Republic of Yemen
Ministry of Higher Education and Scientific Research
International Malaysian University

الجمهوريـــة اليمنيـــة
وزارة التعليم العالي والبحث العلمي
الجامعة الماليزية الدولية
كلية العلوم الطبية

Evaluating Some Bone Related Biochemical Markers in Yemeni Women of Childbearing Age in Ibb Governorate

A research project submitted to the medical laboratory department as partial fulfillment of the requirements for a bachelor's degree in the medical laboratory

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Approved by advisory committee and Examiners

Dr/ Aisha AL-Darasi **Internal Examiners**

Dr

"قال تعالى"

﴿ وَالشَّمْسُ تَجْرِي لِمُسْتَقَرٍّ لَّهَا ۚ ذَٰلِكَ تَقْدِيرُ الْعَزِيزِ الْعَلِيمِ ﴾

Dedication

To whom we love

Our …parents

With love and gratitude

to our ship captain

our…spiritual mother our glorified doctor

Dr/ Fathia salam.

With love and gratitude to our colleagues *T/ Qahera AL-Hilali.*

Prof .Dr/ Fuad AL-Sawadi.

Dr/ Aisha AL-Darasi.

Dr/Ahmed Haidarh

To all our friends , and to all those

sincere and good people

Who helped and supported us throughout the study…

AL-Thawra Hospital (Dr/ sadam AL-Goami).

AL-Noor Hospital (Dr/Eyad Etween).

Blood Bank Center of IBB Governorate .

Alnokhba Laboratories (Olfat AL-Ra'wy) Nurse (Gamila).

Contents

List of Figures

List of Table

List of Abbreviation

Abbreviation	Meaning
WHO	World Health Organization
ALP	Alkaline Phosohatase
CA	Calcium
Ng	Nano Gram
Mg	Mille Gram
UI	Unit International
UV	Ultra Violet
OC	Osteocalcin
PINP	Procollagen Type I N-terminal Propeptide
PICP	Procollagen Type I C-terminal Propeptide
BALP	Bone –specific Alkaline Phosphatase
BSP	Bone Sialoprotein
OP	Osteopontin
HYP	Hydroxyproline
DPD	Deoxypyridinoline
TRAP 5b	Tartrate-resistant Acid Phosohatase 5b
CTX-1	Carboxy-terminal Cross Linked Telo Pepted of Type 1 Collagen
NTX-1	Amino-terminal Cross Linked Telo Pepted of Type 1 Collagen
PTH	Parathyroid Hormone
VDR	Vitamin D Receptor
DM	Diabetes Mellitus

Abstract

Background: During skeletal growth in young females, bone mass is accrued up until the third decade of life. Suboptimal peak bone mass substantially increases the risk of osteoporosis in later life. Thus, strategies aimed at optimizing peak bone mass in younger, premenopausal females are crucial in its prevention.

Aim: This study was carried out to evaluate some biochemical markers related to bone health for Yemeni women of childbearing age, in Ibb governorate.

Method: A total of 81 non-pregnant women, whose ages ranged from (16-43) years old (mean ± SD = 28.08 ± 6.60), were collected during June-July, 2022; and examined for serum Vitamin D_3 (Vit D_3) by Immuno-chemiluminescence, and for serum Calcium (Ca) and Alkaline phosphatase (ALP) by spectrophotometer. About 74.68% of the enrolled subjects were married, and 50.62% of them were lactating. About 37.66% of the enrolled subjects were not used to expose to sunlight, 41.56% of them are used to expose to sunlight for 5-15 minutes, 14.29% of them are used to expose to sunlight for 15-30 minutes, and only 6.49% of them are used to expose to sunlight for 60 minutes and more/daily.

Results: The results showed a moderately decreased mean levels of serum Vit D_3 (13.39 ± 8.02 ng/ml), while the normal range is (30-100 ng/ml). Mean levels of serum Ca was slightly decreased (8.348 ± 0.83 mg/dl), while the normal range is (8.5 - 10.5 mg/dl). Mean levels of serum ALP was slightly high (170.5 ± 69.59 U/L), while the normal range is (38 - 154 U/L), and Vit D_3 levels showed a significant negative correlation with ALP (r = -0.241, P = 0.030). ALP was significantly higher in the married women as compared to the non-married women (P = 0.006), and in the lactating women as compared to the non-lactating women (P = 0.000). Exposure to sunlight showed no effect on any one of the biochemical parameters. Marital state and lactating state showed a significant negative effect on ALP level (P = 0.05, and P = 0.001, respectively).

Conclusion: Yemeni women of childbearing age in Ibb governorate have slight deficiency in serum Ca, and a moderate deficiency in Vit D_3, and a slight elevation in ALP which is negatively correlated significantly with Vit D_3. This suggests a

suboptimal bone mass formation in them, particularly in married and in nursing one.

Chapter 1
Introduction and Literature Review

1. Introduction and literature review

Bone is a dynamic tissue in a constant state of remodeling. In healthy adults, bone resorption is tightly coupled with bone formation. Bone turnover in younger premenopausal women reflects both modeling and remodeling, compared with older women where bone turnover is predominantly a reflection of the latter (Callegari *et al.*, 2017).A variety of factors affect bone health, including genetics, nutrition, physical activity, hormones, and vitamin D levels(Dennison *et al.*, 2014; Seeman 2002; Song *et al.*, 2011; and Cheema *et al.*, 1989). The use of bone turnover markers in clinical practice and research in younger people is limited by the lack of normative data and understanding of common causes of variation in bone turnover marker values in this demographic. To appropriately interpret bone turnover markers, robust reference intervals specific to age, development and sex are necessary (Callegari *et al.*, 2016). The present study evaluates some available bone markers in females of reproductive age in Ibb governorate.

1.1 Bone formation physiology

1.1.1 Bone and its relation to extracellular Calcium

Bone is composed of a tough organic matrix that is greatly strengthened by deposits of calcium salts. Average compact bone contains by weight about 30 per cent matrix and 70 per cent salts. Newly formed bone may have a considerably higher percentage of matrix in relation to salts. The organic matrix of bone is 90 to 95 per cent collagen fibers, and the remainder is a homogeneous gelatinous medium called ground substance. The collagen fibers extend primarily along the lines of tensional force and give bone its powerful tensile strength. The ground substance is composed of extracellular fluid plus proteoglycans, especially chondroitin sulfate and hyaluronic acid. The precise function of each of these is not known, although they do help to control the deposition of calcium salts. The crystalline salts deposited in the organic matrix of bone are composed principally

of calcium and phosphate. The formula for the major crystalline salt, known as hydroxyapatite, is the following: $Ca_{10}(PO_4)_6(OH)_2$. Each collagen fiber of compact bone is composed of repeating periodic segments every 640 angstroms along its length; hydroxyapatite crystals lie adjacent to each segment of the fiber, bound tightly to it. This intimate bonding prevents "shear" in the bone; that is, it prevents the crystals and collagen fibers from slipping out of place, which is essential in providing strength to the bone. In addition, the segments of adjacent collagen fibers overlap one another, also causing hydroxyapatite crystals to be overlapped like bricks keyed to one another in a brick wall (Guyton & Hall, 2007).

1.1.2 Mechanism of bone calcification

The initial stage in bone production is the secretion of collagen molecules (called collagen monomers) and ground substance (mainly proteoglycans) by osteoblasts. The collagen monomers polymerize rapidly to form collagen fibers, the resultant tissue becomes osteoid, a cartilage-like material differing from cartilage in that calcium salts readily precipitate in it. As the osteoid is formed, some of the osteoblasts become entrapped in the osteoid and become quiescent. At this stage they are called osteocytes. Within a few days after the osteoid is formed, calcium salts begin to precipitate on the surfaces of the collagen fibers. The precipitates first appear at intervals along each collagen fiber, forming minute need that rapidly multiply and grow over a period of days and weeks into the finished product, hydroxyapatite crystals. The initial calcium salts to be deposited are not hydroxyapatite crystals but amorphous compounds (no crystalline), a mixture of salts such as $CaHPO_4 \cdot 2H_2O$, $Ca_3(PO_4)_2 \cdot 3H_2O$, and others. Then by a process of substitution and addition of atoms, or reabsorption and reprecipitation, these salts are converted into the hydroxyapatite crystals over a period of weeks or months. A few per cent may remain permanently in the amorphous form. This is important because these amorphous salts can be absorbed rapidly when there is need for extra

calcium in the extracellular fluid. The mechanism that causes calcium salts to be deposited in osteoid is not fully understood. One theory holds that at the time of formation, the collagen fibers are specially constituted in advance for causing precipitation of calcium salts. The osteoblasts supposedly also secrete a substance into the osteoid to neutralize an inhibitor (believed to be pyrophosphate) that normally prevents hydroxyapatite crystallization. Once the pyrophosphate has been neutralized, the natural affinity of the collagen fibers for calcium salts causes the precipitation (Guyton & Hall, 2007).

1.1.3 Deposition and absorption of bone remodeling of bone

1.1.3.1 Deposition of bone by the osteoblasts

Bone is continually being deposited by osteoblasts, and it is continually being absorbed where osteoclasts are active (Figure 1.1). Osteoblasts are found on the outer surfaces of the bones and in the bone cavities. A small amount of osteoplastic activity occurs continually in all living bones (on about 4 per cent of all surfaces at any given time in an adult), so that at least some new bone is being formed constantly.

1.1.3.2 Absorption of bone functions of the osteoclasts

Bone is also being continually absorbed in the presence of osteoclasts, which are large phagocytic, multinucleated cells (as many as 50 nuclei), derivatives of monocytes or monocyte-like cells formed in the bone marrow. The osteoclasts are normally active on less than 1 per cent of the bone surfaces of an adult. Later in the chapter we see that PTH controls the bone absorptive activity of osteoclasts. Histologically, bone absorption occurs immediately adjacent to the osteoclasts. The mechanism of this absorption is believed to be the following: The osteoclasts send out villus-like projections toward the bone, forming a so-called ruffled border adjacent to the bone. The villi secrete two types of substances: (1) proteolytic

enzymes, released from the lysosomes of the osteoclasts, and (2) several acids, including citric acid and lactic acid, released from the mitochondria and secretory vesicles. The enzymes digest or dissolve the organic matrix of the bone, and the acids cause solution of the bone salts. The osteoplastic cells also imbibe by phagocytosis minute particles of bone matrix and crystals, eventually also dissolution these and releasing the products into the blood. Bone Deposition and Absorption Are Normally in Equilibrium. Normally, except in growing bones, the rates of bone deposition and absorption are equal to each other, so that the total mass of bone remains constant. Osteoclasts usually exist in small but concentrated masses, and once a mass of osteoclasts begins to develop, it usually eats away at the bone for about 3 weeks, creating a tunnel that ranges in diameter from 0.2 to 1 millimeter and is several millimeters long. At the end of this time, the osteoclasts disappear and the tunnel is invaded by osteoblasts instead; then new bone begins to develop. Bone deposition then continues for several months, the new bone being laid down in successive layers of con-centric circles (lamellae) on the inner surfaces of the cavity until the tunnel is filled. Deposition of new bone ceases when the bone begins to encroach on the blood vessels supplying the area. The canal through which these vessels run, called the have risen canal, is all that remains of the original cavity. Each new area of bone deposited in this way is called an osteon, as shown in Figure 1.2(Guyton & Hall, 2007).

