FIRAT UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
TÜRKİYE

EVALUATION OF TOTAL SERUM ANTIOXIDANT AND SOME BIOCHEMICAL PARAMETERS IN WOMEN WITH OSTEOPOROSIS

Kawar Farsat Ali HUSSEIN

Master's Thesis

DEPARTMENT OF CHEMISTRY

Biochemistry

AUGUST 2020

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TÜRKİYE

Chemistry Department
Program: Biochemistry

Master's Thesis

Title:	Evaluation of Total Serum Antioxidant and Some Biochemical Parameters in Women with Osteoporosis
Author:	Kawar Farsat Ali Hussein
Submission Date:	29.07.2020
Defense Date:	27.08.2020

THESIS APPROVAL

This thesis, which was prepared according to the thesis writing rules of the Graduate School of Natural and Applied Sciences, Fırat University, was evaluated by the committee members who have signed the following signatures and was unanimously approved after the defense exam made open to the academic audience.

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DECLARATION

I hereby declare that I wrote this master's thesis titled "Evaluation of total serum antioxidant and some biochemical parameters in women wiyh osteoporosis" inconsistent with the thesis writing guide of the Graduate School of Natural and Applied Sciences, Firat University. I also declare that all information in it is correct, that I acted according to scientific ethics in producing and presenting the findings, cited all the references I used, express all institutions or organizations or persons who supported the thesis financially. I have never used data and information. I provide here to get a degree in any way.

August 2020

Kawar Farsat Ali HUSSEIN

PREFACE

First, praises and thanks to the ALLAH, the most merciful, for His showers of blessings throughout our research work to complete the research successfully.

This clinical biochemistry thesis was about antioxidant level in women with osteoporosis was taken from serum patients to know the relationship between osteoporosis and antioxidant, there was no difficulties in my research way.

I would like to thank and express my deep and sincere gratitude to my supervisor Doç.Dr.Mehmet Mürşit TEMÜZ for his continuous support, guidance and encouragement. In addition to being an excellent supervisor.

In addition, I would like to thank Prof. Dr. Mustafa KARATEPE for their advice. The loyal realization for Dr. Dhia Mustafa Suleiman for his guidance.

I would like to express my deep feeling of appreciation to all members and staff of "Duhok Center of Rheumatoid Disease and medical Rehabilitation" where the data of this study was obtained.

Many thanks to the staff of "Dr. Awny lab" and "Mazi Medical Laboratory", where I previously worked on their laboratories machines. Sincere appreciation and thanks to my dear mother and dear late-father that help me in all filed of my life.

Many thanks to my dear sisters and dear brothers for their kindness. Special thanks to my dear wife that aid me and always with me.

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ABSTRACT

Evaluation of Total Serum Antioxidant and Some Biochemical Parameters in Women with Osteoporosis

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Background and objectives: Osteoporosis is a condition of increased bone loss, which leads to increased bone fragility and a marked increase in the risk of fractures. Oxidative stress has been proved involved in bone resorption. Therefore, this research aims at comparing the serum levels of total antioxidative status between women with and without osteoporosis. Moreover, the influence of vitamin D, calcium, vitamin B12, and folic acid were also examined.

Methods: 106 females who attended the Duhok rheumatoid center and conducted bone densitometry participating in this cross-sectional survey. The liver diseases, metabolic bone diseases, chronic kidney diseases, gastrointestinal diseases, chronic inflammation, thalassemia, cancer, and patients who were taking antioxidant or hormone replacement therapy where prevented or excluded from the study. A research questionnaire was used to organize contributors with the required information. By using automated Cobas 6000, serum total vitamin D, serum calcium, vitamin B12, folic acid levels were determined and the total antioxidant level was determined by spectrophotometer.

Results: In osteoporotic individuals, mean values levels for total antioxidant and folic acid were significantly decrease compared with the control group. The vitamin B12 and calcium levels were not changed. Vitamin D did not demonstrate any large variation among the study population in groups with a high incidence of vitamin D insufficiency. The total antioxidant level positively corresponded with total bone mineral density in the entire study population as stated by Pearson correlation analysis. In the sample of women; age was negatively related to total bone mineral density, also in this investigation population body mass index was positively related to total bone mineral density. The folic acid in the patients were positively corresponded.

