**University of Kerbala**

**College of Applied Medical Sciences**

# Relationship between Premature Ovarian Insufficiency and Bones Health in Women of Karbala City

**A Thesis**

Submitted to the council of the

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for the Master Degree in Clinical Laboratories

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بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ

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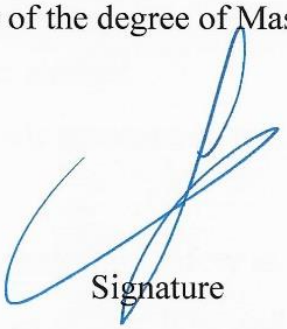

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# Dedication

I dedicate the fruits of my efforts to the one who gave me life, hope, and growth based on a passion for knowledge and learning, my dear father.

To the light of my eyes and the joy of my heart, who taught me to climb the ladder of life with wisdom and patience, whose prayers were his companion, pain ,and excellence my dear mother.

To whom God gave me their presence in my life my brothers and my sisters.

To the pure heart that still guides me safety to those whose heart still embraces me and guides me to giving, love and sacrifice to my dear husband

I thank everyone whose effort has put a stone in building up this work.

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To everyone who even supported me with a smile

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# List of Abbreviations

| Abbreviations | Descriptions/Full forms |
|---|---|
| **AFC** | Antral Follicle Count |
| **$Ag^{+}$** | Silver |
| **AMH** | Anti Mullerian Hormone |
| **ANOVA** | Analysis of one way variation |
| **AOA** | Anti Ovarian Antibodies |
| **$B^{+3}$** | Boron |
| **BALP** | Bone Specific Alkaline Phosphatase |
| **BMD** | Bone Mineral Density |
| **BMI** | Body Mass Index |
| **BTM** | Bone Turnover Markers |
| **Ca** | calcium |
| **CMA** | Chromosomal Microarray |
| **$Co^{+2}$** | Cobalt |
| **COL 1** | Collagen type 1 |
| **$Cu^{+2}$** | Copper II |
| **D2** | ergocalciferol |
| **D3** | Cholecalciferol |
| **E1** | Estrone |
| **E2** | Estradiol |
| **E3** | Estriol |
| **ECM** | Extracellular Matrix |
| **ELISA** | Enzyme-linked immunosorbent assay |
| **$F^{-}$** | Fluoride |
| **FSH** | Follicle stimulating hormone |
| **GCS** | Granulosa Cells |
| **GnRH** | Gonadotropin hormone-releasing hormone |
| **HRP** | Horse Reddish Peroxidase |
| **IVF** | In vitro Fertilization |
| **$K^{+}$** | Potassium |
| **LH** | Luteinizing hormone |
| **$Li^{+}$** | Lithium |
| **$Mg^{+2}$** | Magnesium |

| | |
|---|---|
| **$Nb^{+5}$** | Niobium |
| **P** | Phosphorus |
| **PBM** | Peak Bone Mass |
| **PCOS** | Polycystic Ovary Syndrome |
| **$PO4^{-3}$** | Phosphate |
| **POF** | Premature Ovarian Failure |
| **POI** | Premature Ovarian Insufficiency |
| **PTH** | Parathyroid Hormone |
| **SD** | Standard Deviation |
| **SHBG** | Sex Hormone-binding globulin |
| **$Si^{-4}$** | Silicate |
| **SPSS** | Statistical Package for Social Sciences |
| **TALP** | Total Alkaline Phosphatase |
| **Testo** | Testosterone |
| **TPO** | Thyroid Peroxidase |
| **Vit .D3** | Vitamin D3 |
| **$Zn^{+2}$** | Zinc |
| **FSD** | Female Sexual Dysfunction |
| **HSDD** | Hypoactive Sexual Desire Disorder |

# Summary

This study aimed to investigate the relationship between Premature Ovarian Insufficiency(POI) and bones health and to evaluate the effects of premature ovarian Insufficiency(POI) on women by measuring some parameters including Anti Mullerian Hormone ( AMH), collagen type 1, bone-specific alkaline phophatase(BALP), vitamin D3, Follicular Stimulating Hormone (FSH), Luteinizing Hormone( LH), testosterone ,serum $ca^{+2}$ and Estradiol (E2) in blood.

The study was carried out at private clinics and the infertility unit of the gynecological and obstetric teaching hospital in Karbala, from November 2023 to April 2024. There were 100 women in the study, their ages range from eighteen to forty. Fifty females without POI (control group) and fifty females with POI (patients) were included.

The study's findings showed that was statistically significant decrease ($p<0.007$) in BMI mean values of control group (25.87±2.56) in comparison to the patients groups of POI (27.88±4.52). The results showed a significant increase( $p<0.000$) in levels of FSH (23.56±25.79) in women with POI compared with healthy women (5.39±3.84),as well as significantly increase( $p<0.034$) levels of LH in POI women (12.95±11.46) in comparison with healthy women(8.54±8.92).

The results showed a significantly decreased ( $p<0.000$) in Estradiol levels of

patients group of POI(20.49±16.54) in contrast to control group (87.59± 66.61) as well as there was significantly decrease( $p<0.012$) in the levels of free testosterone of POI group(0.15±0.15) than control group (0.24±0.20).

According to results of this study there was significantly decreased ( $p<0.000$) in Vitamin D3 levels of patients group of POI(4.55± 2.12) in contrast to control group (6.39±1.52) ,also there was significant decrease( $p<0.022$) in concentrations of Calcium of POI patients(8.21±1.34) than healthy group (8.67± 0.41). The study's findings demonstrated that, compared to the control group (4.56±1.09), the patients' group with POI had considerably lower ($p<0.000$) levels of AMH.

Levels of some specific markers of bone health were measured in that study and Results showed that there was significantly decreased( $p<0.000$) in (BALP) levels of patients group of POI(8.63±4.93) in comparison to controls group (15.88±3.60),as well as results showed there was significantly decreased ($p<0.000$) in Collagen type 1 levels of patients group of POI(1.57±0.85) in contrast to control group (2.33±0.74)

There was other correlational links between a subset of research characteristics, such as the existence of positive relationship ($p\leq0.01$) between Collagen 1 and AMH, AMH and BALP, AMH and Vitamin D3, Collagen 1 and BALP, Collagen 1 and Vitamin D3, BALP and Vitamin D3, FSH and LH. also there was positive relationships ($p\leq0.05$) between $Ca^{+2}$ and FSH , $Ca^{+2}$ and LH .

According to research finding above, we conclude that POI has a negative impact on bones health.

# Chapter one

# Introduction

## 1.1 Introduction

Premature ovarian insufficiency (POI) is a clinical disease characterized by an early decline in ovarian function that results in a persistent hypo estrogenic state in women under the age of 40 years (Rahman & Panay,2021). It is distinguished by decreased ovarian follicles and insufficient ovarian sex hormones, which hasten the menopause (Wesevich *et al*, 2020) . About 25% of women who experience spontaneous POI may occasionally resume their ovarian activity (Mishra *et al*, 2019)**.** According to estimates, at least 1% of women have POI, which can lead to long term health issues and psychological stress ( Golezar *et al*,2019).More than 10% of women are affected by POI and early menopause according to recent statistics which suggests that both conditions are more common than previously believed, POI/early menopause or an earlier age at menopause have been linked to a number of biopsychosocial risk factors including genetic, autoimmune, reproductive, lifestyle, early-life, social/environmental, and iatrogenic, Determining a causative role and the underlying mechanisms, however, is still difficult, Comprehending and elucidating these risk factors will enable preventative and risk reduction tactics to enhance the wellbeing of women ( Giri and Vincent, 2020)**.**

The majority of the genetic components linked to POI's genesis are unknown, It is recognized that certain gene mutations and chromosomal abnormalities are the causes of familial POI(Ishizuka, 2021) . At the time of diagnosis, many women with POI experience moderate to severe stress, and they express dissatisfaction with the clinician's level of informational support (Glacken *et al*.,2022 ) .

Bone is a dynamic, highly vascularized natural composite that undergoes continuous remodeling over the course of an individual's lifetime. It can support enough weight for movement and serve as a protective shell for sensitive internal organs to its exceptional mechanical qualities and fracture toughness, Apart from

its structural roles bone tissue also serves as an endocrine organ contributing significantly to the global minerals (particularly Ca and P ions) and nutrition homeostasis(Zhang *et al,* 2021) .

POI typically leads in ovarian hormone insufficiency and is linked to decreased ovarian function, Because osteoclasts are affected by this deficiency in estrogens bone loss is accelerated and surpasses the rate of bone synthesis, Because of this people with POI who are estrogen deficient have poorer bone health which may result in lower bone mineral density and a higher chance of osteoporosis and fractures in later life( Podfigurna *et al*,2020).

Alkaline phosphatase specific to bones (BSALP) BAP a glycoprotein present on the surface of osteoblasts, is a marker of the biosynthetic activity of these cells that produce bone, It has been demonstrated that BAP is a sensitive and trustworthy marker of bone metabolism(Vimalraj ,2020).

Type 1 collagen gives the bone cells mechanical support and growth stimuli, The bone extracellular matrix (ECM) which is mostly made up of type I collagen and noncollagenous proteins is essential to the process of bone remodeling, Any of these processes that are compromised might result in insufficient bone mass accumulation and maintenance which increases the risk of osteoporosis (Costantini *et al*,2020).

The ovaries in females express Anti Mullerian Hormone(AMH) as well and it controls the process of folliculogenesis ,Serum AMH is a measure of the expanding follicle pool in clinical practice and is connected with the size of the primordial follicle pool. establishing serum AMH as an accurate indicator of ovarian reserve (Clemente *et al*., 2021).The corpus luteum, ovarian follicles, and the placenta all produce estrogen, the primary sex hormone in women. Estriol (E3), estradiol (E2), and esttrone (E1) are the three forms and constituents of estrogens that are most frequently observed, with E2 were the utmost potent form

(Kelly *et al*., 2023).It is well known that calcium and phosphorus balance is maintained in large part by vitamin D3,The role of vitamin D3 in the women reproductive system has been extensively studied due to the receptor's widespread distribution in reproductive organs, such as the ovary(Grzesiak , 2020 ). Because 1 in 5 men and 1 in 3 women have osteoporosis, or bone loss and fractures in old age, bone health in the elderly is a major global health concern therefore Bone health is a multifaceted topic influenced by several minerals and hormones(Bhattarai *et al*, 2020).

**Aims of study**

Assessment of relationship between Premature Ovarian Insufficiency and bones health of women in Karbala city by measuring of some parameters (AMH, collagen type 1, bone-specific alkaline phosphatase(BALP), vitamin D3, FSH, LH, testosterone ,serum $ca^{+2}$ and Estradiol (E2).

# Chapter two
# Literatures Review

## 2.1. Literatures Review

The common reproductive endocrine abnormal condition known as premature ovarian insufficiency (POI) is defined as the loss of ovarian function before to reaching 40 years of age, Clinically POI is characterized by decreased estradiol (E2) and elevated gonadotrophins (FSH>25 IU/L) in oligomenorrhea or amenorrhea, Rather than being a single, binary illness, ovarian insufficiency is a continuum of ovarian age or reduced function, It can be temporary or progressive and it typically leads to an early menopause(Jiao *et al*, 2021).

While treatment does not increase life expectancy, appropriate hormone replacement for premenopausal women reduces these harmful health risks and enhances the quality of life for those who have postmenopausal illness, Genetic mutations and chromosomal abnormalities, autoimmune factors, and iatrogenic causes such as radiation therapy, chemotherapy, and surgery are among the possible etiologies of POI(Takahashi *et al*, 2021).

A minimum of four months of oligo/amenorrhea and two high follicle stimulating hormone (FSH) readings (> 25 IU/L) more than four weeks apart are diagnostic criteria for post-ovulation illness (POI), Other sensitive markers used to evaluate ovarian reserves include anti-Mullerian hormone (AMH) and antral follicle count (AFC)(Huang *et al*,2022).

### 2.1.1.Prevalence

At least 1% of women have premature ovarian insufficiency (POI), this can lead to long term health issues and psychological stress so POI related infertility was once thought to be absolute, with little to no benefit from infertility treatments(Ishizuka, 2021) .

Although preterm ovarian failure (PMOF) is linked to a higher risk for the general and mental health of afflicted women, the most concerning symptoms for any affected woman reduce fertility or even infertility(Elhddad , 2020 ). According to

many researches, the prevalence of POI varies based on demographic traits such race, social class, economic status, and lifestyle choices(Golezar *et al*, 2020). In Sweden for example a nationwide register study found that 1.9% of women had POI in 2018 (Lagergren *et al*, 2018). According to another study conducted in Iran, 2.9-3.2 % of women report having POI whereas a meta analysis conducted in 2019 estimated that 3.7% of women worldwide have POI(Golezar *et al*, 2019).

## 2.1.2 . Diagnosis of Premature Ovarian Insufficiency

Life altering effects of a POI diagnosis include decreased fertility and an increased risk of problems from premature estrogen insufficiency, The various etiological causes that contribute to clinical manifestations are diverse including genetic, autoimmune, viral, radiation, and toxin-related factors, The majority of cases are idiopathic, which is most startling in 70–90% of women with POI the underlying reason is still unknown(Webber *et al*, 2016 and Panay *et al*, 2020). POI is frequently accompanied by autoimmune diseases particularly hypothyroidism and primary ad-renal insufficiency (Addison's disease)(Silva *et al*, 2014, Kirshenbaum and Orvieto, 2019). The diagnosis of POI has primarily relied on follicle stimulating hormone (FSH) levels however a sensitive cut off level has not yet been established, Based on earlier reports a number of papers initially utilized FSH values >40, 50, or 20 mIU/ml as the criteria However some patients with POI occasionally had FSH levels below these cut off levels ,As a result there is low diagnostic accuracy for POI despite the fact that treating all stages of POI related issues requires an early diagnosis, The presence of a menstrual problem supported by biochemical evidence should serve as the basis for the diagnosis of POI( Ishizuka, 2021).

Chromosome analysis and testing for FMR1 premutation are recommended diagnostic evaluations to determine the cause of spontaneous POI, Autoantibodies against thyroid peroxidase (TPO) and adrenocortical or 21OH antibodies can be

screened if an immune disorder is suspected (Yeganeh *et al*, 2019). The normal evaluation of POI does not currently include extensive investigations using chromosomal microarray (CMA), panel-based NGS, or other autoantibody techniques (Webber *et al*, 2016 and Yeganeh *et al*, 2019) .

### 2.1.3. Clinical properties

Sometimes even before monthly abnormalities begin women with POI may exhibit classic menopausal symptoms, Infertility or menstrual problems frequently manifest years before they satisfy the aforementioned diagnostic requirements, Patients with secondary amenorrhea may suffer a quick beginning of the condition but oligomenorrhea or polymenorrhea, variations in the menstrual cycle, may also occur before the onset of amenorrhea**(**Ishizuka , 2021) . Women who have post partum illness (POI) may exhibit menopause-like symptoms, occasionally preceded by changes in their menstrual cycle, A deficit in estrogen is characterized by hot flushes and nocturnal sweats(Conway, 2000). Dyspareunia and dryness in the vagina may be extremely upsetting for the patient (Davis and Jane, 2011). Other signs and symptoms include difficulty sleeping, mood swings, dry eyes, stiffness, altered frequency of urination, low libido, and lack of energy(Smith et al, 2004)

### 2.1.4. Pathophysiology

Though there are many other potential causes of POI, idiopathic factors still account for about 90% of cases, The known causes of post-stroke illness (POI) include autoimmune illnesses, environmental factors, iatrogenic causes, and genetics ,The genetic causes of POI have been the subject of extensive research in recent years(Pankiewicz *et al*, 2021). Chromosome abnormalities and genetic mutations, autoimmune factors, and iatrogenic causes such as radiation therapy, chemotherapy, and surgery are among the possible etiologies of POI, It has been claimed that there is a significant correlation between the response genes for the

DNA damage pathway and reproductive lifespan, therefore POI is closely linked to DNA damage and repair in ovarian granulose cells(Takahashi et al, 2021).

One significant mechanism for the faster ovarian follicle degradation is autoimmunity , It has been suggested that antibodies to several ovarian antigens are indicators of ovarian autoimmunity, Nevertheless, there is a deficiency of clinically validated sensitive and specific serum assays to verify autoimmune participation in POI, various molecular antigenic targets in the ovary are identified thanks to a newly developed specific test for anti ovarian antibodies (AOA),so The use of this particular test for AOA has highlighted the necessity of doing autoimmune screening on patients before they receive In vitro Fertilization(IVF) treatment (Khole,2010).

## 2.1.5 .Disturbances of Hormones in Premature Ovarian Insufficiency

### 2.1.5.1 Hypothalamus-pituitary-ovarian axis

Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are the two hormones produced by the pituitary gland in the brain. They are necessary for the gonadal follicles to grow when gonadotropin-releasing hormone (GnRH) is produced by the hypothalamus(Kitano *et al*, 2022).

After attaching receptors of FSH and LH in reproductive gonadal cells, these hormones regulate the synthesis of sexual steroids such progesterone, estrogen, and testosterone (Kishi *et al*, 2018). Follicliculogenesis which affects gonadotropin creation through the hypothalamic-pituitary (H-P) axis, causes the ovary to create estrogens ( Lee *et al*, 2021).

Mid-cycle LH increases due to circulating estrogen, LH binding to the LH receptor induces ovulation and oocyte maturation, Estradiol levels rise at the end

of the follicular phase, Rise of the blood in estradiol causes the pituitary to release more LH(Arroyo *et al*, 2020).

In women, the luteal part of the menstruation cycle is often characterized by a drop in the gonadotropin-releasing hormone (GnRH) pulse rate, Progesterone is the hormone that is accountable for this decrease in the phenomena, Seems necessary for the correct functioning of the persistent cycle ( Kim *et al*, 2022). The frequency of LH pulses has reduced to approximately one pulse every 4-6 hours by the midluteal phase following ovulation, The primary cause of this decline is the negative feedback effects of progesterone (Mccartney *et al*, 2022).

This hormonal imbalance results in anovulatory cycles because there is not enough free (FSH), that is wanted to entice and drive the development of follicles in ovary (Azziz, 2018).

### 2.1.5.2. Anti Mullerian Hormone(AMH)

The testes and ovaries synthesize (AMH), a 140 kDa homodimeric glycoprotein made up of two identical subunits connected by disulphide linkages, Its clinical uses include gonadotropin dose selection during in vitro fertilization and prediction of ovarian response, AMH is utilized to study gonadal function and sexual developmental abnormalities in males(Punchoo and Bhoora, 2021).

Small developed follicles in the ovary produce granulosa cells that release (AMH), AMH has drawn more attention as a measure for ovarian reserve since serum AMH levels substantially correspond with the number of developing follicles, Since AMH levels represent the pool of developing follicles that may be able to ovulate, the term "functional ovarian reserve" which is preferred over "ovarian reserve" is assessed by measuring serum AMH levels (Moolhuijsen and Visser, 2020).

The early onset of menopause is linked to higher risks of osteoporosis and cardio-vascular disease, Serum anti-Mullerian hormone may be useful in the early diagnosis and prediction of age at menopause since a woman's circulating AMH concentration decreases towards the menopause and is a measure of the number of follicles left in the ovary(Nelson *et al* , 2023). Anti Müllerian hormone is raised in patients having polycystic ovarian syndrome (PCOS), A growing body of research suggests that AMH affects the physiology of the female reproductive system(Qiao *et al*, 2020).

AMH appears to be restricted in its ability to predict age at natural menopause, Additionally, there is a dearth of information about the prediction of POI outside of situations in which there is an acute iatrogenic threat to ovarian function, Thus, AMH seems to have a good deal of utility as a POI diagnostic tool, nevertheless, aside from identifying those who are generally more vulnerable, its precision is insufficient to precisely forecast when imminent POI will manifest(Anderson *et al*, 2020). In the early follicular phase, serum AMH levels are typically assessed, much like other hormone indicators of ovarian function like FSH, estradiol, and inhibin B, It has been questioned, though, if variations during the menstrual cycle might account for the fluctuations in serum AMH levels (Moolhuijsen and Visser,2020).