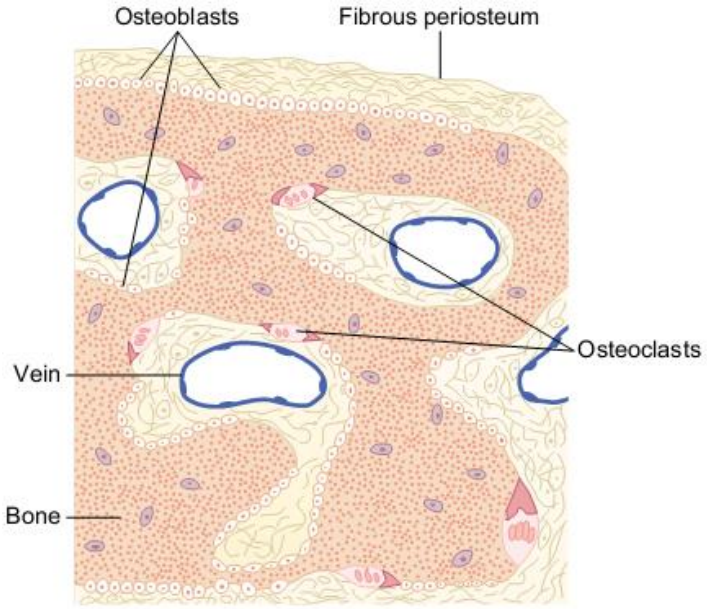

Figure 1.1: Bone structure (Guyton and Hall, 2007)

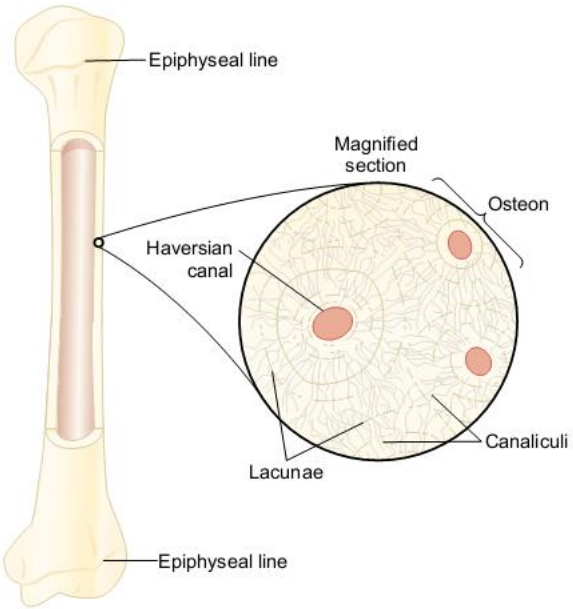

Figure 1.2: Bone structure (Guyton and Hall, 2007)

1.1.3.3 Bones turnover in women

The use of bone turnover markers in clinical practice and research in younger people is limited by the lack of normative data and understanding of common causes of variation in bone turnover marker values in this demographic. To appropriately interpret bone turnover markers, robust reference intervals specific to age, development and sex are necessary (Callegari *et al.,* 2016).

(Glover *et al.,* 2008), mentioned that one turnover is elevated until skeletal maturity is reached, usually in the 3rd decade of life. Women over 45 years of age have fewer ovulatory cycles and a high biological variability of Bone turnover markers (BTMs) as bone turnover is increased in premenopausal women prior to the loss of estrogen in younger females at the end of puberty, high bone turnover is present due to bone modeling, but subsides as bone growth halts and newly formed osteoid undergoes primary and secondary mineralization (Eastell, 2005).During skeletal growth in young females, bone mass is accrued up until the third decade of life (Teegarden *et al.,* 1995; Hansen, 1994). Suboptimal peak bone mass substantially increases the risk of osteoporosis (and related fracture) in later life. Thus, strategies aimed at optimizing peak bone mass in younger, premenopausal females are crucial in its prevention (Hansen *et al.,* 1991).

Oboh *et al.,* (2021), concluded that women who breastfed for > 6 months had significantly reduced Bone mass density (BMD). However, compared to women that breastfed for a ≤ 4 months there was no significant change in BMD .Also, six weeks after delivery (clinically the postpartum phase) and beyond, lactation demands more calcium and is supplied to the infant through breastmilk (Salari and Abdollahi , 2014). About 200 mg/day of calcium is mobilized into breastmilk during lactation (Sawo *et al.,* 2013).

1.1.3.4 Bones Biomarkers

Bone biomarkers are produced from the bone remodeling process included bone formation biomarkers, bone resorption biomarkers and regulators of bone turnover. Various biomarkers are now available for specific and sensitive assessment of the rate for bone formation and bone resorption. For example, the bone formation biomarkers are total alkaline phosphatase (ALP), bone-specific alkaline phosphatase (BALP), osteocalcin (OC), precollege type 1 N-terminal propertied (P1NP) and precollege type 1 C-terminal propertied (P1CP). The bone resorption biomarkers are hydroxyproline (HYP), hydroxylysine (HYL), deoxypyridinoline (DPD), pyridinoline (PYD), bone sialoprotein (BSP), osteopontin (OP), tartrate-resistant acid phosphatase 5b (TRAP 5b), carboxy-terminal crosslinkedtelopeptide of type 1 collagen (CTX-1), amino-terminal crosslinkedtelopeptide of type 1 collagen (NTX-1) and cathepsin K (CTSK). The regulators of bone turnover are precept or activator of NF-kB ligand (RANKL), osteoprotegerin (OPG), dickkopf-1 (DDK-1) and sclerostin. These biomarkers are useful to provide the early assessment of osteoporosis when the BMD measurement of DXA does not offer enough information to make the diagnosis (Tsung-Rong and Chih-Hwa, 2017).

1.2 Vitamin D

Vitamin D comprises a group of fat-soluble compounds that are essential for maintaining the mineral balance in the body. The vitamin D form synthesized in humans is called ‘cholecalciferol’ (vitamin D3). As cholecalciferol synthesized in the skin by the action of ultraviolet light (UV-B), vitamin D does not fit the classical definition of a vitamin; nevertheless, it is recognized as an essential dietary nutrient (Engel, 2010).

Vitamin D has two sources, cholecalciferol (Vitamin D3) and ergocalciferol (Vitamin D2). Cholecalciferol is derived from 7-dehydrocholesterol by the action of sun (UV) rays and is the main source of Vitamin D (Guyton *et al.*, 2007 and

Açıkgöz *et al.*, 2013). Ergocalciferol, which we call Vitamin D2, is derived from foods. Since they have similar metabolisms, they are both called D vitamins (Guyton *et al.*, 2007 and O'Riordan and Bijvoet, 2014). Dietary uptake of vitamin D2 is limited. Vitamin D in foods is mostly found in fatty fish species such as salmon (salmon fish), mackerel, tuna fish, sardine and also in nutrients such as egg yolk, milk, broccoli, green onion, parsley water terrace. But no food contains vitamin D until it supplies the daily vitamin D requirement. In the breast milk there is vitamin D of 10-60 IU/L (Holick, 2007 and Ataş *et al.*, 2008). In America and some European countries, milk, yogurt, orange juice, and cereals are enriched with Vitamin D (Baysal, 2012 andErsoy, 2017). Dietary or endogenously synthesized Vitamin D2 or D3 is stored in fat cells and circulated if necessary (Holick, 2007and Özçelik *et al*., 2012).

Vitamin D obtained from sun exposure, foods, and supplements is biologically inert and must undergo two hydroxylation's in the body for activation. The first hydroxylation, which occurs in the liver, converts vitamin D to 25-hydroxyvitamin D [25(OH)D], also known as "calcidiol." The second hydroxylation occurs primarily in the kidney and forms the physiologically active 1,25-dihydroxyvitamin D [1,25(OH)2D], also known as "calcitriol" (Institute of Medicine, 2010). Parathyroid hormone is involved in the mechanism of the conversion of 25 (OH) D to 1.25(OH)2D. In the absence of this hormone, 1.25(OH)2D cannot occur (Açıkgöz *et al*., 2013 and WHO, 2006).

Although vitamin D status may be influenced by environmental factors such as sun exposure and diet, variation in the genes encoding the vitamin D 25-hydroxylase enzyme C YP2R1, the vitamin D binding protein (DBP) and the vitamin D receptor (VDR) have also been reported to associate with risk of vitamin D deficiency (Wang

1.2.1 Biological effects and physiology of vitamin Din the body

Vitamin D is an important vitamin and its deficiency in our lives in terms of our health cause many health problems. Inadequate sunlight intake, such as long-term stay in indoor environments such as home and office, clothing style, use of sun protection creams with high protection factor, and seasonal changes cause Vitamin D insufficiency to occurs more frequently (Öğüş *et al.*, 2014).Studies conducted in different countries show that Vitamin D deficiency is a worldwide problem and this is a major health problem (Mithal *et al.*, 2009 and Kurt *et al.*, 2011).The main task of Vitamin D is to provide the mineral balance in our bodies. It regulates the metabolism and absorption of minerals and determines serum calcium and phosphorus levels. The target organs that vitamin D affects are the kidneys, small intestines, and bone. In these organs, the effect is caused by the active form of calcitriol. Vitamin D deficiency causes rickets disease in children and osteoporosis in adults (Wranicz and Szostak, 2014 and Genç *et al.*, 2015). Vitamin D deficiency causes a decrease in the efficiency of intestinal calcium absorption and results in a decrease in ionized calcium. The calcium sensor in the parathyroid glands immediately recognizes the decrease causing the parathyroid glands to increase the production and secretion of parathyroid hormone (PTH) (Holick, 2007 and Dusso *et al.*, 2005). PTH maintains serum calcium levels by increasing tubular reabsorption of calcium in the kidneys. Through its receptor on osteoblasts, PTH stimulates the formation of osteoclasts, which in turn dissolves the bone matrix and mineral to release the calcium into the extracellular space (Holick, 2007). This process, known as secondary hyperparathyroidism, can precipitate and exacerbate both osteopenia and osteoporosis, increasing risk of fracture. PTH also causes phosphorus loss in the urine resulting in a low-normal serum phosphorus level (Holick, 2007 and Bischoff *et al.*, 2006).

Vitamin D promotes calcium absorption in the gut and maintains adequate serum calcium and phosphate concentrations to enable normal bone mineralization and to

prevent hypocalcemictetany (involuntary contraction of muscles, leading to cramps and spasms). It is also needed for bone growth and bone remodeling by osteoblasts and osteoclasts (Institute of Medicine, 2010 and Jones *et al.*, 2014).

However, frequent use of tanning beds, which provide artificial UV radiation, can lead to 25(OH)D levels well above 375–500 nmol/L (150–200 ng/mL) (Singh *et al.*, 2014 and Perez *et al.*, 2007).

The combination of high intakes of calcium (about 2,100 mg/day from food and supplements) with moderate amounts of vitamin D (about 19 mcg [765 IU]/day from food and supplements) increased the risk of kidney stones by 17% over 7 years among 36,282 postmenopausal women who were randomly assigned to take 1,000 mg/day calcium and 10 mcg (400 IU)/day vitamin D or a placebo (Jackson *et al.*, 2006). However, other, shorter (from 24 weeks to 5 years) clinical trials of vitamin D supplementation alone or with calcium in adults found greater risks of Hypercalcemia and hypercalciuria, but not of kidney stones (Malihi *et al*., 2019 and Malihi*et al.*, 2016).

1.2.2 Vitamin D deficiency

Recently, scientific researches show that vitamin D has different effects besides the known classical effects. The active form of Vitamin D, the cholecalciferol (Vitamin D3) receptor, has been found in many tissues and cells of the human body. Many important diseases such as cancer, autoimmune diseases, and diabetes mellitus (DM) have been suggested to be related to Vitamin D (Holick, 2007). Vitamin D deficiency, especially seen in Western populations, can lead to serious health problems. Vitamin D is a group of steroids that are hormones and hormone precursors because they can be synthesized in the appropriate biological conditions which is one of the vitamins that are soluble in fat (Champe *et al.*, 2005 and Fidan *et al.*, 2014). Mortality due to fragile fractures due to insufficient 25(OH)D,

especially hip fracture, is common (Gallagher and Sai, 2010and Kuroda *et al.*, 2009).

