D, while vitamin D2-treated patients have blood levels that are equal to those recorded before to therapy.

Serum 25-hydroxyvitamin levels of more than 30 ng/mL (75 nmol/l) are best for improving calcium absorption, decreasing PTH levels, and increasing bone and muscular function, according to current recommendations (Chan and Watts 2006). Treatment for vitamin D insufficiency involves two steps: raising calcifediol levels over 30 ng/mL first, and then maintaining them.

Taking more than 4,200 IU of vitamin D daily is hazardous (Wagner *et al.* 2008). However, no harm was found even in healthy adult patients who were given up to 10,000 IU/day of vitamin D.

Accordingly, specialized research on the advantages of population screening for low vitamin D levels, despite evidence to the contrary, must continue in order to prove their

practicality and cost-effectiveness. It's still too early to advocate broad screening for this reason (National Institute of Statistics 2014).

Cardiovascular diseases (CVDs) are the major cause of mortality in a large number of nations. Preventative and therapeutic strategies can only be successful if they are based on a thorough understanding of the underlying pathophysiology.

Recent studies have linked cardiovascular disease (CVD) to vitamin D deficiency, and the latter has an impact on prognosis. In order to both prevent sickness and improve the prognosis of those who currently have it, scientists have concentrated their efforts on tackling vitamin deficiency as a problem. Supplementing with vitamin D may reduce the risk of cardiovascular disease, however there is insufficient evidence to suggest this.

We should pay attention to the vast range of prevalence of vitamin D insufficiency that occurs around the globe. Sunlight exposure, which influences endogenous vitamin D production and is dependent on latitude, may be one cause for country-to-country variations in blood vitamin D levels.

The disappointing outcomes of vitamin D supplementation study may be due to the vitamin's incapacity to operate in the present situation or the studies' low vitamin D levels.

In order to maintain appropriate levels of 25-hydroxyvitamin D over the follow-up period, it is necessary to know what dosages are needed to keep blood vitamin D levels above the ideal level and to undertake serial assessments of this vitamin.

Scientific interest in vitamin D insufficiency has increased in recent years since it is linked to a number of chronic disorders, including cardiovascular disease. As a result, a number of studies have been conducted to better understand this association. Vitamin D supplementation has not been shown to help many disorders in clinical trials, and the

findings are still disputed. More study is needed to determine the exact relationship between low levels of vitamin D and cardiovascular disease.

People with cardiovascular disease had a higher prevalence of vitamin D insufficiency than the overall population, according to this research.

Women's Vit-D concentrations were likewise shown to be lower than men's, independent of whether or not they had cardiovascular disease.

Vitamin D and the hormone calcifediol are higher in those who don't smoke or exercise, according to research on the relationship between vitamin D and lifestyle. Sedentary individuals are more likely to get the advantages of vitamin D supplementation than those who engage in regular physical activity. According to one research, living a more active lifestyle may lead to higher levels of vitamin D.

Calcifediol levels in the blood did not increase when vitamin D-rich meals were consumed, according to studies. People who spent more time in the sun had higher serum levels of Calcifediol, the main dietary source of vitamin D, as expected. In the winter, vitamin D blood concentrations were lower than in the summer.

A decrease in HDL cholesterol and a rise in total cholesterol, LDL cholesterol, and triglycerides are all associated with getting enough vitamin D from the sun. Vitamin D levels are higher in those who are abundant. A lower APO B, glucose, and insulin concentrations and a lower HA1C score were also seen in individuals who had appropriate vitamin D levels.

Recommendation

Below are some considerations that may be appropriate for the continuation of this work:

- Since differences in the concentration of Vit-D between men and women were observed in this study, it would be interesting to reproduce this analysis by evaluating a sample with a homogeneous proportion of men and women, in order to verify whether this difference between genders is owed to chance or if in fact it exists.

- It also seems pertinent to explore the relationship between the vitamin D status of individuals and the new CVD risk biomarkers, namely APO B, Lp(a), fibrinogen, hsCRP and homocysteine, as the results obtained indicate that Very low concentrations of Vit-D are not favorable for these biomarkers but, even more interestingly, Vit-D concentrations above recommended levels also appear to have a negative relationship with these markers.

- Studying Kirkuk city's population's vitamin D status is necessary because of a high rate of vitamin D deficiency and insufficiency in Madeira, which is more than expected considering the island's geographic position.

- Finally, given the strong relationship found between the serum concentration of Vit-D and the height of individuals, it would be interesting to explore this relationship in order to better understand whether it is the appropriate concentration of Vit-D during the growth phases, which contributes to a greater height of the individuals or if, on the other hand, it is the height that has some influence on the serum concentration of Vit-D and, when verifying this theory, to determine the reasons that are behind this relationship.

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