### 2.1.5.3.Estrogen

The corpus luteum, ovarian follicles, and the placenta all produce estrogen, the primary sex hormone in women, Estriol (E3), estradiol (E2), and Estrogene (E1) are the three utmost prevalent forms and constituents of estrogens, estradiol is the most potent form (Kelly *et al*, 2023). It is not unexpected that during menopause, reduced ovarian estrogen has been associated with a higher danger of osteoporosis and lesser bone mineral density (BMD) (Schmitz *et al*, 2021). Since 17β estradiol is solely formed by the ovary, measuring this hormone is recycled to evaluate ovarian role, (FSH)

and (LH) work together to support regular process of ovulation (Manocha *et al*, 2018).

The ovarian follicle's theca cells are stimulated by LH, which causes them to produce androgen, Certain cells go to nearby granulosa cells (GCs), where they undergo transformation into estrogen upon stimulation by FSH, Others are linked to sex hormone-binding globulin (SHBG) (Gargus *et al*, 2022). This raises levels of the hormone estrogen, which in turn raises the synthesis of LH, increasing LH and initiating ovulation, The corpus luteum grows after ovulation, secretes progesterone and estrogen, and then makes the endometrial lin-ing more ready for pregnancy (Xu *et al*, 2021).

In older postmenopausal women, endogenous estrogen levels have a major physiological impact on the skeleton, Estrogen insufficiency is the reason that postmenopausal women to lose bone so quickly, Increased bone deterioration results from osteoclasts becoming more active in the absence of estrogen (Chandak *et al*, 2017).

Infertile POI patients using estrogen therapy during the transitional phase had lower circulating FSH levels, which may lead to spontaneous pregnancy Furthermore, compared to no pre treatment, estrogen pre treatment in these individuals appears to increase the results of IVF in a GnRH-antagonist short protocol( Pinelli *et al*, 2018).

### 2.1.5.4. Luteinizing hormone and Follicle stimulating hormone

Members of the same family as luteinizing hormone, thyroid stimulating hormone (TSH), follicle stimulating hormone (FSH), and human chorionic gonadotropin (HCG) are among other glycoprotein hormones produced by the pituitary gland, include LH, Two polypeptide subunits that are not chemically bonded to one another make up each molecule (Gounden *et al*, 2021). They all have the same Alpha subunit and a Beta subunit that is unique to a particular hormone, Each of these molecules is made up of 121, 110, and

145 amino acids in the human body, which are the LH, FSH, and HCG subunits, respectively, The alpha subunit of the human common subunit is composed of 92 amino acids (Arroyo *et al*, 2020) .

The hypothalamus initiates ovulation by pulsing the release of GnRH, The anterior pituitary releases LH and FSH in response to this pulsatile release, which in turn stimulates the ovarian follicle, The three main cells in this follicle are the Oocyte, granulosa cells, and theca cells, Theca cells generate androstenedione in response to LH (Ruddenklau & Campbell, 2019). GnRH neurons in the forebrain project fibers to the median eminence to secrete pulsatile GnRH, which activates pituitary secretion of gonadotropin hormones (LH and FSH), in turn driving the synthesis and secretion of gonadal sex steroids [estradiol (E2) and testosterone (T)(Herbison, 2020).

Raised LH leading to improved ovarian theca cell androgen synthesis, excessive androgen inhibits the ability of sex-steroids to afford negative feedback by causing GnRH plasticity to become uncontrolled, which in turn elevates concentrations of LH. and then androgen in a vicious cycle ,which seen in women with PCOS leading to increased testosterone levels (Garg *et al*, 2022) .

steroid hormones secreted by the gonads circulate back to the brain to regulate the neural circuits governing the reproductive neuroendocrine axis, These

regulatory feedback loops ultimately act to modulate gonadotropin releasing hormone (GnRH) secretion, thereby affecting gonadotropin secretion from the anterior pituitary, In females, rising estradiol (E2) during the middle of the menstrual (or estrous) cycle paradoxically “switch” from being inhibitory on GnRH secretion (“negative feedback”) to stimulating GnRH release (“positive feedback”), resulting in a surge in GnRH secretion and a downstream LH surge that triggers ovulation (Kauffman,2022).

## 2.1.5.5. Testosterone

Crucial hormone identified as testosterone in males and females. It serves as a direct androgen in adding to be an essential forerunner for the production of E2 (Alemany, 2022).

Androstenedione and dehydroepiandrosterone, or DHEA, are two examples of pre-androgens, These precursors of testosterone are produced in the ovarian and adrenal glands, and they are also partially transformed to testosterone in the bodies of women(Davis & Jacobsen, 2015) .

Ovulation signifies the beginning of the ovaries' cyclical manufacture of testosterone, which is demonstrated by testosterone concentrations peaking in the middle of the menstrual cycle and continuing to be elevated into the luteal stage, Most of the testosterone in circulation is conjugated with proteins, specifically, about 66% of it is linked to a protein known as sex hormone-binding globulin (SHBG), Thirty percent is combined with albumin(Labrie *et al*, 2017). The percentage of unbound testosterone is only 2–4%, The assumed active form is this, Despite having relatively low plasma levels in women, significant androgen receptor affinity and potent androgenic qualities are exhibited by DHT and testosterone (Ali ,2021).Androgens have been demonstrated to play an important role in sexual motivation in women, As a result many postmenopausal women experience Female Sexual Dysfunction (FSD) which are a group of disorders that pertain to sexual arousal, desire, orgasm, and pain, A prevalent manifestation of FSD is Hypoactive Sexual Desire Disorder (HSDD) or the absence of sexual fantasies, thoughts, and/or desire for or receptivity to sexual activity, There is gaining interest in the use of Testosterone Replacement Therapy (TRT) for the treatment of HSDD in postmenopausal women (Rahman *et al*,2022).

Serum estradiol levels drop by 90% after menopause, but testosterone levels start to go off at age 25 (Johansen et al, 2020).

## 2.1.6. Specific Markers of Bone Health

### 2.1.6.1. Collagen type l

In mammals, type I collagen (Col1) is the most prevalent protein, Ninety percent of the organic component of the bone matrix is made up of Col1, Nevertheless, it is still unclear what exact cellular source Col1 comes from and how it functions during bone development and embryogenesis (Chen *et al*,2021). Collagen is a class of fibrous protein family that is a part of the extracellular matrix (ECM), It is made up of three alpha chains that coil around one another to form the collagen fibers, Collagen is crucial to the structure of connective tissues like cartilage, tendons, and ligaments as well as several organs like skin, heart, liver, kidney, lungs, blood vessels, and bones (Singh &Agrawal, 2023). kind I collagen is the most prevalent kind of collagen found in bone, skin, and connective tissues, It is also crucial for maintaining the integrity of these tissues, An extremely well organized interstitial matrix molecule in the form of fibrils is called type I collagen (Henriksen & Karsdal, 2024).

Premature or not, the loss of ovarian function has a profound effect on the health of the female skeleton, increasing the chance of developing osteoporosis due to the prolonged exposure to low estrogen (Kurtoglu *et al*, 2014). Different collagen maturation processes, resulting from both enzymatic and nonenzymatic activities, take place in bone, Lysyl oxidase is activated during the enzymatic process, resulting in the development of immature and mature crosslinks he collagen fibrils, In type I collagen two types of nonenzymatic that stabilize the processes are reported: the creation of advanced glycation end products as a result of reducible sugar accumulation in bone tissue, and the racemization and isomerization of the collagens telopeptide (Viguet et al,2006).

## 2.1.6.2. Bone-specific alkaline phosphatase(BALP)

A glycoprotein present on the surface of osteoblasts, is a marker of the biosynthetic activity of these cells that produce bone. It has been demonstrated that BAP is a sensitive and trustworthy marker of bone metabolism. One unique feature of the vertebrate skeletal system is the extracellular mineralized matrix (Murshed, 2018, Karpen, 2018). Osteoporosis related loss of bone density is linked to a pathological mineralization deficit (Ensrud and Crandall, 2017).

The most prevalent isozyme type of alkaline phosphatase (ALP), a homodimeric protein with phosphorylating abilities, is tissue nonspecific ALP, Liver-specific ALP and bone-specific ALP (BALP), the two isoforms of tissue non-specific ALP, are found in serum in about similar amounts, In terms of physiology, BALP binds to the membrane of osteoblastic cells and releases very little into the serum, Its content in serum only increases when there is more bone remodeling (Tariq *et al*,2019).

It is still unclear if the more recent biochemical bone markers are clinically beneficial in treating osteoporosis, The most often utilized indicator of bone production is serum total alkaline phosphatase (TALP), yet it is neither very sensitive nor specific, Although the posttranslational drugs for bone and liver alkaline phosphatase are different, the gene product is the same, Despite its uncertain involvement in bone production, bone specific alkaline phosphatase (BSALP) is a well established marker of bone formation, BSALP is present in osteoblast plasma membranes which are released into the bloodstream through an unidentified process, It is a sensitive indicator of higher bone turnover during menopause with a mean rise that is noticeably higher than TALP(Nawawi *et al*, 2001).

## 2.1.6.3 serum calcium $^{+2}$

Two essential elements of the bony inorganic matrix that play a key role in maintaining bone health are calcium and phosphate, It has been demonstrated that a number of ions can either directly induce osteoblast precursor differentiation via growth factor signaling pathways or indirectly activate other processes that aid in the development of bone tissue, These ions include silicate ($Si^{4-}$),phosphate ($PO4^{3-),}$ lithium ($Li^{+}$), magnesium ($Mg^{2+}$), niobium ($Nb^{5+}$), boron ($B^{3+}$), calcium ($Ca^{2+}$), cobalt ($Co^{2+}$), copper(II) ($Cu^{2+}$), fluoride ($F^{-}$), silicate ($Si^{4-}$), silver ($Ag^{+}$), strontium ($Sr^{2+}$), vanadium ($V^{5+}$), and zinc ($Zn^{2+}$), The benefits of employing such ions to promote bone tissue repair over protein growth factors are numerous and include reduced costs, increased simplicity, increased stability, and increased efficacy at low concentrations(O'Neill *et al*, 2018).

Calcium is a mineral that is essential to several bodily processes, though studies on calcium's function have mostly concentrated on bone health, more recent research has directed attention toward various health outcomes from the impacts of calcium supplements or food, Due to their high calcium content, dairy products like milk, yogurt, and cheese are typically consumed in conjunction with other foods, high calcium food content include dairy products, particularly hard cheese, which has a calcium content of 1 g per 100 g, while milk and yogurt have a calcium content of 100–180 mg per 100 g (Cormick & Belizán, 2019).

The bone is a vital organ that stores minerals like calcium and phosphorus in addition to providing mobility and structural support, In addition to use genetics affects bone strength, Bones require continuous mechanical pressure to maintain their strength, The main components of bones are minerals and proteins, both of which are crucial for giving bones their unique characteristics, Proteins by themselves would make the bone too soft and

pliable, and minerals by themselves would make it too brittle, In the collagen matrix the minerals calcium and phosphorus are deposited as hydroxyapatite crystals, Bone cells create collagen (US. Department of Health and Human Services, 2004).

One can acquire vitamin D through diet or by converting dehydrocholesterol, which is present beneath the skin when exposed to sunshine, The active form calcitriol acts on the kidney, gut, and bones to control blood calcium levels by causing the vitamin D receptor to dimerize, In a similar vein low serum calcium levels cause the secretion of parathyroid hormone., Via calcitriol it aids in controlling the blood calcium level, Early life bone modeling and later life bone remodeling are regulated by sex hormones, In older women bone loss is associated with ovarian function loss and a decrease in estrogen production (Bhattarai *et al*, 2020).

### 2.1.6.4 Vitamin D3(VD3)

Crucially, the ovary is an extrarenal location of vitamin D3 metabolism in addition to expressing the vitamin D3 receptor, Studies have looked into howvitamin D3 affects ovarian steroidogenesis and follicular development, Moreover ovarian cancer, premature ovarian failure, and polycystic ovary disease have all been linked to vitamin D3 insufficiency (Grzesiak, 2020).

It is widely acknowledged that a lack of vitamin D3 (VD3) raises the risk of numerous chronic illnesses, The amount of VD3 in the body is influenced by sun exposure, food, supplements, lifestyle, and genetics (DeLuca, 2004). It is commonly recognized that VD3 is essential for bone mineralization and primarily regulates calcium and phosphorus homeostasis ,Nonetheless an expanding corpus of research suggests that it has pleiotropic effects on the body influencing a range of physiological and pathological functions, The kidneys, bones, and intestines are the traditional VD3 target tissues, Notably female reproductive tract tissues are one of the non-classical locations of VD3

activity, The ovary, uterus, fallopian tube, vagina, and placenta of both humans and animals have been shown to contain VD3 receptors (VDRs) and VD3 metabolic enzymes, indicating the direct involvement of VD3 in these organ systems (Lerchbaum &Pietsch 2012).

A growing body of research has revealed links between low levels of vitamin D3 and decreased fertility, endocrine and metabolic diseases, polycystic ovary syndrome, premature ovarian failure , and ovarian cancer (Muscogiuri *et al*, 2017).

## 2.2 Vitamin D Metabolism

In the past, a trace substance known as "vitamin D" was utilized to curecanine rickets. Rickets was a condition that might be cured in dogs confined inside by using cod liver oil. Thus, a molecule found in cod liver oil was classified as vitamin D. These days, cholecalciferol (short for D3) and ergocalciferol (short for D2) are the two compounds that are referred to as "vitamin D". When the steroid nucleus of 7-dehydrocholesterol is broken down, a secosteroid called D3 is created. The cholesterol-derived B ring is broken down by UV radiation from the sun. D3 can be made directly or indirectly by sunshine. The primary source of pre vitamin D3, also known as tachysterol, is sunshine exposure of 7-dehydrocholesterol. Sunlight is frequently the element that limits the synthesis of D3 since 7-dehydrocholesterol is commonly found beneath the skin of humans and other higher animals. (Bhattarai *et al*, 2020).

# Chapter Three
# Materials
# and
# Methods

## 3.1. Subject

### 3.1.1. Study Design

From November 2023 to April 2024, 100 women participated in a case control study. The ethical conduct of the study was approved by the Karbala Health Directorate and the University of Kerbala College of Applied Medical Sciences. After learning the objectives of the study, all patients as well as the management of the gynecological and obstetric teaching hospital gave their consent., as presented in figure (3.1).

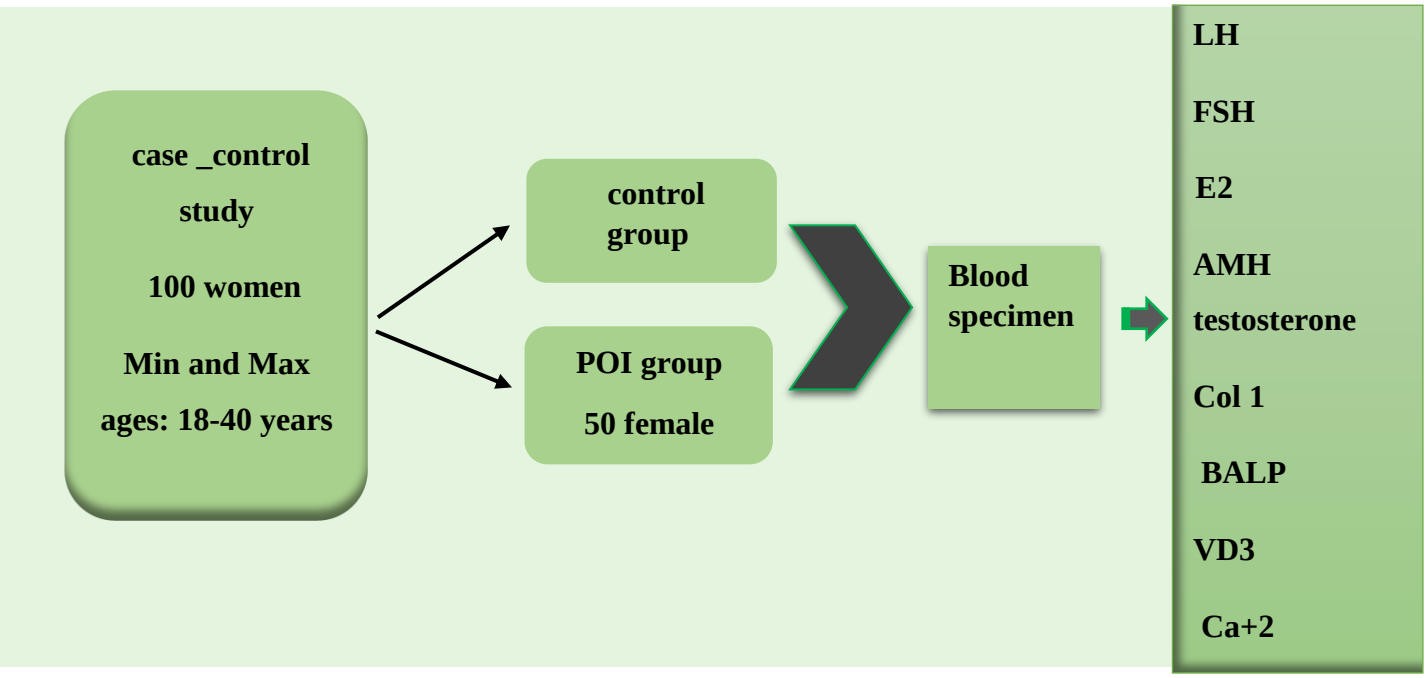


**Figure (3.1) Study Design**

### 3.1.2. Patients

Fifty POI women's entire blood samples age range at which a person can bear children (18–40 years old).The data was gathered from reproductive infertility specialists at the teaching gynecology and obstetrics hospital in Karbala, as well as from a few private clinics. In-depth inquiry on the topic was combined with lab testing. A questionnaire was created to gather more information about the patients, including their age, weight, height circumference, efficacy in reproduction, and regularity of their menstruation. In these studies, measurements of body mass

index (BMI) and other biological parameters were obtained. (Paper of questionnaire was referred in appendix 1).

## 3.1.3. Control

Fifty women, ranging in age from eighteen to forty, who seemed healthy. Weight, age, height, an ordered menstrual cycles, and productiveness were all questioned for the healthy groups. Gynecologists observed that they had healthy ovaries and a typical menstrual cycle. Individuals who do not smoke, have no past medical history of kidney or liver problems, tumors, stroke, auto-immune diseases, severe or long-lasting illnesses, diabetes, type 1 or type 2 diabetes, or cerebrovascular accidents of any kind.

**Inclusion criteria**

Fifty women between the ages of 18 and 40 who were in good health and had regular cycles were selected as the control group. They all had a history of regular menstrual cycles lasting between 28 and 33 days, no signs of thyroid malfunction, and no hyperandrogenism. Fifty women who tested positive for POI were selected as patients; all of them had received a recent diagnosis of POI from gynecologists. Each research subject provided informed consent. Additionally, Each participant underwent a thorough medical investigation to define their body mass index (BMI), and characteristics like coarse facial and body hair, acne, irregular menstruation, and other disorders, and their medical history of various illnesses, gynecological disorders, and infertility.

## Exclusion criteria

Not a single participant in this study was older than 40 years old, nor were any of the individuals smokers. Patient with ovary cancer, autoimmune diseases, removal of ovary, females with any chronic diseases such as diabetes mellitus, heart diseases and kidney diseases, hypothyroidism patients.

### 3.1.4.Blood Samples

A sample of venous blood, about 5 mL, was taken. Gel tubes were filled with the blood. After letting each blood sample clot, it was spun for ten minutes to extract the sera and conduct chemical tests for hormones and biochemical tests (calcium and vitamin D). Next, the remaining blood was transferred into Eppindroff tubes. Until testing .or analyzing, the specimens were saved at -20 °C. The specimens were kept ice-covered in the refrigerators of both private labs and the hospital. An ELISA device was then used to evaluate the samples.

## 3.2. Kits and Chemicals

A description of the kit utilization for the study is given in table (3.1).