In the study of Khalfa and his colleagues, (2019), on females of different age groups, they found that all females of all age groups suffered from hypovitaminosis D3 due to bone disorders. However, they reported that females that were single had lower vitamin D3 levels as well as lower serum calcium levels compared to married females, and they attributed that to their lacking sexual activity and inter hormonal play. Nevertheless, pregnancy and lactating in married females were demonstrated to contribute to vitamin D deficiency (Oboh *et al.,* 2021). Marriage is often considered to have a health-protective effect and studies have found an association between being married and lower rates of chronic illness, fewer physical limitations, and less disability. However, early marriage (often defined as marriage at 20 years of age or younger) is associated with greater psychosocial distress, lower educational attainment, and poorer long-term marital success. All those factors associated with lower bone strength and bone density (Miller-Martinez *et al.*, 2014).

Despite an abundance of sunshine, studies from the Middle East show that there is a high prevalence of vitamin D deficiency in women of childbearing age, mainly attributed to cultural avoidance of sunlight, traditional dress styles that cover most of the body while outdoors, and inadequate vitamin D intake 4. (Dawodu *et al.*, 1998 and Gannagé *et al* ., 2000). Because the vitamin D status of the mother during pregnancy is important in determining the vitamin D status of the infant at birth, the strong relationship between maternal and cord blood vitamin D status (Hollis and Wagner, 2004) and vitamin D deficiency during pregnancy should be of concern, especially if the mother is planning to exclusively breastfeed.

The results of the study of Shaheen *et al*, (2012) on adult females showed that out of 110 samples, 26 had mild (23%), 61 had moderate (55%) and 21 had severe (19.1%) vitamin D deficiencies.

Likewise, in the study of Junaid *et al.*, (2015), they found that risk of vitamin D deficiency was independently associated with illiteracy; in the women of child-bearing age in Lahore Pakistan. They also found that (73 %) of participants were vitamin D deficient. Also, the risk of vitamin D deficiency was independently associated with < 30 min sun exposure per day. However, no correlation was found between the different biochemical tests and the time of exposure to the sunlight in our study . In addition, study of Junaid and his colleagues, (2015), showed that vitamin D deficiency was associated with decreased sunlight exposure, but they did not demonstrate any association between veiling (wearing Nekab) and risk of vitamin D deficiency.

Moreover, Kiran*et al.*, (2014), found a positive correlation between sun light exposure and Vitamin D status in males but not in females; in their study on the healthy adults above the age of 20 living in Potheri village of Kancheepuram district, Tamilnadu, India.

1.3 Calcium

Calcium, the most abundant mineral in the body, is found in some foods, added to others, present in some medicines (such as antacids), and available as a dietary supplement. Calcium makes up much of the structure of bones and teeth and allows normal bodily movement by keeping tissue rigid, strong, and flexible (Institute of Medicine, 2011).

1.3.1 Biological effects and physiology of calcium in the body

Extracellular fluid calcium concentration normally is regulated very precisely, seldom rising or falling more than a few per cent from the normal value of about

9.4 mg/dl, which is equivalent to 2.4 mmol calcium per liter. This precise control is essential, because calcium plays a key role in many physiologic processes, including contraction of skeletal, cardiac, and smooth muscles; blood clotting; and transmission of nerve impulses, to name just a few. Excitable cells, such as neurons, are very sensitive to changes in calcium ion concentrations, and increases in calcium ion concentration above normal (Hypercalcemia) cause progressive depression of the nervous system; conversely, decreases in calcium con-centration (hypocalcaemia) cause the nervous system to become more excited. An important feature of extracellular calcium regulation is that only about 0.1 per cent of the total body calcium is in the extracellular fluid, about 1 per cent is in the cells, and the rest is stored in bones. Therefore, the bones can serve as large reservoirs, releasing calcium when extracellular fluid concentration decreases and storing excess calcium (Guyton and Hall, 2007).

The small ionized pool of calcium has a role in the circulatory system, extracellular fluid, and various tissues mediates blood vessel contraction and dilation, muscle function, blood clotting, nerve transmission, and hormonal secretion (Institute of Medicine, 2011 and Heaney *et al.,* 2010). Calcium from foods and dietary supplements is absorbed by both active transport and by passive diffusion across the intestinal mucosa (Institute of Medicine, 2011 and Weaver and Heaney, 2014). Active transport is responsible for most absorption when calcium intakes are lower, and passive diffusion accounts for an increasing proportion of calcium absorption as intakes rise (Institute of Medicine, 2011 and Weaverand Heaney,2014).

Almost all calcium in the body is stored in the bones, and the body uses the bones as a reservoir for, and source of, calcium to maintain calcium homeostasis (Institute of Medicine, 2011). More than 99% of calcium in the body is in the form of calcium hydroxyapatite, an inorganic matrix of calcium and phosphate that is

stored in the bones and teeth (Institute of Medicine, 2011, Weaver, 2020, WawrzyniakamdSuliburska, 2021). An inverse relationship exists between calcium intake and absorption. Absorption of calcium from food is about 45% at intakes of 200 mg/day but only 15% when intakes are higher than 2,000 mg/day (Fairweather and Teucher, 2002).

1.3.2 Calcium deficiency

Calcium deficiency can reduce bone strength and lead to osteoporosis, which is characterized by fragile bones and an increased risk of falling. Calcium deficiency can also cause rickets in children and other bone disorders in adults, although these disorders are more commonly caused by vitamin D deficiency. In children with rickets, the growth cartilage does not mineralize normally, which can lead to irreversible changes in the skeletal structure. Another effect of chronic calcium deficiency is osteomalacia, or defective bone mineralization and bone softening, which can occur in adults and children (Institute of Medicine, 2011).

For rickets and osteomalacia, the requirements for calcium and vitamin D appear to be interrelated in that the lower the serum vitamin D level (measured as 25-hydroxyvitamin D [25(OH)D]), the more calcium is needed to prevent these diseases (Sempos *et al.*, 2021). Hypocalcaemia (serum calcium level less than 8.5 mg/dL [2.12 mmol/L] or an ionized calcium level below 4.61 mg/dL [1.15 mmol/L]) is usually a result of a vitamin D or magnesium deficiency, impaired parathyroid hormone (PTH) production leading to hyperparathyroidism, impaired bone resorption of calcium, critical illness, or use of certain medications (e.g., bisphosphonates, cisplatin, or proton pump inhibitors) (Fong and Khan, 2012 and Bove and Mannstadt, 2018). The most common symptom is increased neuromuscular irritability, including perioral numbness, tingling in the hands and feet, and muscle spasms (Bove and Mannstadt , 2018).

1.3.3 Hypercalcemia

Hyperkalemia (serum levels greater than 10.5 mg/dL [2.63 mmol/L]) and hypercalciuria (urinary calcium levels higher than 250 mg/day in women and 275 mg/day in men) are rare in healthy people and usually result from cancer, primary hyperparathyroidism, and other conditions (Institute of Medicine, 2011 and Weaver *et al.*, 2020). Severe signs and symptoms can include renal calcification or injury, brain calcification, neurologic symptoms (e.g., depression and bipolar disorder), cataracts, congestive heart failure, paresthesia, seizures, and, in rare cases, coma (Fong and Khan, 2012 and Pepe *et al.*, 2020).

1.4 Alkaline phosphatase

Alkaline phosphatases are a group of isoenzymes, located on the outer layer of the cell membrane; they catalyze the hydrolysis of organic phosphate esters present in the extracellular space. Zinc and magnesium are important co-factors of this enzyme.

1.4.1 Biological effects and physiology of alkaline phosphatase in the body

Alkaline phosphatases are classified as tissue-specific and tissue nonspecific types. Alkaline phosphatases found in the intestine, placenta, and germinal tissue are tissue-specific. This means they are found only in the tissues where they are expressed in physiological conditions. They may also contribute to the circulating pool of serum alkaline phosphatase under specific situations when there is increased stimulation of their production. The tissue-nonspecific alkaline phosphatases form most of the fraction circulating in serum and, therefore, is of clinical interest. A single gene encodes it and is expressed in the liver, bone, and kidneys. Intestinal alkaline phosphatase is coded by a separate gene, which is different from the gene that codes for placental alkaline phosphatase and the Regan isoenzymes (produced in excess amounts in Hodgkin lymphoma). All tissue-

nonspecific alkaline phosphatases have the same amino acid sequence but different carbohydrate and lipid side chains; posttranslational modifications confer their unique physicochemical properties (Castells *et al.*, 2018 and Cristoferi *et al.*, 2018).

Although alkaline phosphatases are present in different body tissues and have different physiochemical properties, they are true isoenzymes because they catalyze the same reaction. In the liver, alkaline phosphatase is cytosolic and present in the canalicular membrane of the hepatocyte. Alkaline phosphatase is present in decreasing concentrations in the placenta, ileal mucosa, kidney, bone, and liver. The majority of alkaline phosphatase in serum (more than 80%) is released from liver and bone, and in small amounts from the intestine. Even though alkaline phosphatases are present in many tissues throughout the body, their precise physiological function remains largely unknown (Pinart *et al.*, 2020 and Doelen *et al.*, 2019).

Serum alkaline phosphatase levels will vary with age in normal individuals. Levels are high during childhood and puberty due to bone growth and development. The decrease in level in the 15 to 50 year age group is slightly higher in men than in women. These levels rise again in old age (significant difference in gender distribution). The reasons for these normal variations are not known. Research has shown a positive correlation with body weight and smoking, and there is an inverse correlation with height (Azpiazu *et al.*, 2019; Brichacek and Brown, 2019; Heinrich *et al.*, 2018).

In individuals with blood groups O and B, serum alkaline phosphatase levels increase after consuming a fatty meal, due to contribution from the intestinal tract. As this elevation can persist for up to 12 hours in the serum, the recommendation is to check the serum enzyme levels in a fasting state (Matsushita *et al.*, 2013).

1.4.2 High alkaline phosphatase blood levels

The liver is the source in most patients with elevated enzyme levels. Increased osteoblast activity seen in disorders of the bone or normally during periods of growth is the next likely contributor. Alkaline phosphatase behaves like any other serum protein. It has a half-life of 7 days, and clearance from serum is independent of bile duct patency or functional cap a governorate of the liver. However, the site of degradation of alkaline phosphatase is not known. Serum alkaline phosphatase levels may remain elevated for up to 1 week after the resolution of biliary obstruction. The influx of placental alkaline phosphatase in the late third trimester contributes to the rise in pregnant women (Masrour and Mahjoub, 2012)

The mechanism of the increase in alkaline phosphatase in hepatobiliary disorders has been a matter of debate. Research has convincingly shown that it is due to increased enzyme synthesis and not to reduced hepatobiliary excretion of the enzyme. Increased hepatic enzyme activity demonstrably parallels the rise in serum alkaline phosphatase activity; this occurs primarily due to increased translation of the mRNA of alkaline phosphatase (mediated by the rising bile acid concentration) and increased secretion of alkaline phosphatase into serum via canalicular leakage into the hepatic sinusoid. The mechanism that precipitates its release into the circulation has not been elucidated. Studies report that vesicles containing alkaline phosphatase, and many such enzymes bound to the sinusoidal membranes, are found in the serum of patients with cholestasis. Because alkaline phosphatase is newly synthesized in response to biliary obstruction, its serum level may be normal in the early phase of acute biliary obstruction even when the serum aminotransferases are already at their peak (Masrour and Mahjoub, 2012 and Pike *et al.*, 2013).

Bellastella*et al,* (2021), found a significant negative correlation between serum vitamin D and ALP; in healthy individuals of both sexes, particularly in females, and at different age groups ($> 18 \leq 40$ and in people $> 40 \leq 65$). In the study of

Shaheen *et al.*, (2012) on adult females, all of the patients in the three groups of vitamin D deficiencies (mild, moderate and sever) had alkaline phosphatase within normal limits and the total mean value of the enzyme was 135.97 ± 68.14I U/L, but the inter group comparison showed highest values of alkaline phosphatase in the moderate vitamin D deficiency group.

