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VITAMIN D LEVELS IN ADULT PATIENTS WITH HEMOPHILIA

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OMEED AKBAR ALI ALI

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Prof. Dr. Vahap OKAN

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OMEED AKBAR ALI ALI

ABSTRACT

VITAMIN D LEVELS IN ADULT HEMOPHILIA

OMEED AKBAR ALI ALI

M. Sc. in Biochemistry Science and Technology

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Hemophilia A and B are hereditary diseases due to X-related recessive recurrence seen in male children of female carriers. The coagulation FVIII and FIX deficiency result in a clinic with hemarthrosis and hematoma bleeds, especially in the joints. In this study, we aimed to measure Vitamin D levels which are thought to be important in bone development and destruction in hemophilia patients and to measure some important parameters in bone metabolism and to show the relation with hemophilia. For this study, 40 male hemophiliacs (32 hemophilia A and 8 haemophilia B) and 40 healthy adults were included in the study between the ages of 18-65. We measured Vitamin D (21,86 ± 8,03 ng/mL for hemophilia, 25,46 ± 6,09 ng/mL for control, Ca, P, PTH and ALP levels in the studied subjects. The age of the cases (33,40 ± 11,44 years for hemophilia, 33,92 ± 10,00 years for control, p> 0,05) and BMI (25,81 ± 5,33 kg/m^2 for hemophilia, 43 ± 3.97 kg/m^2 for control, p> 0.05) were determined. Vitamin D (21,74 ± 5,71 ng/mL for hemophilia A, 25,46 ± 6,09 ng/mL for control, p <0,05) was different between hemophilia A and control groups. There was no significant difference between hemophilia B and Hemophilia A and control in terms of Vitamin D (p> 0.05). In our study Vitamin D values were lower in cases with hemophilia A. In these cases, especially because knee joint bleeds are frequent, these cases act less than normal people. Therefore, Vitamin D levels in these cases should be followed up frequently and should be treated in the absence.

Key words: Hemophilia, Vitamin D, Ca, P, ALP, PTH

ÖZET

ERİŞKİN HEMOFİLİ OLGULARINDA VİTAMİN D DÜZEYLERİ

OMEED AKBAR ALI ALI

Yüksek Lisans Tezi, Biyokimya Bilimi ve Teknolojisi

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Hemofili A ve B, kadın taşıyıcıların erkek çocuklarında görülen X'e bağlı resesif geçen, kalıtsal bir hastalıktır. Koagülasyon FVIII ve FIX eksikliği sonucu kanamalar, özellikle eklemlerde hemarthroz ve hematomlarla seyreden bir kliniğe sahiptir. Biz bu çalışmada hemofili hastalarında kemik gelişim ve yıkımda etkisi olduğunu düşündüğümüz Vitamin D düzeylerini ve kemik metabolizmasında önemli bazı parametreleri ölçerek hemofili ile ilişkisini göstermeyi amaçladık. Bu çalışmaya 18-65 yaş aralığında erkek 40 hemofili olgusu (32 Hemofili A ve 8 Hemofili B) ile 40 sağlıklı yetişkin alınmıştır. Çalışmaya alınan bireylerde Vitamin D (hemofili için 21,86 ± 8,03 ng/mL, kontrol için 25,46 ± 6,09 ng/mL, $p<0,05$), Ca, P, PTH ve ALP düzeylerini saptadık. Olguların yaş (hemofili için 33,40 ± 11,44 yıl, kontrol için 33,92 ± 10,00 yıl, $p>0,05$) ve BMI (hemofili için 25,81 ± 5,33 kg/m^2, kontrol için 26,43 ± 3,97 kg/m^2, $p>0,05$) saptandı. Hemofili A ve kontrol grupları arasında Vitamin D (hemofili A için 21,74 ± 5,71 ng/mL, kontrol için 25,46 ± 6,09 ng/mL, $p<0,05$) farklı bulundu. Hemofili B ile Hemofili A ve kontrol arasında Vitamin D arasında anlamlı farklılık saptanmadı ($p>0,05$). Bizim çalışmamızda Vitamin D değerleri hemofili A olgularında düşük saptandı. Bu olgularda özellikle diz eklemi kanamaları sık görüldüğünden bu olgular normallere göre daha az hareket ederler. Bu nedenle bu olguların Vitamin D düzeyleri sık aralıklarla takip edilmeli ve eksikliğinde tedavi edilmelidir.

Anahtar Kelimeler: Hemofili, Vitamin D, Ca, P, ALP, PTH

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LIST OF SYMBOLS/ABBREVIATIONS

WFH	World Federation of hemophilia
FVII	Factor VII
VWD	Von Willebrand disease
BC	Before Christ
HCV	Hepatitis C virus
HIV	Human Immunodeficiency Virus
25(OH)D	25-Hydroxy Vitamin D
7-DHC	7-Dehydrocholesterol
PRED3	Vitamin Provitamin D3
BMD	Bone Mineral Density
BMI	Body Mass Index
PTH	Parathyroid Hormone
Ca-R	Calcium-sensing Receptor
HCl	Hydrochloride
CKD	Chronic kidneys Disease
CLD	Chronic Liver Disease
Na	Sodium
Pi	Inorganic phosphor
ALP	Alkaline Phosphatase
GGT	Gamma Glutamyl Transferase
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
mRNA	Messenger Ribonucleic Acid
V.D	Vitamin D
VDR	Vitamin D Receptor

P	Phosphorus
Ca	Calcium
DEXA	Dexamethasone
CVD	Cardiovascular Diseases
MI	Myocardium Infarction
TRIS	Tris (hydroxymethyl) aminomethane
UV	Ultra Violet Rays
pNPP	p-Nitro Phenyl Phosphatase
pNP	p-Nitro Phenol
AMP	2-Amino-2-methyl-1-propanole
PO_4	Phosphate

CHAPTER I

INTRODUCTION

1.1 Hemophilia

Hemophilia is a genetic disorder that affects the blood function. Parents' genes are passed along to children. There're many proteins known as clotting factors included in blood that controls the hemorrhage. As there is deficiency in clotting factor Blood comes to the hemophilia [1]. A hemophilia refers to lack of clotting factors in the blood where blood don't clot normally, hemorrhage differs from patient to another one according to the severity of hemophilia. Hemophilia comes in second order after Von Willebrand's disease of the most severe bleeding disorders. In 2009, the World Hemophilia Federation (WFH) stated that 153,251 people worldwide have a hemophilia [2].

Hemophilia A and B are having the same symptoms. Hemophilia A is the most common form, it caused by the decreasing of clotting factor VIII's level (FVIII). Whereas, haemophilia B known as Christmas disease, has a deficient coagulation factor IX level (FIX). Clinical manifestations of hemophilia A and hemophilia B are indiscernible and occur in mild, moderate and severe forms. Besides, acquired hemophilia is another bleeding disorder, which is not hereditary, such as classical hemophilia. This is a too rare condition in which one's immune system develops antibodies against body's coagulation factors and the factor level in their blood is decreased [3].

The most important clinical manifestation of hemophilia is hemorrhage in the large joints of the upper and lower extremities. This condition usually begins once the affected child reaches the toddler stage and increases in frequency as the child becomes more active. Often, 1 or 2 joints become the principal targets of repeated hemarthroses.

With time, this situation can result in a chronic synovitis, destruction of carrilage and bone, and a progressive flexion contracture at the target joint [4]. Bleeding into muscle (iliopsoase, gastrocnemius, and flexor muscles of the arm) or soft tissues with the formation of large spreading hematomas is also common. Damage to muscle results in for the muscle atrophy and contractures. Wound healing is also abnormal in hemophilia, demonstrating excessive persistent angiogenesis and increased iron deposition, similar to hemophilic arthropathy. Other side, mucosal bleeding, haematuria, and intracranial hemorrhage and prolonged bleeding following surgery or minor trauma, are less common of bleeding. Milder hemophiliacs (>5% factor Vlll or IX activity) may not be detected until surgery is performed or the patient has a dental extraction. Usually, the procedure is completed without evidence of unusual bleeding because of normal primary (platelet-based) hemostasis. However, within a few hours, the surgical wound or tooth socket begins to ooze, wound healing is disrupted, and blood seeps into surrounding tissues. Hematoma formation in the pharyngeal and retropharyngeal areas can threaten airway patency and present a medical emergency [5]. Large sub-periosteal or muscle bleeds can on occasion lead to the formation of a hemophilic pseudotumor. These are cyst-like structures containing serosanguinous or dark brown viscous material bound to a fibrous membrane. Over time, pseudotumors expand and impinge on adjacent structures. Those that arise from a sub-periosteal bleed, usually involving the pelvis or femurs, can eventually erode adjacent bone to form large cystic lesions [6].

Individuals are suffering from hemophilia experience with similar fate around the world before the 1960s when there was no adequate treatment. In the early stages of youth, the severe joint injuries have occurred and most patients die before reaching 20. At early years, the hemophilia was centering on the treatment with fresh blood transfusions. The discovery of cryoprecipitate followed by the development of clotting factor concentrations has significantly increased the clinical management options. The concentrates can be easily stored, when they are given at home and while they are being transported by the patients during the journey, the patients have begun to take home therapy [7]. Hemophilia and management of it have a significant influence on society, including community integration and the economy. In fact, the important problem for all patients with hemophilia disease is not an active part of society and that the cost of medical care and is high makes this. In the developed ten countries,

early treatment of bleeding events and home remedies rapidly developed as a primary management choice. The main objective of this study was to identify useful suggestions and recommendations in Turkey for clinics and hospitals about these diseases.

1.2 History of Hemophilia

Due to clotting FVIII or FIX deficiency, hemophilia is a scarce inherited bleeding disorder. While the history of hemophilia dates back to the second century BC, a modern description of hemophilia appeared only at the beginning of the 19th century. The discovery of "antihemophilic globulin" in the mid-20th century led to the production of cryoprecipitate and later FVIII and FIX concentrates. After the tragic consequences of infection of blood-borne viruses by virus-neutralized factor concentrates during the 1970s and 1980s were hampered by the traumatic consequences of the haemophilia community, first plasmid-derived products and recombinant products then revolutionized haemophilia treatment by the widespread adoption of home treatment and prophylactic regimens has significantly increased the quality of life and lifespan of people with hemophilia over the past decade. [8]. Hemophilia was known in the ancient world, although effective treatment was only available in the last decade. The earliest written references to things that appear haemophilia, BC It is seen in Jewish texts of the 2nd century. According to rabbi rules, children who died after the previous two male circumcisions were exempted from circumcision. The first modern explanation for defining hemophilia belongs to John Conrad, and John Conrad considers three main characteristics for haemophilia: males tend to have a hereditary hemorrhage. However, the first use of the word "hemophilia" arises in texts written in 1904 [9].

1.3 Incidence

Hemophilia is the most severe bleeding disorder after von Willebrand's disease which is the most common congenital bleeding disorder over the world. In 2016, the World Hemophilia World Federation (WFH) identified 304,362 people with hemorrhagic disorders worldwide [10]. This estimate included 105 countries and about 91% of the world's population. Approximately 61.5% of documented total hemophilia patients are severely affected cases [11]. People with impaired bleeding are classified into three

groups: 187,183 people with hemophilia and 74,819 people with von Willebrand disease (VWD) and 42,360 people with other bleeding disorders [12]. The incidence of hemophilia in the world is 1 from 10,000 and in males, this ratio is 1 from 5,000. Hemophilia A occurs in approximately 5,000-10,000 male births, with 85% of all hemophilia are Hemophilia A and 15% are hemophilia B. The incidence of disease is the same for all geographies It does not differ racially [13]. The proportion of people with hemophilia in Turkey, according to the report of the Turkish Society of Hematology in 2012 about 5000 people, 80% of which are hemophilia A and 20% hemophilia B [14].

1.4 Types of Hemophilia

Hemophilia is a scarce hereditary hemorrhage disorder and is divided into two types resulting from a deficiency of factor: this blood disorder has two forms Hemophilia A and Hemophilia B (FVIII and FIX). Also, third type of Hemophilia is Hemophilia C which is depend on the FXI deficiency in blood [15].

1.4.1 Hemophilia A

Hemophilia A is a severe bleeding disorder due to lack or absence of FVIII. Haemophilia is a hereditary disorder associated with X, but men are predominantly affected by carrier women. However, there are no family history of hemorrhagic failure in nearly three-thirds of the patients because they have a spontaneous mutation in the near past [16]. Because the plasma level of FVIII is inadequate for clotting. Factor VIII is an indispensable protein in blood coagulation and wraps around in an inactive form bound to the von Willebrand factor and protects it from proteolytic degradation. In response to injury, factor VIII is activated by thrombin and is separated from the von Willebrand factor. Factor VIII serves as a cofactor for factor IX (A) and hemostasis is contributed by thrombin expansion and expansion of fibrin formation. Factor VIII deficiency leads to suboptimal thrombin formation and fragile blood clots that can be broken easily [17]. In healthy subjects, normal factor VIII plasma levels (FVIII: c) range from 50 to 150 International Units (IU dL-1). Hemophilia occurs as a mild, moderate, or severe bleeding disorder due to the plasma level of factor VIII [18,19]. There is no measurable factor VIII levels (FVIII: c, <1 IU dL-1) that cause spontaneous hemorrhage in joints and muscles in severe hemophilia A patients; whereas in moderate haemophilia A patients (FVIII: c, 1-5 IU dL-1) usually only after

mild trauma and in mild haemophilia A patients only large trauma or postoperative bleeding occurs. Approximately half of the patients have severe form [20].

Severe hemophilia can cause serious morbidity and mortality; Spontaneous bleeding, unless treated with mainly joints and muscles, results in permanent disability. Bleeding episodes in moderate or mild haemophilia are rare and usually associated with trauma or surgery [21].

1.4.2 Hemophilia B

Hemophilia B is a severe hemorrhagic disorder caused by a deficiency of a substance called FIX, a clotting factor, which is required for blood clotting. FIX deficiency, spontaneous hemorrhage and blood after injury may cause inadequate normal clotting. A clotting factor is a protein that controls bleeding [22,23]. Factor IX is also known as the Christmas factor because of the association of hemophilia B with the period [24]. While males are primarily affected, their teeth may carry affected genes, but usually do not tend to bleed. Since the sex is linked, this gene is on the X chromosome and is almost always carried by women without symptoms and passes on to their daughters and their sons. A daughter becomes an asymptomatic carrier when she is widespread from her mother, but when a boy is inherited extensively, it is affected by hemophilia B. Up to 30% of cases occur in a new gene mutation that does not exist in the mother. If your father has hemophilia and is not a mother carrier, all of your daughters will be carriers. None of the men get wide from their father. If the father is a hemophilia patient and the mother is a carrier, the girls can inherit two X chromosomes and become hemophilia. Hemophilia B is clinically indistinguishable from hemophilia A and at the same time is inherited by the inheritance of the X chromosome. However, the treatment of both diseases is quite different and the diagnosis of both diseases should be determined by coagulation factor tests [25].

Hemophilia B suffers from recurrent, often life-threatening, bleeding events that can occur without significant damage. The seriousness of the situation depends on the degree of lack of FIX. 45% Hemophilia B has less than 1% FIX level and they have Christmas disease. The remaining individuals have FIX levels between 1% and 5% and are in moderate condition or more than 5% and mild condition. [26]. The most

common places for hemorrhages are joints (ankles, elbows, and knees) and muscles and mucous membranes.