**Table (3.1): Kits and chemicals**

| No | Kits and Chemicals | Company and Country |
|---|---|---|
| 1 | Anti Mulle rian Hormone (AMH) kit | Pars Biochem\ China |
| 2 | Bone-specific alkaline phosphatase kit | Pars Biochem\ China |
| 3 | Collagen type 1 kit | Pars Biochem\ China |
| 4 | Estradiol kit | Roche\Germany |
| 5 | Follicular Stimulating Hormone reagent kit | Roche \Germany |
| 6 | Free Testosterone Kit | Nanshan,Shenzhen\China |
| 7 | Luteinizing Hormone reagent kit | Roche\Germany |
| 8 | Serum calcium kit | Roche\Germany |
| 9 | Vitamin D3 Kit | Roche \ Germany |

## 3.3. Instruments and lab supplies

**A summary of the instruments and supplies used in this study showed in table (3.2)**.

**Table (3.2) : Tools and apparatus**

| No. | Devices &Tools | Companies | Origins |
|---|---|---|---|
| 1 | Centrifuges | Kokusan | Japan |
| 2 | Cobas e 411 equipment | Roche | Germany |
| 3 | freezers | ALS | Italy |
| 4 | ELISA (EL x 800 Auto Reader) | BIOTEK | U.S.A |
| 5 | ELx50 Auto strip washer | EASY MED | China |
| 6 | Eppendrof tubes | Carl ROTH | Switzerland |
| 7 | Gel tubes | Carl ROTH | Switzerland |
| 8 | Micropipettes | Micropipettes | Germany |
| 9 | Refrigerators | LG | South Korea |
| 10 | spectrophotometer | Apel | Japan |
| 11 | Syringes, 10 ml, single-usage | BIOTEK | U.S.A |
| 12 | Syringes, 5 ml, single-usage | ULTRA HEALTH | China |
| 13 | Tourniquets | Volltaren | China |

## 3.4. The Methods

### 3.4.1.Body Mass Index calculations

(BMI, or body mass index) In order to categorize obesity, this was calculated using the methods (Keys et al., 1972),as show in table(3-3) .BMI (kg/$m^2$) is calculated as Weight (kg) / Height ($m^2$).

**Table 3-3: BMI categories**

| Weight Condition | (BMI)(kg/$m^2$) WHO |
|---|---|
| Under weight | Less than 18.50 |
| Normal | 18.50 to 24.90 |
| weight Over | 25 to 29.90 |
| Obese | 30 or above |

## 3.4.2. Measurement Luteinizing Hormone Levels

In ELISA LH level test, two specific monoclonal antibodies that detect human LH are sandwiched together.

**The reagents:**

The LH tag can be founded on the reagent rack pack.(Components of the kit were referred in appendix 2).

## 3.4.3. The measurement of Follicular Stimulating Hormone Levels

In the ELISA FSH level test, two specific monoclonal antibodies that detect human FSH are sandwiched together.

**Reagent and Operational Solution**

There is FSH tag on the rack pack of substances.( Components of the kit were referred in appendix 3).

## 3.4.4. Measuring of Anti-Mullerian Hormone Levels

**Principle of the assay**

The kit measures the amount of human AMH in the sample by coating microtiter plate wells with purified humanoid AMH antibody, creating a solid-phase antibody, adding AMH to the wells, and combining the AMH antibody with HRP labeling to form an antibody-antigen-enzyme-antibody complex. After thoroughly washing, the TMB substrate solution is added, and the TMB substrate turns blue. A sulphuric acid solution is added to stop the HRP enzyme-catalyzed process, and the color shift is detected spectrophotometrically at 450 nm. The O.D. of the samples is then compared to the standard curve to determine the concentration of AMH in the samples.

**Materials provided with the kit**

(Components of the kit were referred in appendix 4).

**procedure**

1. Dilution and sample addition: arrange 10 standard wells on coated ELISA plates; fill the first and second wells with 100μl of standard; add the first and second wells with 50μl of Standard dilution; stir; remove 100μl from the first and second wells and add it to the third and fourth wells independently. then mix in 50μl of standard dilution for the third and fourth wells; remove 50μl from the third and fourth wells and discard; add 50μl for the fifth and sixth wells; mix in 50μl of standard dilution for the fifth and sixth wells. Remove 50μl from the fifth and sixth wells and add it to the seventh and eighth wells. Add standard dilution 50μl to the seventh and eighth well, mix. Remove 50μl from the seventh and eighth well and add it to the ninth and tenth well. Add standard dilution 50μl to the ninth and tenth well, mix. Remove 50μl from the ninth and tenth well discard (add Sample 50μl to each well after Diluting,(density: 18 ng/ml□12 ng/ml, 6 ng/ml □3 ng/ml□1.5 ng/ml)

2.add sample ： Set aside the blank wells (blank comparison wells do not contain the sample or the HRP-Conjugate reagent; all other step operations are the same). evaluating the sample thoroughly. Pour 40μl of the sample dilution into the testing sample well, followed by 10μl of the testing sample (the sample final dilution is five times). Fill the wells with the sample, being careful not to touch the well wall. Gently mix.

3.Incubate: At 37°C for 30 minutes after covering the plate with the Closure plate membrane.

4. Configure liquid: dilute 30-fold (or 20-fold) wash solution with purified water, then set aside.

5. Washing: Take off the cover plate membrane, throw away the liquid, swing

dry, add washing buffer to each well, wait 30 seconds, then drain, repeat 5 times, and pat dry.

6.add enzyme: Fill every well—aside from the blank well—with 50μl of HRP-Conjugate substance.

7.Cultivate： Working with 3.

8. Cleaning / Working with 5.

9. Color ：Fill each well with 50 ul of Chromogen Solution A and B, then leave the light preservation on for 15 minutes at 37°C.

10. Stop the reaction: Put 50 μl of the Stop Solution into each well, then stop the reaction when the blue hue turns yellow.

11. Assay: Take the blank well as zero. Within 15 minutes of adding the stop solution, read the absorbance at 450 nm. .

**Calculate**

Draw the standard curve on graph paper using the OD value for the perpendicular and the typical density for the parallel. The specimens density can be found by multiplying the sample curve by the dilution multiple to find the corresponding density based on the sample OD value or the sample density can be calculated by using the sample OD value in the straight line regression equation of the standard curve with the standard density and the OD value.

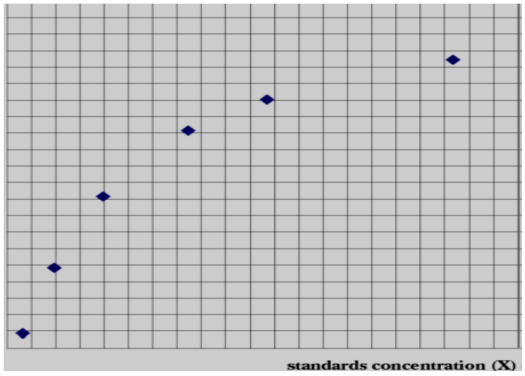


**Figure 3.2:standard curve for** (AMH)

## 3.4.5 .Measuring of free testosterone concentrations

### A. Test principle

Label FITC with a pure TEST antigen and ABEI with a monoclonal antibody in contradiction of the assessment. To create antibody-antigen complexes, the specimens, FITC, displacing reagent, attractive microbeads coated with anti-FITC, and the calibrator or control are all labeled with ABEI after thorough mixing and incubation at 37°C. Afterwards Following sedimentation caused by a magnetic field, the supernatant is poured off, and the sample is then washed once. After applying the initial chemicals, a flash chemiluminescent reaction is initiated. The light signal is measured as Relative Light Units using a photomultiplier in 3 seconds, and the value of the relationship between Relative Light Units and f-TEST concentration in the samplings is proportionate.

### B. The components

(Components of the kit were referred in appendix 5).

## 3.4.6. Measuring of serum Estradiol (E2) levels

Results of the estradiol levels were obtained by ELISA. The antigen of 17-estradiol in specimen competes with the 17-estradiol antigen coupled to(HRP) for binding to the few 17-estradiol-specific antibodies coated on the micrplate (solid phase) of the Estradiol ELISA Kit. Boundless and destined parts are disjointed by straightforward solid-phase washing following incubation. The bound-fraction enzyme HRP reacted with substrate 35 ($H_2O_2$) and TMB substrate when the stop solution ($H_2SO_4$) addition, resulting in a blue color that eventually turns yellow. There is a negative correlation between the color intensity and the amount of 17-estradiol in the sample.

To ascertain the concentration of 17-estradiol in a sample, a calibration curve is employed.

## 3.4.7. Measuring of Bone-specific alkaline phosphatase Level

### Principle of test

The kit measures the amount of human BALP in the sample, coats microtiter plate wells with purified humanoid BALP antibody to generate a solid-phase antibody, and then increases BALP to the wells. After thoroughly washing, the combined BALP antibody with HRP labeling became an antibody-antigen-enzyme complex. When TMB substrate solution is added, TMB substrate turns blue. A sulphuric acid solution is added to stop the HRP ezyme-catalyzed process, and the color shift is detected spectrophotometrically at 450 nm. The O.D. of the samples is then compared to the standard curve to determine the concentration of BALP in the samples.

### The constituents of the kit

(Components of the kit were referred in appendix 6).

### Procedure

1. Dilution and sample addition: arrange 10 Standard wells on coated ELISA plates; fill the first and second wells with 100μl of Standard; add the first and second wells with 50μl of Standard dilution; stir; remove 100μl from the first and second wells and add it to the third and fourth wells independently. Next, mix in 50μl of Standard dilution for the third and fourth wells. After that, remove 50μl and discard it from the third and fourth wells. Next, mix in 50μl of Standard dilution for the fifth and sixth wells; take out 50μl from the fifth and the sixth well and add to the seventh and the eighth well, then add Standard dilution 50μl to the seventh and the eighth well ,mix ; take out 50μl from the seventh and the eighth well and add to the ninth and the tenth well, add Standard dilution 50μl to the ninth and the tenth well, mix , take out 50μl from the ninth and the tenth well

discard(add Sample 50μl to each well after Diluting ,(density: 90 ng/L，60 ng/L，30 ng/L，15 ng/L，7.5 ng/L)

2. Add sample ◒ Set blank wells apart (the other step operations are the same, but the blank comparison wells do not contain the sample or the HP-Conjugate reagent). evaluating the sample thoroughly. Add 40μl of the diluted sample to the testing sample well, followed by 10μl of the final 5-fold dilution of the sample. Fill the wells with the sample, being careful not to touch the well wall, and gently mix.

3.Incubate: At 37°C for 30 minutes after covering the plate with the Closure plate membrane.

4. Configure liquid: distilled water is used to dilute a 30- or 20-fold wash solution, then set aside.

5. Cleaning: Uncover the membrane of the closure plate, remove the liquid, dry with a brush, add the washing buffer to each well, wait 30 seconds, then drain, repeat five times, and pat dry.

6.Add enzyme: Pour 50μl of HRP-Conjugate reagent into each well—apart from the blank well.

7.Cultivate ：Working with 3.

8. Cleaning / Working with 5.

9. Color: Fill each well with 50 ul of Chromogen Solution A and 50 ul of Chromogen Solution B. Evacuate the light preservation for 15 minutes at 37°C.

10. Stop the reaction: Fill each well with 50 μl of the Stop Solution, then stop the reaction when the blue color turns yellow.

11. Assay: Take the blank well as zero, and within 15 minutes after adding the stop solution, read the absorbance at 450 nm.

**Calculate**

Take the standard density as the horizontal, the OD value for the vertical, draw the standard curve on graph paper, find out the corresponding density according to the sample OD value by the Sample curve, multiplied by the dilution multiple, or calculate the straight line regression equation of the standard curve with the standard density and the OD value ,with the sample OD value in the equation, calculate the sample density, multiplied by the dilution factor, the result is the sample actual density.

## 3.4.8. Collagen type 1 Level Determination

**Principle**

The kit measures the amount of human Collagen in the sample by covering microtiters plate wells with purified Humanoid Col Ⅰ antibody, creating a solid phase antibody, and then adding Col Ⅰ to the wells. Col Ⅰ and HRP-labeled Col Ⅰ antibody combine to form an antibody-antigen-enzyme-antibody complex. After completely washing, TMB substrate solution is added, and TMB substrate turns blue. The addition of a sulfuric acid solution at HRP ends the enzyme-catalyzed process, and the color shift is detected spectrophotometrically at 450 nm. The O.D. of the samples is then compared to the standard curve to determine the concentration of Col 1 in the samples.

**The constituents of the kit**

(Components of the kit were referred in appendix 7).

**Assay procedure**

1. Dilution and sample addition: arrange 10 standard wells on coated ELISA plates; fill the first and second wells with 100μl of standard; add the first and second well mix with 50μl of standard dilution; remove 100μl from the first and second wells and add it to the third and fourth wells independently. Next, mix in

50µl of the standard dilution and add it to the third and fourth wells. After that, remove and discard 50µl from each well, Add 50µl to the fifth and sixth well, followed by the addition of standard dilution 50µl to the fifth and sixth well, mix; remove 50µl from the fifth and sixth well and add it to the seventh and eighth well, followed by the addition of standard dilution 50µl to the seventh and eighth well, mix; remove 50µl from the eighth and seventh well and add it to the ninth and tenth well, add standard dilution 50µl to the ninth and tenth well, mix, take out 50µl from the ninth and the tenth well discard(add sample 50µl to each well after Diluting ,(density: 30 µg/L, 20 µg/L ,10µg/L 5µg/L, 2.5µg/L).

2.Add sample: Assign blank wells individually (the HRPConjugate reagent and sample are not added to blank comparison wells; all other step operations are the same). evaluating the sample thoroughly. Add 40µl of the dilutedsample to the testing sample well, followed by 10µl of the final 5-fold diluted sample. Fill the wells with the sample, being careful not to touch the well wall, and gently mix.

3.Incubate: At 37°C for 30 minutes after covering the plate with the Closure plate membrane.

4. Set up a liquid: Dilute the 30-fold (or 20-fold) wash solution with distilled water and set aside.

5. Cleaning: Uncover the membrane of the closure plate, remove the liquid, dry with a brush, add the washing buffer to each well, wait 30 seconds, then drain, repeat five times, and pat dry.

6.Add enzyme: Fill every well—aside from the blank well—with 50µl of HRP-Conjugate substance.

7.Cultivate ： Working with 3.

8. Cleaning / Working with 5.

9. Color: Fill each well with 50 ul of Chromogen Solution A and 50 ul of Chromogen Solution B. Evacuate the light preservation for 15 minutes at 37°C.

10. Stop the reaction: Put 50 µl of the stop solution into each well, then stop the reaction when the blue hue turns yellow.

11. Assay: Take the blank well as zero. Within 15 minutes of adding the stop solution, read the absorbance at 450 nm.

## Calculate

Draw the standard curve on graph paper using the standard density as the horizontal and the OD value as the vertical. Determine the density that corresponds to the sample OD value by multiplying the sample curve by the dilution multiple, or compute the straight line regression equation of the standard curve using the sample OD value and the standard density. Once the sample OD value is entered into the equation, compute the sample density and multiply it by the dilution factor. The output is the sample density.

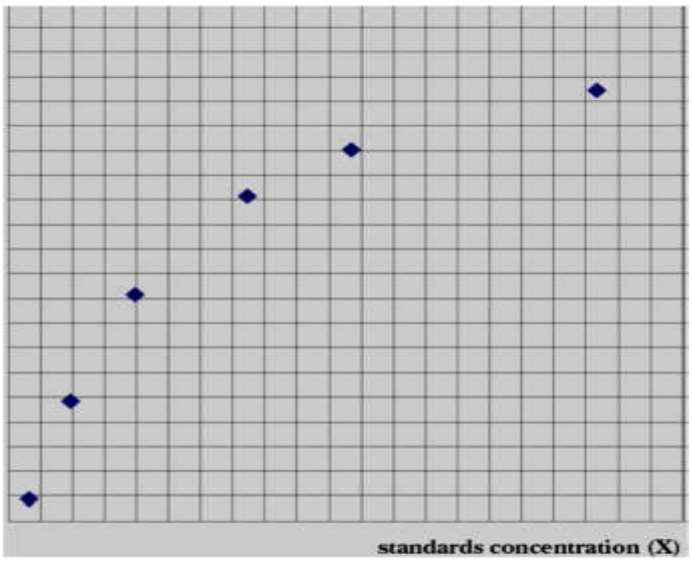


**Figure 3.3:standard curve for** (Human Col Ⅰ)

## 3.4.9. Vitamin D3 levels Determination

**Principle of the assay**

The kit creates a solid-phase antibody by covering microtiters plate wells with cleansed humanoid VD3 antibody and quantifies the amount of human VD3 in the sample, adds VD3 to the wells, and then combines the VD3antibody with HRP labeling to form an antibody-antigen-enzyme-antibody complex after thoroughly washing the plate. When TMB substrate solution is added, TMB substrate turns blue. A sulphuric acid solution is added to stop the HRP enzyme-catalyzed process, and the color shift is detected spectrophotometrically at 450 nm. The O.D. of the samples is then compared to the standard curve to determine the concentration of VD3 in the samples.

**The constituents of the kit**

(Components of the kit were referred in appendix 8).

**Assay procedure**

1. Dilute and add sample to standard: arrange 10 standard wells on coated ELISA plates; fill the first and second wells with 100µl of standard; add the first and second wells with 50µl of standard dilution; mix; remove 100µl from the first and second wells and add it to the third and fourth wells separately. Then mix in 50µl of the standard dilution to the third and fourth wells; after that, remove 50µl and discard it from the third and fourth wells; add 50µl to the fifth and sixth wells; and last, mix in 50µl of the standard dilution to the fifth and sixth wells.; 50µl should be removed from the fifth and sixth wells and added to the seventh and eighth wells. standard dilution should then be added to the seventh and eighth wells, mixed. Similarly, 50µl should be removed from the seventh and eighth wells and added to the ninth and tenth wells. standard dilution should be added to the ninth and tenth wells, mixed. Finally, 50µl should be removed from the ninth and tenth wells and discarded(add Sample 50µl toeach well after Diluting

,(density:48ng/ml, 32 ng/ml, 16ng/ml, 8ng/ml, 4ng/ml).

2.Add sample: Assign blank wells individually (the HRP-Conjugate reagent and sample are not added to blank comparison wells; all other step operations are the same) .evaluating the sample thoroughly. Gently mix the testing sample (10μl) after adding 40μl of the sample dilution (the sample final dilution is five times). Avoid touching the well wall as much as possible when adding the sample to the wells.

3.Incubate: At 37°C for 30 minutes after covering the plate with the Closure plate membrane.

4. Configure liquid: dilute 30-fold (or 20-fold) wash solution with ditilled water, then reserve.

5. Cleaning: Uncover the membrane of the closure plate, remove the liquid, dry with a swing, add washing buffer to each well, wait 30 seconds, then drain, repeat 5 times, and dry with a pat.

6. Add enzyme: Add 50μl of HPP-Conjugate reagent to every well, excluding the blank well.

7.Cultivate : Working with 3.

8. Cleaning / Working with 5.

9. color: Fill each well with Chromogen Solutions A50ul and B, then leave the light preservation on for 15 minutes at 37°C.

10. Stop the reaction: Put 50 μl of the Stop Solution into each well, then stop the reaction when the blue hue turns yellow.

11. Assay: Take the blank well as zero. Within 15 minutes of adding the stop solution, read the absorbance at 450 nm.

**Calculate**

Draw the standard curve on graph paper using the standard density as the horizontal and the OD value as the vertical. Determine the density that corresponds to the sample OD value by multiplying the sample curve by the dilution multiple. Alternatively, compute the straight line regression equation of the standard curve using the sample OD value and the standard density. Then, using the sample OD value as an input, compute the sample density multiplied by the dilution factor, yielding the sample density.

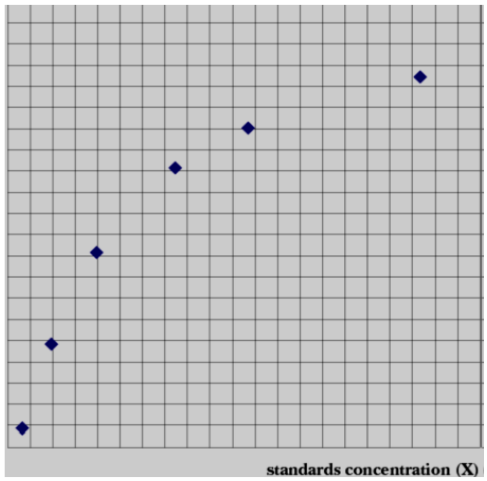


**Figure 3.4 : standard curve for** (Vitamin D3)

## 3.4.10. Measuring of serum calcium levels

**Principle**

Total calcium content in blood, plasma, or urine can be measured using the Moorehead and Briggs-developed CPC (O-Cresol Phtalein Complexone) technology.