To our knowledge, studies on bone markers in the young Yemeni women are lacking. This study will investigate some available bone related biochemical markers in Yemeni women of reproductive age, in Ibb governorate, considering the marital and lactating states of them.

Chapter 2

Objectives

2. Objectives

2.1 General Objective

The general objective of this study is to Evaluate Some Bone Health Related Biochemical Markers (serum vitamin D, Alkaline Phosphatase, Calcium) in Yemeni Women of Reproductive Age, in Ibb Governorate.

2.2 Specific Objectives

- To estimate the biochemical markers in the married and non-married, Yemeni women of reproductive age, in Ibb governorate.
- To investigate the biochemical markersin the lactating and non-lactating, Yemeni women of reproductive age, in Ibb governorate.
- To examine the correlations between the different biochemical parameters with each other.
- To study the correlations between the different biochemical parameters and other factors such as age, number of children, exposure to the sun and the education level.

Chapter 3
Materials And Methods

3. Method and Materials:

3.1 Type of study:

This study is a cross-sectional one.

3.2 Study area, population and sample size:

The study was conducted on 81 non-pregnant women of reproductive age (range: 18-43 years),from different areas in Ibb governorate, from June – July, 2022. Samples were tested for serum alkaline phosphatase and calcium in the laboratories of Al-Thowra hospital. Vitamin D test was performed in the laboratories of Al-Noor hospital, Ibb governorate.

3.3 Inclusion Criteria:

- Being women of child-bearing age.
- Resident in Ibb governorate.
- Free from physiological conditions (e.g. pregnancy), or systemic diseases that may affect the studied parameters (e.g. fractures, hepatic diseases, etc.).

3. Exclusion Criteria:

- Being women of post-menopausal age.
- Not resident in Ibb governorate.
- Pregnant women
- Evidence of the presence of systemic diseases that may affect the studied parameters (e.g. fractures, hepatic diseases, etc.).

3.5 Ethical considerations:

All participants gave an informed consent to use their information and blood. The proposal of the study was approved by the dean of the college of medical sciences, The International Malaysian University, Ibb governorate ".

3.6 Data collection:

3.6.1 Questionnaire:

Face to face interviews were used to collect data from enrolled subjects. All of the volunteers in the study who agreed to participate in this study were asked to complete a personal data and questionnaire which was designed to meet the study needs. It included issues about and socio-geographic parameters: personal information like name, age, gender, address, and marital status, number of children, pregnancy and lactation. In addition, it included other information about exposure to sunlight, bone diseases and drugs received. Most questions were yes or no question which offers a dichotomous choice.

3.6.2 Sampling and sample collection:

Blood samples were collected by withdrawing 3ml of venous blood into new plastic tubes with a tight -fitting lid. The tubes were left at room temperature until coagulation was complete. Then, they were centrifuged for 10 min. at 3000 rpm. Serum was separated and frozen in Eppendorf's tubes at –20℃ until being examined for the biochemical tests.

3.6.3 Biochemical analysis:

For the biochemical tests (Serum ALP, Ca, vitamin D), all reagents, calibrators, controls and samples were brought to room temperature prior to starting the test run. A spectrophotometer (Copra BTS-350 , Bio systems ,Spain) was used to measure the absorbance of the ALP and Ca tests for each sample. Vitamin D was tested by reagent produced by (Roche Diagnostics, Germany) on an instrument of Immune - chemiluminescence technique (COBAS e411, Hitachi, Japan).

3.6.3.1 Serum calcium measuring:

- Procedure

1. Reagents and samples were brought to room temperature.
2. Into labeled test tubes, it was pipetted:

TUBES	Blank	Sample	CAL. Standard
Working reagent	1.0 mL	1.0 mL	1.0 mL
Sample	–	10 µL	–
CAL.Standard	–		10 µL

3. The tubes were mixed and let stand for 5 minutes at room temperature.
4. The absorbance (A) of the samples and the standard was read in the spectrophotometer at660 nm against the reagent blank.

- Calculations:

$$\frac{A_{Sample}}{A_{Standard}} \times C_{Standard} = mg/dL \text{ total calcium}$$

(standard conc. = 10 mg/dL)

- Reference values in adult female serum

8.5-10.5 mg/dL

3.6.3.2 Alkaline phosphate

- Procedure

- Working reagent, samples and controls were brought to room temperature (25ºC).
- A pill was taken from the first reagent container (R1) and dissolved in a 15 ml of (R2) which was pulled from the second container, and they were mixed in an empty container.
- The spectrophotometer was set to 0 absorbance with distilled water at 404 nm.
- 1 ml of the readily prepared reagent was pipetted into a new test tube.
- 20 ul of serum was added into to the same tube, and mixed gently.
- Mixture was poured to a cuvette. Then, cuvette was inserted into the cell holder and stopwatch was started.
    - Cuvette was incubated for 1 minute and initial absorbance reading was recorded.
    - The absorbance readings were repeated exactly after 1, 2 and 3minutes.
    - The differences between absorbance were calculated.

- The mean of the results was calculated to obtain the average change in absorbance per minute (▲A/min).

- Calculations:

U/L = ▲A/min x 2745 (37ºC)

- Reference values in adult non-pregnant, none post-menopausal female serum

38-154 U/L (25ºC))

3.6.3.3 Vitamin D

- Intended use

This assay is intended for the quantitative determination of total 25 hydroxyvitamin D in human serum. This assay is to be used as an aid in the assessment of vitamin D sufficiency. The electrochemiluminescence binding assay is intended for use on Elecsys and COBAS e immunoassay analyzers.

- 3rd incubation: After addition of streptavidin coated micro particles and 25 hydroxyvitamin D labeled with biotin, unbound ruthenylated labeled vitamin D binding proteins become occupied. A complex consisting of the ruthenylated vitamin D binding protein and the biotinylated 25 hydroxyvitamin D is formed and becomes bound to the solid phase via interaction of biotin and streptavidin.

Their action mixture is aspirated into the measuring cell where the micro particles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Results are determined via a calibration curve which is instrument-specifically generated by 2 point calibration and a master curve provided via the reagent barcode or e barcode.

- Procedure

1. Reagent cartridge was put in the reagent tray of the COBAS e 411 instrument.
2. Scan button was pressed to identify the information from the barcode of the reagent.
3. Sample were put in the in the sample tray of the COBAS e 411 instrument.
4. Data related to the test (name and number of patient, and sample position) were inserted, and register button was pressed.
5. Start button was pressed, and the analysis was initiated.
6. The result were appeared in the display after 27 minutes and recorded.

3.7 Data analysis:

Data were analyzed using the Statistical Package for Social Sciences program (SPSS-IBM, version 20) and were introduced in columns and tables patterns. Means ± SD, percentages and ranges were collected. T-test was used to compare the differences between the means of the continuous parameters of each two groups (married vs. non-married, and lactating vs. lactating). Pearson correlation test was used to study the relationship between any two continuous parameters in each one of the two groups. Spearman correlation test was used to study the relationship between the categorical parameters and the continuous parameters. Kruskal-Wallis test was used to study the effect of the categorical variables on the continuous variables. Mann-Whitney test was used to study the effect of the nominal variables on the parametric variables.

Statistical significance was determined at a level of $P \leq 0.05$.

3.8 Material and reagent:

3.8.1 Material:

Glass test tubes, Plain tubes, Automated COBAS E411 immunoassay analyzers(Roche Diagnostics, Germany),spectrophotometer (Copra BTS-350,Biosystems, Spain), centrifuge (ROTOFIX 32 A , China), syringes ,Eppendorf's tubes, cotton, micro- pipettes, cuvette and general laboratory equipment.

3.8.2 Reagents:

- Calcium commercially available kit (supereact, Spain).
- ALP commercially available kit (supereact, Spain).
- Vitamin D3 commercially available kit (Reagent of elecsys and COBAS E411 immunoassay analyzers, Roche Diagnostics, Germany)
- Working reagents of automated COBAS E411 immunoassay analyzers, Roche Diagnostics, Germany (Wash solution, Pro-cell, Clean-cell, cups, tips)
- Other covenantal laboratory reagents.

Chapter 4
Results

4 Results:

This research which performed to study some biochemical biomarkers related to bones health in females of reproductive age (mean ±SD = 28.08 ±6.60 years, Range: between 16 and 43 years) in Ibb governorate. The total number of enrolled subjects in this study was 81 women. The geographic parameters show that most of enrolled women are married (74.68%) (as shown in figure 4.1), and from all the participants; nursing (lactating) females are 50.62% (figure 4.2).