1.4.3 Hemophilia C

Hemophilia C is a rare bleeding disorder caused by the development of autoantibodies (inhibitors) directed against plasma coagulation factors, most commonly factor XI (FXI). Hemophilia C (deficiency of FXI) was first described in an American Jewish family in two sisters and their maternal uncle [27].

Acute reduction in FXI causes mild bleeding unlike bleeding in haemophilia A or haemophilia B which is clearly linked to the factor level, the bleeding risk in heamophilia C is not always influenced by severity of the deficiency, especially in the individuals with the partial deficiency. Indeed, some patients with severe deficiencies do not have a tendency to bleed, and some patients with mild deficits are over blooded. This unpredictability, which is not fully understood, makes hemophilia C more difficult than hemophilia A or B [28].

Serious insufficiency is defined as FXI c activity of 15-20 U / dL or less. However, this is now a suitable terminology since bleeding disorders are not clinically severe even at very low FXI levels. Spontaneous bleeding occurs infrequently, but bleeding may occur postoperatively, more commonly at the lowest level of bleeding. Levels in this range define the individuals with a gene mutation of less than about 15 U / dL, usually 2 (FXI). [29,30].

1.5 Clinical Diagnosis of Hemophilia

If a family history is available, the hemophilia can be diagnosed before, during, or after birth. There are many options for parents. If there is no family haemophilia story, it is often diagnosed only when a child starts walking or crawling [31]. The clinical signs and symptoms of hemophilia A and B are the same and are associated with prolonged and excessive bleeding tendency. The bleeding tendency in hemophilia is determined in large part by the baseline level of the deficient or defective clotting factor. The presence of easy ecchymosis in early childhood, especially intra-articular and intramuscular spontaneous hemorrhage, and interventions should suggest haemophilia in the presence of long-standing bleeding episodes that are expected after trauma [32].

To ensure adequate procoagulant function in healthy individuals, the circulating FVIII concentration should be as low as 200 ng / mL; whereas FIX should be at about 5000 ng / mL. The severity of bleeding findings depends directly on the degree of FIX or FVIII deficiency. Patients with factor activity <1% showed "severe hemophilia", whereas those with 1-5% showed "moderate hemophilia" and those with> 5% showed "mild hemophilia" In mild hemophilia, the disease may remain silent for many years, and occasionally a new diagnosis of hemophilia may be made in those older than 60 years of age when challenged with a surgical procedure [33]. The bleeding pattern in severe hemophilia is distinct and is not often seen in other bleeding disorders. Severe hemophilia can cause severe mortality and morbidity; Spontaneous bleeding, mainly in joints and muscles, results in permanent disability if left untreated. Bleeding episodes are rare in moderate or mild hemophilia and are usually associated with trauma or surgery [34].

1.6 Causes of Hemophilia

Hemophilia disease occur as a result of abnormality of the genes which are responsible for blood clotting factors either of abnormality genes inherited from one of the parents which translate appear on it symptoms of Hemophilia or as a result of sporadic genes formats of clotting mechanism for the child, so it appears on a person has no family history of such disease, and maybe has results [35].

Hemophilia may be existed in the family for successive generations without knowing it and even without symptoms' appearance of the disease. As a result, hemophilia may infect the family's males. In most of cases, we cannot determine if these family's females are carrier to the disease or not. But it will only be diagnosed when a new born is a male. [36].

Hemophilia effects male (x) mainly as affected when the of (x) chromosome abnormal mother, When the chromosome x moves from mother to son where the son lacks the second X chromosome, which can compensate for its defective genes (ie, those genes that have a problem). While the females carry the pathogen but they remain non-infected with the disease, so that the female infection of this disease must be an abnormal gene on the X chromosomes, which is very rare [37].

The genetic mutation may occur during pregnancy as a result of the mother's exposure to several factors that may affect her pregnancy, and then the genes pass from the pregnant mother to the two genes with hemophilia. Although most cases are inherited, a person can acquire hemophilia through an automatic genetic mutation, and can occur if the body forms antibodies or antibodies to the coagulation factors that prevent it from acting (acquired hemophilia) [38].

1.7 Complications of Hemophilia

Bleeding can cause many problems, including neurological deficits; however, a very common finding is that it causes destruction of joints due to recurrent bleeding. Feeling that the joints are painful as they move and their range of motion is limited. Anyone who has received blood from a patient with hemophilia A or B will probably find that the patient cannot fully expand the elbow. Prior to the appearance of recombinant FIX, patients were using the plasma-derived FIX concentration of many donors. Prior to 1985, there was a significant risk that this product could be contaminating with hepatitis B or C or HIV [39]. The risk for patients with hemophilia is even greater because it is usually a heavier condition, and treatment is probably needed more often, and donors are also needed for each treatment. Due to the shortage, the majority of the product was imported from the United States, where the incidence of these diseases was higher and the tendency for the lifestyle to differ in terms of lifestyle was due to the use of paid donors. In a study published in 1998, 41% of hemophilia A patients, HIV antibodies were found in 6% of the patients. For more severely affected patients, there were 59% and 11%, respectively. [40] In 1985, a viral inactivation process made the product safer, but the appearance of the recombinant 'genetically engineered' FIX made a big difference. Otherwise, we may still be concerned about the contamination of other agents, such as the new variant Creutzfeldt-Jakob disease [41].

Many people with hemophilia A, including patients with hemophilia B, are both infected with both hepatitis C virus (HCV) and HIV, and two viruses together cause quite poor prognosis [42]. It continues to generate a significant amount of deaths due to liver disease and hepatocellular carcinoma [43]. However, people who have hemophilia and are not infected with HIV appear to have a lower mortality rate from the general population due to cancer; this requires further research [44].

Factor VIII replacement therapy is the basis of hemophilia therapy and is effective as long as a patient does not develop an alloyant (inhibitor) against exogenous FVIII. Inhibitor development is the most important treatment complication in patients with hemophilia today and is associated with severe morbidity and reduced quality of life. The development of an inhibitor is the result of a complex interaction between a patient's immune system and genetic and environmental risk factors. The mainstay of treatment is the removal of the inhibitor with the immunity tolerance [45].

1.8 Vitamin D

Vitamin D is a critical for cardiovascular health and musculoskeletal system and also for phosphorus and calcium homeostasis. When vitamin D had shown to play an important role in many acute and chronic illnesses, it came to the center of attention. Based on Funk's ideas, vitamin D was discovered 100 years ago and was first described by Mc Collum [55-57]. In 1952, vitamin D was shown to affect serum calcium and is necessary for neuromuscular function [55]. By the end of the 1960's experiments with radioactive 3H vitamin D3, a biologically active form of vitamin D, which was very important for the metabolism of organisms, was thought to exist [56]. Subsequently, it took some more years to isolate and name these active metabolites (eg, 25 (OH) D and 1,25 (OH) D) [57,58].

It is most important to maintain a balanced level of calcium by increasing the absorption of vitamin D in the intestine and protecting the level of phosphate and calcium in the bone [59]. It is well known that vitamin D exerts multiple functions in vitamin D, autoimmune diseases, cell growth, bone biology, neuromuscular or inflammatory and other immunological functions [60].

1.8.1 Sources of Vitamin D

There are two sources of vitamin D supplementation: (1) cutaneous synthesis and (2) diet. The majority is provided by skin synthesis [52].

7-Dehydrocholesterol (provitamin D3, 7-DHC), which is synthesized from cholesterol in the liver, turns into provitamin D3 (ultraviolet B wave length: 290-315 nm) when it absorbs solar energy. Under heat effect Vitamin D immediately converts to vitamin D3 (cholalciferol). Although direct sunlight exposure depends on the season, latitude

and time, it is responsible for the total amount of D3 vitamins production and over-exposure can also lead to the formation of ineffective photoproducts [53].

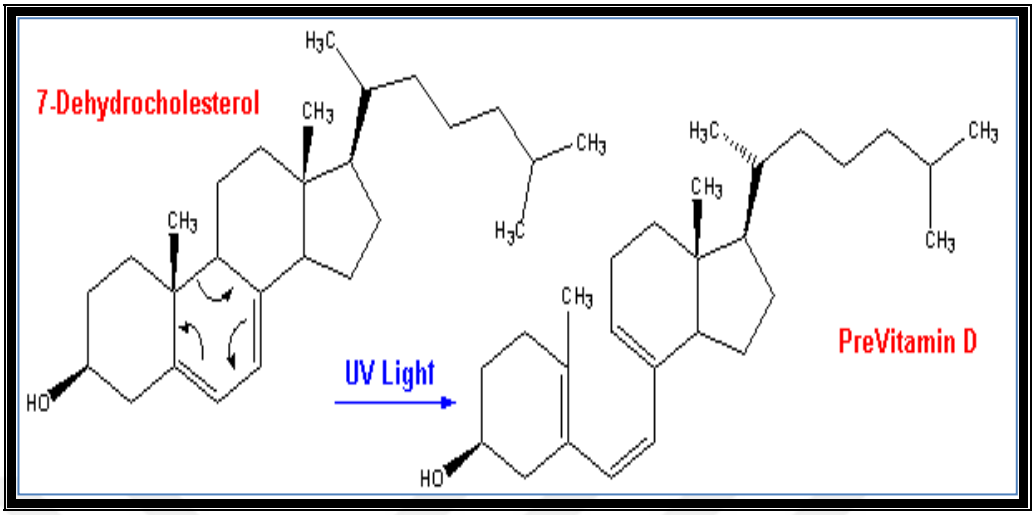


Figure 1.1 Illustrates the transformation of 7-Dihydrocholesterol to Colcalrorol vitamin D_3

A smaller portion of vitamin D is taken from the diet [60,63], along with phosphate and calcium. While Ergocalciferol (vitamin D2) is found in plants and plant products, cholecalciferol (vitamin D3) is also found in cod liver oil as well as other animal products such as mackerel, fresh and canned salmon and tuna fish [46,47].

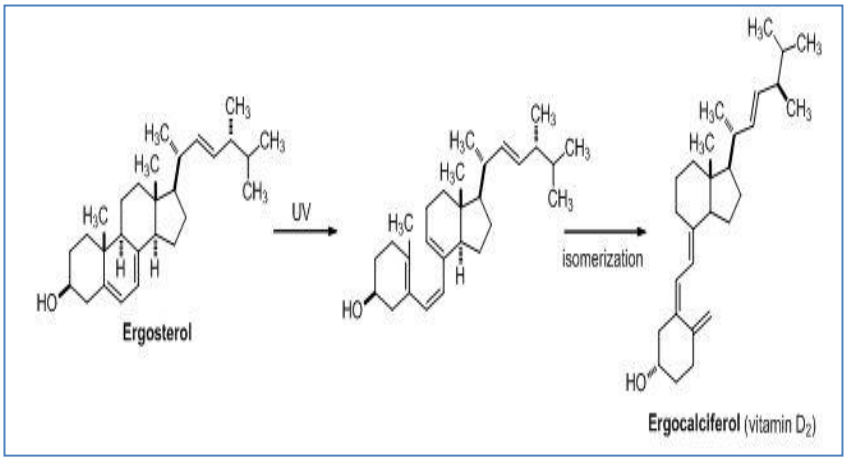


Figure 1.2 Shows the transformation of the Arcticol to the Arccocyclol vitamin D_2.

The both vitamin D3 exporters both under the skin or the food are ineffective and are converted into effective form by way of metabolic processes in the liver to 25-OHD3 by the 25-hydroxylase enzyme [46].

1.8.2 Synthesis of Vitamin D

Synthesis of vitamin D in nature is either produced in the skin by exposure to UVB radiation or is ingested in the diet, and subsequent activation in liver and in kidney, when steroid, which is found in some plants and is found in some fungi, is exposed to the sun, ergosterol produces vitamin D2 [54]. Vitamin D3 results in a 7-Dehydrocholestrol (7-DHC) compound, which is found under the skin. This compound is added to Vitamin Provitamin D3 (PRED3) and then the latter is converted to vitamin D3. After sun exposure, preD3 and vitamin D3 are converted into ineffective products. Vitamin D3 enters the skin or food into the bloodstream and is transformed by metabolic processes into the liver, (25 (OH) D3 by 25-OHASE enzyme). And then enter 25 (OH) D3 again into the circulatory system and transfer to the kidney to be converted to Cholecalciferol (1,25) OH 2D3-mediated enzyme, 25 (OH) D3 1 -hydroxylase (1-OHASE) Kidney production of 1,25 (OH) 2D3 is mediated by several factors, including the level of phosphorus in blood Pi and the thyroid hormone PTH, which in turn regulates the metabolism of calcium through its intervention or interaction with major tissues such as the bones and intestines [55]. Also, 1,259 (OH) 2D3 drives the process of rupture of (25-OH) D 24-hydroxylase (24-OHase) metabolizes 25 (OH) D in other tissues to regulate the cell growth process as shown in the figure: -

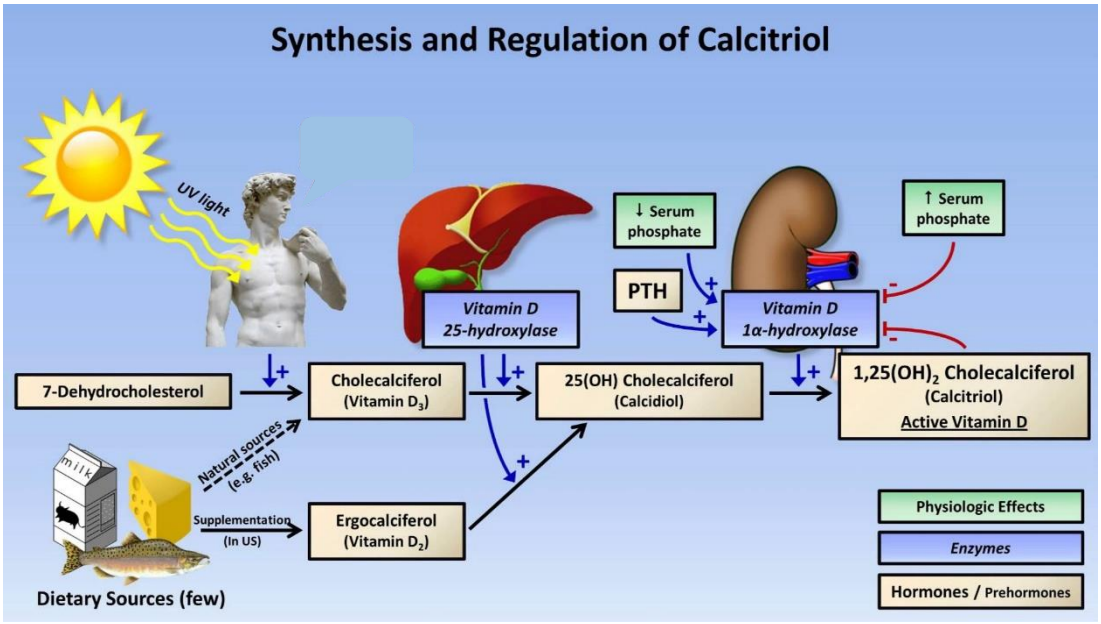


Figure 1.3 Synthesis of vitamin D. Schematic presentation of vitamin D intake and metabolism. Vitamin D is provided by diet intake or cutaneous synthesis. Vitamin D

is taken up by bloodstream into the liver where the chemical structure of Vitamin D is changed by hydroxylation. Then it is send to the kidneys for another hydroxylation. Finally, the active metabolite 1.25 (OH) D is given to circulation to be active.