When calcium and CPC combine in an alkaline solution, a dark-red complex is produced, and the amount of calcium in the material is indicated by its absorbance at 570 nm.

**Procedure**

A thorough Kenza 240TX procedure is supplied upon request, wavelength of 570 nm were used.37°C is the temperature.

It is important to maintain a steady temperature because the dye's absorption is temperature-sensitive.

| | **Automated Analyzer** | **Manuals procedure** |
|---|---|---|
| Reagents | R1 120 µL<br>R2 120 µL | WR ,1000 µL |
| Stander ,Control, Sample | 6 µL | 25 µL |

Association thoroughly. For five minutes, incubate at room temperature. Compare the 570 nm absorbance (550-590) to the reagent blank. When the color is exposed to light, it stays that way for an hour. Mix well. For five minutes, incubate at room temperature. Compare the 570 nm absorbance (550-590) to the reagent blank. When the color is exposed to light, it stays that way for an hour.

**Calculations**

Therefore, calculate the following: Result=$\frac{Abs\ (Assay)}{Abs\ (Stander)}$ x concentration of standard

## 3.5- Statistical Analysis

This examination is best described as a case-control study. SPSS (Statistical Package for the Social Sciences, version 24) was utilized for statistical Assessment , and T-test with the variation in data measurement that is least substantial was employed. The data is displayed as mean with standard deviation(SD) attached. At the ($p< 0.05$) threshold, statistical significance was considered to exist. The comparison between the groups yielded the P value, or the least significant difference (Armitage *et al*, 2008).

# Chapter four

# Results

# and

# Discussions

## 4.1. Research on clinical cases

### 4.1.1. A BMI comparison between POI patients and control

The relationship between patients and control according to their BMI was estimated by examining of 50 POI women and 50 healthy women working as a control group. Rendering to results showed in table (4.1), and figure (4.1). There was statistically significant increase in BMI mean values of the POI patients group (27.88±4.52) in comparison to control group (25.87±2.56)

**Table (4.1):The mean and standard deviation of body mass index for POI patients and control .**

| | Sample | Mean | Std. Deviation | P Value |
|---|---|---|---|---|
| **BMI Kg/ m2** | Controls | 25.87 | 2.56 | 0.007 |
| | Patients | 27.88 | 4.52 | |

**The data represented as mean ± SD**

Women with a high BMI are more prone than those of normal weight to have irregular menstrual periods, ovulatory dysfunction, and disruptions in the hypothalamic pituitary ovarian axis, which can result in greater rates of infertility. Additionally, research has demonstrated that a woman's high BMI is a significant risk factor for unfavorable pregnancy outcomes (Ribeiro *et al* ,2022).

The World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) define overweight, obese, and severely obese as having a body mass index (BMI) of more than 25 kg/m2, 30 kg/m2, and 40 kg/m2, respectively(Chooi *et al*, 2019).

Numerous studies have demonstrated the effects of obesity on the oocyte, Oocyte competence and maturation may be affected by changes in a variety of hormones, including those that initiate oocyte maturation, in individuals who are obese, Adipose tissue is overproduced in obesity because it is an essential location for the synthesis of steroid hormones and metabolism (Al-yasiry *et al*,2022). Overweight and obesity are on the rise globally and have a negative impact on

many bodily processes, including reproductive health, Specifically women who are obese experience disruptions to the "hypothalamic pituitary ovarian axis" and often experience irregular menstruation that results in anovulation and infertility, Negative pregnancy outcomes such gestational diabetes, hypertension, and early labor are also linked to high BMI (Silvestris *et al*,2018).

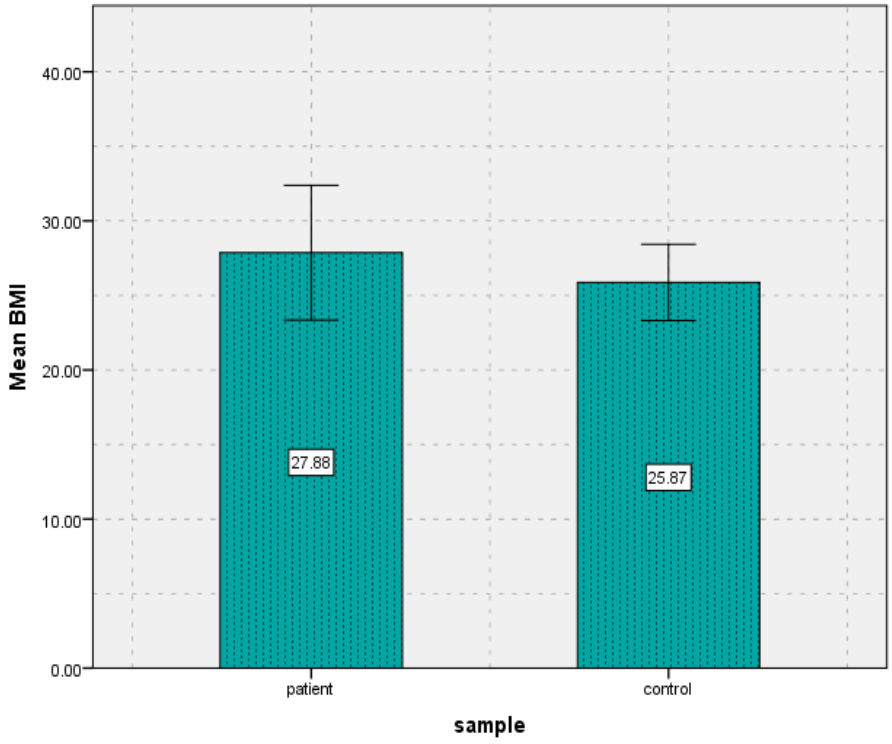


**Figure (4.1) : Distribution of BMI in POI and control groups**

## 4.1.2. The biochemical parameters

### 4.1.2.1. Measuring of LH and FSH levels

Table (4.2) , figure (4.2A) and figure (4.2B) provided the results, which indicated a significant increase in FSH levels. (23.56±25.79) in women with POI compared with healthy women (5.39±3.84),also there is significantly increase levels of LH in POI patients (12.95±11.46) in comparison with healthy women(8.54±8.92).

**Table (4.2): LH and FSH concentrations of the POI patients and control group**

| Parameters | Control group N=50 Mean | SD | Patient group N=50 Mean | SD | P value |
|---|---|---|---|---|---|
| **LH (m.IU/mL)** | 8.54 | 8.92 | 12.95 | 11.46 | 0.034 |
| **FSH (m.IU/mL)** | 5.39 | 3.84 | 23.65 | 25.79 | 0.000 |

**The information displayed as mean ± SD**

The pulsatile release of gonadotropin releasing hormone by the hypothalamus typically stimulates the pituitary gland to release gonadotropins, such as LH and FSH, Luteinizing hormone acts primarily on ovarian theca cells that have LH receptors, which enhances the production of androgens (Ashraf *et al*, 2019) . The granulosa cells of ovary are impacted by FSH, which converts androgens made in the theca cells into estrogens, mainly estradiol, which is necessary for the development of follicles ( Liu *et al*, 2021) .

In comparison to healthy control women, women with POI exhibited significantly higher levels of FSH, luteinizing hormone (LH), and BMI (Wu,2021).

Patients with POI presented with significantly higher FSH and decreased AMH levels(Liu,2020).

The current study's findings show that the levels of the hormones FSH and LH differ significantly ($P<0.05$) between the two study groups (patients and healthy individuals), The patients' FSH and LH values were higher than Normal, in comparison to healthy individuals ($4.75 \pm 0.651$ and $4.04 \pm 0.94$), respectively, were $6.780 \pm 15.54$ and $5.44 \pm 12.40$ (Khudhair *et al*, 2024).

An increased FSH level is one of the diagnostic criteria for POI, Low levels of FSH and LH might raise suspicions of hypogonadotrophic hypogonadism (Fernando *et al*,2023).Patients in the POI group presented with characteristic

hypergonadotropic hypogonadism ,significantly elevated serumconcentrations of FSH and LH (Maciejewska *et al*. 2020).

The FSH/LH ratio greatly raise during the pre POI stage because FSH increased substantially earlier and more steeply than LH, Previous research has demonstrated that the FSH/LH ratio is a reliable indicator of a poor ovarian response and is linked to unfavorable results during in vitro fertilization (IVF) treatments (Jiao *et al*, 2021).

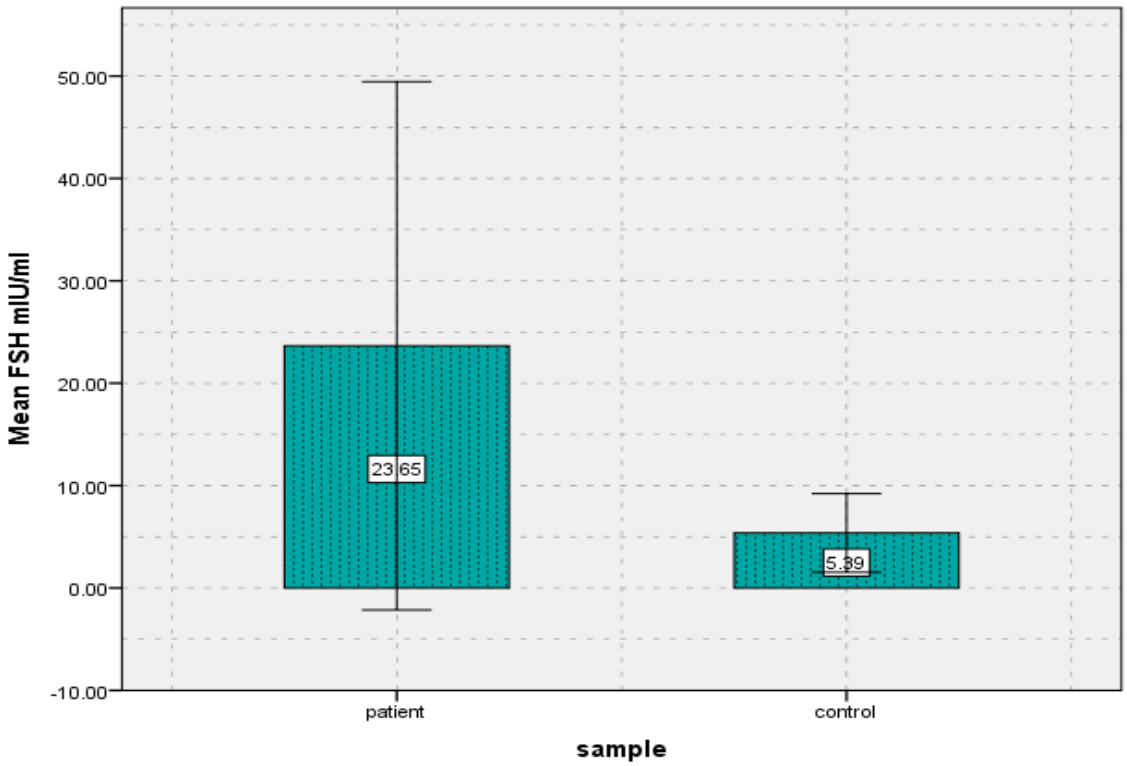


**Figure (4.2A) : The average of FSH concentrations in the control and POI groups**

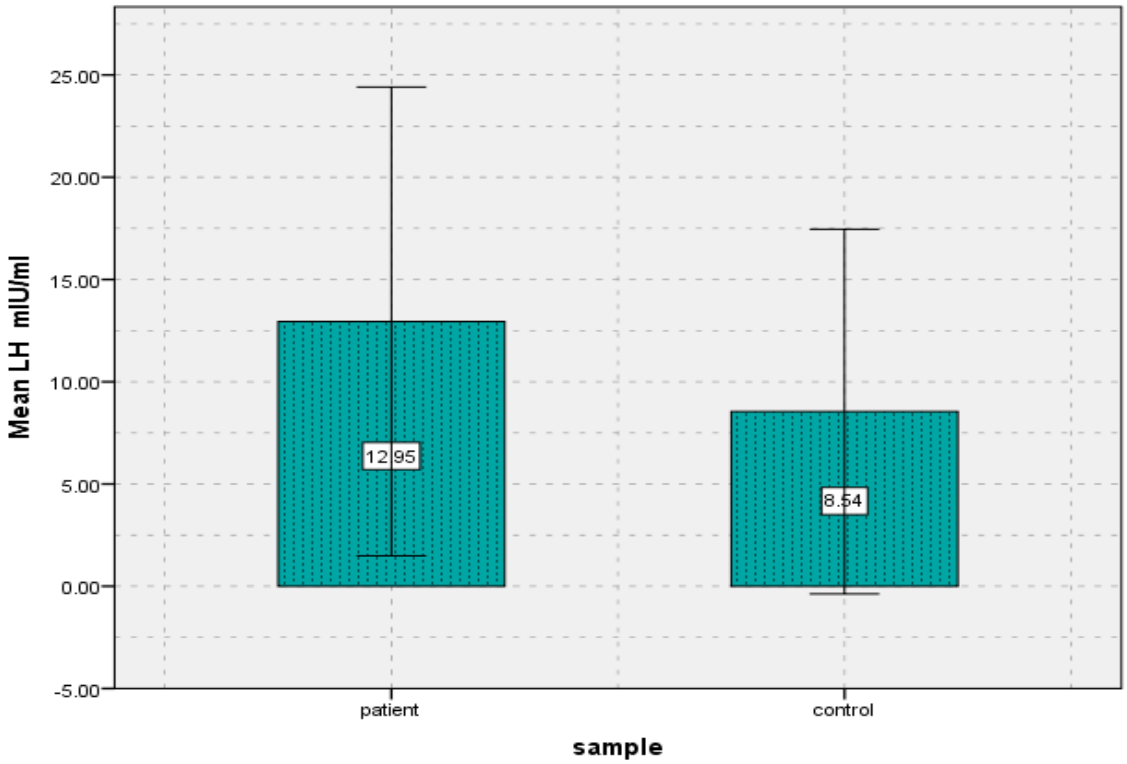


**Figure (4.2B) : The average of LH concentrations in the control and POI groups**

## 4.1.2. 2. Estradiol and Free Testosterone

The offered records presented in the table( 4.3) and appearing significantly decreased in Estradiol concentrations of POI group (20.49±16.54) in contrast to control group (87.59±66.61) as in figure( 4.3 ),as well as there was significantly decrease in the levels of free testosterone of POI groups(0.15±0.15) than healthy groups (0.24±0.20) as presented in figure (4.4) .

**Table (4.3): Estradiol and Free testosterone in control and POI groups**

| **Parameters** | **Control group N=50 Mean** | **SD** | **Patient group N=50 Mean** | **SD** | **P value** |
|---|---|---|---|---|---|
| Estradiol (pg/mL) | 87.59 | 66.61 | 20.49 | 16.54 | 0.000 |
| Free testosterone (ng/mL) | 0.24 | 0.20 | 0.15 | 0.15 | 0.012 |

**The data represented as mean ± SD**

Measuring estradiol levels when POI is suspected is a helpful adjunct to confirm that it is low, validating the POI diagnosis (Fernando *et al*, 2023). Many physiological processes are negatively impacted by low estrogen including moisture, wrinkles, atrophy of the skin, poor wound healing and barrier function, a decrease in the perception of one's own beauty and even psychological health, women experiencing changes in their skin due to menopause look for medical and cosmetic procedures that will improve their self-image and prevent their skin from aging, especially on exposed areas like the hands, neck, and face (Lephart and Naftolin, 2021).

The majority of osteoporotic postmenopausal women suffer bone loss associated with low estrogen levels, An imbalance between bone production and resumption and an increase in bone turnover lead to the rapid loss of bone mass, Excessive use of glucocorticoids can also cause osteoporosis by demineralizing bone and altering its microscale spatial heterogeneities, which increases the risk of fracture (Cheng et al, 2022).

Recent studies have revealed alterations in osteocytes during estrogen deprivation, which may be crucial in the development of the illness, Advanced knowledge of these alterations is necessary to determine the effectiveness of osteocyte targeted therapeutics in preventing restart and secondary mineralization, which would shift the paradigm for osteoporosis treatment (McNamara, 2021).

Androgens include compounds that can be turned to testosterone, dihydrotestosterone, and testosterone itself. The production of circulating testosterone comes from peripheral conversion of adrenal androgens (50%) and the ovaries (25%) as well as the adrenal cortex (25%), Furthermore the intracellular synthesis of testosterone in target tissues plays a major role in the female androgen action, Reduced testosterone production after menopause has been connected to sexual issues(Johansen *et al*,2020).

No cost biologically active androgens include testosterone, It is a substance that

is secreted, and according to (Sun *et al*, 2021) its origins could be connected to the adrenal zona fasciculate and the ovary.

Only 1% of testosterone is free, the rest is attached to albumin and globulin, which binds sex hormone (about 33% and 66%, correspondingly) (Njoroge *et al*, 2022). It's exciting to reminder that the only form of testosterone that has biotic action is unbound testosterone, the inert version of the hormone is testosterone linked to proteins(Zamir *et al*, 2021) .

The use of physiologic testosterone dosages for brain and breast protection, osteoporosis prevention, and sexual function is becoming more and more supported by research, For the past 80 years studies have assessed the safety of testosterone use in females, Long-term safety was established by a recent paper on the consequences of subcutaneous hormone pellet therapy, which examined a sizable patient cohort over a 7-year period, Furthermore two sizable, long-term, peer-reviewed studies have demonstrated a noteworthy decline in the incidence of invasive breast cancer in women receiving testosterone therapy (Donovitz, 2022).