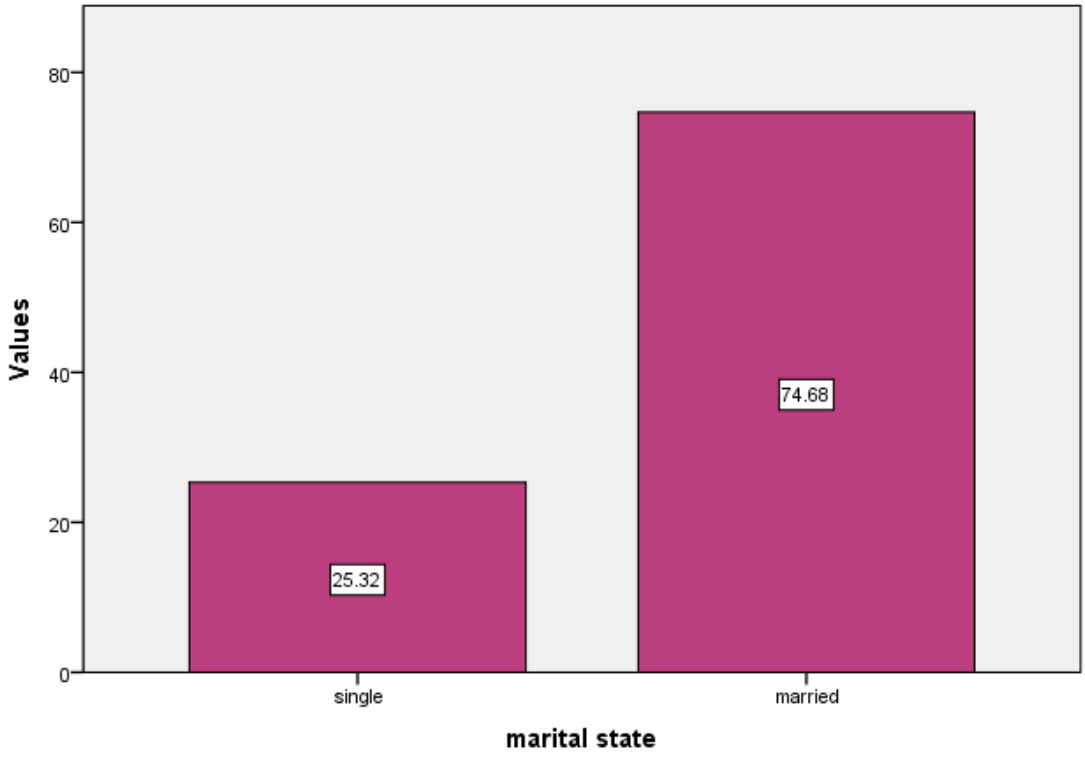

Figure (4.1): Percent of marital state in the women subjects

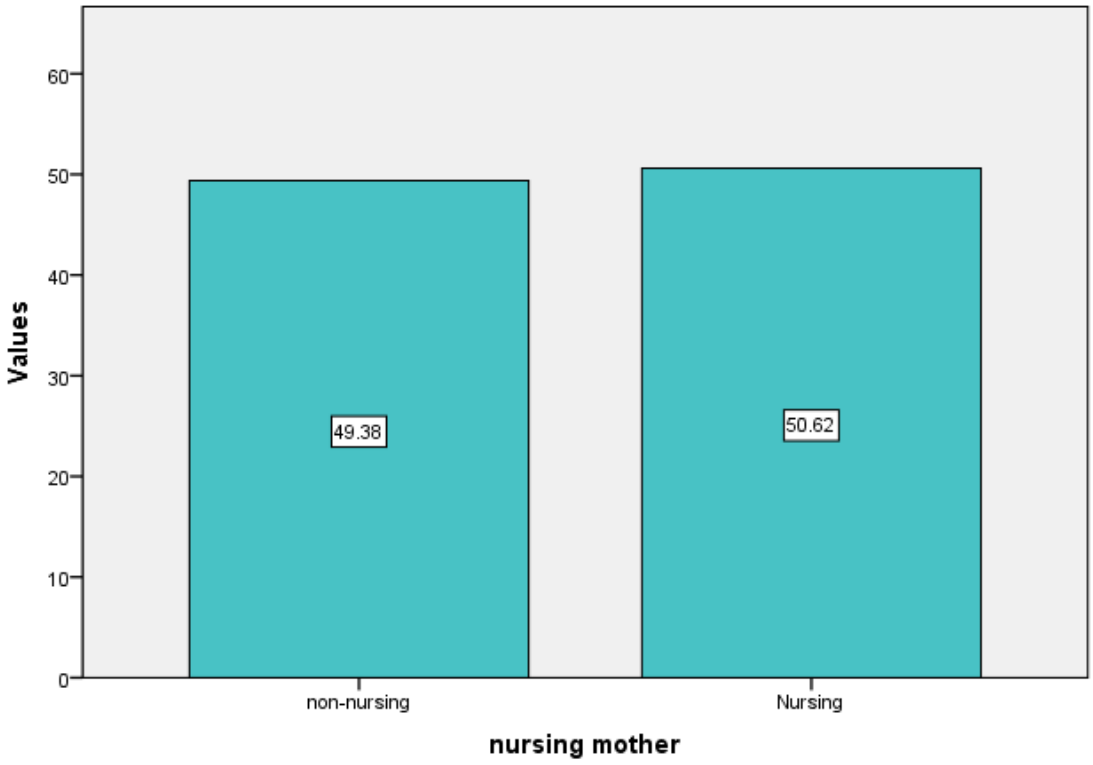

Figure 4.2:Percent in lactating and non-lactating women subjects

As presented in figure 4.3, study level of the female subjects in this study showed a wide variation between them. About 36.23% of them are graduated, 28.99% had completed their secondary education, 26.09% had completed their primary study, 7.25% are illiterate and only 1.45% are post-graduated.

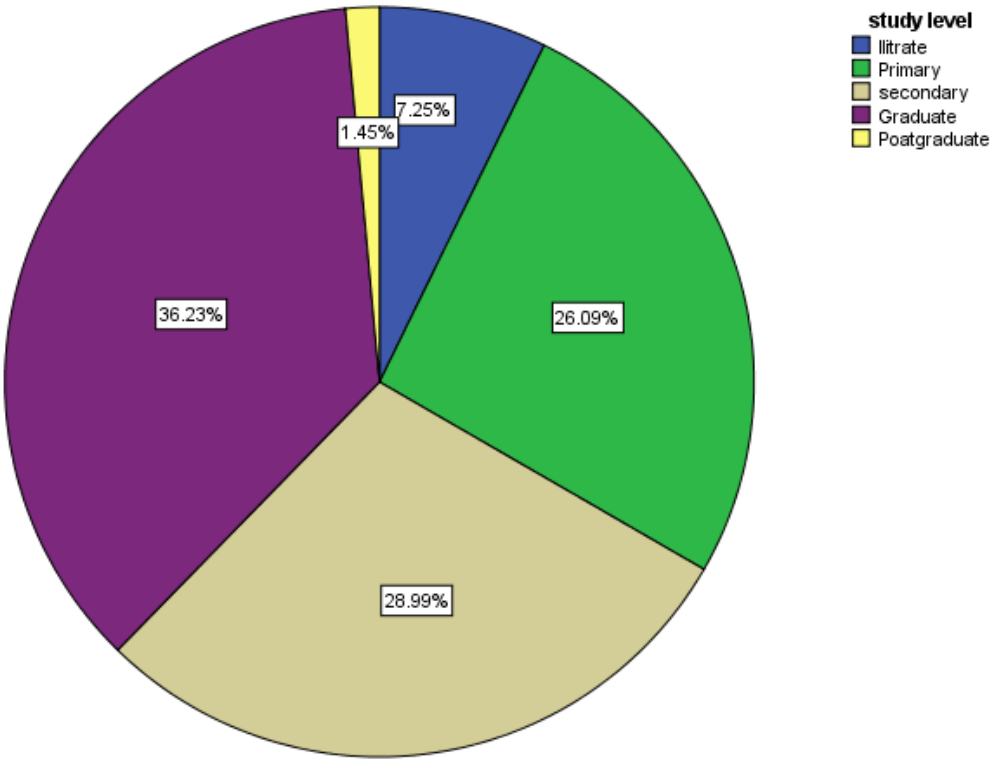

Figure (4.3): Percent of the study level in the women subjects

For number of children of the study population, figure 4.4 showed that most (31.65%) of the volunteer females in this study have no babies (most of them are single females). However, 12.66% of the subjects have 1 baby, 15.19% have 2 babies, 12.66% have 3 babies, 12.66% have 4 babies, 7.59% have 5 babies, 3.79% have 6 babys,2.53% have 7 babies, and 1.26% have 8 babies (figure 4.4).

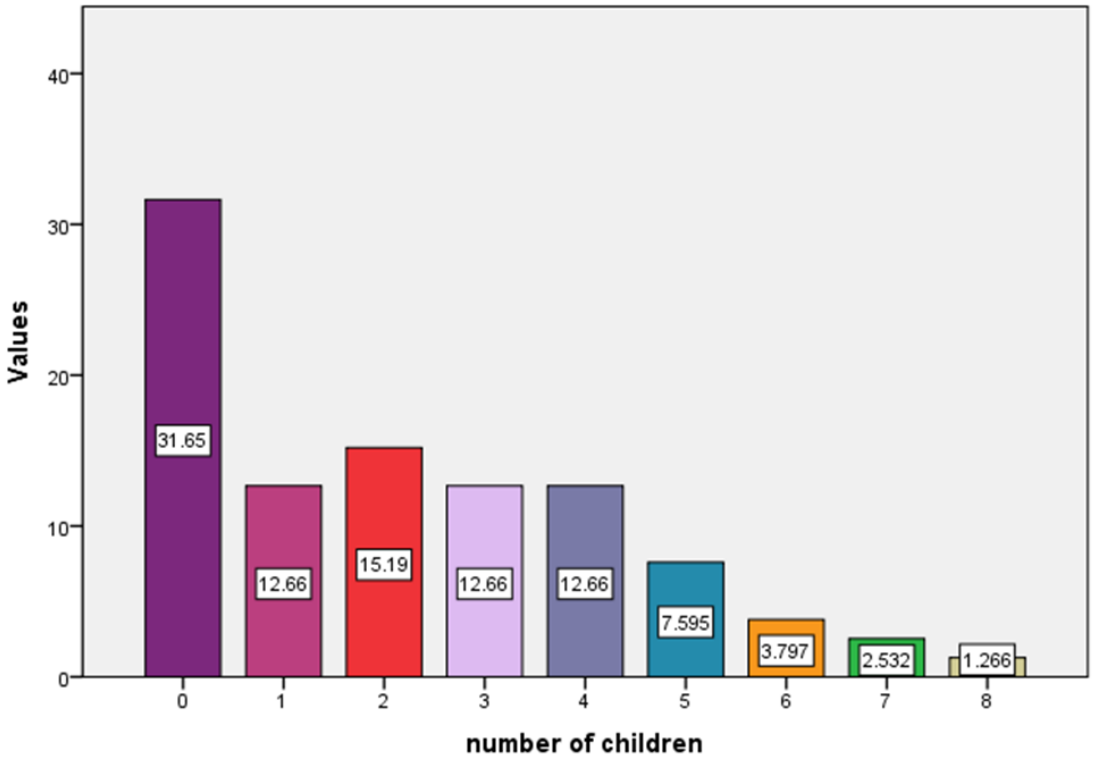

Figure 4.4:Percent of number of children in the women subjects

For the time of exposure to sun light per day by the subjects, 37.66% of them are not exposed to the direct sunlight daily. However, 41.56% of them are exposed to the direct sunlight for 5-15 minutes daily, and 14.29% of them are exposed to the direct sunlight for 15-30 minutes daily, and the minority of them (6.49%) are exposed to the direct sunlight for 60 minutes and more daily (figure 4.5).

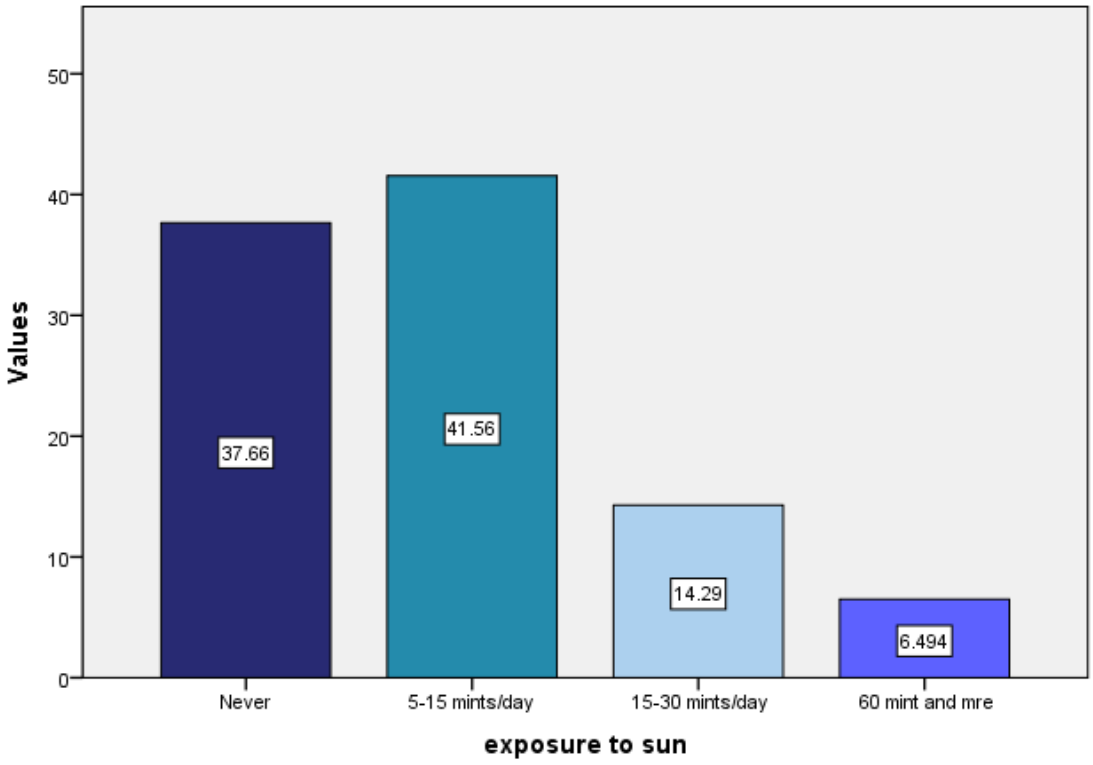

Figure 4.5: Percent of the time of daily exposing to the direct sunlight in the women subjects

Table 4.1 shows the biochemical parameters of the participants in this study. Mean serum vitamin D_3 level in the subjects is 13.39 ng/ml; which is within the moderate deficiency, while the optimum value is ranged from 25-80 ng/ml. Mean serum Calcium level is 8.48 mg/dl; which is slightly lower than the lower limit of the normal range (8.5-10.5 mg/dl), however, mean serum alkaline phosphatase level is 170.5 U/L; which is fairly higher than the normal range (38-154 U/L).