1.8.3. Vitamin D Deficiency

Deficiency of Vitamin D is a widespread and global problem. 25 (OH) D in the blood is measured to determine the D vitamin status. Inadequate vitamin D levels are between 20 ng / mL and 29 ng / mL, and levels below 20 ng / mL are defined as deficiency [56.57]. Recent evidence shows that; 25 (OH) D levels are circadian rhythms and therefore require special care during taking blood samples [58]. An admission survey and a health interview for adults showed that more of the German population had a level of 25 (OH) D below 20 ng / mL [59]. It was reported that in 2012, approximately 80% of European residents were conducting an investigation showing a serum concentration of vitamin D below 30 ng / mL. The likelihood of complaints of vitamin D deficiency is increasing with age [60]. There is plenty of evidence that vitamin D deficiency may be associated with a variety of chronic diseases such as diabetes, cardiovascular disease, cancer, obesity, and depression. The aim of this study was to investigate the prevalence of vitamin D deficiency and its correlation with Haemophilia patients [61].

Lack of exposure to sunlight in hemophilic patients due to inactivity and restriction of movement may lead to deficiency of vitamin D, as sunlight is the principal source of vitamin D production. Arthropathy in hemophilic children together with immobilization makes them more susceptible to vitamin D deficiency and osteoporosis that can affect parathormone level, calcium, and phosphorus metabolism [62].

1.8.4 Relationship of Vitamin D with Hemophilia

Osteoporosis is a major problem in hemophilic patients. The causes of osteoporosis in hemophilic patients are inadequate exercise, inflammation, low vitamin D levels and multiple bleeding. Children with hemophilia A are under the risk of reducing bone mineral density (BMD) and developing osteoporosis and later life-time fractures [63]. Low BMD pathogenesis in the hemophilia is multifactorial and includes hemophilic arthropathy with immobilization [64]. Patients with decreased hemophilia in BMD may have a higher risk of fracture and osteoporosis in later life [63]. The aim of this

study was to compare serum vitamin D levels of healthy people with hemophilia patients and to evaluate serum vitamin D levels and various factors in hemophilia patients.

1.9 Calcium

Calcium is the most abundant substance in the body (25 to 35 mol) [1.0 to 1.4 kg] in the body. More than 98% are found in teeth and bones, which provide a large reserve that can be drawn as old as necessary [65]. One percent of bone calcium can be rapidly replaced by extracellular calcium; this calcium is equally distributed between extracellular and intracellular fluids. Extracellular calcium acts as a cofactor for many of the extracellular enzymes, most importantly for the enzymes of the clotting cascade, along with the basic substrate for mineralization of cartilage and bone [66]. The calcium requirement depends on the calcium metabolism state regulated by the three main mechanisms of renal reabsorption, bone turnover, and intestinal absorption. These are regulated by a range of interacting hormones including parathyroid hormone (PTH), 1,25-dihydroxy D vitamin (1,25 (OH) 2D) and ionized calcium and their corresponding receptors in the bone, gut, and kidney [67]. As shown in Figure:

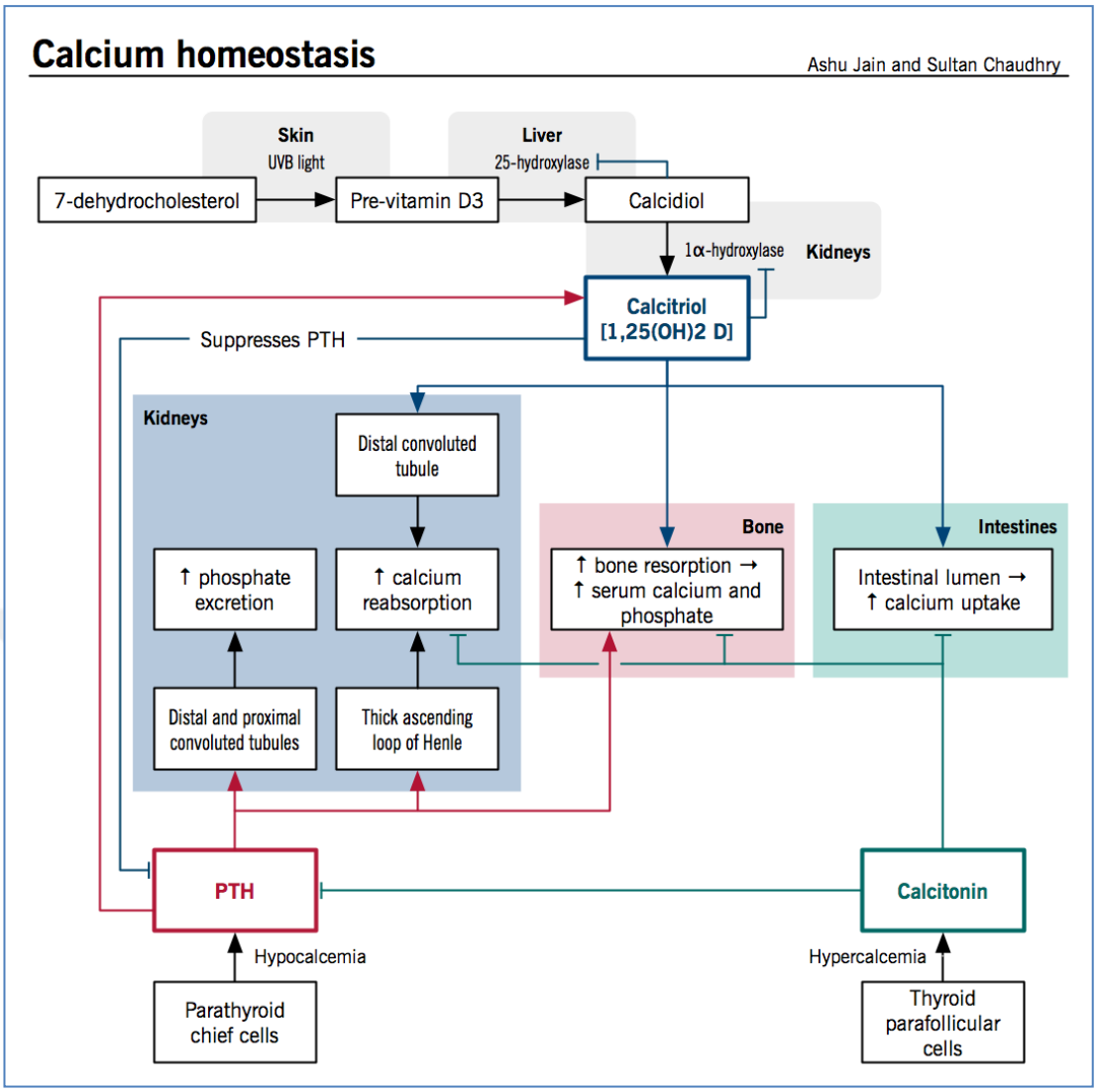


Figure 1.4 Reflects the effect of PTH, calcium and vitamin D on the sodium level.

Calcium serves two basic purposes: to provide skeletal strength and, at the same time, to provide a dynamic reservoir to preserve extra- and intracellular calcium pools. Also, it is important and necessary for muscle contraction, blood clotting and permeability of cell walls and control of the heart [68]. Calcium in serum exists in three fractions:

1-Calcium is associated with plasma protein albumin and accounts for about (40%) of the amount of calcium in the blood.

2-Calcium united with ions in the serum (phosphate, bicarbonate, sulfate) This part forms about [13%] of the amount of calcium.

3-Calcium, which is a free ionic form which accounts for about (47%) [65], and Calcium is a biologically active fraction that is closely controlled by hormonal mechanisms.

Changes in the ionized calcium concentration in the blood modulate PTH release in minutes per minute in vivo, affecting the level of calcium-sensing receptor (Ca-R) activation in parathyroid cells. Hypocalcemia directly stimulates PTH release by inactivating Ca-R, and plasma PTH concentrations rise within a few minutes as the blood ionized calcium concentration decreases [69]. Decreases in the extracellular calcium concentration that lasts for several hours also promote genetic transcription of Pre-Pro-PTH, which increases thyroid hormone PTH release [70]

1.9.1 Calcium Function

Calcium plays a vital role in a large variety of biological functions in the form of bound complexes or free ions. One of the most significant functions of bound calcium is skeletal mineralization [71]. The basic function of calcium is the formation of teeth and bones, and it contributes to the blood coagulation process and plays a role in muscle contraction and in its duration and helps to lower blood pressure (hypertension) [72]. Calcium is the activator of many enzymes such as adenosine triphosphatase (ATPase), lipase, and succinic dehydrogenase. It is also necessary for membrane permeability involved in muscle contraction [73]. Calcium absorption requires calcium binding protein and is regulated by sunlight, vitamin D, thyrocalcitonin, and parathyroid hormone. Thyrocalcitonin reduces plasma phosphate and calcium levels, while parathyroid hormone increases them. Phosphorus and calcium are predominantly absorbed in the upper small intestine, especially in the duodenum, and the amount absorbed depends on the source [74]. It also plays many of roles within the body and is included in a diverse range of processes including bone growth and remodeling, secretion (exocytosis), excitation contraction coupling, stabilization of membrane potentials, enzyme co-factor, second messenger and intracellular signaling. The amount of calcium in the blood is tightly controlled by signaling pathways and molecules, including PTH and vitamin D. Insufficient intake, bad calcium absorption, and excessive calcium loss contribute to a reduction in bone mineralization. For example, children who do not have enough calcium, such as osteoporosis, osteoporosis, calcium deficiency disease (hypocalcemia), and rickets cannot grow to

full potential height as in adults [75]. Deficiency of Vitamin D causes poor absorption of calcium and a decrease in bone mineralization, which results in soft, flexible bones that are easily deformed [76]. A decrease in the absorbed calcium causes the serum ionized calcium concentration to decrease [65]. It stimulates parathyroid hormone (PTH), which is effective in three ways to increase and maintain serum calcium levels [77]. Important factors that promote calcium absorption: Vitamin K and growth hormone, vitamin D, lactose sugar and acidic HCl coming from the stomach to the intestines [78]. There is also evidence of low plasma calcium along with chronic diseases such as colon cancer and hypertension [72,79].

1.9.2 Calcium–Phosphate Interactions

Phosphate [inorganic phosphorus] and calcium interact in several basic processes. Skeletal calcium and phosphate metabolism work in cohort with osteoblasts, osteocytes and extracellular matrix proteins, making the osteoid mineralized [80]. On the other hand, there is a less understood regulatory system in skeletal tissues that prevents harmful deposition of calcium phosphate complexes in the soft tissue [81,82]. Soft tissue classification is common in CKD. Classification in blood vessels is associated with increased mortality predicted from serum phosphate and calcium phosphate levels [84].

The serum PTH level, which is central to calcium homeostasis, also plays an important role in phosphate homeostasis. Increased serum PTH [85] in renal Na / Pi II co-transporters decreases renal phosphate reabsorption and serum phosphate while reduced PTH, renal phosphate reabsorption and serum phosphate increase.

1.10 Alkaline Phosphatase

ALP is a member of hydrolase enzyme class responsible for removing phosphate groups (dephosphorylation) from many types of molecules, involving proteins, alkaloids, and nucleotides, and it is most effective in an alkaline environment. ALP is present as several iso-enzymes including liver, bone, placental, intestinal and kidney [86].

Serum ALP is principally liver and bone isoforms derived from the biliary canicular membrane and osteoblasts respectively and as such primarily reflects changes in bone

and liver function. ALP can be raised in a number of conditions including hepatobiliary disease (often seen with elevated Gamma Glutamyl Transferase GGT), bone disease associated with increased osteoblast activity (Paget's disease, osteomalacia), disorders of calcium homeostasis and malignancy [87].

Decreased levels of ALP in hypophosphatasia, an autosomal recessive inherited disorder associated with low ALP activity and characterized by dental and bone abnormalities. Clinical symptoms range from the rapidly fatal perinatal variant with profound skeletal hypo mineralization and respiratory compromise to a milder, progressive osteomalacia later in life due to tissue-nonspecific alkaline phosphatase deficiency in osteoblasts and chondrocytes impairing bone mineralization [88].

Biochemical findings in osteomalacia include low serum and urinary calcium, low serum phosphate, elevated serum ALP and elevated PTH. Hence, markedly high levels often associated with Paget's disease. Moderate increases occur in osteomalacia [89].

1.11 Phosphorus

Phosphorus is the second most important mineral after calcium in the human body. Phosphorus plays a role in numerous biological processes, including energy metabolism and bone mineralization, while also providing a structural framework for RNA and DNA. It is synthesized by various biochemical pathways such as beta-oxidation and glycolysis [90,91]. Phosphorus is a component of enzymes, phosphorylation is responsible for activation of many hormones, and phosphorus is involved in acid-base regulation as a buffer at the surface of bone. Approximately 80% of phosphorus is present in the teeth and bones [92,93]. Plasma and serum contain only a small portion of the total body phosphorus in the form of inorganic phosphate, lipid phosphorus, and phosphoric ester. Changes in serum phosphate levels do not reflect the total phosphorus deposition of the body. [94].

A normal diet provides approximately 20 mg/kg/day of phosphorus, which is absorbed at 16 mg/kg/day in both intracellular and intercellular processes [predominantly jejunum] in the small intestine [92]. The intracellular process is mediated by the Sodium Phosphate co-migration, which is found in the villi of the small intestine [95].

Phosphorus is absorbed by the small intestine through passive diffusion through an electrochemical gradient and is also actively transported between cells using a type 2B

sodium phosphate carrier [96]. Between 60-70% of the phosphorus consumed is absorbed via the intestines [97,98]. 70% of the phosphorus is excreted by the kidneys and 30% is excreted through the gastrointestinal tract [99]. In adults, the serum phosphorus concentration is about 3-4 mg / dL, which is expressed as elemental phosphorus, but most are present as phosphate [92]. The quantity of phosphorus absorbed by the intestines is directly related to the quantity present in the diet and its bioavailability [96].The maintenance of calcium homeostasis is responsible for exchanges between phosphorus in the extracellular space and bone [100].

The kidney is the primary or primary regulator of phosphate concentration in blood plasma. The thyroid hormone (PTH) has a major effect, which reduces the process of retinal absorption. This effect of the hormone is made with some contribution by active vitamin D3 [101].

Decrease in serum phosphorus is seen in rickets, hyperparathyroidism and Toni-Fanconi Syndrome. Deficit disease or symptoms in children cause rickets and osteomalacia in adults. Increase in serum phosphorus is seen in chronic nephritis and hypoparathyroidism. Toxicology disease or symptoms include low serum Ca2 +: P ratio. It can also cause bone loss [102, 103]. Phosphorus deficiency is rare. However, there are several phosphate metabolism disorders that may cause altered calcium metabolism [104].

The increase in nutrient phosphorus leads to a small amount of phosphate absorption, with little evidence of an upper limit or saturation of the absorption process [93]. Three mg/kg/day of phosphorus is released into the intestines via pancreatic and intestinal secretions and a net phosphorus uptake is around 13 mg/kg/day [90].The high level of phosphates in the blood promotes the development of thyroid gland hyperplasia where high concentrations of phosphorus facilitate the manufacture of thyroid hormone PTH by regulating the completion of genetic expression of the formation of PTH mRNA of the hormone to facilitate the process of translation [105]. High phosphate levels in the blood also reduce the effectiveness of vitamin D3 therapy in patients with hyperthyroidism [106]. There is a strong correlation between increased cardiovascular disease mortality and factors that help precipitate calcium such as blood hyperphosphatemia [107,108].