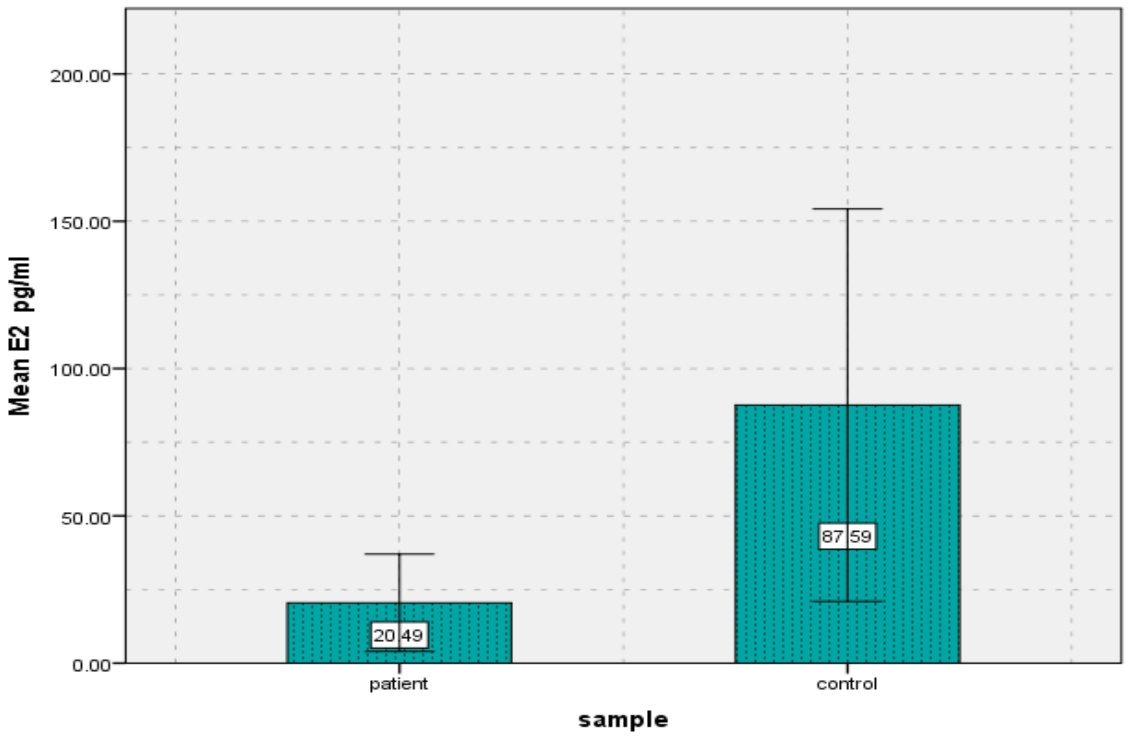


**Figure (4.3) : Mean of Estradiol concentrations in control and POI groups**

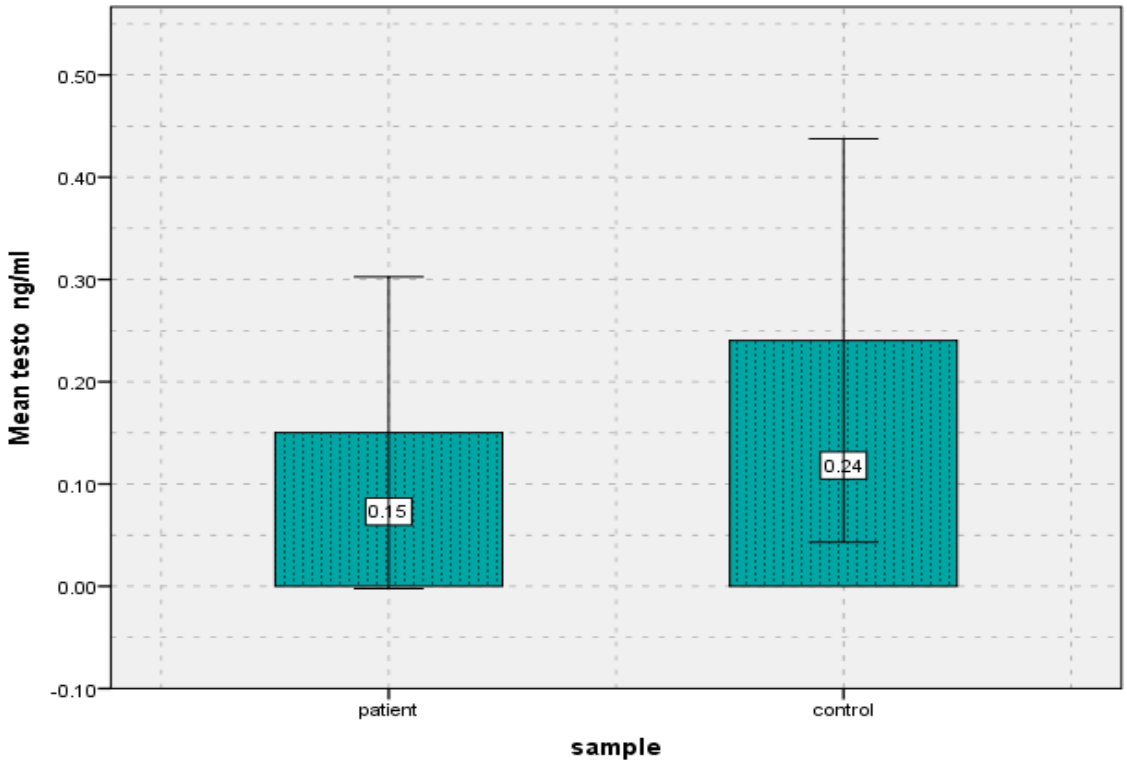


**Figure (4.4) : Mean of Free testosterone concentrations in control and POI groups**

## 4.1.2. 3. Vitamin D3 and serum Calcium

As per the findings displayed in table (4.4), the POI patients' Vitamin D3 levels were significantly lower (4.55±2.12) compared to the control group (6.39±1.52), as depicted in figure (4.5). Additionally, the POI patients' Calcium levels were significantly lower (8.21±1.34) than those of the control group (8.67±0.41), as illustrated in figure (4.6).

**Table (4.4): levels of Vitamin D3 and calcium in control and POI patients**

| Parameters | Control group N=50 Mean | SD | Patient group N=50 Mean | SD | P value |
|---|---|---|---|---|---|
| Vitamin D3 (ng/dL) | 6.39 | 1.52 | 4.55 | 2.12 | 0.000 |
| calcium (mg/dL) | 8.67 | 0.41 | 8.21 | 1.34 | 0.022 |

**The information displayed as mean ± SD**

These results were similar to the findings of a study in Baqubah , Iraq ( Al-Hamdany *et al*,2019).

A decrease in the concentration of 25 (OH) D in serum below optimal values is known as vitamin D deficiency, This condition can potentially result in suboptimal absorption of calcium in the intestine, the development of secondary hyperparathyroidism, and an increased risk of fractures particularly in elderly individuals (Salokhiddinovna ,2023).

Purdue Smithe *et al* conducted a prospective assessment of the correlation between the risk of POI and the serum levels of total and free 25(OH)D, as well as its binding protein (VDBP), The 656 women in this nested case-control study ranged in age from 25 to 42, Serum 25(OH)D quartiles were measured for both the control and case groups, The probability of POI was inversely correlated with the serum level of VDBP (OR = 1.80, 95% CI for OR [1.09, 2.98]), but not significantly correlated with the serum levels of total (OR = 1.04, 95% CI for OR [0.60, 1.80]) or Free 25(OH)D (OR = 0.70, 95% CI for OR [0.41, 1.20]) (Purdue-Smithe *et al*,2018).

Ersoy *et al*. conducted a cross-sectional study with 48 women with POI and 82 women as the control group to assess the prognosis of vitamin D for POI, Serum 25(OH)D levels were measured using the ELISA technique, The serum vitamin

D level and POI did not significantly correlate (P = 0.477) (Ersoy *et al*,2016).

evaluated serum vitamin D in POI women in a case-control study of 63 women, including 35 women with POI, in 2023 in Turkey, They reported a significant and inverse relationship between serum vitamin D level and the risk of POI (9.5 ± 4.05 ng/mL in the POI group and 18.5 ± 7.5 ng/mL in the control group( P < 0.001) (Dashti *et al*, 2023) .

It has been demonstrated that calcium in humans can promote egg maturation either naturally or by the release of LH, This is thought to be caused by calcium modifying intra cytoplasmic cAMP concentrations (Ahuja &Parmar , 2017). Sperm-derived substances also play a role in egg activation, The egg can therefore grow into an embryo, Furthermore reproduction happens when calcium is present(Chen, 2020).

Ninety-nine percent of the Ca in the body is found in bone, which also serves as a calcium storage and gives the skeletal system stiffness and shape, The residual portion engages in metabolic activities such as contraction of muscles and vessels, transmission of nerve signals, transmembrane transport, activation of enzymes, and hormone regulation, Insufficient calcium consumption has been linked to bone health in most studies, particularlyrickets in children and fractures, osteopenia, and osteoporosis in older adults(Cormick & Belizán. 2019).

A tiny preventive impact against bone loss is provided by calcium supplements but this effect vanishes when use stopped, The antifracture benefit of calcium supplements is restricted to elderly, fragile women or community dwelling individuals who have low calcium intake from their diet and low vitamin D levels (Heidari *et al*,2020).

The control of parathyroid hormone (PTH) is influenced by the interaction between calcium and vitamin D Specifically the blood 25(OH)D concentration indicates the vitamin D status, and adequate calcium intake determines the suppression of PTH to low (normal) amounts, Populations lacking in viamin D may have increased PTH which raises calcium requirements and bone turnover(

Shlisky *et al*,2022).

According to WHO guidelines maintaining a minimum recommended calcium intake of 1000 mg/day and a vitamin D intake of 800IU/day is essential to maintaining bone health and reducing the risk of fractures(Podfigurna *et al*, 2020).

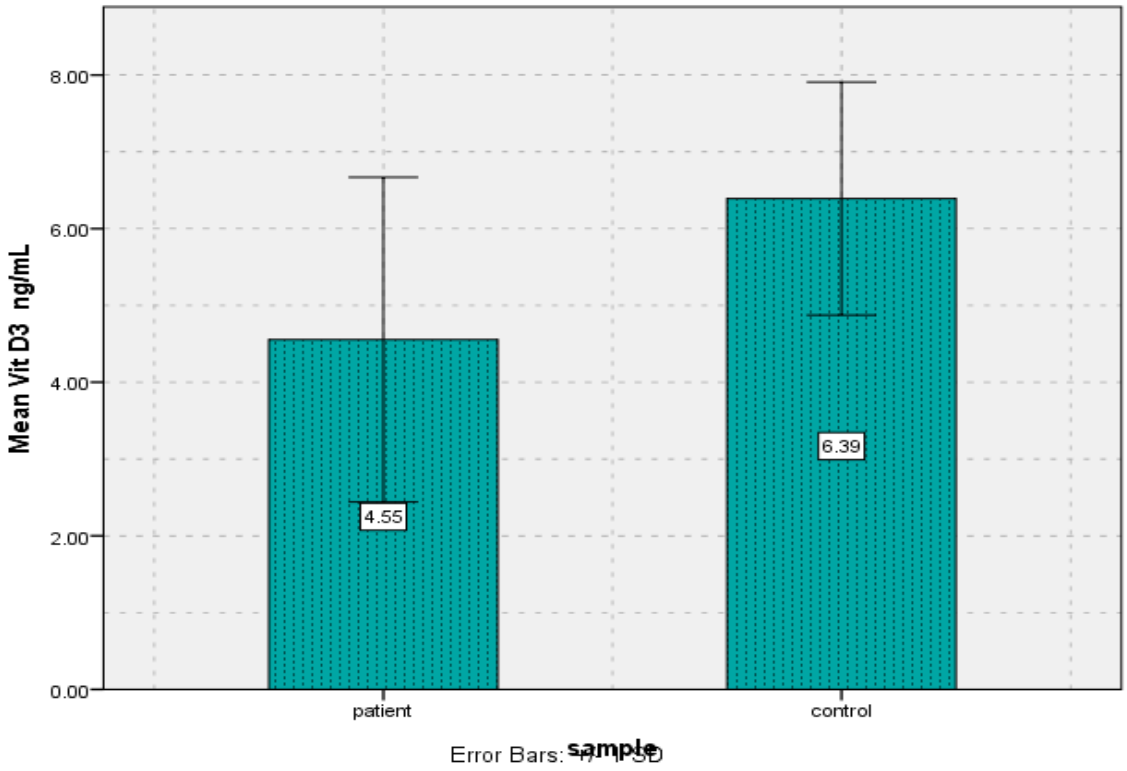


**Figure (4.5) : Mean of Vitamin D3 concentrations in controls and POI groups**

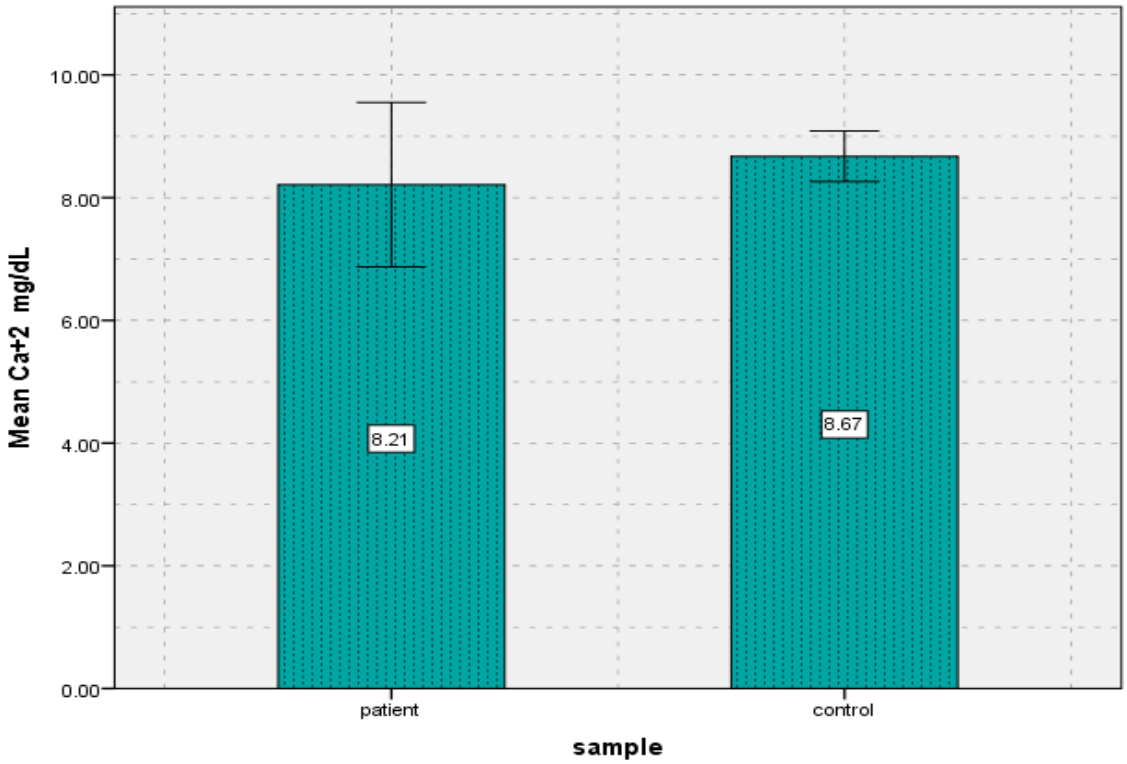


**Figure (4.6) : Mean of Calcium concentrations in controls and POI groups**

## 4.1.3 Anti Mullerian Hormone(AMH) levels

The information displayed as mean ± SD the results shown in table (4.5) show that the patients' group with POI had considerably lower (AMH) levels (2.31±1.03) compared to the control group (4.56±1.09), as shown in figure (4.7).

**Table (4.5):AMH levels for women with and without POI .**

| | Sample | Mean | Std. Deviation | P Value |
|---|---|---|---|---|
| **AMH** (ng/ml) | Control | 4.56 | 1.09 | 0.000 |
| | Patients | 2.31 | 1.03 | |

**The data represented as mean ± SD**

Anti-Müllerian hormone levels in serum are frequently employed as indicators of ovarian reserve, Since AMH is created from tiny antral follicles, the quantity of primordial follicles that remain is substituted for it ( Iwase *et al*,2018). POI patients reported lower levels of AMH and greater basal FSH ($P < 0.001$) in

comparison to control women , Compared to control women POI patients had higher baseline FSH and lower levels of AMH ($P < 0.001$)
( Wang &Sun, 2022).our results are agree with these researches findings .In patients with POI, serum AMH levels measured using conventional techniques are extremely low or fall below the lower limit of measurement, Conversely, POI cases in cycles with tiny antral follicles may manifest very low serum AMH levels, This could help predict cycles of follicular growth and promote more effective use of limited follicle development(Vimalraj, 2020).

Serum AMH levels determined with standard methods are very low or below the lower limit of measurement in patients with POI. Very low serum AMH levels, on the other hand, may show up in POI instances in cycles with small antral follicles, This might assist anticipate cycles with follicular growth to encourage more efficient utilization of scarce follicle development (Iwase *et al*. 2018).

One biomarker of ovarian reserve is anti-Müllerian hormone . Menopause between the ages of 40 and 45 may be linked to a low AMH level prior to the age of 39, Premature ovarian insufficiency (POI) is associated with a risk that is not well understood (Desongnis *et al*,2021).

Further study is required to increase the likelihood of successful follicular development and conception in patients with POI, even though AMH levels may predict follicle growth ( Osuka *et al*, 2023).

Chromosome analysis and testing for FMR1 pre mutation are recommended diagnostic assessments to determine the etiology of spontaneous POI, If an immunological problem is suspected, autoantibodies against thyroid peroxidase (TPO), 21OH, or adrenocortical antibodies may be screened for (Yeganeh *et al*, 2019).

The normal evaluation of POI does not currently include extensive investigations using chromosomal microarray (CMA), panel-based NGS, or other autoantibody techniques (Webber *et al*, 2016 &Yeganeh *et al*, 2019) .

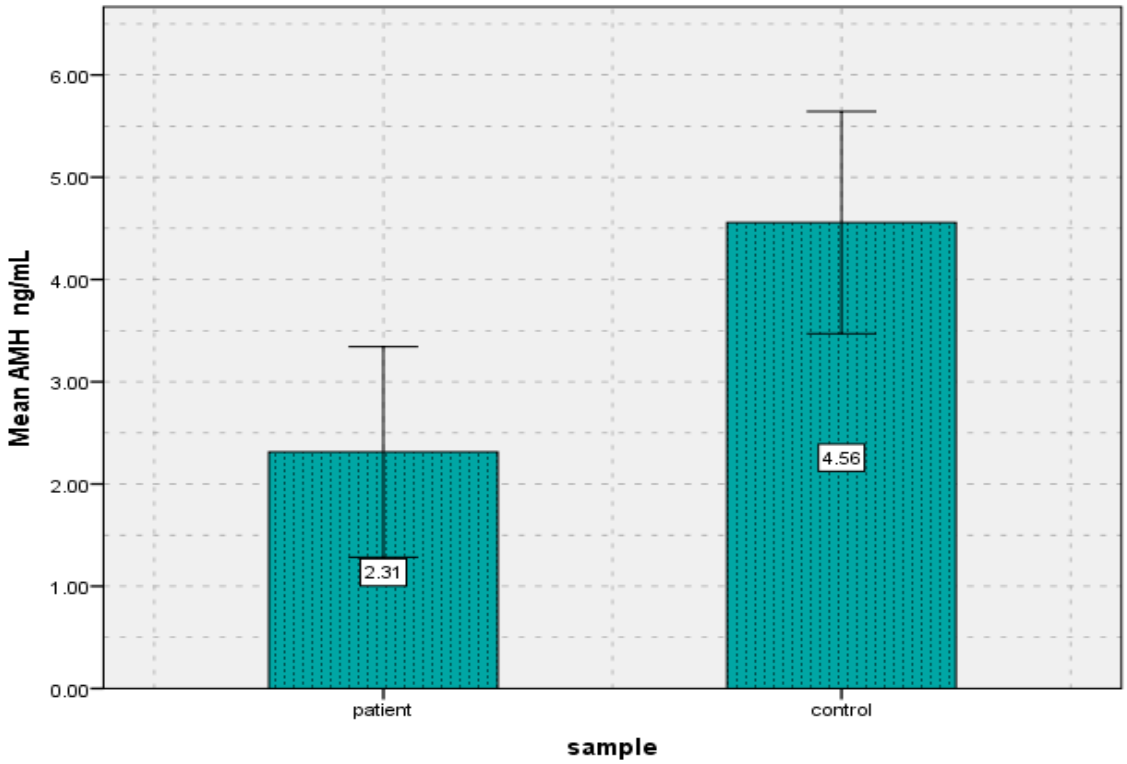


**Figure (4.7) : Mean of AMH concentrations in control and POI groups**

## 4.1.4. Levels of Specific Markers of Bone Health in POI

### 4.1.4.1 Bone Specific Alkaline Phosphatase (BALP) levels

The results shown in table (4.6) show that the BALP levels of the POI patient group are considerably lower (8.63±4.93) than those of the control group (15.88±3.60), as shown in figure (4.8).

**Table (4.6):The average and standard deviation of BALP levels for POI and control groups**

| | Sample | Mean | Std. Deviation | P Value |
|---|---|---|---|---|
| **BALP** (ng/L) | Control | 15.88 | 3.60 | 0.000 |
| | Patients | 8.63 | 4.93 | |

**The data represented as mean ± SD**

The total amount of alkaline phosphatase (ALP), a nonspecific enzyme that hydrolyzes phosphate in alkaline media, is a reflection of the combined activity of many isozymes that are present in the kidney, liver, intestinal lining, and bone, When bone healing activity occurs such as bone metastases osteoblasts produce substantial amounts of bone specific alkaline phosphatase (BAP) the enzyme's bone specific isoform (Aqeel *et al*,2021).

The largest quantity of bone tissue remaining at the conclusion of skeletal maturation is referred to as peak bone mass (PBM), In order to reduce the chance of developing osteoporosis later in life, PBM must be attained from childhood, About 85–90% of total adult bone mass is gained by the age of 18 for girls and 20 for boys, though the age at which peak bone mass is obtained may vary depending on an individual's circumstances(Meczekalski *et al*,2023).