Table 4.1: Some bone related biomarkers in the women subjects

	Minimum	Maximum	Mean	Std. Deviation
Vitamin D3 (ng/ml)	3.00	47.95	13.39	8.02
S. Calcium (mg/dl)	6.30	11.70	8.48	0.83
Alk. Phosphatase (U/L)	100.00	540.00	170.50	69.59

The Pearson's correlation test between the parametric variables shows only significant negative correlation ($P< 0.05$) between Vitamin D3 levels (ng/ml) and Alk. Phosphatase levels (U/L) as seen in table 4.2 and in figure 4.6.

However, Spearman's correlation shows no significant relationship between the parametric and the non-parametric variables.

Table 4.2: Correlation between the different bone related biochemical parameters in the women subjects

	Age		**Children**		**Alk. phosphatase**		**S. Calcium**	
	r	P-value	r	P-value	r	P-value	r	P-value
Vitamin D3	-0.055	0.630	-0.186	0.101	-0.241	0.030*	0.018	0.872
S. Calcium	-0.169	0.137	-0.184	0.192	0.009	0.938	-	-
Alk. Phosphatase	-0.132	0.247	0.164	0.149	-	-	-	-

*: $P \leq 0.05$

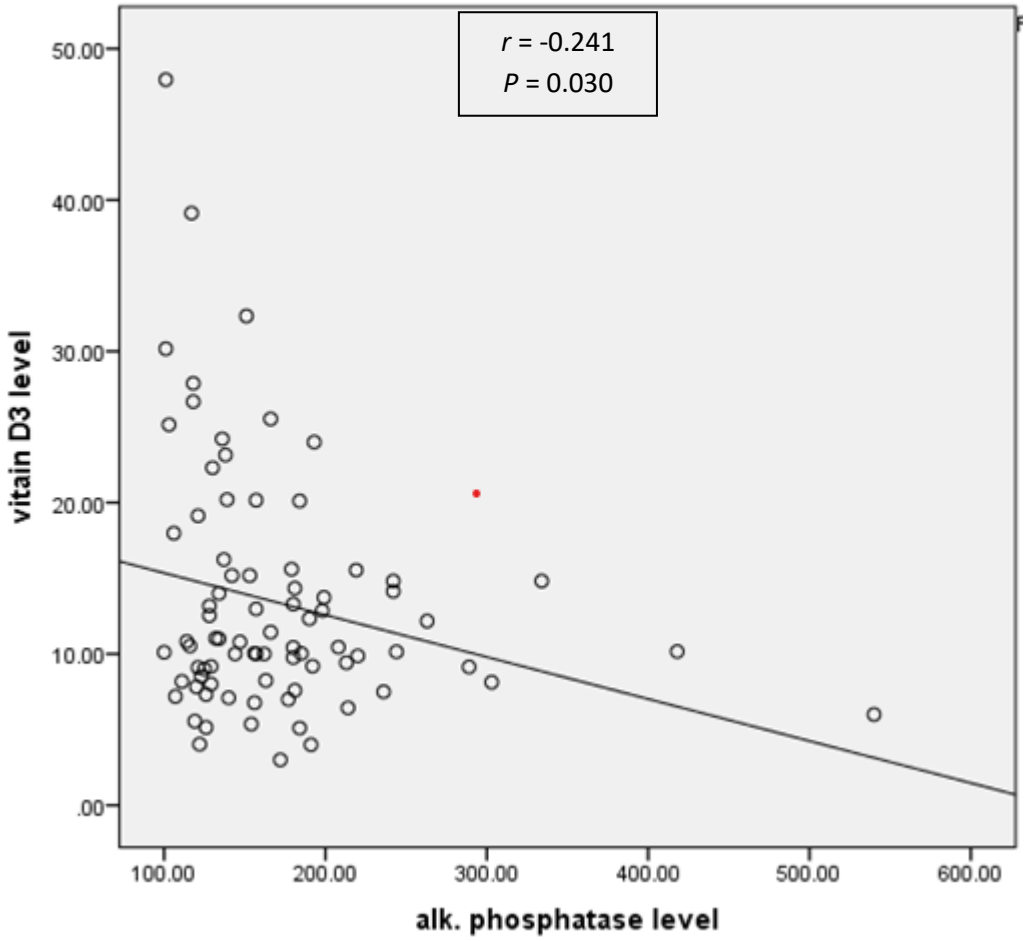

Figure 4.6: Correlation between vitamin D3 and alkaline phosphatase in the subjects

To study the differences between the married women and non- married women in their biochemical parameters, unpaired t-test was performed. The results show a significantly higher ($P < 0.01$) mean of Alk. Phosphatase (U/L) levels in the married women as compared to the non-married women (table 4.3).

Table 4.3: A comparison between the bone related biomarkers in the married and non-married women

	Single (Mean±SEM) (n =20)	**Married (Mean±SEM) (n = 59)**	**t-test**	**P-value**
Vitamin D3	16.03±2.14	12.73±0.95	1.401	0.173
S. Calcium	8.61±0.20	8.45±0.10	0.696	0.492
Alk. Phosphatase	143.80±8.13	180.40±9.94	-2.850	0.006**

**: $P \leq 0.01$

To study the differences between the lactating women and non-lactating women in their biochemical parameters, unpaired *t*-test was performed. The results show a significantly higher ($P< 0.001$) mean of Alk. Phosphatase (U/L) levels in the lactating women as compared to the non-married women (table 4.4).

Table 4.4: A comparison between the bone related biomarkers in the lactating and non-lactating women.

	Non-lactating women (Mean±SEM) (n =40)	**Lactating women (Mean±SEM) (n = 41)**	***t*-test**	***P*-value**
Vitamin D3	14.83±1.55	11.97±0.86	1.607	0.113
S. Calcium	8.53±0.71	8.42±0.147	0.558	0.579
Alk. Phosphatase	142.12±5.25	189.19±13.08	-3.976	0.000***

***: $P \leq 0.001$

Table 4.5 shows the relationship between some non-parametric variables related to the study and the performed biochemical tests. Kruskal- Wallis test shows no significant effects of the different periods of time of exposure to the sunlight on each one of the biochemical tests. In the contrary, Mann-Whiteny test shows that marital state and lactating state have a significant effect on mean serum Alk. Phosphatase levels, in which mean serum Alk. phosphatase levels are significantly higher in the married and in the lactating subjects ($P \leq 0.05$, and $P \leq 0.001$, respectively).

Table 4.5: The relationship between some non-parametric variables and the bone related biochemical markers in the subjects

	Vitamin D3	**Calcium**	**Alk. Phosphatase**
	P-value	*P*-value	*P*-value
Exposure to sunlight[(a)]	0.132	0.226	0.326
Marital state[(b)]	0.169	0.852	0.018*
Lactating[(b)]	0.395	0.974	0.00***

(a): Kruskal- Wallis test
(b): Mann- Whiteny test
*: $P \leq 0.05$
***: $P \leq 0.01$

Chapter 5

Discussions

5. Discussion

The use of bone turnover markers in clinical practice and research in younger people is limited by the lack of normative data and understanding of common causes of variation in bone turnover marker values in this demographic. To appropriately interpret bone turnover markers, robust reference intervals specific to age, development and sex are necessary (Callegari *et al*., 2016). The present study evaluates some available bone markers females of reproductive age in Ibb governorate.

In the present study which included eighty one women of childbearing age (mean ± SD = 28.08 ± 6.60; range = 16-43 years), the percent of married women was 74.68% while the percent of single women was 25.32%. In contrast, in the study of Khalfa and his colleagues, (2019), on females of different age groups, they found that all females of all age groups suffered from hypovitaminosis D3 due to bone disorders. However, they reported that females that were single had lower vitamin D3 levels as well as lower serum calcium levels compared to married females, and they attributed that to their lacking sexual activity and inter hormonal play. Nevertheless, pregnancy and lactating in married females were demonstrated to contribute to vitamin D deficiency (Oboh *et al.,* 2021). Marriage is often considered to have a health-protective effect and studies have found an association between being married and lower rates of chronic illness, fewer physical limitations, and less disability. However, early marriage (often defined as marriage at 20 years of age or younger) is associated with greater psychosocial distress, lower educational attainment, and poorer long-term marital success. All those factors associated with lower bone strength and bone density (Miller-Martinez *et al.,* 2014). Glover *et al.,* (2008), mentioned that one

turnover is elevated until skeletal maturity is reached, usually in the 3rd decade of life. Women over 45 years of age have fewer ovulatory cycles and a high biological variability of Bone turnover markers (BTMs) as bone turnover is increased in premenopausal women prior to the loss of estrogen in younger females at the end of puberty, high bone turnover is present due to bone modeling, but subsides as bone growth halts and newly formed osteoid undergoes primary and secondary mineralization (Eastell, 2005). During skeletal growth in young females, bone mass is accrued up until the third decade of life (Teegarden *et al.,* 1995; Hansen, 1994). Suboptimal peak bone mass substantially increases the risk of osteoporosis (and related fracture) in later life. Thus, strategies aimed at optimizing peak bone mass in younger, premenopausal females are crucial in its prevention (Hansen *et al*, 1991).

On the other hand, the percent of lactating women in our study was 50.62% and the percent of non-lactating women was 49.38%, and 31.65% of involved women in our study have no children as 25.32% of them were non-married. Oboh *et al.,* (2021), concluded that women who breastfed for > 6 months had significantly reduced Bone mass density (BMD). However, compared to women that breastfed for a ≤ 4 months there was no significant change in BMD. Also, six weeks after delivery (clinically the postpartum phase) and beyond, lactation demands more calcium and is supplied to the infant through breastmilk (Salari and Abdollahi, 2014). About 200 mg/day of calcium is mobilized into breastmilk during lactation (Sawo *et al,* 2013).

Also, as shown in the figure (4.3) for the classification of subjects according to the educational level, the result showed that most subjects are graduated (36.23%), and 28.99% have completed their secondary

school, and 26.09% have completed their primary school, and only 1.45% are post-graduated. In accordance, in the study of Junaid *et al.,* (2015), they found that risk of vitamin D deficiency was independently associated with illiteracy; in the women of child-bearing age in Lahore Pakistan.

Furthermore, in this study, mean serum calcium (8.348 ± 0.83 mg/dl) is lower than the normal range (8.5 - 10.5 mg/dl), and serum level of vitamin D show a moderate deficiency (mean ± SD = 13.39±8.2 ng/ml), while the normal range is between 30 and 100 ng/ml. Similarly, in the study of Junaid *et al.,* (2015), in the women of child-bearing age in Lahore Pakistan, they found that (73 %) of participants were vitamin D deficient. Likewise, the results of the study of Shaheen et al, (2012) on adult females showed that out of 110 samples, 26 had mild (23%), 61 had moderate (55%) and 21 had severe (19.1%) vitamin D deficiencies.

Moreover, in the present study, about 37.66% of the subjects are never exposed to the sunlight daily, and most of them (41.56%) are exposed to sunlight for only 5-15 minutes. Likewise, Junaid *et al.,* (2015), found that risk of vitamin D deficiency was independently associated with < 30 min sun exposure per day; in the women of child-bearing age in Lahore Pakistan. However, no correlation was found between the different biochemical tests and the time of exposure to the sunlight in our study. In similar, Kiran *et al.,* (2014), found a positive correlation between sun light exposure and Vitamin D status in males but not in females; in their study on the healthy adults above the age of 20 living in Potheri village of Kancheepuram district, Tamilnadu, India.

Vitamin D is a secosteroid synthesized in the skin when exposed to sun light. Sun light is the major source of this vitamin in the body.