1.12 Parathyroid Hormone

PTH is the main regulator of some electrons, if it affects several types of cells directly and indirectly. The hormone level increases in both bone building and osteoporosis. It regulates bone resorption to release calcium and compensate for the lack of calcium in the blood. Lining cells [109]. Calcium has a role in regulating the biological synthesis of PTH hormone. The rise in blood calcium level may diminish or decrease in the process of genetic copying of the genetic code of the hormone, so the thyroid gland is ready to respond or respond to the reduction in calcium much more easily than its height. In the case of hypercalcemia hypercalcemia, PTH secretion is greatly reduced and most of what is produced is fragments or fragments scattered from the peptide carboxylic endpoint of the hormone carboxylic-terminal fragments [110].

1.12.1 Role of Parathyroid Hormone

The functions of parathyroid hormone are the stimulation to increase the association of calcium with protein and also by increasing the production of vitamin D3. 51 PTH also regulates the levels of calcium and phosphorus in blood and their response is according to the level of calcium [111].

The action of PTH in the bone is a multiplier that affects directly and indirectly several types of cells and increase the level of hormone in the case of bone building and bone resorption as PTH regulates resorption to release calcium and compensate for the lack of calcium in the blood [112].

Thyroid gland activity is increased due to hypercalcemia. It also rises as a result of the chronic decrease in the level of serum calcium. This decrease increases the activity of the thyroid gland or because of the high level of phosphate in the serum which leads to a direct decrease in the level of serum calcium which in turn stimulates Activation of thyroid gland. Hyperthyroid hyperthyroidism is also caused by hyperplasia of the same gland, and in rare cases, activity is increased due to carcinoma of the carcinoma. The symptoms of this type of hyperactivity may be severe and the disease may continue without side effects for several years or life span, which is commonly known as hyperthyroidism asymptomatic hyperparathyroidism [91].

The activity of the PTH is sometimes reduced due to surgical ablation, in which the removal of PTH in the treatment of the hyperthyroidism of thyroid glands, and the decrease in activity of the autoimmune hypoparathyroidism, as the immune system forming antibodies that attack the PTH Like objects that are alien to the body so that the glands cannot produce enough of the hormone [113].

CHAPTER II

LITERATURE REVIEW

Vitamin D is one of the major factors for normal phosphate and calcium homeostasis, as well as important factors in bone mass preservation and bone development. Albayrak and Davut, [2015]. were found that low vitamin D levels in hemophilic patients is the highest during winter and autumn [114].

Sanadhya et al, [2016] compare the difference in serum vitamin D levels of pediatric hemophilic patients and healthy children, the result was significant correlation between severity of Hemophilia and Vitamin D insufficiency [61].

Hanaa et al, [2017] were found that vitamin D deficiency is an essential cause of decreased bone mineral density in hemophilic children [115].

AlIoglu et al, [2012] were found that serum parathormone levels were significantly increased, BMD was significantly reduced in severe haemophilics [116].

Some studies were showed that vitamin D prevents osteopenia, rickets, and fractures and Vitamin D plays important roles in the absorption and bone deposition. Low calcium absorption generates a number of physiological problems, since calcium is important for most metabolic functions, as well as the muscular activity [117,118].

Thiele et al, [2013] maternal lack of sun exposure and a minimal intake of vitamin D in the diet contributes to low vitamin D in breast milk and therefore to vitamin D deficiency in the baby [119].

Holick, [2012] the kidneys and liver are two key organs for the production of vitamin D. All patients with kidney or liver disease will likely have problems with the production of VD and therefore should receive vitamin D supplements [120].

Caroline S et al, [2012] vitamin D in patients with Chronic liver disease CLD and inversely correlated with disease severity. Vitamin D supplementation improves response to antiviral therapy and reduces rejection rates after liver transplantation [121]. G.Pongron, [1969] conducted a study on rats and found that The osteodystrophy and the higher requirements for vitamin D observed in hepatic insufficiencies may be due to an inability of the liver to transform vitamin D into its metabolically active form [122].

Diana Mager et al, [2016] adults with diabetes and chronic kidney disease (CKD) are at risk for vitamin D [123]. Testa A., et al, [2010]. Vitamin D deficiency is an independent risk factor for the development of end-stage renal failure. It is demonstrated that there is a relation between bad VD condition and endothelial dysfunction in hemodialysis patients [124].

Drechsler C et al, [2010] found the It has been demonstrated that there is a relation between VDR gene polymorphism and left ventricle bulk in in patients with end-stage renal failure, and this indicates ventricle hypertrophy progression [125] Molinari et al, [2011] found the has been suggested that low 25(OH)D3 levels are related with sudden cardiac death in diabetic hemodialysis patients [126].

Takiishi T et al, [2012] effects of vitamin D on the pathophysiology of type 1 diabetes have been described, autoimmune destruction of pancreatic islet beta cells [127].

Nunlee-Bland G et al, [2011] investigated the in observational studies, it has been shown that there is a relation between low serum 25(OH)D3 levels, and diabetes mellitus and metabolic syndrome [128].

De Boer IH et al, [2008] in a prospective cohort study titled Nurses' Health Study presented by Pittas et al, 25(OH)D3 levels and glucose intolerance in females have been monitored for 20 years and it has been found that VD and calcium intake is negatively correlated with the risk of developing type 2 diabetes [129].

Deleskog et al, [2012] report that there is a decrease in the risk of becoming diabetic faced by prediabetic patients with high 25(OH)D3 level [130].

Snijder R. et al, [2007] it should not be forgotten that high hyperparathyroid hormone levels are a result of VD deficiency and related to myocardial hypertrophy and high blood pressure [131].

Kunutsor et al, [2013] in their wide meta-analysis, Kunutsor et al. show that there is a negative correlation between basal free serum VD levels and hypertension [132]. Larsen et al, [2012] investigated the in recent studies, there are different results assessing VD's effect on blood pressure. [133].

Yetley et al, [2008] in one study, there some measurements have been made on 25[OH]D3, anthropometric values, body fat and peak bone mass, and it has been detected that there is a very strong negative correlation between DEXA measurements of visceral, subcutaneous fat and total body fat [134].

When vitamin D deficiency and epidemiological data is considered together, it has been understood that there is a negative correlation between all elements of metabolic syndrome and $25(OH)D_3$ (levels De Luis DA, 2008) [135].

Scragg R. et al, [1981] found the relationship between cardiovascular diseases (CVD) and vitamin D is one of the most popular subjects for research. It was Robert Scragg who first inferred in 1981 that cardiovascular mortality varies seasonally, and that UVB rays have positive and protective effects on cardiovascular risk [136].

Burgaz A et al, [2011] found the A number of observational studies and prospective meta-analyses have demonstrated the probable interconnection between Vitamin D deficiency, cardiovascular diseases and risk factors [137].

Nibbelink KA et al, [2007] found the It has been demonstrated in culture cardiomyocytes that VD metabolites have anti-hypertrophic and anti-proliferative effects [138].

Pittas et al, [2010] reported a meta-analysis including 9 prospective observational studies. According to this report, in 5 out of these 9 studies there is a correlation between low VD levels and high CVD risk [139].

Wang TJ et al, [2008] CVD history have been monitored for 5.4 years, and it has been detected that the percentage of incidents related to CVD was higher by 53 to 80 in patients with low 25(OH) D levels [140].

CHAPTER III

MATERIAL AND METHOD

3.1 Material

Our study has started in Gaziantep University, Medical Faculty, Department of Internal Diseases, Hematology Clinic, in accordance with the decisions of Helsinki Declaration and the approval 2017/227 given by the Gaziantep University Ethics Committee, given on 19.06.2017. Moreover, this study is supported by Gaziantep University Scientific Research Projects Commission (TF.YLT.17.36).

40 patients between the ages of 18 and 65, who applied to the Hematology Clinic of Gaziantep University and were diagnosed with hemophilia have been chosen as the subjects of this study. As for the control group, 40 healthy adults within the same age range who consulted the ward of internal diseases for routine controls were chosen. The ages of the members of the control group have been carefully chosen in accordance with the ages of the members of the study group.

The process of drawing blood was conducted in the Venesection Unit of Gaziantep University Medical Faculty. The blood samples for the routine analysis were drawn from the volunteering patients in sitting position after the forearm antecubital area was sterilized with a piece of cotton soaked in alcohol. 2 ml of blood from each patient was drawn into the coagulation tubes filled with 32% sodium citrate for the coagulation tests, and 5 ml from each patent was drawn into the tubes without anticoagulant for the ALP, calcium, phosphorus, vitamin D and parathormone analysis.

Once the blood sample is obtained, it should be stored in room temperature for at least 30 minutes for it to coagulate and centrifuge must be performed after the coagulation occurred. This process should take place no later than 2 hours after the bloodletting process and the samples should not be stored in +4 ^{o}C in order not to accelerate the heamolysis during the waiting period. Afterwards, the blood samples were centrifuged

in 4000 rpm for 10 minutes (Nuve NF800R, Turkey) and the blood serum was separated. The blood serum, then, was stored in Eppendorf tubes in -80 ºC. The patients were checked for factor VIII and factor IX for hemophilia diagnosis. All the analysis of all the parameters included in the study were made in the central laboratory of Gaziantep University Medical Faculty.

3.1.1. Exclusion Criteria

Patients who have a factor deficiency except for factor VIII and factor IX

Female patients (diagnosed or unhealthy)

Volunteers who are not between the ages 18 and 65

3.1.2 The Evaluation of The Parameters

Inhibitor, FVIII and FIX levels were measured by using a coagulation device (Stago Star Evolution, USA) on the blood samples in tubes with sodium citrate. Also, levels of Vitamin D, parathormone (Beckman Coulter Unicel DxI800, USA), alkaline phosphatase, calcium and phosphor (Beckman Coulter AU5800, USA) levels were measured in the blood samples without anticoagulants.

3.2 METHOD

3.2.1 Measuring the Vitamin D Levels

Vitamin D total assay is a two-step competitive binding immunoenzymatic test. In the first incubation, the sample is added to a reaction vessel with paramagnetic particles coated with a DBP release agent and sheep monoclonal anti-25 (OH) vitamin D antibody. 25 (OH) vitamin D is released from the DBP and is bound to the immobilized monoclonal anti-25 (OH) vitamin D in the solid phase. Then, a competitive 25 (OH) vitamin D analog-alkaline phosphatase conjugate is added to bind to the immobilized monoclonal anti-25 (OH) vitamin D. After a second incubation, the solid-phase bound materials are held in a magnetic field. The unbound material is washed. Subsequently, the chemiluminescent substrate Lumi-Phos * 530 is added to the vessel and the light produced by the reaction is measured with a luminometer. Light production is inversely proportional to the 25 (OH) vitamin D concentration in the sample. The

amount of analyte in the sample is determined from a stored multi-point calibration curve.

3.2.1.1 Expected Values

Table 2.1 Optimal values of 25(OH) Vitamin D in serum.

Vitamin D Status	25 (OH) Vitamin D Concentration Range (ng/mL)	25 (OH) Vitamin D Concentration Range (nmol/L)
Deficient	< 20	< 50
Insufficient	20 to < 30	50 to < 75
Sufficient	30 – 100	75 – 250
Upper Safety Limit	>100	> 250

3.2.2 Measuring the Parathormone Levels

PTH test kit is a paramagnetic particle chemiluminescence analysis made by using immunoassay systems in order to determine the quantitive levels of human serum and parathyroid hormone with an intact plasma (parathyrin, PTH).

The PTH kit is a bi-zonal enzymatic ("sandwich") examination. A sample with monoclonal anti-PTH anticore conjugated with alkaline phosphate, saline buffed with TRIS including protein and paramagnetic particles covered with polyclonal anti-PTH anticore are added into the reaction container. After the incubation in a reaction container, the materials are brought to a solid phase and when the materials that have not been brought to a solid phase were being washed, they are held in a magnetic area. Afterwards, chemiluminescence substrate Lumi-Phos* 530 is added to the container and the light produced by the reaction is measured using a luminometer. The light production levels are directly proportional with the PTH concentration in the sample. The amount of analyte in the sample is calculated based on the multipoint calibration curve.

3.2.2.1 Expected Values

Median values and 95% non-parametric reference ranges are shown below:

Table 2.2 Optimal values of PTH in serum

n	Avarage Age	Age Range	Median Value	Reference Range
289	40	19-67	37,8 pg/mL (4,0 pmol/L)	12-88 pg/mL (1,3-9,3 pmol/L)

3.2.3 Measuring the Calcium Levels

The test kit for calcium is based on calcium ions (Ca2+) which react with Arsenazo III (2,2'-[1,8-Dihydroxy 3,6-disalfonaftilene-2,7-bisazote]-bisbenzenarsonic asid) in a way that it produces an intense purple complex. In this method, the increase in the absorbance of the Ca-Arsenazo III complex is measured bichromatically in 660/700 nm. The increase in the absorbance of the reaction mixture is directly proportional with the calcium concentration in the sample [141,142].

3.2.3.1 The Reaction Principle

Ca^{+2} + Arsenazo III $\xrightarrow[\text{pH 6,9}]{}$ Ca-Arsenazo III complex (purple)

3.2.3.2 The Expected Values

Table 2.3 Optimal values of Ca in serum

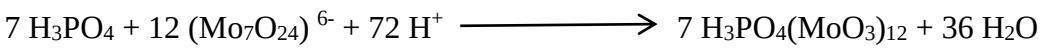
Sample Type	Group	Reference Range
Serum, plasma	Adults	2,20-2,65 mmol/L (8,8-10,6 mg/dL)

3.2.4 Measuring the Phosphorus Levels

The phosphorus kit is a photometric UV test which is used to determine the amount of the inorganic phosphorus in human serum, plasma and urine in Beckman Coulter analysis devices. Inorganic phosphorus reacts with molybdate and creates a heteropolyanion complex. Using surface-active agent annihilate the need to prepare a

filtrate that does not contain protein. The absorbance in 340/380 nm is directly proportional to the inorganic phosphor concentration in the sample [143,144].

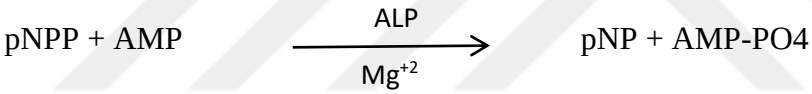
3.2.4.1 The Reaction Principle



3.2.5 Measuring Alkaline Phosphatase Levels

The alkaline phosphate in human serum and plasma is detected and measured in quantity. The alkaline phosphate activity is determined by measuring the ratio of p-nitrophenylphosphatase (pNPP) turning into p-nitrophenol (pNP) in the presence of 2-methyl-1-propanole (AMP), zinc ions and magnesium which is a phosphate acceptor in pH 10,4. Depending on the pNP occurrence, the absorbance change rate is measured in 410/480 nm dichromatically and it is directly proportional with the ALP activity in the sample [145].