A decline in bone mineral density poses a threat to POI , It could be difficult for them to achieve their ideal peak bone mass (PBM), The PBM attained by young girls is roughly 30% less than that of young boys, Furthermore PBM is attained in around 50% of cases throughout puberty, Enough serum estradiol plays a major role in determining normal bone Metabolism, It is anticipated that a serum estradiol level of 40 pg/ml or more is the minimal threshold needed to guarantee appropriate bone metabolism, Serum estradiol levels in untreated POI patients are known to be extremely low, typically less than 40 pg/ml, Therefore osteopenia and osteoporosis pose a concern to POI patients(Meczekalski *et al*, 2023).

Hormone therapy has been shown to improve the bone health of women with premature ovarian insufficiency, as these individuals typically have reduced bone mineral density.( Costa *et al*, 2022).

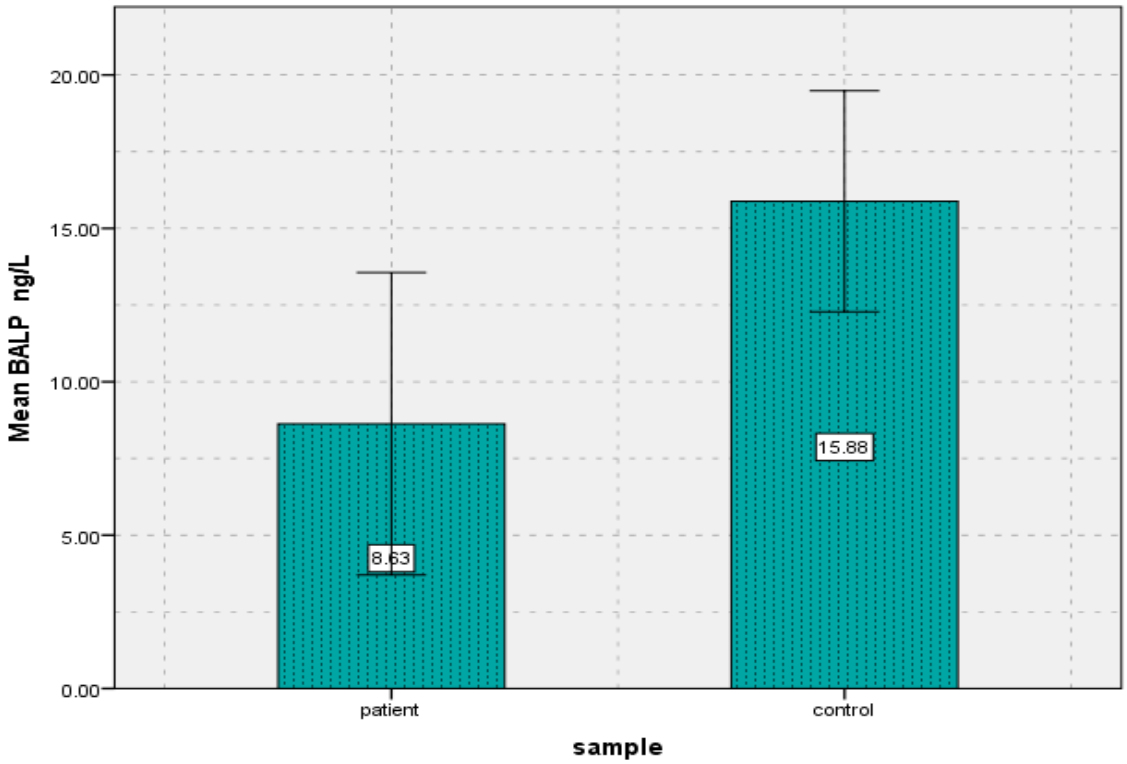


**Figure (4.8) : Mean of BALP concentations in control and POI groups**

### 4.1.4.2 Collagen type 1 levels

As indicated by the results in table (4.7), the POI patient group's Collagen type 1 levels are considerably lower (1.57±0.85) than those of the control group (2.33±0.74), as shown in figure (4.9).

**Table (4.7):The average and standard deviation of Collagen type 1 levels for POI and control groups .**

| | Sample | Mean | Std. Deviation | P Value |
|---|---|---|---|---|
| **Collagen type 1** (ng/L) | Control | 2.33 | 0.74 | 0.000 |
| | Patients | 1.57 | 0.85 | |

**The data represented as mean ± SD**

Type 1 collagen is present in tiny quantities in non-skeletal tissues like skin and makes up a large portion of the bone matrix, but it is not limited to bone tissue. With the probable exception of bone, other organs do not significantly

contribute to the concentration of bone turnover markers (BTM) in the blood stream since their mass is smaller than that of bone and they turnover more slowly than bone (Brown et al,2022).

It is evident that compared to normal healthy women, women with POF typically have lower bone mineral density (BMD) As a result, BMD measurement is necessary, How frequently the tests to assess BMD should formed is unclear at this time, To create guidelines, further prospective research is needed. Nonetheless, it would seem sensible to periodically measure BMD and to yearly check for any endocrine abnormalities in women with POF(Takahashi *et al*,2021).this study agree with our research findings.

Approximately 94% of the organic bone matrix is composed of type I Collagen, Bone remodeling and modeling happen at the same time that the skeleton develops, Since bones are not completely formed at birth, bone modeling is the gradual and ongoing process by which connective tissues build bones until adolescence, Another ongoing process that replaces mature bone tissues with newly generated bone is called "bone remodeling", Bone turnover is another term for this process (Seema *et al*,2020).

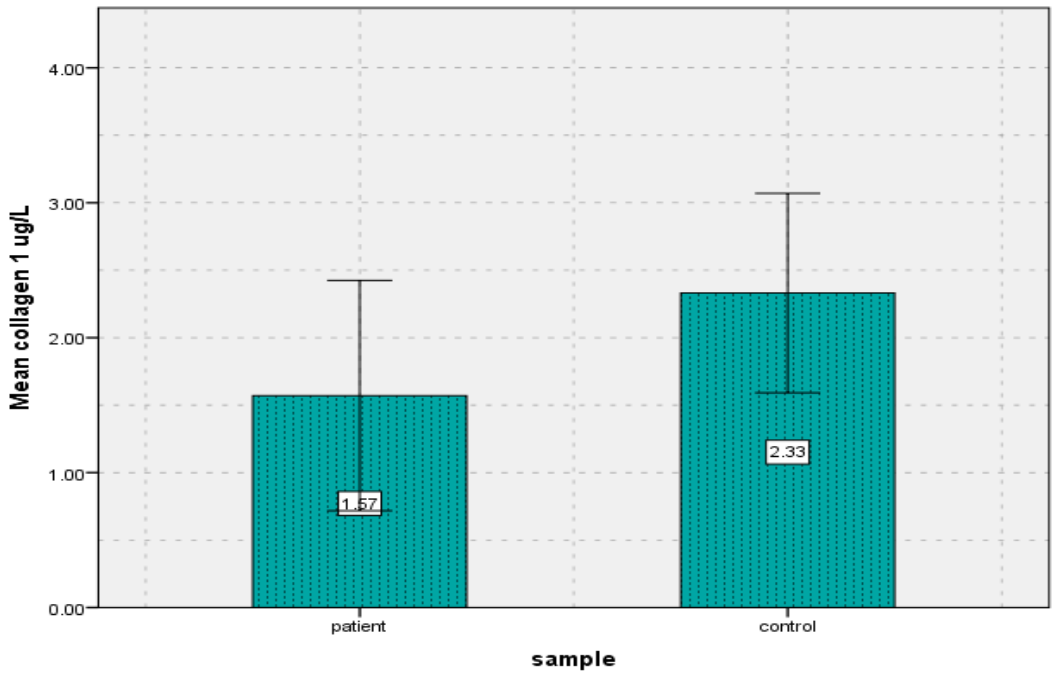


**Figure (4.9) : Mean of Collagen type 1 concentrations in control and POI groups**

## 4.2. Correlation of parameters of study

**Table 4.8 :The correlations between Parameters of the study**

| variable | AMH ng/mL | E2 pg/ml | collagen 1 ug/L | BALP ng/L | Vit D3 ng/mL | Ca+2 mg/dL | FSH mIU/ ml |
|---|---|---|---|---|---|---|---|
| | r | | | | | | |
| **E2 pg/ml** | -.149 | | | | | | |
| **collagen 1 ug/L** | .751** | -.143 | | | | | |
| **BALP ng/L** | .514** | -.079 | .583** | | | | |
| **Vitamin D3 ng/mL** | .586** | -.095 | .461** | .736** | | | |
| **Ca+2 mg/dL** | .053 | .187 | .099 | .131 | -.084 | | |
| **FSH mIU/ml** | .236 | -.337* | .247 | .202 | .086 | .300* | |
| **LH mIU/ml** | .203 | -.160 | .094 | .056 | .024 | .287* | .837** |
| **testo ng/ml** | -.009 | .179 | .166 | .145 | .050 | -.060 | -.150 |

***--significant correlations p≤0.05 , r: correlation coefficient**

****-- significant correlation p≤0.01**

According to results presented in table( 4.8) there is an extrusive relationships (p≤0.01) between AMH and Collagen 1 , AMH and BALP,AMH and Vitamin D3, Collagen 1 and BALP, Collagen 1 and Vitamin D3, BALP and Vitamin D3, FSH and LH .also there is a**n** extrusive relationships (p≤0.05) between Ca+2 and FSH , Ca+2 and LH .That meaning when one of these markers increase another increase and the converse is true.

Based on findings showing serum AMH levels gradually decline with age and have a high correlation with both the size of the pool of primordial follicles and the number of antral follicles, AMH has been recognized as a useful measure for ovarian reserve (Yoon *et al*,2013). BAP, a glycoprotein present on the surface of osteoblasts, is a marker of the biosynthetic activity of these cells that produce bone, It has been demonstrated that BAP is a sensitive and trustworthy marker of bone metabolism, One unique feature of the vertebrate skeletal system is the extracellular mineralized matrix (Karpen, 2018).

When bone healing activity occurs such as bone metastases, osteoblasts produce substantial amounts of bone specific alkaline phosphatase (BAP), the enzyme's bone specific isoform (Aqeel *et al*,2021). The primary constituents of the organic matrix found in bone are proteins, lipids, collagen, and blood cells, Whereas the organic matrix's collagen fiber arrangement gives the bones strength, the non organic matrix's mineral component gives the bones their hardness and stiffness, Reduced bone mineral density (BMD), the breakdown of bone microarchitecture (BMA), and alterations in the overall quantity and variety of proteins, particularly collagen 1, are the hallmarks of osteoporosis, which impacts the skeletal system (Feng *et al*, 2021).This agree with our research findings.

Since a lack of this vitamin has been linked to a number of diseases, including those that affect bone health, the relationship between 25(OH)D and bone health in adults is biologically plausible, Vitamin D is important throughout development because it facilitates greater absorption of calcium in the intestine, which also positively contributes to bone health (Segheto *et al*,2021). The table also showed a negative reverse relationship ($p \leq 0.05$) between estrogen and FSH ,meaning when FSH increase there is decline in estrogen level and the converse is true.

High FSH concentration is indicative of either hyperthyroidism or hypothyroidism, or ovarian malfunction, A pituitary or hypothalamic defect is indicated by either low or normal FSH levels, The present study's results reveal a loss in early ovarian reserve or early impotence, which is correlated with a high concentration of FSH, Consequently the woman's fertility declines with time( Al-Hamdany &Jaffer,2019)

It has been observed that in patients with POI, serum E2 levels are a useful predictor of ovarian function. On the other hand, follicle growth leads to a high serum E2 level, indicating that the follicles are already developing.

Anti-Müllerian hormone levels in serum are frequently employed as indicators of ovarian reserve, Since AMH is created from tiny antral follicles, the amount of primordial follicles that remain is replaced by it, When serum AMH levels are determined using standard methods, they are very low or below the lower limit of quantification in patients with POI(Osuka *et al*, 2023).

These results were similar to the findings of a study by Al-Hamdany *et al*,2019, which showed increased FSH and LH levels ,decreased AMH, vitamin D, and calcium (Al-Hamdany et al,2019).With difference in estrogen level ,our study showed significantly low levels in POI in compared with healthy women ,but in their study there was no differences in E2 levels between both groups.

The relationships between other parameters in study are found but they were statistically non-significant.

# Conclusions

## And

# Recommendations

## Conclusions

The Current study concludes the following:

1. The BMI group between (25-29.5 kg/m2) is the most category predispose to have Premature Ovarian Insufficiency(POI) among the BMI groups in the study.
2. The results concludes decreased of Estradiol, Testosterone, Calcium, VD3, BALP, and Collagen 1 in POI patients.
3. The results concludes increased of FSH and LH in POI patients.
4. There is a positive extrusive relationship between AMH and Collagen 1 AMH and BALP, AMH and Vitamin D3, Collagen 1 and BALP, Collagen 1 and Vitamin D3, BALP and Vitamin D3, FSH and LH ,Ca+2 and FSH Ca+2 and LH in women with POI .
5. There is a negative reverse relationship between estrogen and FSH levels in women with POI.
6. The correlational relationships between other parameters in study are found but they were statistically non-significant

## Recommendations

1. Studying the genetic parameters such as chromosome analysis and testing for FMR1 pre mutation to investigate POI in further studies.
2. Studying the immune parameters including screening for autoantibodies against thyroid peroxidase (TPO), and 21OH or adrenocortical antibodies to investigate POI in further studies.
3. It is necessary to add other parameters for women with POI, such as a vitamin D3 test and E2.
4. Administration of hormonal therapy like E2 to compensate its deficiency
5. Make a calendaring to follow up increasing in FSH levels to assets respond to therapy.

# References

**Ahuja**, A., & Parmar, D. (2017). Role of minerals in reproductive health of dairy cattle: a review. Int. J. Livest. Res, 7(10), 16-26

**Alemany**, M. (2022). The Roles of Androgens in Humans: Biology, Metabolic Regulation and Health. International Journal of Molecular Sciences, 23(19). https://doi.org/10.3390/ijms231911952

**Al-Hamdany**, W. A. S. & Gaffar, M. K. (2019). A Comparison of the Effect of Dysmenorrhea and Secondary Amenorrhea on the Concentration of Some Biochemical and Hormonal Factors in Women in Tikrit City. Tikrit Journal of Pure Science 24(2) http://dx.doi.org/10.25130/tjps.24.2019.028.

**Al-yasiry** RZ, Rabab Zahir; Jwad, Mufeda Ali[1]; Hasan, Muhjah Falah[2]; Alsayigh, Haythem Ali(2022), How Obesity Affects Female Fertility, Medical Journal of Babylon19(2):p 111-114, Apr–Jun 2022. | DOI: 10.4103/MJBL.MJBL_8_22

**Anderson** RA, SM Nelson , 2020, Anti-Müllerian hormone in the diagnosis and prediction of premature ovarian insufficiency ,Seminars in Reproductive Medicine, Semin Reprod Med 2020; 38(04/05): 263-269 DOI: 10.1055/s-0040-1722319 .

**Aqeel** Rashid F, Mahdi S, Abd-Alkader Mahdy S, et al. Effect of obesity on plasma alkaline phosphatase activity in breast cancer. Rep Biochem Mol Biol 2021; 10: 307–313.

**Aramesh**, S., Alifarja, T., Jannesar, R., Ghaffari, P., Vanda, R., & Bazarganipour, F. (2021). Does vitamin D supplementation improve ovarian reserve in women with diminished ovarian reserve and vitamin D deficiency: a before-and-after intervention study. BMC Endocrine Disorders, 21(1), 1-5.

**Armitage** P, G Berry, JNS Matthews - 2008, Statistical methods in medical research,https://books.google.com/books?hl=ar&lr=&id=OevV49Dhn_YC&oi=

fnd&pg=PR5&dq=armitage+2008&ots=3MmjBt_p5q&sig=IbICTh2cqJzJQS_i5hv8y7OhKlw .

**Arroyo A**., Kim, B., & Yeh, J. (2020). Luteinizing Hormone Action in Human Oocyte Maturation and Quality: Signaling Pathways, Regulation, and Clinical Impact. Reproductive Sciences, 27(6), 1223–1252. https://doi.org/10.1007/s43032-019-00137-x

**Ashraf S ,** Mudasar Nabi, Shayaq ul Abeer Rasool, Fouzia Rashid & Shajrul Amin, (2019), Hyperandrogenism in polycystic ovarian syndrome and role of *CYP* gene variants: a review in Egyptian Journal of Medical Human Genetics Volume 20, article number 25

**Azziz**, R. (2018). Reproductive endocrinology and infertility: Clinical expert series polycystic ovary syndrome. Obstetrics and Gynecology, 132(2), 321–336. https://doi.org/10.1097/AOG.0000000000002698

**Bhattarai**, H. K., Shrestha, S., Rokka, K., & Shakya, R. (2020). Vitamin D, calcium, parathyroid hormone, and sex steroids in bone health and effects of aging. Journal of Osteoporosis, https://doi.org/10.1155/2020/9324505.

**Bhattarai,** S Shrestha, K Rokka - Journal of osteoporosis, 2020 , Vitamin D, calcium, parathyroid hormone, and sex steroids in bone health and effects of aging, https://doi.org/10.1155/2020/9324505.

**Bosch**, C Alviggi, M Lispi, A Conforti, 2021, Reduced FSH and LH action: implications for medically assisted reproduction , Journal of Human Reproduction, Pages 1469–1480, https://doi.org/10.1093/humrep/deab065

**Brown** JP, Andrew Don Wauchope b c, Pierre Douville a, Caroline Albert d, SamuelD. Vasikaran e, Current use of bone turnover markers in the management of osteoporosis,2022,https://doi.org/10.1016/j.clinbiochem.2022.09.002

**Chandak**, L. G., Lohe, V. K., Bhowate, R. R., Gandhi, K. P., & Vyas, N. V. (2017). Correlation of periodontitis with mandibular radiomorphometric indices, serum calcium and serum estradiol in postmenopausal women: A case–control study. Indian Journal of Dental Research, 28(4), 388.

**Chen** Y, Sujuan Yang, Sara Lovisa, Catherine G. Ambrose, Kathleen M. McAndrews, Hikaru Sugimoto & Raghu Kallur,2021, Type-I collagen produced by distinct fibroblast lineages reveals specific function during embryogenesis and Osteogenesis Imperfecta Nature Communications volume 12, Article number: 7199

**Chen**, C., Li, S., Hu, C., Cao, W., Fu, Q., Li, J., et al. (2020). Protective effects of puerarin on premature ovarian failure via regulation of Wnt/β-catenin signaling pathway and oxidative stress. Reprod. Sci. 28, 982–990.

**Chen, Q**., Ke, H., Luo, X., Wang, L., Wu, Y., Tang, S., ... & Chen, X. (2020). Rare deleterious BUB1B variants induce premature ovarian insufficiency and early menopause. Human Molecular Genetics, 29(16), 2698-2707.

**Cheng** CH ,Li-Ru Chen and Kuo-Hu Chen ORCID, Osteoporosis Due to Hormone Imbalance: An Overview of the Effects of Estrogen Deficiency and Glucocorticoid Overuse on Bone Turnover, Int. J. Mol. Sci. 2022, 23(3), 1376; https://doi.org/10.3390/ijms23031376

**Chooi YC**, Ding C, Magkos F. The epidemiology of obesity Metabolism. 2019;92:6–10 , https://doi.org/10.1016/j.metabol.2018.09.005

**Clemente** N**,** Chrystèle Racine, Alice Pierre, Joëlle Taieb ,2021,Endocrine Reviews, Anti-Müllerian Hormone in Female Reproduction 42(6), 753–782, https://doi.org/10.1210/endrev/bnab012

**Conway GS**. Premature ovarian failure. *Br Med Bull* 2000;56: 643-649.

**Cormick G**, JM Belizán , Calcium: More Than Bone? Implications for Clinical Practice and Theory, J Clin Med Res. 2021; 13(5): 253–25,doi: 10.14740/jocmr4505.