Vitamin D is collective term given to Vitamin D_3 (Cholecalciferol) and Vitamin D_2 (ergocalciferol). Cholecalciferol is synthesized from the skin when exposed to UV radiation. Ergocalciferol is obtained from fungi. Although vitamin D status may be influenced by environmental factors such as sun exposure and diet, variation in the genes encoding the vitamin D 25-hydroxylase enzyme C YP2R1, the vitamin D binding protein (DBP) and the vitamin D receptor (VDR) have also been reported to associate with risk of vitamin D deficiency (Wang *et al.*, 2010; McGrath *et al.,* 2010). Also, Junaid *et al.,* (2015), showed that vitamin D deficiency was associated with decreased sunlight exposure, they did not demonstrate any association between veiling (wearing Nekab) and risk of vitamin D deficiency; this finding is consistent with that from a recent study in Bangladesh which was performed by Islam *et al.,* (1996), who reported that vitamin D deficiency was common among women in that setting irrespective of veiling. The relative contribution of environmental vs. genetic determinants of risk of vitamin D deficiency has not previously been described in Yemeni women.

In addition, serum ALP in the conducted study is slightly higher (mean±SD = 170.5±69.59 U/L) than the normal range (38-154 U/L). Besides, serum ALP in our study is negatively correlated with serum vitamin D levels (P <0.05) but not with serum Ca levels. This is in agreement with the results of a recently published study which was carried out by Bellastella *et al.*, (2021), who found a significant negative correlation between serum vitamin D and ALP; in healthy individuals of both sexes, particularly in females, and at different age groups ($> 18 \leq 40$ and in people $> 40 \leq 65$). In the study of Shaheen *et al.*, (2012) on adult females, all of the patients in the three groups of vitamin D

deficiencies (mild, moderate and sever) had alkaline phosphatase within normal limits and the total mean value of the enzyme was 135.97 ± 68.14I U/L. But in parallel to our study, the inter group comparison showed highest values of alkaline phosphatase in the moderate vitamin D deficiency group.

Alkaline phosphatase (ALP) is an enzyme found in several tissues throughout the body. It can originate from the liver, bone, intestines, or kidneys, but bone ALP and liver ALP constitute about 95% of the total ALP activity in human serum. Elevated levels of ALP in the blood are most commonly caused by liver disease or bone disorders. ALP levels will rise in the serum following increased production by osteoblasts in a state of high bone turnover. Throughout life, bone continuously remodels itself through bone resorption and replacement (Bellastella *et al.*, 2021).

In this study, phosphate, estrogen, parathyroid hormone, several bone markers, genetic factors, socio-geographic, anthropometric, physiologic and life style factors were not investigated. However, the reduced budget and the unavailability of most of the needed reagents are the cause. Further studies including them should be conducted to study their inter-correlation with the bone health in the Yemeni women.

Chapter 6
Conclusion and Recommendations

6. Conclusion and Recommendations:

6.1 Conclusion:

Yemeni women of childbearing age who are resident in Ibb governorate have slight deficiency in serum Ca, and a moderate deficiency in Vit D_3, and a slight elevation in ALP, which is negatively correlated significantly with Vit D_3. This suggests a suboptimal bone mass formation in them, particularly in married and in nursing one.

6.2 Recommendations

It is recommended that:

1. Yemeni women expose their bodies to sunlight for more time or use external vitamin D supplement.
2. Perform in health educational lectures in the schools and universities, and in the television and social media that focus on the importance of exposure to sunlight and that highlight the sources of food rich in vitamin D and Calcium, particularly in pregnancy and lactation.
3. 3-Conducting further studies with large sample size that study socio-geographic, anthropometric, physiologic and life style factors that are related to the bone health in the Yemeni women.
4. 4-Carrying out further studies including further biochemical bone markers and genetic factors that are related to the bone health in the Yemeni women.

Reference

Reference

Açıkgöz A, Günay T, Uçku R. (2013). Gebelikte D Vitamini gereksinimi ve desteklenmesi. TAF Prev Med Bull 2013;12:595–608.

Ataş A, Çakmak A, Soran M. (2008). Vitamin D metabolizması ve rikets hastalığı. Bakırköy Tıp Derg 2008;4:1–7.

Azpiazu D, Gonzalo S, Villa-Bellosta R. (2019). Tissue Non-Specific Alkaline Phosphatase and Vascular Calcification: A Potential Therapeutic Target. Curr Cardiol Rev. 15(2):91-95.

Baysal A.A. (2012). Beslenme. Vol. 14. Ankara: Hatipoğlu Yayınları; 2012. p. 176–83.

Bellastella G, Scappaticcio L, Longo M, et al.(2021). New insights into vitamin D regulation: is there a role for alkaline phosphatase?. Journal of Endocrinological Investigation. 2021; 44:1891–1896.

Bischoff-Ferrari H.A, Giovannucci E, Willett W.C, Dietrich T, Dawson-Hughes B. (2006). Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr. 2006;84:18-28.

Bove-Fenderson E, Mannstadt M. (2018). Hypocalcemic disorders. Best Pract Res Clin Endocrinol Metab 2018;32:639-56.

Reference

Brichacek A.L, Brown C.M. (2019). Alkaline phosphatase: a potential biomarker for stroke and implications for treatment. Metab Brain Dis. 2019 Feb;34(1):3-19.

Callegari ET, Gorelik A, et al. (2016). Bone turnover marker reference intervals in young females. Annals of Clinical Biochemistry: International Journal of Laboratory Medicine. 2016. 45:4.

Callegari ET, Gorelik A, et al. (2017). Bone turnover marker determinants in young women: results from the Safe-D study. Annals of Clinical Biochemistry: International Journal of Laboratory Medicine. 2017; 55:3.

Castells L, Cassanello P, Muñiz F, de Castro M.J, Couce M.L. (2018). Neonatal lethal hypophosphatasia: A case report and review of literature. Medicine (Baltimore). 2018 Nov;97(48):e13269.

Champe P.C, Harvey R.A, Ferrier D.R. (2005). Vitamins Biochemistry. 3rd ed. New York: Lippincott Williams and Wilkins; 2005. p. 371–93.

Cheema C, G rant BF, Marcus R. (1989). Effects of estrogen on circulating " free" and total 1,25-dihydroxyvitamin D and on the parathyroid-vitamin D axis in postmenopausal women. J Clin Invest. 1989; 83:537– 42.

Cristoferi L, Nardi A, Ronca V, Invernizzi P, Mells G, Carbone M. (2018). Prognostic models in primary biliary cholangitis. J Autoimmun. 2018 Dec;95:171-178.

Dawodu A, Absood G, Patel M, Agarwal M, Ezimokhai M, et al. (1998). Biosocial factors affecting vitamin D status of women of

childbearing age in the United Arab Emirates. J Biosoc Sci. 1998; 30: 431-437. Ref.: https://goo.gl/6K5osP

Dennison EM, Jameson KA, Edwards MH, Denison H J, Aihie Sayer A, Cooper C. (2014). Peripheral quantitative computed tomography measures are associated with adult fracture risk: the Hertfordshire Cohort Study. Bone. 2014; 64:13-7.

Dusso A.S, Brown A.J, Slatopolsky E. (2005). Vitamin D. Am J Physiol Renal Physiol. 2005; 289:F8–28.

Eastell R. (2005). Role of oestrogen in the regulation of bone turnover at the menarche. J Endocrinol 2005; 185: 223–234.

Ersoy N, Ersoy G. (2017). D Vitamini yetersizliği ve depresyon: Ne yapabiliriz. Hacettepe Üniv Sağlık Bilimler Fakültesi Derg 2017;4:1–14

Fairweather-Tait SJ, Teucher B. (2002). Iron and calcium bioavailability of fortified foods and dietary supplements. Nutr Rev 2002;60:360-7. Prev Med Bull 2013;12:595–608.

Fong J, Khan A. (2012). Hypocalcemia: updates in diagnosis and management for primary care. Can Fam Physician 2012;58:158-62.

Gallagher J.C, Sai A.J. (2010). Vitamin D insufficiency, deficiency, and bone health. J Clin Endocrinol Metab 2010;95:2630–3.

Gannagé-Yared MH, Chemali R, Yaacoub N, Halaby G. (2000). Hypovitaminosis D in a sunny country: relation to lifestyle and bone markers. J Bone Miner Res. 2000; 15: 1856-1862.Ref.: https://goo.gl/TuJeEu

Reference

Genç A, Sevim D.G, Özen A.T, Yılmaz G.D. (2015). Vitamininin D Çeşitli hastalıklardaki nadir kullanım alanları. Ankara Üniv Fak Mecm 2015;68. DOI: 10.1501/Tıpfak_000000890.

Glover SJ, Garnero P, Naylor K, Rogers A, Eastell R. (2008). Establishing a reference range for bone turnover markers in young, healthy women. Bone. 2008; 42(4):623-630.

Guyton A.C, Hall J.E. Bölüm. (2007). Paratiroid hormonu, kalsitonin, kalsiyum ve fosfat mekanizması, D Vitamini, kemik ve dişler. In: Çavuşoğlu H, Yeğen BC, editors. Tıbbi Fizyoloji. Vol. 11. İstanbul: Nobel Tıp Kitabevleri; 2007. p. 978–94.

Hansen M. (1994). Assessment of age and risk factors on bone density and bone turnover in healthy premenopausal women. Osteoporosis Int 1994; 4: 123–128.

Hansen MA, Overgaard K, Riis BJ, et al. (1991). Role of peak bone mass and bone loss in postmenopausal osteoporosis: 12 year study. BMJ 1991; 303: 961–961.

Heaney R.P. (2010). Calcium. In: Coates PM, Betz JM, Blackman MR, et al., eds. Encyclopedia of Dietary Supplements. 2nd ed. London and New York: Informa Healthcare; 2010:101-6.

Heinrich D, Bruland Ø, Guise T.A, Suzuki H, Sartor O. (2018). Alkaline phosphatase in metastatic castrationresistant prostate cancer: reassessment of an older biomarker. Future Oncol. 2018 Oct;14(24):25432556.

Reference

Holick M.F. (2007). Vitamin D deficiency. N Engl J Med 2007;357:266–81.

Hollis BW, Wagner CL. (2004). Assessment of dietary vitamin D requirements during pregnancy and lactation. Am J Clin Nutr. 2004; 79: 717-726.Ref.: https://goo.gl/jGRCDZ

Institute of Medicine, Food and Nutrition Board. (2010). Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC: National Academy Press, 2010.

Institute of Medicine, Food and Nutrition Board. (2011). Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC: National Academy Press, 2011.

Islam MZ, Akhtaruzzaman M, Lamberg-Allardt C. (2006). Hypovitaminosis D is common in both veiled and nonveiled Bangladeshi women. Asia Pac J Clin Nutr. 2006;15(1):81– 7.

Jackson R.D, LaCroix A.Z, Gass M, Wallace R.B, Robbins J, Lewis C.E. (2006). Calcium plus vitamin D supplementation and the risk of fractures. N Engl J Med 2006;354:66983.

Jones G., Ross A.C, Caballero B, Cousins R.J, Tucker K.L, Ziegler T.R. (2014). Vitamin D. In: Modern Nutrition in Health and Disease, 11th ed. Philadelphia: Lippincott Williams & Wilkins, 2014.)

Junaid K, Abdul Rehman, et al. (2015). High prevalence of vitamin D deficiency among women of child-bearing age in Lahore Pakistan, associating with lack of sun exposure and illiteracy. BMC Women's Health (2015) 15:83.

Khalfa HM, Al-Msaid HFL, Alsahlanee R. (2019). Relationship between vitamin D3 levels and marital status: A random study in females suffering from bone disorders in Al-Najaf province. Journal of Medical and Life Science. 2019; 1(3):65 -70

Kiran B, Prema A, Thilagavathi R, Jamuna Rani R. (2014). Serum 25-Hydroxy Vitamin D, Calcium, Phosphorus and Alkaline Phosphatase Levels In Healthy Adults Above the age of 20 Living in Potheri Village of Kancheepuram District, Tamilnadu. J App Pharm Sci. 2014; 4 (12): 030-034.