3.2.5.1 The Reaction Principle



3.2.5.2 The Expected Values

Table 2.4 Optimal values of ALP in serum

Sample Type	Group	Reference Range
Serum, plasma	Adults	30-120 U/L

3.3 Statistical Analysis

Whether the quantitive data is in normal distribution is tested with the Wilk test. Student t test was used to compare the variables, which are in normal distribution, in two groups, and ANOVA tests were used to compare the same variables in three groups. Mann Whitney U test was used to compare the variables, which are not in normal distribution, in two groups and the Kruskal Wallis test was used to compare

them in three groups. The relations between the variables in normal distribution were tested using Pearson correlation coefficient, whereas the relations between the variables that are not in normal distribution were tested useing Spearman rank correlation coefficient. SPPS 22.0 programmer was used in the analysis. $P<0,05$ was taken to be significant.

CHAPTER IV

RESULTS

Table 4.1 The comparison of the quantitive variables between the groups

	Hemophilia	Control	*P
Number samples	40	40	
Age *(years)*	33 ± 11	33 ± 10	0,828
BMI *(kg/m^2)*	25,81 ± 5,33	26,43 ± 3,97	0,575

*: p<0,05 is statistically significiant level

80 patients participated to this study and the number of participants in the patients group is equal to those in the control group. It was observed that age, height, weight BMI and the general distribution of the parameters involved in the study were in normal distribution in patient and control groups. The 80% of the patients are Hemophilia A, whereas the 20% of them are Hemophilia B (Table 4.2).

Table 4.2 Descriptive Statistic

		N	%
Group	Control	40	50,0%
	Hemophilia	40	50,0%
Hemophilia	Hemophilia A	32	80,0%
subgroups	Hemophilia B	8	20,0%
Place work	Indoor	43	57,3%
	outdoor	32	42,7%

Table 4.3 Factor levels of hemophilia patients

	hemophilia	Total
Number samples	40	40
Factor	1,22 ± 1,07	1,21 ± 1,06

Table 4.4 The comparison of the quantitive variables between the groups

	Hemophilia	Control	*P
Number samples	40	40	
VitD *(ng/mL)*	21,86 ± 8,03	25,46 ± 6,09	0,027*
Ca *(mg/dL)*	9,97 ± 0,38	10,20 ± 0,38	0,011*
P *(mg/dL)*	3,45 ± 0,84	3,46 ± 0,46	0,961
PTH *(pg/mL)*	44,47 ± 33,81	38,44 ± 16,60	0,314
ALP *(U/L)*	115,85 ± 36,64	93,12 ± 22,86	0,001*

*: p<0,05 is statistically significiant level

Table 4.5 Vitamin D distribution in hemophilia patients

	(Severe vitamin D deficiency) <10 ng/mL	**(Vitamin D deficiency) 10-20 ng/mL**	**(Vitamin D insufficiency) 20-30 ng/mL**	**Normal > 30 ng/mL**	**Total ng/mL**
N	2 (% 5)	10 (% 25)	25 (% 62,5)	3(% 7,5)	40
Age *(Years)*	41 ± 4	36 ± 10	33 ± 11	19 ± 2	33 ± 11
Vit.D *(ng/mL)*	7,45 ± 3,13	14,9 ± 3,21	23,77 ± 2,84	38,91 ± 13,42	21,87 ± 8,04

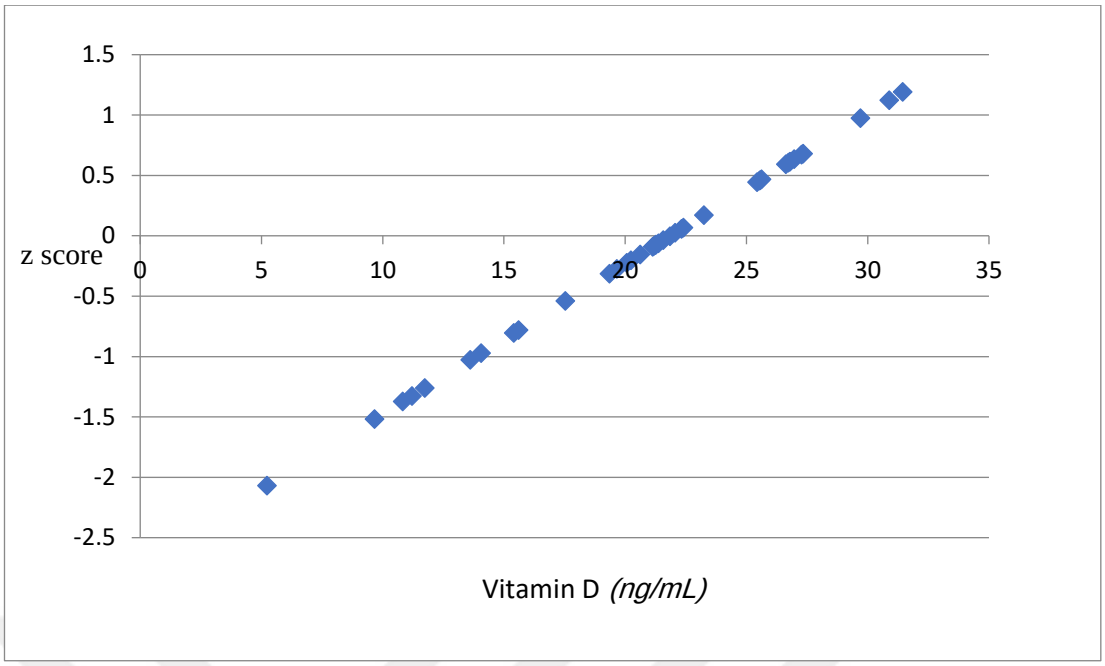


Figure 4.1 Z score for vitamin D levels in hemophilia patients

No significant difference regarding age, BMI, P and PTH was found between the control group and the group with hemophilia diagnosis when the quantitive variables were compared among groups ($p>0.05$), however significant differences were observed with regard to Vitamin D and Ca^{2+} levels ($p<0.05$). Both parameters increased in the control group more in comparison with the group with hemophilia (Table 4.7). Moreover, ALP levels were observed to have increased significantly in the group with hemophilia ($p<0.001$).

Table 4.6 Vitamin D distribution in healthy control group

	(Severe vitamin D deficiency) <10 ng/mL	(Vitamin D deficiency) 10-20 ng/mL	(Vitamin D insufficiency) 20-30 ng/mL	Normal >30 ng/mL	Total ng/mL
N	-	7 (% 17,5)	22 (% 55)	11 (% 27,5)	40
Age *(Years)*	-	34 ± 5	35 ± 9	30 ± 11	33 ± 9
Vit.D *(ng/mL)*	-	18,67 ± 0,52	22,6 ± 2,31	33,51 ± 4,76	25,46 ± 6,09

Table 4.7 The comparison of the quantitive variables between control and hemophilia A

Factor	Group	N	Mean	P
Age *(Years)*	Control	40	33 ± 9	0,985
	hemophilia A	32	33 ± 11	
BMI *(kg/m^2)*	Control	35	26,43 ± 3,97	0,695
	hemophilia A	32	25,98 ± 5,40	
Vit.D *(ng/mL)*	Control	40	25,46 ± 6,09	0,010*
	hemophilia A	32	21,74 ± 5,71	
Ca *(mg/dL)*	Control	40	10,20 ± 0,38	0,086
	hemophilia A	32	10,05 ± 0,33	
P *(mg/dL)*	Control	40	3,46 ± 0,46	0,509
	hemophilia A	32	3,38 ± 0,53	
PTH *(pg/mL)*	Control	40	38,44 ± 16,60	0,916
	hemophilia A	32	38,02 ± 16,15	
ALP *(U/L)*	Control	40	93,12 ± 22,86	0,006*
	hemophilia A	32	111,62 ± 32,16	

*: p<0,05 is statistically significiant level

Table 4.8 The comparison of the quantitive variables between control and hemophilia B

Factor	Group	N	Mean	P
Age *(Years)*	Control	40	33 ± 9	0,535
	hemophilia B	8	31 ± 10	
BMI *(kg/m^2)*	Control	35	26,43 ± 3,97	0,447
	hemophilia B	8	25,16 ± 5,36	
Vit.D *(ng/mL)*	Control	40	25,46 ± 6,09	0,109
	hemophilia B	8	22,34 ± 14,66	
Ca *(mg/dL)*	Control	40	10,20 ± 0,38	0,005*
	hemophilia B	8	9,67 ± 0,44	
P *(mg/dL)*	Control	40	3,46 ± 0,46	0,648
	hemophilia B	8	3,73 ± 1,62	
PTH *(pg/mL)*	Control	40	38,44 ± 16,60	0,146
	hemophilia B	8	70,27 ± 65,29	
ALP *(U/L)*	Control	40	93,12 ± 22,86	0,016*
	hemophilia B	8	132,75 ± 49,91	

*: p<0,05 is statistically significiant level

As a result of the comparison between the factor groups and the control groups, the Vitamin D levels were observed to have decrease significantly in Hemophilia A patients (p= 0,010), whereas ALP increased significantly (p=0,006) **(Table 4.8).**

As a result of the comparison between the control group and the group with Hemophilia B, it was observed that the Ca^{2+} levels have decreased significantly in the group with Hemophilia B (p=0,005*). Contrary to this, ALP levels have increased in the same group (p=0,016) **(Table 4.9).** When the parameters between the groups of Hemophilia A and B patients are compared, the Ca^{2+} levels were found high is Hemophilia A patients and the difference is statistically significant (p=0,012).

Table 4.9 The comparison of the quantitive variables between hemophilia A and hemophilia B

	Factor Group	N	Mean	P
Age *(Years)*	hemophilia A	32	33 ± 11	0,636
	hemophilia B	8	31 ± 10	
BMI *(kg/m^2)*	hemophilia A	32	25,98 ± 5,40	0,800
	hemophilia B	8	25,16 ± 5,36	
Vit.D *(ng/mL)*	hemophilia A	32	21,74 ± 5,71	0,853
	hemophilia B	8	22,34 ± 14,66	
Ca *(mg/dL)*	hemophilia A	32	10,05 ± 0,33	0,012*
	hemophilia B	8	9,67 ± 0,44	
P *(mg/dL)*	hemophilia A	32	3,38 ± 0,53	0,562
	hemophilia B	8	3,73 ± 1,62	
PTH *(pg/mL)*	hemophilia A	32	38,02 ± 16,15	0,128
	hemophilia B	8	70,27 ± 65,29	
ALP *(U/L)*	hemophilia A	32	111,62 ± 32,16	0,147
	hemophilia B	8	132,75 ± 49,91	

*: p<0,05 is statistically significiant level

The relation between age, BMI, Vitamin D, P, PTH and ALP have been studied in the correlation analysis.

4.2 The Correlation Analysis of the Control Group

When the control group was analyses based on age, it was determined that there is intermediate positive correlations between age and BMI and weak positive correlation between age and PTH. Moreover, a weak negative relation was found between age and P and ALP. When the relation between BMI and other parameters in this group was examined, it was seen that there was a positive intermediate correlation between age and BMI. Also, it was found that there is negative weak correlation between BMI and Vitamin D. Ca^{+} did not display any significant correlations with any parameter. When the correlation relations between the parameters P, ALP, PTH and other parameters, it was observed that P and ALP were in weak negative correlation with age among all the other parameters, and PTH was in positive correlation with only age, likewise.

4.3 The Correlation Analysis of the Hemophilia Group

In this group, an intermediate positive correlation was observed between BMI and age, whereas an intermediate negative correlation was found between Vitamin D and age. It was also seen that BMI is in intermediate negative correlation with Vitamin D. It was also reported that Vitamin D showed a weak negative relation with PTH, apart from age and BMI. Moreover, when the relation between Ca^{+} and other parameters were examined, it was found that it is in intermediate negative correlation with PTH only. Another parameter with which PTH displays a significant relation is parameter P; P and PTH are in positive correlation and there is a strong relation between them. Another parameter with which P shows a significant correlation is ALP, and these two parameters are in intermediate positive correlation. ALP and PTH were also observed to be in intermediate positive correlation. Lastly, there was no other parameter than P and PTH with which ALP was in a significant correlation observed.

4.4 The Correlation Analysis of the Hemophilia A Group

It was observed that there was an intermediate positive correlation between Vitamin D and age and a intermediate negative correlation between BMI and Vitamin D. Moreover, the relation between BMI and Vitamin D was revealed to be a negative weak correlation. Also, it was found that Vitamin D is in intermediate negative correlation with PTH. Another finding within this group is that Ca^{+}, P and ALP displayed no significant correlation with any parameter. Lastly, PTH evaluated within

this group was seen to Show an intermediate negative correlation only with Vitamin D.

4.5 The Correlation Analysis of the Hemophilia B Group

In this group, a strong negative correlation between age and Vitamin D, and a very strong negative correlation with age and Ca^{+}. The only parameter with which Vitamin D displays a significant relation is found to be age. Moreover, it was observed that there was a strong negative relation between BMI and P. It was also observed that Ca^{+} was in a very strong negative correlation with age and a strong negative correlation with PTH. Ca^{+} was found to be the only parameter with which PTH displays a significant correlation and these two were determined to be in strong negative correlation with each other. On the other hand, P was found to display a strong negative correlation with only BMI. Lastly, our findings showed that ALP displayed no significant correlation with any other parameter.

Table: 4.10 Correlations of parameters in control group

Factor Group	Age	BMI	Vit.D	Ca	P	PTH	ALP
Age *(years)*	1,000	0,463**	-0,160	-0,236	-0,326*	0,340*	-0,398*
BMI *(kg/m^2)*	0,463**	1,000	-0,382*	-0,242	0,029	0,290	-0,247
Vit.D *(ng/mL)*	-0,160	-0,382*	1,000	0,219	0,239	-0,171	0,183
Ca *(mg/dL)*	-0,236	-0,242	0,219	1,000	-0,038	-0,146	0,275
P *(mg/dL)*	-0,326*	0,029	0,239	-0,038	1,000	-0,128	0,215
PTH *(pg/mL)*	0,340*	0,290	-0,171	-0,146	-0,128	1,000	0,012
ALP (*U/L*)	-0,398*	-0,247	0,183	0,275	0,215	0,012	1,000

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table: 4.11 Correlations of parameters in Hemophilia A group

Factor Group	Age	BMI	Vit.D	Ca	P	PTH	ALP
Age *(years)*	1,000	0,487**	-0,410*	-0,345	-0,230	0,157	-0,107
BMI *(kg/m²)*	0,487**	1,000	-0,371*	-0,048	-0,167	0,095	-0,250
Vit.D *(ng/mL)*	-0,410*	-0,371*	1,000	0,173	-0,070	-0,412*	0,114
Ca *(mg/dL)*	-0,345	-0,048	0,173	1,000	0,229	0,056	0,101
P *(mg/dL)*	-0,230	-0,167	-0,070	0,229	1,000	0,298	-0,051
PTH *(pg/mL)*	0,157	0,095	-0,412*	0,056	0,298	1,000	-0,029
ALP *(U/L)*	-0,107	-0,250	0,114	0,101	-0,051	-0,029	1,000

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table: 4.12 Correlations of parameters in Hemophilia B group

Factor Group	Age	BMI	Vit.D	Ca	P	PTH	ALP
Age *(years)*	1,000	0,310	-0,714*	-0,922**	0,286	0,690	0,238
BMI *(kg/m²)*	0,310	1,000	-0,667	-0,120	-0,762*	0,286	-0,262
Vit.D *(ng/mL)*	-0,714*	-0,667	1,000	0,527	0,238	-0,524	-0,095
Ca *(mg/dL)*	-0,922**	-0,120	0,527	1,000	-0,455	-0,778*	-0,287
P *(mg/dL)*	0,286	-0,762*	0,238	-0,455	1,000	0,286	0,333
PTH *(pg/mL)*	0,690	0,286	-0,524	-0,778*	0,286	1,000	-0,095
ALP *(U/L)*	0,238	-0,262	-0,095	-0,287	0,333	-0,095	1,000

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table: 4.13 Correlations of parameters in Hemophilia group

Factor group	Age	BMI	Vit.D	Ca	P	PTH	ALP
Age *(years)*	1	0,548**	-0,422**	-0,288	0,021	0,224	-0,061
BMI *(kg/m²)*	0,548**	1	-0,433**	-0,007	-0,287	0,089	-0,194
Vit.D *(ng/mL)*	-0,422**	-0,433**	1	0,244	-0,066	-0,367*	0,024
Ca *(mg/dL)*	-0,288	-0,007	0,244	1	-0,272	-0,481**	-0,150
P *(mg/dL)*	0,021	-0,287	-0,066	-0,272	1	0,644**	0,404**
PTH *(pg/mL)*	0,224	0,089	-0,367*	-0,481**	0,644**	1	0,480**
ALP *(U/L)*	-0,061	-0,194	0,024	-0,150	0,404**	0,480**	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

When the relation between Vitamin D levels and working conditions, it was observed that the number of participants whose workplace is outdoors was higher than the number of participants whose workplace is indoors and the difference between the two is significant (p=0.003) (Table 4.14).