**Cormick** G. & J.M. Belizán. Nutrients 2019, 11(7), 1606; https://doi.org/10.3390/nu11071606.

**Costa** GPO, Edson Santos Ferreira-Filho, Ricardo dos Santos Simoes, José Maria Soares-Junior, Edmund Chada Baracat, Gustavo Arantes Rosa Maciel, Impact of hormone therapy on the bone density of women with premature ovarian insufficiency: A systematic review, https://doi.org/10.1016/j.maturitas.2022.09.01, 167, 105-112

**Costantini**, RE Mäkitie, MA Hartmann, 2020 , Early-onset osteoporosis: rare monogenic forms elucidate the complexity of disease pathogenesis beyond type I collagen، Journal of Bone and Mineral Research, 37(9), Pages 1623–1641, https://doi.org/10.1002/jbmr.4668.

**Dashti** S, ATN Tabrizi, ZFA Maleki, 2023,The Relationship Between Fat-Soluble Vitamins and Premature Ovarian Failure: A Systematic Review Journal Of Nursing And Midwifery Sciences.10,3, : https://doi.org/10.5812/jnms-138923

**Davis SR**, Jane F. Sex and perimenopause. *Aust Fam Physician* 2011;40: 274-278.

**Davis**, S. R., & Wahlin-Jacobsen, S. (2015). Testosterone in women-the clinical significance. The Lancet Diabetes and Endocrinology, 3(12), 980–992. https://doi.org/10.1016/S2213-8587(15)00284-3

**Deluca HF**. Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr. 2004;80:1689–1696. doi: 10.1093/ajcn/80.6.1689S. E 2018

**Desongnis** S, G Robin, D Dewailly, P Pigny, 2021, AMH assessment five or more years after an initially low AMH level , European Journal of Obstetrics & Gynecology and Reproductive Biology 256, 70-74 .

doi: 10.1007/s43032-020-00325-0

**Donovitz** GS, A Personal Prospective on Testosterone Therapy in Women—What We Know in 2022, - Journal of MDPI, 12(8), 1194; https://doi.org/10.3390/jpm12081194 .

**Edwin** D. Lephart & Frederick Naftolin (2021),Menopause and the Skin: Old Favorites and New Innovations in Cosmeceuticals for Estrogen-Deficient Skin, Review 11, 53–69

**Elhddad** AS, 2020 ,The Prevalence of Premature Ovarian Failure among Subfertile Patient in Albayda Fertility Centre/A Public Center in Libya, Scholars International Journal of Anatomy and Physiology, 10.36348/sijap.2020.v03i05.002

**Ensrud**, K.E., Crandall, C.J., 2017. Osteoporosis , Annals of Internal Medicine, 167(3).

**Ersoy E**, Ersoy AO, Yildirim G, Buyukkagnici U, Tokmak A, Yilmaz N. Vitamin D Levels in Patients with Premature Ovarian Failure. Ginekol Pol. 2016;87(1):32-6. [PubMed ID: 27306466]. https://doi.org/10.17772/gp/57839

**Feng**, Y Ge, Y Xie, W Xie, C Liu, L Li, D Ta, Q Jiang - Photoacoustics, 2021 Detection of collagen by multi-wavelength photoacoustic analysis as a biomarker for bone health assessment , Volume 24, 100296, https://doi.org/10.1016/j.pacs.2021.100296.

**Fernando** WD , Amanda Vincent Karen Magraith(2023), Premature ovarian insufficiency and infertility, doi: 10.31128/AJGP-08-22-6531.

**Gao,** X., Yamazaki, Y., Tezuka, Y., Onodera, Y., Ogata, H., Omata, K., Morimoto, R., Nakamura, Y., Satoh, F., & Sasano, H. (2019). The crosstalk between aldosterone and calcium metabolism in primary aldosteronism: a possible calcium metabolism-associated aberrant "neoplastic" steroidogenesis in adrenals. The Journal of Steroid Biochemistry and Molecular Biology, 193, 10543

**Garg A**., Patel, B., Abbara, A., & Dhillo, W. S. (2022). Treatments targeting neuroendocrine dysfunction in polycystic ovary syndrome (PCOS). Clinical Endocrinology, 97(2), 156–164. https://doi.org/10.1111/cen.14704

**Gargus E**. S., Bae, Y., Chen, J., Moss, K. J., Ingram, A. N., Zhang, J., Montgomery, N. T., Boots, C. E., Funk, W. E., & Woodruff, T. K. (2022). An Ovarian Steroid Metabolomic Pathway Analysis in Basal and Polycystic Ovary Syndrome (PCOS)-like Gonadotropin Conditions Reveals a Hyperandrogenic Phenotype Measured by Mass Spectrometry. Biomedicines, 10(7). https://doi.org/10.3390/biomedicines10071646

**Giri** R, AJ Vincent -, 2020, prevalence and risk factors of premature ovarian insufficiency/early menopause Seminars in Reproductive Medicine , Semin Reprod Med; 38(4): 237-246, DOI: 10.1055/s-0040-1722317.

**Glacken** SM -Byrne, GS Conway , Best Practice & Research Clinical Obstetrics & Gynaecology, Volume 81, Pages 98-110, https://doi.org/10.1016/j.bpobgyn.2021.09.011Get rights and content

**Golezar S**, Ramezani TF, Khazaei S, Ebadi A, Keshavarz Z. The global prevalence of primary ovarian insufficiency and early menopause: a meta-analysis. Climacteric (2019) 22:403–11. doi:10.1080/13697137.2019.1574738

**Golezar**, S., Keshavarz, Z., Tehrani, F. R., & Ebadi, A. (2020). An exploration of factors affecting the quality of life of women with primary ovarian insufficiency: a qualitative study. BMC women's health, 20(1), 1-9

**Gounden V**., Ellberg, C. C., & Jialal, I. (2021). Laboratory investigation of disorders of the pituitary gland. In Handbook of Diagnostic Endocrinology. INC. https://doi.org/10.1016/B978-0-12-818277-2.00002-9

**Grzesiak** M, 2020, Vitamin D3 action within the ovary–an updated reviewPhysiological research 69(3): 371–378 doi: 10.33549/physiolres.934266.

**Heidari** B, K Hajian-Tilaki, M Babaei - Diabetes & Metabolic Syndrome , 2020, Effectiveness and safety of routine calcium supplementation in postmenopausal women. A narrative review , 14( 4), 435-442.

**Henriksen** K, MA Karsdal ,2024, Biochemistry of collagens, laminins and elastin Chapter 1 - Type I collagen , Pages 1-11, https://doi.org/10.1016/B978-0-443-15617-5.00047.

**Herbison**, A. E. (2020). A simple model of estrous cycle negative and positive feedback regulation of GnRH secretion, Front Neuroendocrinol. 57:100837. doi: 10.1016/j.yfrne.2020.100837 .

https://doi.org/10.1016/j.biochi.2020.04.025

**Huang** Q, S Chen, J Chen, Q Shi, S Lin, 2022, Reproductive Biology and Endocrinology ,therapeutic options for premature ovarian insufficiency, , Volume 20, article number 28

**Ishizuka** B- Frontiers in endocrinology, 2021 ,Current understanding of the etiology, symptomatology, and treatment options in premature ovarian insufficiency (POI), https://doi.org/10.3389/fendo.2021.626924

**Iwase A**, Osuka S, Goto M, Murase T, Nakamura T, Takikawa S, et al. 2018, Clinical application of serum anti-Müllerian hormone as an ovarian reserve marker: a review of recent studies. J Obstet Gynaecol Res.; 44(6): 998–1006

**Jiao** X, T Meng, Y Zhai, L Zhao, W Luo, P Liu ,2021-Ovarian reserve markers

in premature ovarian insufficiency, within different clinical stages and different etiologies , Volume 12 ,| https://doi.org/10.3389/fendo.2021.601752

**Johansen** N, Angelica Lindén Hirschberg, Mette H. Moen, 2020, Journal of Obstetrics and Gynaecology Research,The role of testosterone in menopausal hormone treatment. What is the evidence? https://doi.org/10.1111/aogs.13819

**Kanbou**r, S. A., & Dobs, A. S. (2022). Hyperandrogenism in Women with Polycystic Ovarian Syndrome: Pathophysiology and Controversies. Androgens, 3(1), 22–30. https://doi.org/10.1089/andro.2021.0020

**Karpen** H**,** (2018)Mineral homeostasis and effects on bone mineralization in the preterm neonate health systematic review and meta-analysis, https://doi.org/10.1016/j.clp.2017.11.005.

**Kauffman** S ,2022, Neuroendocrine mechanisms underlying estrogen positive feedback and the LH surge, Neuroendocrine Science, Volume 16 https://doi.org/10.3389/fnins.2022.953252 .

**Kelly**, D. S., Sabharwal, S., Ramsey, D. J., & Morkin, M. I. (2023). The effects of female sex hormones on the human cornea across a woman ' s life cycle. BMC Ophthalmology, 1–10. https://doi.org/10.1186/s12886-023-03085-y

**Keys** A ,Karvonen N, Kimura N ,Taylor HL. Indices of relative weight and obesity. J Chronic Dis 1972;25:329–43. Reprinted Int J Epidemiol 2014;doi:10.1093/ije/dyu058

**Khole** V. J Midlife Health. (2010), Does ovarian autoimmunity play a role in the pathophysiology of premature ovarian insufficiency? Journal of Mid-life Health 1(1):p 9-13, Jan–Jun 2010. | DOI: 10.4103/0976-7800.66986

**Khudhair NY**, NK Saleh, MF Nazzal, Measuring the Levels of AMH, FSH, LH, TSH, Progesterone, Estrogen, Vitamin D, Calcium, and Magnesium in Women

with Premature Ovarian insufficiency - Journal of Pioneering Medical Sciences, 2024, 13( 1), p34, 10.61091/jpms202413106.

**Kim**, S. H., Lundgren, J. A., Patrie, J. T., Solorzano, C. M. B., & Mccartney, C. R. (2022). Acute progesterone feedback on gonadotropin secretion is not demonstrably altered in estradiol- - pretreated women with polycystic ovary syndrome. November 2021, 1–16. https://doi.org/10.14814/phy2.15233

**Kirshenbaum** M, Orvieto R. Premature ovarian insufficiency (POI) and autoimmunity-an update appraisal. J Assist Reprod Genet 2019;36:2207–2215

**Kish**i, H., Kitahara, Y., Imai, F., Nakao, K., & Suwa, H. (2018). Expression of the gonadotropin receptors during follicular development. Reproductive Medicine and Biology, 17(1), 11–19. https://doi.org/10.1002/rmb2.12075

**Kitano**, T., Takenaka, T., Takagi, H., Yoshiura, Y., Kazeto, Y., Hirai, T., Mukai, K., & Nozu, R. (2022). Roles of Gonadotropin Receptors in Sexual Development of Medaka. Cells, 11(3), 1–11. https://doi.org/10.3390/cells11030387

**Kurtoglu**-Aksoy N, Akhan SE, Bastu E, Gungor-Ugurlucan F, Telci A, Iyibozkurt AC, Topuz S Clin Exp Obstet Gynecol. 2014; Implications of

**Labrie** F., Martel, C., Bélanger, A., & Pelletier, G. (2017). Androgens in women are essentially made from DHEA in each peripheral tissue according to intracrinology. Journal of Steroid Biochemistry and Molecular Biology, 168(2016), 9–18. https://doi.org/10.1016/j.jsbmb.2017.12.007

**Lagergren** K., Hammar, M., Nedstrand, E., Bladh, M., & Sydsjö, G. (2018). The prevalence of primary ovarian insufficiency in Sweden; a national register study. BMC women's health, 18(1), 1-4

**Lee**, E. B., Chakravarthi, V. P., Wolfe, M. W., & Rumi, M. A. K. (2021). ER β Regulation of Gonadotropin Responses during Folliculogenesis., Reviews in

Endocrine and Metabolic Disorders, Int. J. Mol. Sci. 2021, 22(19), 10348; https://doi.org/10.3390/ijms221910348.

**Lerchbaum** E, OBERMAYER-PIETSCH B. Vitamin D and fertility: a systematic review. Eur J Endocrinol. 2012;166:765–778. doi: 10.1530/EJE-11-0984

**Liu** P**,** Xiruo Zhang, Jingmei Hu, Linlin Cui, Shidou Zhao, Xue Jiao, Yingying Qin,( 2020 ), Dysregulated cytokine profile associated with biochemical premature ovarian insufficiency, https://doi.org/10.1111/aji.13292

**Liu,** K**.,** Guo, C., Lao, Y., Yang, J., Chen, F., Zhao, Y., & Yang, Y. (2020). A fine-tuning mechanism underlying self-control for autophagy : deSUMOylation of BECN1 by SENP3. Autophagy, 16(6), 975–990. https://doi.org/10.1080/15548627.2019.1647944

**Manocha A**., Kankra, M., Singla, P., Sharma, A., Ahirwar, A. K., & Bhargava, S. (2018). Current Medicine Research and Practice Clinical signi fi cance of reproductive hormones. https://doi.org/10.1016/j.cmrp.2018.05.006

markers to maximize the ability to conceive with residual follicles,Reproductive Medicine and biology, 22(1) https://doi.org/10.1002/rmb2.12556.

**Mccartne**y, C. R., Campbell, R. E., Marshall, J. C., & Moenter, S. M. (2022). The role of gonadotropin- - releasing hormone neurons in polycystic ovary syndrome. August 2021, 1–13. https://doi.org/10.1111/jne.13093

**McNamara** LM, Current Osteoporosis Reports, Osteocytes and Estrogen Deficiency, 19, 592–603

**Meczekalski B**,Olga Niwczyk ,Gregory Bala and Anna Szeliga , Managing Early Onset Osteoporosis: The Impact of Premature Ovarian Insufficiency on Bone Health,2023, J. Clin. Med. 2023, 12(12), 4042; https://doi.org/10.3390/jcm12124042

**Mishra** GD, Hsin-Fang Chung a, Antonio Cano b, Peter Chedraui c, Dimitrios G. Goulis d, Patrice Lopes e f, Alfred Mueck g, Margaret Rees h, Levent M. Senturk i, Tommaso Simoncini j, John C. Stevenson k, Petra Stute l, Pauliina Tuomikoski m, Irene Lambrinoudaki n, EMAS position statement: Predictors of premature and early natural menopause,2019 https://doi.org/10.1016/j.maturitas.2019.03.008

**Moolhuijsen** LME, JA Visser -, 2020, Anti-Müllerian hormone and ovarian reserve: update on assessing ovarian function, The Journal of Clinical Endocrinology & Metabolism, Volume 105(11), Pages 3361–3373, https://doi.org/10.1210/clinem/dgaa513

**Muscogiuri** G, ALTIERI B, De ANGELIS C, PALOMBA S, PIVONELLO R, COLAO A, ORIO F. Shedding new light on female fertility: The role of vitamin D. Rev Endocr Metab Disord. 2017;18:273–283. doi: 10.1007/s11154-017-9407-2.

**Nagham H**. Ali, Madlen Qassm Abbas (2021). ARTICLE REVIEW : HORMONAL CHANGES IN WOMEN WITH PCOS, Journal NX 7(3),29–39.

**Nawawi**, TN Yazid, NM Ismail, 2001 -s erum bone specific alkaline phosphatase and urinary deoxypyridinoline in postmenopausal osteoporosis

**Nelson** N, Susan R Davis, Sophia Kalantaridou , Mary Ann Lumsden , Nick Panay, Richard A Anderson,2023, Anti-Müllerian hormone for the diagnosis and prediction of menopause, Human Reproduction Update, Volume 29, Issue 3, Pages 327–346, https://doi.org/10.1093/humupd/dmac04

**Njoroge** J, Tressel, W., Biggs, M. L., Matsumoto, A. M., Smith, N. L., Rosenberg, E., Hirsch, C. H., Gottdiener, J. S., Mukamal, K. J., & Kizer, J. R. (2022). Circulating Androgen Concentrations and Risk of Incident Heart Failure in Older Men: The Cardiovascular Health Study. Journal of the American Heart Association, 11(21). https://doi.org/10.1161/JAHA.122.026953

**O'Neill E**, G Awale, L Daneshmandi, O Umerah - Drug discovery today, 2018, The roles of ions on bone regeneration, 23(4), , Pages 879-890, https://doi.org/10.1016/j.drudis.2018.01.049.

**Osuka** S, Y Kasahara, S Iyoshi ,2023,Follicle development and its prediction in patients with primary ovarian insufficiency: Possible treatments and

**Panay N**, Anderson RA, Nappi RE, Vincent AJ, Vujovic S, Webber L, Wolfman W. Premature ovarian insufficiency: an international menopause society white paper. Climacteric 2020;23:426–446

**Pankiewicz** K, P Laudański, T Issat, 2021, the role of noncoding RNA in the pathophysiology and treatment of premature ovarian insufficiency- MDPI journal ,Int. J. Mol. Sci. 2021, 22(17), 9336; https://doi.org/10.3390/ijms22179336.

**Pinelli** S, Paolo Giovanni Artini, Stefano Basile, Maria Elena Rosa Obino, Claudia Sergiampietri, Diana Giannarelli, Giovanna Simi & Vito Cela,2018, Estrogen treatment in infertile women with premature ovarian insufficiency in transitional phase: a retrospective analysis, Journal of Assisted Reproduction and Genetics, Volume 35, pages 475–482 .

**Podfigurna A** 1, Marzena Maciejewska-Jeske ,Malgorzata Nadolna ,Paula Mikolajska-Ptas ,Anna Szeliga ,Przemyslaw Bilinski,Paulina Napierala and Blazej Meczekalski , 2020, impact of hormonal replacement therapy on bone mineral density in premature ovarian insufficiency patients J. Clin. Med., 9(12), 3961; https://doi.org/10.3390/jcm9123961

premature ovarian failure on bone turnover markers and bone mineral41(2):14-53, ,

**Punchoo** R, S Bhoora - Frontiers in Endocrinology, 2021, variation in the Measurement of Anti-Müllerian Hormone–What Are the Laboratory Issue? 2021

May;99(5):637-650. doi: 10.1007/s00109-021-02055-5. Epub 2021 Feb 27.PMID: 336410662021, 35, 101600.

**Purdue-**Smithe AC, Whitcomb BW, Manson JE, Hankinson SE, Troy LM, Rosner BA, et al. Vitamin D Status Is Not Associated with Risk of Early Menopause. J Nutr. 2018;148(9):1445-52. [PubMed ID: 30107585]. [PubMed Central ID: PMC6454521]. https://doi.org/10.1093/jn/nxy129.

**Qiao** J ,KL Hu, FT Liu, H Xu, R Li, -, 2020, High antimüllerian hormone levels are associated with preterm delivery in patients with polycystic ovary syndrome, Journal of Fertility and Sterility,113(2),Pages 444-452.e1, https://doi.org/10.1016/j.fertnstert.2019.09.039

**Rahman** F, Maria Uloko Leah Ibrahim Puri & Rachel S. Rubin,2022, The clinical management of testosterone replacement therapy in postmenopausal women with hypoactive sexual desire disorder, International Journal of Impotence Research volume 34, pages635–641

**Rahman**, R.; Panay, N. Diagnosis and management of premature ovarian insufficiency. Best Pract. Res. Clin. Endocrinol. Metab, 35(6), https://doi.org/10.1016/j.beem.2021.101600.

**Ribeiro** Larissa M, Lizandra M.P. Sasaki, Adelino A. Silva c, Elivan S. Souza d, Amanda Oliveira Lyrio d, Ana C.M.G. Figueiredo d, Leila B.D. Gottems d,2022, Overweight, obesity and assisted reproduction: A systematic review and meta-analysis, Pages 117-127 . https://doi.org/10.1016/j.ejogrb.2022.01.019

**Ruddenklau,** A., & Campbell, R. E. (2019). Neuroendocrine Impairments of Polycystic Ovary Syndrome. Endocrinology, 160(10), 2230–2242. https://doi.org/10.1210/en.2019-00428

**Salokhiddinovna**, X. Y. (2023). Clinical Features of the Course of Vitamin D Deficiency in Women of Reproductive Age. EUROPEAN JOURNAL OF

INNOVATION IN NONFORMAL EDUCATION, 3(11), 28–31. Retrieved from http://www.inovatus.es/index.php/ejine/article/view/2049

**Schmitz** D., Ek, W. E., Berggren, E., Höglund, J., Karlsson, T., & Johansson, Å. (2021). Genome-wide Association Study of Estradiol Levels and the Causal Effect of Estradiol on Bone Mineral Density. The Journal of Clinical Endocrinology & Metabolism, 106(11), e4471–e4486.