Kuroda T, Shiraki M, Tanaka S, Ohta H. (2009). Contributions of 25-hydroxy Vitamin D, co-morbidities and bone mass to mortality in Japanese postmenopausal women. Bone 2009;44:168–72.

Kurt M, Cmertoğlu İ, Yalın P, Diner G. (2011). Osteoporozlu hastalarda D Vitamini dzeyleri. Turk J Osteoporos 2011;17:68–70.

Malihi Z, Lawes CMM, Wu Z, Huang Y, Waayer D, Toop L, et al. (2019). Monthly highdose vitamin D supplementation does not increase kidney stone risk or serum calcium: Results from a randomized controlled trial. Am J Clin Nutr 2019;109:1578-87 .

Malihi Z, Wu Z, Stewart AW, Lawes CMM, Scragg R. (2016). Hypercalcemia, hypercalciuria, and kidney stones in long-term studies of vitamin D supplementation: A systematic review and meta-analysis. Am J Clin Nutr 2016;104:1039-51.

Masrour Roudsari J, Mahjoub S. (2012). Quantification and comparison of bone-specific alkaline phosphatase with two methods

in normal and paget's specimens. Caspian J Intern Med. 2012 Summer;3(3):478-83.

Matsushita M, Harajiri S, Tabata S, Yukimasa N, Muramoto Y, Komoda T. (2013). Alkaline phosphatase activity in blood group B or O secretors is fluctuated by the dinner intake of previous night. Rinsho Byori. 2013 Apr;61(4):307-12

McGrath JJ, Saha S, Burne TH, Eyles DW. (2010). A systematic review of the association between common single nucleotide polymorphisms and 25-hydroxyvitamin D concentrations. J Steroid Biochem Mol Biol. 2010; 121(1– 2):471– 7.

Miller-Martinez D, Seeman T, Karlamangla AS, et al. (2014). Marital histories, marital support, and bone density: findings from the Midlife in the United States Study. Osteoporos Int. 2014; 25(4): 1327–1335.

Mithal A, Wahl D.A, Bonjour J.P, Burckhardt P, Dawson-Hughes B, Eisman J.A. (2009). Global Vitamin D status and determinants of hypovitaminosis D. Osteoporos Int 2009;20:1807–20.

O'Riordan J.L, Bijvoet O.L. (2007). Rickets before the discovery of Vitamin D. Bonekey Rep 2014;3:478. [CrossRef}.

Oboh I, Coleman C, Cremona A. (2021). The in flue nce of lactation and its duration on bone mineral density in pregnancy and postpartum: A systematic review with meta-analysis. Clinical Nutrition Clinical Nutrition ESPEN. 2021; 46:121-132.

Öğüş E, Sürer H, Kılınç A, Fidancı V, Yılmaz G, Dindar N. (2014). Vitamini D düzeylerinin aylara, cinsiyete ve yaşa göre değerlendirilmesi. Ankara Med J 2014;15:1–5.

Reference

Özçelik D.C, Koçer H, Kasım İ, Şencan İ, Kahveci R, Özkara A. (2012). Vitamini D. Turk Med J 2012;6.

Pepe J, Colangelo L, Biamonte F, Sonato C, Danese VC, Cecchetti V, et al. (2020). Diagnosis and management of hypocalcemia. Endocrine 2020;69:485-95.

Perez-Castrillon JL, Vega G, Abad L, Sanz A, Chaves J, Hernandez G, Duenas A. (2007). Effects of atorvastatin on vitamin D levels in patients with acute ischemic heart disease. Am J Cardiol 2007;99:903-5.

Pike A.F, Kramer N.I, Blaauboer B.J, Seinen W, Brands R. (2013). A novel hypothesis for an alkaline phosphatase 'rescue' mechanism in the hepatic acute phase immune response. Biochim Biophys Acta. 2013 Dec;1832(12):2044-56.

Pinart M, Kunath F, Lieb V, Tsaur I, Wullich B, Schmidt S. (2020). German Prostate Cancer Consortium (DPKK). Prognostic models for predicting overall survival in metastatic castration-resistant prostate cancer: a systematic review. World J Urol. Mar;38(3):613-635.

Salari P, Abdollahi M. T. (2014). he infl uence of pregnancy and lactation on maternal bone health: a systematic review. J Fam Reprod Health 2014;8(4):135e 48.

Sawo Y, Jarlou L, Goldberg G, Laskey M, Prentice A. (2013). Bone mineral density Changes after lactation in Gambian women accustomed to a low calcium intake. Eur J Clin Nutr 2013;67:1142 e6. https://doi.org/10.1038/ejcn.2013. 162 available:.

Seeman E. (2009). Pathogenesis of bone fragility in women and men. Lancet. 2002; 359:1841-50.

Sempos C.T, Durazo-Arvizu R.A, Fischer P.R, Munns C.F, Pettifor J.M, Thacher T.D. (2021). Serum 25-hydroxyvitamin D requirements to prevent nutritional rickets in Nigerian children on a low-calcium diet—a multivariable renanalysis. Am J Clin Nutr 2021;114:231-7.

Shaheen S, Noor SS, Barakzai Q. (2012). Serum Alkaline Phosphatase Screening for Vitamin D Deficiency States. Journal of the College of Physicians and Surgeons Pakistan. 2012; 22 (7): 424-427.

Singh P, Trivedi N. (2014).Tanning beds and hypervitaminosis D: A case report. Ann Intern Med 2014;160:810-1 .

Song L, Zhang X, Zhou Y. (2011). A synergetic role of 1,25-dihydroxyvitamin D(3) in 17beta-estradial induced-proliferation and differentiation of osteoblastic MC3T3-E1 cells. Eur J Pharmacol. 2011;659:273 –80.

Teegarden D, Proulx W.R, Martin B.R, et al. (1995). Peak bone mass in young. J Bone Miner Res 1995; 10: 711–715.

Tsung-Rong K, Chih-Hwa C. (2017). Bone biomarker for the clinical assessment of osteoporosis: recent developments and future perspectives. Biomarker Research. 2017; 5:18.

van der Doelen M.J, Mehra N, Hermsen R, Janssen M.J.R, Gerritsen W.R, van Oort I.M. (2019). Patient Selection for Radium-223 Therapy in Patients With Bone Metastatic Castration-Resistant

Prostate Cancer: New Recommendations and Future Perspectives. Clin Genitourin Cancer. 2019 Apr;17(2):79-87.

Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, et al. (2010). Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet. 2010; 376(9736):180 – 8.

Wawrzyniak N, Suliburska J. (2021). Nutritional and health factors affecting the bioavailability of calcium: a narrative review. Nutr Rev 2021.

Weaver C.M, Heaney R.P. (2014). Calcium. In: Ross AC, Caballero B, Cousins RJ, Tucker KL, Ziegler TR, eds. Modern Nutrition in Health and Disease. 11th ed. Baltimore, MD: Lippincott Williams & Wilkins; 2014:133-49.)

Weaver C.M. (2020).Calcium. In: Marriott BP, Birt DF, Stallings VA, Yates AA, eds. Present Knowledge in Nutrition. 11th ed. Cambridge, Massachusetts: Wiley-Blackwell; 2020:321-48.

Weaver C.M. Marriott B.P, Birt D.F, Stallings V.A, Yates A.A .(2020). Calcium. In: Present Knowledge in Nutrition. 11th ed. Cambridge, Massachusetts: Wiley-Blackwell; 2020:321-48.

Reference

World Health Organization.(2006). Food and Agriculture Organization. Guidelines on Food Fortification with Micronutrients. Geneva: World Health Organization; 2006.

Wranicz J, Szostak-Węgierek D. (2014). Health outcomes of Vitamin D. Part I. Characteristics and classic role. Rocz Panstw Zakl Hig 2014;65:1

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تقييم بعض المؤشرات البيوكميائية المرتبطة بصحة العظام عند النساء اليمنيات اللاتي في سن الانجاب بمحافظة اب

بحث مقدم الى قسم المختبرات الطبية – كلية الطب والعلوم الصحية – الجامعة الماليزية الدولية كجزء من متطلبات نيل درجة البكالوريوس في علوم المختبرات الطبية

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1444-2022

الملخص العربي

خلفية علمية: خلال فترة نمو الهيكل العظمي في النساء الشابات؛ يزيد تراكم كتلة العظام حتى العقد الثالث من العمر. فإذا حدث نقص في وصول كتلة العظام الى الحالة المثالية, فإن هذا سيزيد من احتمال الإصابة بهشاشة العظام في الأعمار اللاحقة. ولهذا, تعتبر الاستراتيجيات التي تهدف الى ايصال كتلة العظام الى المستوى الأمثل في النساء الشابات قبل سن اليأس؛ حاسمة في الوقائية من الإصابة.

الهدف من الدراسة: تم إجراء هذه الدراسة لتقييم بعض المؤشرات البيوكيماوية المتعلقة بصحة العظام لدى النساء اليمنيات في سن الانجاب؛ في مدينة إب.

الطريقة: تم أخذ عينة مكونة من 81 امرأة غير حامل, وكان مدى أعمارهن (من 16 الى 43) سنة؛ وبمتوسط عمر (mean ± SD = 28.08 ± 6.60)؛ وذلك خلال الفترة من يونيو الى يوليو 2022م؛ وتم قياس مستويات فيتامين د$_3$ بتقنية الانبعاث الضوئي المناعي, وقياس كالسيوم وانزيم الفوسفاتيز القاعدية في المصل بتقنية قياس طيف الامتصاص الضوئي. وقد كانت حوالي 74.68% من النساء اللواتي شملتهن الدراسة متزوجات؛ و 50.62% منهن مرضعات. كما أن حوالي 37.66% منهن لا يتعرضن للشمس عادة؛ و 41.56% منهن يتعرضن للشمس لمدة 5-15 دقيقة يوميا؛ و 14.29% منهن يتعرضن للشمس لمدة 30-15 دقيقة يوميا؛ و 6.49% منهن يتعرضن للشمس لمدة 30 دقيقة الى ساعة فأكثر يوميا.

النتائج: أظهرت النتائج انخفاضا متوسطا في مستويات فيتامين د$_3$ وبمتوسط (13.39 ± 8.02 ng/ml) بينما المعدل الطبيعي هو (30-100 ng/ml). كما أن متوسط مستويات كالسيوم المصل كان منخفضا قليلا (8.348 ± 0.83 mg/dl)؛ بينما المعدل الطبيعي هو (8.5 - 10.5 mg/dl). وكان متوسط مستويات انزيم الفوسفاتيز القاعدية مرتفعا قليلا (170.5 ± 69.59 U/L), بينما المعدل الطبيعي هو (38 - 154 U/L). وكذلك أظهرت مستويات فيتامين د$_3$ ارتباطا سلبيا معنويا مع مستويات انزيم الفوسفاتيز القاعدية (r = - 0.241, P = 0.030). كما كان متوسط مستويات انزيم الفوسفاتيز القاعدية أعلى معنويا في النساء المتزوجات مقارنة بغير المتزوجات (P = 0.006)؛ وفي النساء المرضعات مقارنة بغير المرضعات (P = 0.000). ولم تظهر مدة التعرض اليومي للشمس أي تأثير معنوي على أي من المؤشرات البيوكيميائية. بينما وجد أن الزواج والرضاعة تؤثر سلبيا بشكل معنوي على مستويات انزيم الفوسفاتيز القاعدية (P = 0.05 و P = 0.001, على التوالي).

الاستنتاج: النساء اليمنيات في سن الانجاب في مدينة إب لديهن انخفاض خفيف في مستويات كالسيوم المصل, و انخفاض متوسط في مستويات فيتامين د$_3$, وارتفاع خفيف في مستويات انزيم الفوسفاتيز القاعدية؛ والذي يرتبط سلبيا بشكل معنوي مع مستويات فيتامين د$_3$. وهذا يجعلنا نفترض بأن تكوين كتلة العظام لديهن تحت المستوى المثالي؛ خصوصا عند المتزوجات والمرضعات منهن.