When the effect of whether their workplace was indoors or outdoors was examined with regard to Vitamin D levels, no significant difference between the participants of the control group was found, however it was also observed that among the participants of the group with hemophilia, those whose workplace is outdoors showed higher Vitamin D levels in a statistically meaningful amount compated to those whose workplace is indoors p=0.04) (Table 4.15).

Table 4.14 The Comparison of workplace and vitamin D

Place work		N	Mean	P
Vit.D	Indoor	43	25,14 ± 7,90	0,003*
	outdoor	32	20,12 ± 5,50	

*: p<0,05 is statistically significiant level

Table 4.15 The effect of working indoors or outdoors on the vitamin D levels

Group		Place work	N	Mean	P
Control	Vit.D	Indoor	21	25,82 ± 7,00	0,096
		outdoor	14	22,03 ± 4,76	
Hemophilia	Vit.D	Indoor	22	24,50 ± 8,79	0,004*
		outdoor	18	18,64 ± 5,71	

*: p<0,05 is statistically significiant level

CHAPTER V

DISCUSSION AND CONCLUSION

5.1. DISCUSSION

Hemophilia is a genetic blood disorder, which is usually inherited. The gene is passed down from a parent to a child. Hemophilia happened when blood does not have enough clotting factor [1]. The most characteristic clinical manifestation of the hemophilias is bleeding into large joints of upper and lower extremities [4].

Calcium plays a key role in a wide range of biologic functions. One of the most important functions as bound calcium is in skeletal mineralization [71]. Calcium absorption requires calcium-binding proteins and is regulated by vitamin D, sunlight, parathyroid hormone and thyrocalcitonin. The amount of calcium in the blood is tightly controlled by signaling pathways and molecules, including PTH and vitamin D [75]. PTH level increases in both bone building and osteoporosis. It regulates bone resorption to release calcium and compensate for the lack of calcium in the blood. Lining cells [109]. Calcium has a role in regulating the biological synthesis of PTH hormone.

Phosphorus plays a role in numerous biologic processes, including energy metabolism and bone mineralization [102]. High phosphate levels in the blood also reduce the effectiveness of vitamin D3 therapy in patients with hyperthyroidism [106]. Biochemical findings in osteomalacia include low serum and urinary calcium, low serum phosphate, elevated serum ALP and elevated PTH. Hence, markedly high levels often associated with Paget's disease. Moderate increases occur in osteomalacia [89].

Vitamin D is to maintain a balanced level of calcium in the blood by promoting absorption in the intestines and maintain the level of calcium and phosphate in the bone [59]. Osteoporosis in hemophilic patients is a significant problem. The causes of osteoporosis in hemophilic patients are lack of adequate exercise, multiple hemorrhage and inflammation, and low vitamin D levels. Children with Haemophilia A have

reduced bone mineral density (BMD) and are at risk for developing osteoporosis and fractures later in life. A total of 80 volunteers were enrolled in our study, including 40 hemophiliacs and 40 healthy controls. Numbers of patient and control were determined by performing Power analysis before the study was started. Thirty-two of the hemophiliacs are Hemophilia A (80%) and eight of the hemophiliacs are Hemophilia B (20%) (Table: 4.2). In our region and globally number of hemophilia patients especially numbers of hemophilia B patients are very low, so there is an unbalanced distribution between Hemophilia A and B [14]. Only adult male patients and healthy controls were involved in this study. When the age and BMI values of the patient and healthy control group were examined, no statistically significant difference was found between groups ($p > 0.05$) (Table: 4.1). The parameters that we examined in this study are the factors that can be affected by age and BMI. Therefore, no statistically significant difference between the two groups indicates that the results are not affected by age or BMI [57].

Patients and healthy people who had not previously received Vitamin D supplementation and did not have hepatitis C or HIV were included in this study. In this respect, it was ensured that Vitamin D levels did not change depending on these conditions [149,150].

There are two types of hemophilia, both have the same symptoms. Hemophilia A is the most common form and is caused by having reduced levels of clotting FVIII. Hemophilia B, also known as Christmas Disease, is caused by having reduced levels of clotting FIX. The age and BMI of hemophilia A and hemophilia B patients were examined with each other and separately healthy control group and no statistically significant difference was found ($p>0,05$) (Table: 4.9). This shows that there is a balanced distribution among the groups and that the results are not affected by age and BMI [57].

Mean values and standard deviations for Ca, phosphorus, alkaline phosphatase and parathormone levels of hemophilia patients and healthy control group were calculated (Table: 4.4). When the results were examined, the mean values of both the patients and the healthy control group were within the reference ranges (Reference intervals; Ca: 8,8-10,6 mg/dL, phosphorus: 2,5-4,5 mg/dL, ALP: 30-120 U/L ve PTH: 12-88 mg/dL) [151]. These results indicate that there is no pathological condition that causes

a significant change in Ca, P, ALP and PTH levels in the patient and healthy control group. And these results show that our results are not affected by any other diseases.

The mean value of factor values of hemophilia patients was found 1.22 ± 1.07% (reference 60-150% catalytic activity). This result shows that laboratory findings are compatible with disease groups. [18-20].

When the Vitamin D distributions in the healthy control group were examined, Vitamin D deficiency was found in the majority of the control group (55%). According to the World Health Organization (WHO), over the world, one out of every three people, or one out of every two people have Vitamin D deficiency (Table: 4.6). So our findings support this expression [152].

When vitamin D distribution of the hemophilia group was examined, 5% of the patients had severe vitamin D deficiency, 25% had vitamin D deficiency, 62.5% had vitamin D insufficiency and 7.5% had normal vitamin D levels. A great majority of hemophiliacs (92.5%) had vitamin D levels lower than normal. These results indicate that hemophilia patients are suffering from Vitamin D deficiency. These findings conform with results of previous studies that are studies of Albayrak *et al.* in Hemophilia A children and Sanadhya *et al.* in hemophilia children [62,114].

Z scores were calculated in hemophilia patients (Figure: 4.1). When Table 1 was examined, it was shown that z scores of a majority (77.5%) of hemophiliac patients were in ± 1 SS. Z scores of 95% of the patients were in ± 2 SD. This result shows that the Vitamin D levels of patients are uniformly distributed.

Vitamin D, Ca, P, PTH and ALP levels of patient and control groups were measured (Table: 4.4). There was a statistically significant increase ($p < 0,05$) in ALP in patients as compared to controls. In contrast, statistically, significantly lower levels of calcium and vitamin D were detected among patients as compared to controls ($p < 0,05$).

There are 7 published studies on vitamin D levels in hemophiliacs. In the 5 of these studies Vitamin D levels were found to be significantly lower in the hemophilia patients than in the control group, and this is consistent with our results [62,146-148]. In contrast, in two of these studies, Vitamin D levels in hemophilia patients were not different from control group (Gallacher et al., Katsarou et al). Vitamin D levels can vary with age, BMI, season, ethnicity, living area, feeding habits, socioeconomic

status, and especially exposure to sunlight. In these two studies, Vitamin D levels could be found insignificant compared to the control group due to any of these factors. In order to minimize the effects of these situations in our work; all examples of the volunteers who participated in the study collected in a short period of 10 days in the autumn season and volunteers were selected from those living in the province of Gaziantep. Also, it was noted that all the individuals were citizens of Turkey. It was also noted that there was a balanced distribution of working places (open or closed area) of volunteers participating in the study (Table: 4.14).

Vitamin D has a key role in calcium absorption. Because of this, Vitamin D levels directly affect calcium levels. In our study, the reason why the low levels of Ca levels in hemophilia patients compared to the control group is low levels of Vitamin D. Moreover, this result is consistent with many previous studies [115,116]

In our study, ALP was found significantly higher in hemophilia group than control group ($p<0,05$). Costa *et al.* and Dhanwal *et al.* found that ALP levels were elevated in some disease groups which they had low Vitamin D levels in relation to vitamin D levels [154,155]. Our findings are consistent with these results. In addition, intraosseous hemorrhages are very common in hemophilia patients. This may have resulted in too much ALP release to the blood and elevated serum ALP levels [32].

In our study, there was no difference in PTH and phosphorus between hemophilia and control ($p> 0.05$). However, Alioglu *et al.* found that parathormone levels decrease in hemophiliacs [116].

Hemophilia A and hemophilia B patients were compared with each other and separately healthy control group (Table: 4.7, 4.8, 4.9). When hemophilia A compared with control group, Vitamin D was significantly lower in hemophilia A group but ALP was found significantly higher ($p <0.05$). These results also correspond to the results of Albayrak's study on children with Hemophilia A [114].

When hemophilia B compared with control group, Ca was significantly lower in hemophilia group and ALP was found to be significantly higher ($p <0.05$).

When Hemophilia A compared with and Hemophilia B, there was a significant difference only in Ca levels ($p <0.05$). Vitamin D levels were higher in hemophilia B group than hemophilia A, but there was no statistically significant difference ($p> 0.05$).

Hemophilia A patients show more hemorrhage due to genetic reasons, even though they have the same factor value as Hemophilia B patients [32]. For this reason, lower levels of Vitamin D were seen in patients with Hemophilia A. However, probably there was no significant difference because of the low number of Hemophilia B.

Correlation analyses were made between the parameters in the control and hemophilia groups. In the control group, there were significant positive correlation between age and BMI ($p < 0,05$, r: 0,463) and PTH ($p < 0,05$, r: 0,340). However, there were significant inverse correlation between age and phosphorus ($p<0,05$, r:-0,326) and ALP ($p < 0,05$, r: -0,398). There was also a statistically significant inverse correlation between vitamin D and BMI ($p < 0.05$, r: -0.382). In other words, the more increase BMI, the more decrease in Vitamin D levels. As age increases, exercise and metabolism decrease. As a result, we can observe increases in weight and, consequently, BMI. An inverse correlation between BMI and Vitamin D was mentioned. With the increase in weight, the rate of people getting sunlight is decreasing. As the sunlight is the main source for vitamin D synthesis, there may have been reductions in Vitamin D levels.

In the control group, there were significant inverse correlation between Vitamin D and age ($p<0,05$, r:-0,422), BMI ($p<0,05$, r:-0,433) and PTH ($p < 0,05$, r: r:-0,367). However, there was no significant correlation between Vitamin D and calcium, phosphorus and ALP ($p > 0.05$). In other words, the more decrease Vitamin D, the more increase in age, BMI and PTH levels. Vitamin D is expected to decline with age and BMI. Depending on age and weight gain, people are less exposed to sunlight. This leads to a decrease in the synthesis of vitamin D. However, Eldash and colleagues found no significant association between vitamin D and BMI in hemophiliacs (115). There are publications that examine the association of vitamin D with PTH, and our findings are consistent with the results in these publications. Eldash et al. and Jahnsen et al. found a negative correlation between vitamin D and PTH in hemophilia patients. In addition, Dhanwal et al. found a negative correlation between vitamin D and PTH in patients with hip fractures and also Costa et al. found a negative correlation between vitamin D and PTH in obese patients (115-155).

Sunlight is the most influential factor for Vitamin D levels. For this reason, the study places of the patients and controls taken into the study were also examined (Table:

4.15). Vitamin D levels were higher in open-field workers in both control and hemophilia groups. However, this increase was not significant in the control group ($p > 0.05$). Vitamin D levels in the open field workers were significantly higher in the hemophilia group ($p < 0.05$). These results show us the effects of exposure to sunlight on Vitamin D. It also shows that sunlight is the main source for Vitamin D.

5.2 Conclusion

The present study includes evaluation of different factors affecting bone metabolism in people with hemophilia including the following: vitamin D, calcium, phosphorous, Alkaline Phosphatase, and parathormone levels and comparing them with healthy people.

In conclusion, the issue of vitamin D deficiency in hemophilic people should be revisited. The high percentage (96%) of vitamin D deficiency in patients with hemophilia compared with healthy people and It was vitamin D levels in patients with hemophilia were very low. This decrease leads to osteoporosis and Arthropathic disease.

Plasma Vitamin D levels should be measured twice a year. In case of deficiency Vitamin D should be supplemented. Vitamin D deficiency is a problem that always escapes the attention of doctors. This affects the lifestyles of the patients. Therefore, vitamin D deficiency must continue to be investigated in the decades to come. Programs should be developed to prevent patients spending their lives with vitamin D deficiency. Vitamin D and calcium supplementation may be a cheap and relatively safe early intervention for people with hemophilia to prevent bone loss and low peak bone mass. Maintenance dosage of vitamin D supplementation may be >1000 U per day.

Hemophilia care providers should promote adequate bone formation during childhood and reduce bone loss during adulthood as well as identify patients with low BMD that would benefit from therapy.

In the next studies researcher will study effect of Vitamin D on BMD in children with hemophilia. Children with hemophilia who have Vitamin D deficiency can be examined for the protective effect of Vitamin D on BMD by measuring BMD before and after treatment with Vitamin D supplementation.

REFERENCES

[1] Rosendaal FR, Brit E (1990). The Increasing Prevalence of Hemophilia. *Thrombosis and Haemostasis*, **63**, 145.

[2] World Federation of Haemophilia, Global Incidence and Prevalence Rates Approved by the President's Strategic Council. (2009).

[3] White GC II, Rosendaal F., Alendrot LM, Lusher JM, Ingersley J, (2001). Factor VIII and factor IX subcommittee, *Thromb Haemost*, **85**, 560 .

[4] Peyvandi F, Garagiola I, Young G (2016). The past and future of haemophilia: diagnosis, *treatments, and its complications*. **388**, 187–97.

[5] Dorine B., Marilyn H., Information booklet on mild hemophilia (2007). The Atlantic Hemophilia Nurses Group, *Canadian Hemophilia Society J.Bayer Health Care*, **1stEd**, 20- 30.

[6] Kelly L. Vanderhave, MD, Michelle S. Caird, MD, Mark Hake, MD, Robert N. Hensinger, MD, Andrew G. Urquhart, MD, Selina Silva, MD, Frances A. Farley, MD (2010). Musculoskeletal care of the hemophilic patients. *J Am. Acad. Orthop. Surg*; **20**, 553-63.