Conclusions: The study findings of this survey demonstrate that oxidative stress may be a consequential indicator of bone loss in postmenopausal women.

Key word: Antioxidant, Oxidant, Oxidative stress, Vitamin D, Calcium, Vitamin B12, Folic Acid, Osteoporosis.

ÖZET

Osteoporozlu Kadinlarda Total Serum Antioksidan ve Bazi Biyokimyasal Parametrelerin Değerlendirilmesi

Kawar Farsat Ali HUSSEIN

Yüksek Lisans Tezi

FIRAT ÜNİVERSİTESİ
Fen Bilimleri Enstitüsü

Kimya Anabilim Dalı

Biyokimya
Ağustos 2020, Sayfa: xiii+51

Giriş ve hedefler: Osteoporoz, kemik kırılganlığının artmasına ve kırık riskinde belirgin bir artışa yol açan artmış kemik kaybının durumudur. Oksidatif stresin kemik rezorpsiyonu ile ilişkili olduğu kanıtlanmıştır. Bu nedenle, bu araştırma osteoporozlu ve osteoporozsuz kadınlar arasındaki total antioksidatif durumun serum düzeylerini karşılaştırmayı amaçlamaktadır. Ayrıca D vitamini, kalsiyum, B12 vitamini ve folik asidin etkisi de incelenmiştir.

Yöntemler: Duhok romatoid merkezinde kemik dansitometrisi uygulayan 106 kadın bu araştermaya katıldı. Karaciğer hastalıkları, metabolik kemik hastalıkları, kronik böbrek hastalıkları, gastrointestinal hastalıklar, kronik inflamasyon, talasemi, kanser ve antioksidan veya hormon replasman tedavisi alan hastalar çalışmaya dahil edilmedi.Katılımcıları düzenlemek için gerekli bilgileri içeren bir araştırma anketi kullanıldı. Otomatik Cobas 6000 ile serum toplam D vitamini, serum kalsiyum, B12 vitamini, folik asit seviyeleri tayin edildi ve toplam antioksidan seviyesi spektrofotometre ile değerlendirildi.

Sonuçlar: Osteoporotik bireylerde, toplam antioksidan ortalama değerleri ve folik asit için seviyeleri kontrol grubu ile kayaşlandığende önemli ölçüde azalmıştır. B12 vitamini ve kalsiyum seviyeleri düzeyleri değişmedi. D vitamini yetmezliği insidansının yüksek olduğu gruplarda çalışma popülasyonu arasında büyük bir değişiklik göstermedi. Toplam antioksidan seviyesi Pearson korelasyon analizi ile belirtildiği gibi tüm çalışma popülasyonunda toplam kemik mineral yoğunluğuna pozitif olarak karşılık geldi. Kadın örnekleminde; yaş toplam kemik mineral yoğunluğu ile negatif ilişkiliydi, bu araştırmada da popülasyonun vücut kitle indeksi toplam kemik mineral yoğunluğu ile pozitif ilişkiliydi. Hastalaraledi folik asit seviyeleri pozitif yünde karşılık geldi.

Sonuçlar: Bu araştırmanın bulguları, oksidatif stresin postmenopozal kadınlarda kemik kaybının bir sonucu olabileceğini göstermektedir.

Anahtar Kelimeler: Antioksidan, Oksidan, Oksidatif stres, D Vitamini, Kalsiyum, B12 Vitamini, Folik Asit, Osteoporoz.

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ABBREVIATIONS

Abbreviations

BMD	: Bone Mineral Density
BMI	: Body mass index
DXA (DEXA)	: Densitometry X-ray (Dual-energy X-Ray absorptiometry)
FA	: Folic Acid
MTHFR	: Methylene Tetrahydrofolate Reductase
NS	: No Significant
OP	: Osteoporosis
OS	: Oxidative Stress
PBM	: Peak Bone Mass
PMO	: Post-Menopausal Osteoporosis
RNS	: Reactive nitrogen species
ROS	: Reactive oxygen species
SD	: Standard deviation
TAS	: Total antioxidant status
WC	: Waist circumference
WHI	: Women's Health Initiative
WHO	: World Health Organization