**Seema** Rani ,Sanchita Bandyopadhyay-Ghosh, ORCID, Subrata Bandhu Ghosh and Guozhen Liu, Advances in Sensing Technologies for Monitoring of Bone Health,2020 , 10(4), https://doi.org/10.3390/bios10040042., 10(4).

**Segheto** KJ, M Pereira, DCG Silva… - Ciência & Saúde …, 2021 Vitamin D and bone health in adults: a systematic review and meta-analysis, https://doi.org/10.1590/1413-81232021268.15012020 .

**Shlisky** J, R Mandlik, S Askari, S Abrams, JM Belizan – 2022, The New York Academy of Sciences, Calcium deficiency worldwide: prevalence of inadequate intakes and associated health outcomes1512(1), Pages 10-28.

**Silva** CA, Yamakami LY, Aikawa NE, Araujo DB, Carvalho JF, Bonfa E. Autoimmune primary ovarian insufficiency. Autoimmune Rev 2014;13:427–430

**Silvestri**s,G De Pergola, R Rosania, (2018). Obesity as disruptor of the female fertility, Reproductive biology and Endocrinology, Volume 16, article number 22.

**Singh D**, V Rai, DK Agrawal – National Library of Medicine, Regulation of Collagen I and Collagen III in Tissue Injury and Regeneration, 2023; 7(1): 5–16, doi: 10.26502/fccm.92920302

**Singh** S., Pal, N., Shubham, S., Sarma, D. K., & Verma, V. (2023). Polycystic Ovary Syndrome : Etiology , Current Management , and Future Therapeutics, J. Clin. Med. 2023, 12(4), 1454; https://doi.org/10.3390/jcm12041454.

**Smith** JA, Vitale S, Reed GF, Grieshaber SA, Goodman LA, Vanderhoof VH, Calis KA, Nelson LM. Dry eye signs and symptoms in women with premature ovarian failure. *Arch Ophthalmol* 2004;122: 151-156.

**Sun,** Y., Li, S., Liu, H., Bai, H., Hu, K., Zhang, R., Liu, Q., & Fan, P. (2021). Oxidative stress promotes hyperandrogenism by reducing sex hormone-binding globulin in polycystic ovary syndrome. Fertility and Sterility, 116(6), 1641–1650. https://doi.org/10.1016/j.fertnstert.2021.07.1203 .

**Takahashi**, A Yousif, L Hong, II Chefetz - Journal of Molecular Medicine, 2021 , premature ovarian insufficiency: pathogenesis and therapeutic potential of mesenchymal stem cell,41(13), 1734–1743.

**Takahashi,** Abdelrahman Yousif, Linda Hong & IIana Chefetz, Premature ovarian insufficiency: pathogenesis and therapeutic potential of mesenchymal stem cell Review 99, 637–650, (2021)..

**Tariq** S, S Tariq, KP Lone, S Khaliq -, 2019, Alkaline phosphatase is a predictor of Bone Mineral Density in postmenopausal female ,Pakistan journal of medical, Pak J Med Sci. 2019 May-Jun; 35(3): 749–753. doi: 10.12669/pjms.35.3.188

**Tosi** F., Villani, M., Migazzi, M., Faccin, G., Garofalo, S., Fiers, T., Kaufman, J. M., Bonora, E., & Moghetti, P. (2021). Insulin-Mediated Substrate Use in Women with Different Phenotypes of PCOS: The Role of Androgens. Journal of Clinical Endocrinology and Metabolism, 106(9), E3414–E3425. https://doi.org/10.1210/clinem/dgab380

**US** Department of Health and Human Services, Bone Health and Osteoporosis: A Report of the Surgeon General, vol. 437, US Department of Health and Human Services, Washington, DC, USA, 2004.

**Viguet** S-Carrin, P Garnero, PD Delmas, 2006 ,The role of collagen in bone strength, , Osteoporosis international, Volume 17, pages 319–336, (2006).

**Vimalraj** S**,** 2020, Alkaline phosphatase: Structure, expression and its function in bone mineralization, https://doi.org/10.1016/j.gene.2020.144855 .

**Wang C,**Yun Sun**,** Induction of Collagen I by CXCL10 in Ovarian Theca–Stroma Cells via the JNK Pathway, ORIGINAL RESEARCH article 13 - 2022 | https://doi.org/10.3389/fendo.2022.823740.

**Webber** L, Davies M, Anderson R, Bartlett J, Braat D, Cartwright B, Cifkova R, de Muinck Keizer-Schrama S, Hogervorst E, Janse F et al. ESHRE guideline: management of women with premature ovarian insufficiency. Hum Reprod 2016;31:926–937

**Wesevich,** V., Kellen, A. N., and Pal, L. (2020). Recent advances in understanding primary ovarian insufficiency. F1000Res 9::1101.doi: 10.12688/f1000research.26423.1

**Wu** J, Y Zhuo, Y Liu, Y Chen, Y Ning, J Yao -, **a**ssociation between premature ovarian insufficiency and gut microbiota , BMC Pregnancy and Childbirth , Volume 21, article number 418, (2021)

**Xu**, X., Deng, S., & Lian, Z. (2021). Estrogen Receptors in Polycystic Ovary Syndrome, 10(2), 459; https://doi.org/10.3390/cells10020459

**Yeganeh** L, Boyle JA, Wood A, Teede H, Vincent AJ,2019, Menopause guideline appraisal and algorithm development for premature ovarian insufficiency. Maturitas ;130:21–31

**Yoon** SH, YM Choi, MA Hong, JJ Kimstudy of anti-Müllerian hormone and anti-Müllerian hormone type II receptor polymorphisms with idiopathic primary ovarian insufficiency Human Reproduction, Volume 28, Issue 12, December 2013, Pages 3301–3305.

**Zamir** Amit , Tavor Ben-Zeev and Jay R. Manipulation of Dietary Intake on Changes in Circulating Testosterone Concentrations Hoffman Nutrients 2021, 13(10), 3375; https://doi.org/10.3390/nu13103375

**Zhang** T, M Chen, K Yao, X Huang, B Zhang, 2021 , Bone physiological microenvironment and healing mechanism: Basis for future bone-tissue engineering scaffolds, - Bioactive materials ,Volume 86, pp. 119-130, https://doi.org/10.1016/j.bioactmat.2021.03.043.

# Appendices

# Appendix 1

**Questioner for participants**

**Name: _____. Age:.....**

**Number of telephone:...**

**Sports:... smoking:...**

**How long have you been wed?**

**Height:... Weight:...**

**Do you experience difficulties becoming or staying pregnant?**

**Is there a fetus miscarriage:….**

**What is the average number of days that a period bleeds?**

**Exists dysmenorrhea in you? Yes \No**

**Do you suffer from dyspareunia? Sure \Nay**

**Do you currently have a kidney, bladder, or vaginal infection?Yes/No**

**Have you ever had abnormal hair growth? Sure/No**

**Have you gained or lost weight?**

**Have your tolerance to heat or cold, your skin tone, the color of your nails, or the growth of body hair changed in any way?**

**Do you have depressive symptoms like melancholy, incessant crying or wrath, or emotional instability?**

**Have you ever used medication to control your menstrual cycle?**

**When was the latest instance?**

**Do you use any medications to control your sex hormones? In agreement or disagreement**

**Which of the following have you ever experienced?**

**Diabetes mellitus: Sure/No**

## Appendix 2

**Components of LH kit**

| Reagents | Contents | Concentration |
|---|---|---|
| M | Crystal transparent cap streptavidin-coated microparticles, 0.72 mg/mL, 1 container (6.5 mL), preservation | 6.5 mL. |
| R1 | Preservative, 8.0-pH TRIS buffer, and biotinylated mouse monoclonal anti-LH antibody are the ingredients. vial of R1 Anti-LH-Ab-Biotin with a gray cap. | 10 mL |
| R2 | Anti-LH-AbRu(bpy) in a container with a black cap; ruthenium complex containing 0.3 mg/ml of monoclonal anti-mouse LH antibody; pH 8.0; TRIS buffer 50 mmol/L; preservative | 10 mL |

## Appendix 3

**Components of Follicular Stimulating Hormone kit**

| Reagents | Contents | Concentration |
|---|---|---|
| M | Microparticles coated with a preservation agent and streptavidin (clear cap), 0.72 mg/mL, 1 container (6.5 mL). | 6.5 mL. |
| R1 | One vial of anti-FSH-Abbiotin (gray cap); one bottle of biotinylated monoclonal anti-FSH antibody (mouse); buffer, pH 6.0, 50 mmol/L; preservatives; | 10 mL |
| R 2 | mouse anti-FSH monoclonal antibody containing 0.8 mg/L of ruthenium compound (anti-FSH-Ab-Ru(bpy)) in a black-capped container with 50 mmol/L of MES buffer at pH 6.0 and preservatives | 10 mL |

## Appendix 4

**components of Anti Mullerian Hormone kit**

| Materials provided with the kit | 48determinations | 96 determinations | Storage |
|---|---|---|---|
| User manual | 1 | 1 | |
| Closure plate membrane | 2 | 2 | |
| Sealed bags | 1 | 1 | |
| Microelisa stripplate | 1 | 1 | 2-8℃ |
| Standard: 27ng/ml | 0.5ml×1 bottle | 0.5ml×1 bottle | 2-8℃ |
| Standard diluent | 1.5ml×1 bottle | 1.5ml×1 bottle | 2-8℃ |
| HRP-Conjugate reagent | 3ml×1 bottle | 6ml×1 bottle | 2-8℃ |
| Sample diluent | 3ml×1 bottle | 6ml×1 bottle | 2-8℃ |
| Chromogen Solution A | 3ml×1 bottle | 6ml×1 bottle | 2-8℃ |
| Chromogen Solution B | 3ml×1 bottle | 6ml×1 bottle | 2-8℃ |
| Stop Solution | 3ml×1 bottle | 6ml×1 bottle | 2-8℃ |
| wash solution | （20ml×20 fold）×1bottle | （20ml×30 fold）×1bottle | 2-8℃ |

## Appendix 5

**Free testosterone kit ingredients**

| Integral of the Reagents Used in 100 tests | |
|---|---|
| Polyclonal antibody against sheep FITC (1.2% (W/V) TRIS, 0.2% (NaN3) NaN3) coated nano magnetic microbeads. | 2.5 ml |
| A calibrator with limited precision is made from cow serum containing 0.2 percent sodium nitrite. | 2.5 ml |
| Utilizing 0.2% sodium nitrite in cow serum serves as a high-precision calibrator. | 2.5 ml |
| FITC-labeled TEST antigen, 0.2% sodium nitrite, bovine serum albumin, and 0.2% sodium nitrite | 6.5ml |

| | |
|---|---|
| Bovine serum albumin, anti-TEST monoclonal antibody labeled with ABEI, and 0.2% sodium nitrite. | 6.5ml |
| Reagents of every kind are available and prepared for use | |

## Appendix 6

**The constituents of Bone-specific alkaline phosphatase(BALP) kit**

| **Materials provided with the Kit** | **48 Determinations** | **96 Determinations** | **storage** |
|---|---|---|---|
| User manual | **1** | **1** | |
| Closure plate membrane | **2** | **2** | |
| Sealed bags | 1 | 1 | |
| Microelisa stripplate | 1 | 1 | 2-8 ℃ |
| Standard：135 ng/L | (0.5ml×1 bottle) | (0.5ml×1 bottle) | 2-8 ℃ |
| Standard diluent | (1.5ml×1 bottle) | (1.5ml×1 bottle) | 2-8 ℃ |
| HRP-Conjugate reagent | (3ml×1 bottle) | (6ml×1 bottle) | 2-8 ℃ |
| Sample diluent | (3ml×1 bottle) | (6ml×1 bottle) | 2-8 ℃ |
| Chromogen Solution A | (3ml×1 bottle) | (6ml×1 bottle) | 2-8 ℃ |
| Chromogen Solution B | (3ml×1 bottle) | (6ml×1 bottle) | 2-8 ℃ |
| Solution to Stop | (3ml×1 bottle) | (6ml×1 bottle) | 2-8 ℃ |
| washing solution | (20ml×20 fold) ×1bottle | (20ml×30 fold) ×1bottle ×1bottle | 2-8 ℃ |

# Appendix 7

### Constituents of the Human Col Ⅰ kit

| Materials provided with the Kit | 48 Determinations | 96 Determinations | storage |
|---|---|---|---|
| User manual | **1** | **1** | |
| Closure plate membrane | **2** | **2** | |
| Sealed bags | 1 | 1 | |
| Microelisa stripplate | 1 | 1 | 2-8 ℃ |
| Standard：45 ug/L | (0.5ml×1 bottle) | (0.5ml×1 bottle) | 2-8 ℃ |
| Standard diluent | (1.5ml×1 bottle) | (1.5ml×1 bottle) | 2-8 ℃ |
| HRP-Conjugate reagent | (3ml×1 bottle) | (6ml×1 bottle) | 2-8 ℃ |
| Sample diluent | (3ml×1 bottle) | (6ml×1 bottle) | 2-8 ℃ |
| Chromogen Solution A | (3ml×1 bottle) | (6ml×1 bottle) | 2-8 ℃ |
| Chromogen Solution B | (3ml×1 bottle) | (6ml×1 bottle) | 2-8 ℃ |
| Solution Stop | (3ml×1 bottle) | (6ml×1 bottle) | 2-8 ℃ |
| washing solution | (20ml×20 fold) ×1bottle | (20ml×30 fold) ×1bottle ×1bottle | 2-8 ℃ |

# Appendix 8

### The constituents of Vitamin D3 kit

| Materials provided with the Kit | 48 Determinations | 96 Determinations | storage |
|---|---|---|---|
| User manual | 1 | 1 | |
| Closure plate membrane | 2 | 2 | |
| Sealed bags | 1 | 1 | |
| Microelisa stripplate | 1 | 1 | 2-8 ℃ |
| Standard：45 ug/L | (0.5ml×1 bottle) | (0.5ml×1 bottle) | 2-8 ℃ |
| Standard diluent | (1.5ml×1 bottle) | (1.5ml×1 bottle) | 2-8 ℃ |
| HRP-Conjugate reagent | (3ml×1 bottle) | (6ml×1 bottle) | 2-8 ℃ |

| | | | |
|---|---|---|---|
| Sample diluent | (3ml×1 bottle) | (6ml×1 bottle) | 2-8 ℃ |
| Chromogen Solution A | (3ml×1 bottle) | (6ml×1 bottle) | 2-8 ℃ |
| Chromogen Solution B | (3ml×1 bottle) | (6ml×1 bottle) | 2-8 ℃ |
| Solution Stop | (3ml×1 bottle) | (6ml×1 bottle) | 2-8 ℃ |
| washing solution | (20ml×20 fold) ×1bottle | (20ml×30 fold） ×1bottle ×1bottle | 2-8 ℃ |

# الخلاصة

هدفت هذه الدراسة إلى دراسة العلاقة بين قصور المبيض المبكر (POI) وصحة العظام وتقييم آثار قصور المبيض المبكر (POI) على النساء في محافظة كربلاء من خلال قياس بعض المعالم (مؤشر كتلة الجسم ، الهرمون المضاد لمولر، الكولاجين نوع 1، الفوسفاتيز القلوي الخاص بالعظام (BALP) ، فيتامين D3، الهرمون المنبه للجريبات، الهرمون اللوتيني، التستوستيرون، الكالسيوم Ca+2، والإستراديول (E2) في الدم.

تم إجراء الدراسة في عدة عيادات خاصة ووحدة العقم في مستشفى أمراض النساء والتوليد التعليمي في كربلاء بين نوفمبر 2023 وأبريل 2024. تم تضمين 100 امرأة في الدراسة تتراوح أعمارهن بين الثامنة عشرة والأربعين. تم تضمين 50 أنثى بدون مرض قصور المبايض المبكر (مجموعة سيطرة) 50 أنثى مع مرض قصور المبايض (المرضى). تم قياس المعايير الكيموحيوية التي تم قياسها وهي (AMH، الكولاجين نوع 1، الفوسفاتيز القلوي الخاص بالعظام (BALP)، فيتامين D3، FSH، LH، التستوستيرون، الكالسيوم ca+2 واستراديول (E2). تم استخدام أمصال المرضى ومجموعة السيطرة لتحديد هذه المعالم

أظهرت نتائج هذه الدراسة وجود تغيرات ذات دلالة إحصائية (P<0.007)) في متوسط قيم مؤشر كتلة الجسم بين مجموعة السيطرة (25.87±2.56) ومجموعة المرضى (27.88±4.52).كما أظهرت النتائج زيادة معنوية (P<0.000) في مستويات الهرمون المنبه للجريبات( FSH23.56±25.79) لدى النساء ذوات قصور المبايض المبكر مقارنة بمجموعة السيطرة (5.39±3.84)، وكذلك زيادة معنوية (P<0.034 )في مستويات الهرمون اللوتيني LHعند النساء اللاتي يعانين من قصور المبايض المبكر. (12.95±11.46) مقارنة بمجموعة السيطرة (8.54±8.92).

أظهرت النتائج انخفاضا معنويا (P<0.000) في مستويات الاستراديول لمجموعة مرضى قصور المبايض المبكر(16.54 ±20.49) مقارنة بمجموعة السيطرة (87.59±66.61) وكذلك هناك انخفاض معنوي (P<0.012) في مستويات التستوستيرون الحر لمجموعة المرضى (0,15± 0,15) مقارنة بمجموعة السيطرة (0.24 ± 0.20).

وفقا لنتائج هذه الدراسة هناك انخفاض معنوي (P<0.000) في مستويات فيتامين D3 لمجموعة المرضى (4.55±2.12)بالمقارنة مع مجموعة السيطرة (6.39±1.52)، كما كان هناك انخفاض معنوي (P<0.022). في مستويات الكالسيوم لمجموعة المرضى (1.8.21±1.34) مقارنة بمجموعة السيطرة (8.67±0.41).

كما أظهرت نتائج الدراسة أنه بالمقارنة مع مجموعة السيطرة (4.56 ± 1.09)، فإن مجموعة المرضى لديهم انخفاض معنوي في مستويات AMH (P<0.000) .

تم قياس مستويات بعض المعالم المحددة لصحة العظام في هذه الدراسة لدراسة تأثير قصور المبايض المبكر على هذه المعالم. أظهرت النتائج وجود انخفاض معنوي (P<0.000) في مستويات (BALP) للمرضى (8.63±4.39)مقارنة بمجموعة السيطرة (15.88±3.60)، كما أظهرت النتائج وجود انخفاض معنوي (P<0.000) ) في مستويات الكولاجين من النوع 1 لدى مجموعة المرضى (1.57 ± 0.85) مقارنة مع مجموعة السيطرة (2.33 ± 0.74)

واظهرت الدراسة وجود علاقات ارتباطية أخرى بين مجموعات فرعية داخل الدراسة، مثل وجود علاقة طردية (P<0.01) بين الكولاجين 1 وAMH، AMH وBALP، AMH وفيتامين D3،

الكولاجين 1 وBALP، الكولاجين 1 و فيتامين D3 و BALP و فيتامين D3 و FSH و LH .كما توجد علاقة طردية ($P<0.05$) بين Ca+2 و FSH و Ca+2 و LH.

كما أظهرت الدراسة وجود علاقة عكسية ($P<0.05$) بين هرمون الاستروجين وهرمون FSH، أي أنه عند زيادة هرمون FSH يحدث انخفاض في مستوى هرمون الاستروجين وبالعكس.

توجد علاقات بين المعالم الأخرى في الدراسة ولكنها كانت غير دالة إحصائياً.

**جامعة كربلاء**

**كلية العلوم الطبية التطبيقية**

# العلاقة بين قصور المبايض المبكر وصحة العظام لدى النساء في محافظة كربلاء

**رسالة مقدمة**

**الى مجلس كلية العلوم الطبية التطبيقية _جامعة كربلاء**

**وهي جزء من متطلبات نيل درجة الماجستير في علوم التحليلات المرضية**

**من قبل**

**حنان نجدي كاظم**

بكالوريوس تحليلات مرضية

كلية العلوم الطبية التطبيقية/جامعة كربلاء،2019

**بأشراف**

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الدكتور الاختصاص

**حميدة هادي عبد الواحد**

**2024 م**