[7] Rosendaal FR, Smit C, Briºt E (1991). Hemophilia treatment in historical perspective: a review of medical and social developments. *Ann Hematol*, **62**, 5-15.

[8] Massimo Franchini, Pier Mannuccio Mannucci (2014). The History of Hemophilia. *Semin Thromb Hemost*, **40**, 571–576.

[9] Stevens RF (1999). The history of haemophilia in the royal families of Europe. *Br J Haematol*, **105(1)**, 25–32.

[10] World Federation of Haemophilia, Report on the Annual Global Survey (2016). 14.

[11] World Federation of Haemophilia, Global Incidence and Prevalence Rates Approved by the President's Strategic Council. (2015).

[12] Stonebraker, J.S., et al., (2010). A study of variations in the reported haemophilia A prevalence around the world. Haemophilia : *the official journal of the World Federation of Hemophilia*,. **16(1),** 20-32.

[13] Prevalence around the world (2010). Haemophilia: the official journal of the World Federation of Hemophilia, **16(1)**, 20-32.

[14] Hemophilia Society in Turkey: http://www.turkhemoder.org/sayfa/10/hemofili-nedir.

[15] Manuel Carcao, Paul Moorehead, David Lillicrap, HEMOPHILIA A and B (1940). C H A P T E R 137.

[16] Antonarakis SE (1995). Molecular genetics of coagulation factor VIII gene and hemophilia A. *Thromb. Haemost*,**74**, 322-328.

[17] Gouw SC, van den Berg HM, le CS, van der Bom JG (2007). Treatment characteristics and the risk of inhibitor development: a multicenter cohort study among previously untreated patients with severe hemophilia A. J *Thromb Haemost*,**5,** 1383-90.

[18] Oldenburg J, El Maarri O, Schwaab R (2002). Inhibitor development in correlation to factor VIII genotypes. *Haemophilia,* **2**, 23-9.

[19] Schwaab R, Brackmann HH, Meyer C (1995). Haemophilia A: Mutation type determines risk of inhibitor formation. *Thromb Haemost*, **74**, 1402-6.

[20] Gouw SC, van den Berg HM, Fischer K (2013). Intensity of factor VIII treatment and inhibitor in children with severe hemophilia *A: the RODIN study. Blood*,**121**, 4046-4055.

[21] Larsson SA, Wiechel B (1983). Deaths in Swedish hemophiliacs, *Acta Med Scand* **214**, 199-206.

[22] Chitlur M, Warrier I, Rajpurkar M, Lusher JM (2009). Inhibitors in factor IX deficiency a report of the ISTH-SSC international FIX inhibitor registry. *Haemophilia,* **15(5)**, 1027-1031.

[23] Nathwani AC, Tuddenham EG (1992). Epidemiology of coagulation disorders. *Baillieres Clin Haematol*, **5**, 383-439.

[24] Biggs R, Douglas AS, Macfarlane RG, (1952). Christmas disease: a condition previously mistaken for haemophilia. *Br Med J. 27***, 2(4799)**, 1378-82.

[25] Simioni P, Tormene D, Tognin G, (2009). X-linked thrombophilia with a mutant factor IX (factor IX Padua). *N Engl J Med*, **361**, 1671–5.

[26] Plug I, Mauser-Bunschoten EP, Bröcker-Vriends AH, van Amstel HK, van der Bom JG, van Diemen-Homan JE, Willemse J, Rosendaal FR. Bleeding in carriers of hemophilia. *Blood* 2006; 108: 52-6.

[27] Zucker M, Seligsohn U, Salomon O, Wolberg AS (2014). Abnormal plasma clot structure and stability distinguishing bleeding risk in patients with severe factor XI deficiency. *J Thromb Haemost*, **12 (7),** 1121-30 .

[28] Brenner B, Laor A, Lupo H, Zivelin A, Lanir N, Seligsohn U (1997). Bleeding predictors in factor-XI-deficient patients. *Blood Coagul Fibrinolysis*. **8(8)**, 511-5 .

[29] Peyvandi F, Di Michele D, Bolton-Maggs PH, Lee CA, Tripodi A, Srivastava A (2012). Classification of rare bleeding disorders (RBDs) based on the association between coagulant factor activity and clinical bleeding severity. *J Thromb Haemost*, **10 (9)**, 1938-43.

[30] Guella I, Solda G, Spena S, (2008). Molecular characterization of two novel mutations causing factor XI deficiency: A splicing defect and a missense mutation responsible for a CRM + defect. *Thromb Haemost*. **99 (3)**, 523-30.

[31] Hay CR, Brown S, Collins PW, Keeling DM, Liesner R (2006). The diagnosis and management of factor VIII and IX inhibitors. a guideline from the United Kingdom Haemophilia Centre Doctors Organisation. *Br J Haematol*. **133**, 591–605.

[32] Ljung R, Petrini P, Nilsson IM (1990). Diagnostic symptoms of severe and moderate haemophilia A and B. A survey of 140 cases. *Acta Paediatr Scand*, **79**, 196.

[33] Berg MH, Fischer K (2003). Prophylaxis for severe hemophilia: experience from Europe and the United States. *Seminars in Thrombosis and Hemostatis*, **29**, 49-54.

[34] Triemstra A.H.M, Rosendaal F.R, Smit C, Van der Ploeg H.M, Briºt E (1995). Mortality in patients with haemophilia. *Annals of Internal Medicine*, **123,** 823-827.

[35] Veltkamp JJ, Meilof J, Remmelts HG, (1970). Another genetic variant of haemophilia B: haemophilia B Leyden. *Scand J Haematol***, 7(2),** 82-90.

[36] Peyvandi F, Jayandharan G, Chandy M, Srivastava A, Nakaya SM, Johnson MJ, (2006). Genetic diagnosis of haemophilia and other inherited bleeding disorders. *Haemophilia*, **12(3),** 82–9.

[37] Renault NK, Dyack S, Dobson MJ, Costa T, Lam WL, Greer WL (2007). Heritable skewed X-chromosome inactivation leads to haemophilia A expression in heterozygous females. *Eur J Hum Genet*, **15(6)**, 628–37.

[38] Picketts, DJ. Mueller CR, Lillicrap D (1994). Transcriptional control of the factor FIIV, IX gene: Analysis of five cis-acting elements and the deleterious effects of naturally occurring hemophilia B Leyden mutations. *Blood* **84,** 2992.

[39] Hilgartner, MW, Giardina, P. Liver dysfunction in patients with hemophilia A, B and von Willebrand's disease. *Transfusion*. **1977,17**:495–499.

[40] Sabin CA, Phillips AN, Yee TT, (2005). Twenty-five years of HIV infection in haemophilic men in Britain: an observational study.*BMJ*. **29, 331(7523),** 997-8.

[41] Dolan G (2006). Clinical implications of emerging pathogens in haemophilia: the variant Creutzfeldt-Jakob disease experience. *Haemophilia.* **12 (1**), 16-20.

[42] Bierhoff E, Fischer HP, Willsch E, (1997). Liver histopathologyin patients with concurrent chronic hepatitis C and HIV infection. *Virchows Arch*, **430(4)**, 271-7.

[43] Quintana M, del Amo J, Barrasa A, Perez-Hoyos S, Ferreros I, Hernandez F, et al,(2003). Progression of HIV infection and mortality by hepatitis C infection in patients with haemophilia over 20 years. *Haemophilia*; **9**: 605-12.

[44] DunnAL (2010). Malignancyin patients with haemophilia: a review of the literature. *Haemophilia.* **16(3)**, 427-36.

[45] Char Witmer and Guy Young (2013). Factor VIII inhibitors in hemophilia A: rationale and latest evidence. *Ther Adv Hematol*, **4(1)**, 59–72.

[46] Lund, J, Deluca, H.F (1966). Biologically active metabolite of vitamin D3 from bone liver and blood serum. *J. Lipid. Res*, **7**, 739–744.

[47] Deluca, H.F; (2014). History of the discovery of vitamin D and its active metabolites. *Bonekey Rep*, **3**, 479.

[48] Wacker, M.; Holick, M.F. (2013). Vitamin D—Effects on skeletal and extraskeletal health and the need for supplementation. *Nutrients*, **5**, 111–148.

[49] Yahav, S., Buffenstein, R. (1993). Cholecalciferol supplementation alters gut function and improves digestibility in an underground inhabitant, the naked mole rat (Heterocephalusglaber), when fed on a carrot diet. *The Bri. J. nutrition*, **69 (1)**, 233–41.

[50] Elke Wintermeyer, Christoph Ihle, Sabrina Ehnert, Ulrich Stöckle, Gunnar Ochs, Peter de Zwart, Ingo Flesch, Christian Bahrs and Andreas K. (2016). Crucial Role of Vitamin D in the Musculoskeletal System, *Nussler* **8(6),** 319

[51] Munns, C.F.; Shaw, N.; Kiely, M.; Specker, B.L.; Thacher, T.D.; Ozono, K.; Michigami, T.; Tiosano, D.; Mughal, M.Z.; Makitie, O. (2016). Global consensus recommendations on prevention and management of nutritional rickets. J. *Clin. Endocrinol. Metab*. **101**, 394–415.

[52] Holick, M.F. (2005). Vitamin D. In Modern nutrition in health and disease. 10th edition.M. Shils, *Baltimore, Maryland*, 329–345.

[53] MacLaughlin, J.A., Anderson, R.R., and Holick, M. F (1982). Spectral character of sunlight modulates photosynthesis of previtamin D3 and its photoisomers in human skin. Science, **216,** 1001–1003.

[54] Hess, A.F., and Weinstock, M. (1924). Antirachitic properties imparted to inert fluids and to green vegetables by ultraviolet irradiation. J. Biol. *Chem*. **62**, 301–313.

[55] Matsuoka, L.Y., Ide, L., Wortsman, J., MacLaugh lin, J., and Holick, M.F. (1987). Sunscreens suppress cutaneous vitamin D3 synthesis. J. Clin. Endocrinol. *Metab*. **64**, 1165–1168.

[56] Gultekin, A., Ozalp, I., Hasanoglu, A., and Unal, A. (1987). Serum-25-hydroxycholecalciferol levels in children and adolescents. *Turk. J. Pediatr*. **29**, 155–162.

[57] Ensrud KE, Ewing SK, Fredman L, Hochberg MC, Cauley JA, Hillier TA (2010). Circulating 25-hydroxyvitamin D levels and frailty status in older women. *The Journal of Clinical Endocrinology & Metabolism*, **95(12)**, 5266-73.

[58] Masood, T.; Kushwaha, R.S.; Singh, R.; Sailwal, S.; Pandey, H.; Varma, A.; Singh, R.K.; Cornelissen, G. (2015). Circadian rhythm of serum 25 (OH) vitamin D, calcium and phosphorus levels in the treatment and management of type-2 diabetic patients. *Drug Discov*. *Ther*, **9**, 70–74.

[59] Rabenberg, M.; Scheidt-Nave, C.; Busch, M.A.; Rieckmann, N.; Hintzpeter, B.; Mensink, G.B.M. (2015). Vitamin D status among adults in Germany—Results from the German health interview and examination survey for adults (DEGS1). *BMC Public Health*, 15: 641.

[60] Amling, M (2015). Calcium and vitamin D in bone metabolism. Clinical importance for fracture treatment. Unfallchirurg, **118**, 995–999.

[61] Holick, M.F (2004). Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. *AM J Clin Nutr*. **80(6),** 1678S-1688S.

[62] Sanadhya1. A, Singh. J. (2016). Comperative study of vitamin D levels in Haemophilia and healthy children. *Haemophilia* (PP 01-04; 2279-0861.

[63] Paschou, S.A., Anagnostis, P., Karras, S., Annweiler, C., Vakalopoulou, S., Garipidou, V. & Goulis, D.G(2014). Bone mineral density in men and children with haemophilia A and B: a systematic review and meta-analysis. *Osteoporosis International*, **25**, 2399–2407.

[64] Tlacuilo-Parra A, Morales-Zambrano M, Tostado-Rabago N, Esparza-Flores MA, Lopez-Guido B, Orozco-Alcala J. (2008). Inactivity is a risk factor for low bone mineral density among haemophilic children. *Br J Haematol*, 140: 562–7

[65] Robertson WG, Marshall RW. (1979). Calcium measurements in serum and plasma total and ionized. *CRC Crit Rev Clin Lab Sci*, **11**,271–305.

[66] Brown EM, MacLeod RJ. (2001). Extracellular calcium sensing and extracellular calcium signaling. *Physiol Rev*, **81(1),** 239–297.

[67] Egbuna O. (2009). The full-length calcium-sensing receptor dampens the calcemic response to 1alpha, 25(OH)2 vitamin D3 in vivo independently of parathyroid hormone. *Am J Physiol Renal Physiol*, **297(3)**, 720–728.

[68] Campbell AK. (1990). Calcium as an intracellular regulator. *Proc Nutr Soc* **49**, 51–56.

[69] Kos CH. (2003). The calcium-sensing receptor is required for normal calcium homeostasis independent of parathyroid hormone. *J Clin Invest*, **111(7)**, 1021–1028.

[70] Canaf. L, Hendy. GN. (2002). Human calcium-sensing receptor gene. Vitamin D response elements in promoters P1 and P2 confer transcriptional responsiveness to 1,25-dihydroxyvitamin D. *J Biolo Chem*, **277(33),** 30337–30350.

[71] Wang. L, Nancollas. GH, Henneman. ZJ. (2006). Nanosized particles in bone and dissolution insensitivity of bone mineral. *Biointerphases* **1**, 106–111.

[72] Allender, PS. Cutler. JA, Follmann. D. (1996). Dietary calcium and blood pressure. *Ann Intern Med*, **124**, 825-831.

[73] Murray. RK, Granner. DK, Mayes. PA, Rodwell. VW. (2000). Harper's Biochemistry, 25th Edition, McGraw-Hill, Health Profession Division, USA.

[74] Hays VW, Swenson MJ (1985). Minerals and Bones. *In: Dukes' Physiology of Domestic Animals*, 449-466.

[75] Ensrud. KE, Doung. T, Cauley. JA, Heaney RP, Wolf RL, Harris E & Cummings SR (2000). Low fractional calcium absorption increases the risk for hip fracture in women with low calcium intake. *Study of Osteoporotic Fractures Research Group*. **132**, 345-353.

[76] Baraton, S. Marianor. L, Arnold. JF. (2014). Sarum Calunm and Pona; Effect of phosphate, vitamin D and uremid *Nefroiogia*, **34(5)**,658-69.

[77] Bouhtiauy. I, LaJeunesse. D, Brunette. M.G. (1991). The mechanism of PTH action on calcium reabsorption by the distal tubule. *Endocrinology*, **128**, 251-258.

[78] Grodner. M, Long. S, Deyoung. S. (2004). Foundation and clinical application of nutrition. **210**, 212-213.

[79] Slattery. M, Edwards, S. Boucher, K. (1999). Lifestyle and colon cancer: An assessment of factors associated with risk. *Am J Epidemiol*, **150**, 869 – 77.

[80] Qin C, Baba O, Butler WT (2004). Post-translational modifications of sibling proteins and their roles in osteogenesis and dentinogenesis. *Crit Rev Oral Biol Med* **15**, 126–136.

[81] Kirsch T (2006). Determinants of pathological mineralization. *Curr Opin Rheumatol* **18,** 174–180.

[82] Giachelli CM 2005. Inducers and inhibitors of biomineralization: Lessons from pathological calcification. *Orthod Craniofac* **8**, 229–231,

[83] London GM, Guerin AP, Marchais SJ, Metivier F, Pannier B, Adda H (2003). Arterial media calcification in end-stage renal disease: Impact on all-cause and cardiovascular mortality. *Nephrol Dial Transplant* **18**, 1731–1740,.

[84] Kestenbaum B, Sampson JN, Rudser KD, Patterson DJ, Seliger SL, Young B, Sherrard DJ, Andress DL (2005). Serum phosphate levels and mortality risk among people with chronic kidney disease. *J Am Soc Nephrol* **16**, 520 –528,.

[85] Kronenberg HM (2002). NPT2a—The key to phosphate homeostasis. *N Engl J Med* **347**, 1022–1024.

[86] Reichling JJ, Kaplan MM (1988). Clinical use of serum enzymes in liver diseases. *Dig Dis Sci*, **33**,1601-1614.

[87] Backstrom MC, Kouri T, Kuusela AL, (2000). Bone isoenzyme of serum alkaline phosphatase and serum inorganic phosphate in metabolic bone disease of prematurity. *Acta Paediatr*, **89**, 867–73.

[88] Whyte MP (1994). Hypophosphatasia and the role of alkaline phosphatase in skeletal mineralization. *Endocr Rev*, **15**, 439–461.

[89] Maldonado O, Demasi R, Maldonado Y, (1998). Extremely high levels of alkaline phosphatase in hospitalized patients. *J Clin Gastroenterol*, **27**, 342-345.

[90] Golub EE (2011) Biomineralization and matrix vesicles in biology and pathology. *Semin Immunopathol* **33**, 409-417.

[91] Larsen, P.; Henry, M.; Kenneth, S. ; shlomo, M. (2003) . Williams Textbook of Endocrinology. 10th ed. USA, 1303-1371.

[92] Knochel J, Shils ME, Olson JA, Shike M, Ross AC. (1999). Modern nutrition in health and disease. *9th ed. Philadelphia, Pennsylvania: Lippincott Williams & Wilkins,* Chapter 8: 157-167.

[93] Wagner CA. (2007) Novel insights into the regulation of systemic phosphate homeostasis and renal phosphate excretion. *J Nephrol* **20**, 130-134.

[94] Raina R, Garg G, Sethi SK, Schreiber MJ, Simon JF, et al. (2012) Phosphorus Metabolism. J Nephrol Therapeutic S3:008. doi:10.4172/2161- 0959.S3-008.

[95] Murer H, Forster I, Biber J. (2004) The sodium phosphate cotransporter family SLC34. *Pflugers Arch* **447**, 763-767.

[96] Uribarr. J. (2007). Phosphorus homeostasis in normal health and in chronic kidney disease patients with special emphasis on dietary phosphorus intake. *Seminars in Dialysis,* **20(4),** 295-301.

[97] Albaa. F, Hutchison. AJ. (2003). Hyperphosphatemia in Renal Failure: Causes, consequences, and current management. *Drugs*, **63(6),** 577-496.

[98] O'Callaghan CA, Brenner BM, (2000). Kidney at a glance. Williston, Vermont: Blackwell Publishing.

[99] Slatopolsky E, Bricker NS. (1973). The role of phosphorus restriction in the prevention of secondary hyperparathyroidism in chronic renal disease. *Kidney International***, 4(2)**, 141-145.

[100] Uribarri J, Calvo MS (2003). Hidden sources of phosphorus in the typical American diet, *Seminars in Dialysis*, **16(3)**, 186-188.

[101] Alan, H. ; Donald, M. ; Janet, R. (1988) . Varley´s Practical Clinical Biochemistry, 601-621.

[102] Malhotra VK. (1998). Biochemistry for Students. Tenth Edition. Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, India.

[103] Murray RK, Granner DK, Mayes PA, Rodwell VW (2000). Harper's Biochemistry, 25th Edition, McGraw-Hill, Health Profession Division, USA.

[104] Knochel. J.Shils ME, Olson JA, Shike M, Ross AC, (1999). Modern nutrition in health and disease. *9th ed. Philadelphia, Pennsylvania: Lippincott Williams & Wilkins,* Chapter 9: 157-167.

[105] Moallem E.; Kilav R.; Silver J.; Naveh. (1998). Many T. RNA protein binding and post-transcriptional regulation of parathyroid hormone gene expression by calcium and phosphate. *J Biol Chem*, **273**: 5253-5259.

[106] Llach, F.; Yudd, M. (1988). The importance of hyperphosphatemia in the severity of hyperparathyroidism and its treatment in patients with chronic renal failure. *Nephrol Dial Transplant*, **13**, 57-61.

[107] Ganesh, S.K.; Hulbert-Shearon, T.; Levin, N.W.; Port, F.K.; Stack, A.G. (2001). Association of elevated serum PO4, Ca PO4 product, and parathyroid hormone with cardiac mortality risk in chronic hemodialysis patients. *J Am Soc Nephrol*, **12**, 2131-2138.

[108] Llach, F. (1999). Calcification: Dealing with another risk factor in patients with renal failure. *Semin* Dial, **12**, 293-295.

[109] Vinay, K.; Ramzi, S.; Stanley, L. (2003). Robbins basic pathology, 7th ed. 738.

[110] Canalis, E.; Centrella, M.; Burch, W.; et al. (1989) . Insulin-like growth factor 1 mediates selective anabolic effects of parathyroid hormone in bone cultures. *J Clin Invest*, **83**, 60-65.

[111] Mayer, G.P.; Keaton, J.A. Hurst, J.G, (1979). Effects of plasma calcium concentration on the relative proportion of hormone and carboxyl fragments in parathyroid venous blood. *Endocrinology*, **104**, 1778-1784.

[112] Hemmingsen, C; Lewin, E; Staun, M, (1996). Effect of PTH on renal calbindin-D28K.*J Bone Miner Res*, **11**, 1086-1093.

[113] Marx S. (2000). Hyperparathyroid and hypoparathyroid disorders. *NEJM*, **343**, 1863–1875.

[114] Albayrak. C, Albayrak. D. (2015). Vitamin D levels in children with severe hemophilia A: an underappreciated deficiency, *Blood Coagulation and Fibrinolysis*, **26**:285–289.

[115] Hanaa H. Eldash, Zeze Th. Atwaa and Mohammad A. Saad (2017). Vitamin D deficiency and osteoporosis in hemophilic children: *an intermingled comorbidity*. **28**, 14–18.

[116] Alıoğlu, B. Selver, B. Ozsoy, H. Koca, G. Ozdemır. M, Dallar, Y. (2012). Evaluation of bone mineral density in Turkish children with severe haemophilia A: Ankara hospital experience. *Haemophilia*, **18**, 69–74.

[117] Premaor MO, Furlanetto TW. (2006). Hipovitaminose D em Adultos: Entendendo Melhor a Apresentação de uma Velha Doença. *Arq Bras Endocrinol Metab*, **50(1),** 25-37.

[118] Pedrosa MAC, Castro ML (2005). Papel da vitamina D na função neuro-muscular. *Arq Bras Endocrinol Metab*; **49(4)**, 495-502.

[119] Thiele Dk, Senti JL, Anderson CM. (2013). Maternal vitamin D supplementation to meet the needs of the breastfed infant: a systematic review. *J Hum Lact*, **29 (2),** 163-70.

[120] Holick MF. Vitamina D. 1.ed. São Paulo (2012). Fundamento Educacional,

[121] Caroline S. et al. (2013). Vitamin D in chronic liver disease, *Liver Int*, **33**, 338–352.

[122] Pongron, A. Kennan, L. Deluca, F. (1969). "Activation" of Vitamin D by the Liver. *The Journal of Clinical Investigation* **48**.

[123] Diana R. Mager, Stephanie T. Jackson, Michelle R. Hoffmann, Kailash Jindal, Peter A. Senior (2017). Vitamin D3 supplementation, bone health and quality of life in adults with diabetes and chronic kidney disease: Results of an open label randomized clinical trial. *Clinical Nutrition and Metabolism*. 36..686-695.

[124] Testa A, Mallamaci F, Benedetto FA (2010). Vitamin D receptor (VDR) gene polymorphism is associated with left ventricular (LV) mass and predicts left ventricular hypertrophy (LVH) progression in end-stage renal disease (ESRD) patients. *J Bone Miner Res*, **25**, 313-319.

[125] Drechsler C, Pilz S, Obermayer-Pietsch B (2010). Vitamin D deficiency is associated with sudden cardiac death, combined cardiovascular events, and mortality in haemodialysis patients. *Eur Heart J*, **31**, 2253- 2261.

[126] Molinari, C, Uberti F, Grossini E (2011). 1α,25-Dihydroxycholecalciferol induces nitric oxide production in cultured endothelial cells. Cell. Physiol. *Biochem*, **27**, 661–668.

[127] Takiishi T, Gysemans C, Bouillon R, (2012). Vitamin D and diabetes. *Rheum Dis Clin North Am*, **38**:179–206.

[128] Rosen CJ, Adams JS, Bikle DD (2012). The Nonskel- etal effects of Vitamin D: an Endocrine Society Scien- tific Statement. *Endocrine Reviews*, **33**, 456-492.

[129] Dutta D, Mondal SA, Choudhuri S (2014). Vitamin-D supplementation in prediabetes reduced progression to type 2 diabetes and was associated with decreased insulin resistance and systemic inflammation: An open label randomized prospective study from Eastern India. *Diabetes Res Clin Pract*. **8227(13)**, 00478-6.

[130] Deleskog A, Hilding A, Brismar K (2012). Low serum 25-hydroxyvitamin-D level predicts progression to type-2 diabetes individuas with prediabetes but not normal glucose tolerance. *Diabetologia*, **55**, 1668-78.

[131] Snijder. MB. Lips. P, Seidell JC. (2007). Vitamin D status and parathyroid hormone levels in relation to blood pressure: A population-based study in older men and women. *J. Intern. Med*, **261**, 558–565.

[132] Kunutsor. S.K, Apekey. T.A, Steur. M. (2013). Vitamin D and risk of future hypertension: Meta-analysis of 283,537 participants. *Eur. J. Epidemiol*, **28**, 205–221.

[133] Larsen. T, Mose. F.H, Bech. J.N. (2012). Effect of cholecalciferol supplementation during winter months in patients with hypertension: A randomized, placebo-controlled trial. *Am. J. Hypertens*, **25,** 1215–1222-33.

[134] Yetley. EA. (2008). Assessing the vitamin D status of the US population. *Am J Clin Nutr*, **88**, 558-64.

[135] Deluis. DA, Pacheco. D, Izaola. O. (2008). Clinical results and nutritional consequences of biliopancreatic diversion: three years of follow-up. *Ann Nutr Metab*, **53**, 234-9.

[136] Scragg. R. (1981). Seasonality of cardiovascular disease mortality and the possible protective effect of ultra-violet radiation. *Int. J. Epidemiol*, **10**, 337–341.

[137] Burgaz. A, Orsini. N, Larsson. S. (2011). Blood 25-hydroxyvitamin D concentration and hypertension: A meta-analysis. J. *Hypertens*, **29**, 636–645.

[138] Nibbelink. KA, Tishkoff. DX, Hershey. SD (2007). 1,25(OH)2Vitamin D3 actions on cell proliferation, size, gene expression, and receptor localisation, in the HL-1 cardiac myocyte. *J Steroid Biochem Mol Biol*, **103**,533-537.

[139] Pittas. AG, Chung. M, Trikalinos. T, (2010). Systematic review: Vitamin D and cardiometabolic outcomes. *Ann Intern Med*, **152**, 307-314.

[140] Wang. TJ, Pencina. MJ, Booth. SL (2008). Vitamin D defi- ciency and risk of cardiovascular disease. *Circulation*, **117**, 503-511.

[141] Bauer. PJ (1981). Affinity and stoichiometry of calcium binding by arsenazo III. *Anal Biochem*, **110**, 61-72.

[142] Michaylova. V, Ilkova. P (1971). Photometric determination of micro amounts of calcium with arsenazo III. *Anal Chim Acta*, **53**, 194-198.

[143] Daly. JA, Ertingshausen. G (1972). Direct method for determining inorganic phosphate in serum with the "CentrifiChem". *Clin Chem*, **18 (3),** 263-5.

[144] Gamst. O, Try. K (1980). Determination of serum-phosphate without deproteinization by ultraviolet spectrophotometry of the phosphomolybdic acid complex. *Scand J Clin Lab Invest*, **40 (5),** 483-6.

[145] Tietz. NW, Rinker. D, Shaw. LM. (1983). IFCC methods for the measurement of catalytic concentration of enzymes Part 5. IFCC method for alkaline phosphatase. *J Clin Chem Clin Biochem*, **21**, 731-48.

[146] Gerstner. G, Damiano. ML, Tom. A, Worman. C, Schultz. W, Recht. M, Stopeck. AT. (2009). Prevalence and risk factors associated with decreased bone mineral density in patients with haemophilia. *Haemophilia*, **15**, 559–565.

[147] Linari. S, Montorzi. G, Bartolozzi. D, Borderi. M, Melchiorre. D, Benelli. M, Morfini. M. (2013). Hypovitaminosis D and osteopenia/osteoporosis in a haemophilia population: a study in HCV/HIV or HCV infected patients. *Haemophilia*, **19**, 126–133.

[148] Anagnostis. P, Vakalopoulou. S, Slavakis. A, Charizopoulou. M, Kazantzidou. E, Chrysopoulou T, (2012). Reduced bone mineral density in patients with haemophilia A and B in Northern Greece. *Thromb Haemost*, **107**, 545–551.

[149] Schiefke. I, Fach. A, Wiedmann. M, Aretin. AV, Schenker. E, Borte. G. (2005). Reduced bone mineral density and altered bone turnover markers in patients with noncirrhotic chronic hepatitis B or C infection. *World J Gastroenterol;* **11**, 1843–1847.

[150] Liel. MS, Greenberg. DL, Recht. M, Vanek. C, Klein. RF, Taylor. JA (2012). Decreased bone density and bone strength in a mouse model of severe factor VIII deficiency. *Br J Haematol*, **158**, 140–143.

[151] Burtis. CA, Ashwood. ER, Bruns. DE (2006). Tietz textbook of clinical chemistry and molecular diagnostics. Missouri, Elsevier.

[152] Hollick. MF, Chen. TC (2008). Vitamin D deficiency a worldwide problem with health consequences. *Am J Clin Nutr*, **87**, 10805-68.

[153] Dhanwal. DK, Sahoo. S, Gautam. VK, Saha. R. (2013). Hip fracture patients in India have Vitamin D deficiency and secondary hyperparathyroidism. *Osteoporos Int* **24**, 553–557.

[154] Costa. TL, Paganotto. M, Radominski. RB, Kulak. CM, Borba. VC. (2015). Calcium metabolism, vitamin D and bone mineral density after bariatric surgery. *Osteoporos Int***, 26**,757–764.

[155] Jahnsen. J, Hewitt. S, Søvik. TT, Aasheim. ET, Kristinsson. J, Birketvedt. GS, Bøhmer. T, Eriksen. EF, Mala. T (2013) Secondary hyperparathyroidism, vitamin D sufficiency, and serum calcium 5 years after gastric bypass and duodenal switch. *Obes Surg* **23(3),** 384–390.