1. INTRODUCTION

Osteoporosis is an endless and multifactorial confusion that described via low bone mass and micro-architectural weakening of bones [1]. It has been documented that bone break is an important consequence of osteoporosis [2].The Osteoporosis pathologic process is imperfectly understood. Furthermore essential factors are aging and genetic factors, in addition to these factors there are many factors for instance inhalation of cigarette, disable to move, inadequate consumption of calcium, problems of the thyroid gland, and complaint in the task of parathyroid, troubles in the tract of (GI) gastrointestinal, renal sickness [3], race, sex, previous fractures, long-standing laziness, microgravity, more than is necessary physical activity, underweight, hormones, drugs, oxidative stress-linked factors, low antioxidant level, more than necessary eating lipid, food inadequacy give rise to osteoporosis (OP) [4, 5]. Osteoporosis is the fourth-factor minatory human health after heart failure, brain failure, and cancer; according to the WHO document in 1991 [6].

New research subjects focused on metabolism that affected by free radicals of bone below pathologic cases and physiologic [7]. Aging pathophysiology process is affected by oxidative stress according to the evidence of survey results [8] or weak antioxidant rotating concentration levels were suspected hold connected accompanied by diminished BMD (bone mineral density) and triggered by laboratory tests or animal researches osteoporosis [9].

It is demonstrated evidence that reactive oxygen species (ROS) are involved in bone resorption, with a direct contribution of osteoclast generated superoxide to bone degradation [10]. A several years ago, tremendous steps have been taken in analyzing appropriate experimental tests to determine such oxidative stress biological marker; like the standard of glutathione peroxidase, lipid peroxidation, catalase enzyme, superoxide dismutase, DNA damage, total antioxidant level, minerals such as selenium and zinc and vitamins of antioxidant like A, C, and E. To evaluate age-related disease like osteoporosis, OS biomarkers have been examined in various tissues. Consequently, the utilize of biological markers facing distinguish long-suffering persons with OS can frequently beneficial for the diagnosis of osteoporosis [11].

Low level of cobalamin (B12) and vitamin B9 (Folic Acid) concentrations along with the increasing of homocysteine concentration was associated in a few trials with a lower bone mineral density which occurred in an increased likelihood of a bone break in older women [12]. An elevated plasma homocysteine concentration (more than 15μmol/L) predominates in people between the ages 50-60years comparison to young people. The mechanism of this is a synthesis of manufactured and inherited components, nutrition, behavior and hormonal components [13]. Vitamin B12 and folic acid are crucial elements of homocysteine metabolism and was shown to be effective in balancing homocysteine concentrations [14]. The theory was that the changing of enhanced levels

of homocysteine by taking supplements folate and vitamin B12 may preserve healthy bone tissue problems [15].

Calcium and vitamin D3 has also been documented to provide a significant position inside the health protection of tissue of bones. Steady calcium ingestion and vitamin D3 in a patient with osteoporosis are necessary to appropriate medication. Likewise, it has been confirmed that almost 50% of women attending bone loss treatment have included the insufficient level of vitamin D3 along with about 90% of women who do not take enough calcium. New researches show that a majority of drugs promote healing from osteoporotic illness can correspond to reducing the risk of bone breaks. This boost can be supported by the existence of adequate levels of calcium and vitamin D3 in these treatments [16].

1.1. Aims of the study

The target of this survey is for measurement consequence of total serum levels of antioxidant status, serum folic acid, serum vitamin B12, vitamin D3 and calcium on the prognosis and even as a part of the risk for osteoporosis among menopause women in Duhok city\Iraq. The specific objectives are:

- To estimate total antioxidant status in healthy and osteoporotic women.
- Measure the serum level of vitamin B9 (folic acid) and cobalamin (B12) for participant.
- Measure the serum levels of both vitamin D3 and calcium.
- Evaluate the relationship between total serum antioxidant levels and other nutritional elements as a danger factor for developing osteoporosis women enrolled in this study.
- To show the relationship between total antioxidant status and BMD

1.2. Bone Definition and Function

Bone is a metabolically active tissue formed of the organic mineralized bone matrix produced by specialized bone cells. In teams , these cells work to eliminate and replace bone tissue [17]. Bones have so many tasks; they give the whole body it is architecture [18], provide assistance and soft tissue safety [19], and has an essential metabolic function acting as a mineral reservoir for storage of calcium, magnesium and phosphates that are required for the maintenance of blood homeostasis [20]. Furthermore, they operate with the muscles to keep the body moving. They contain bone marrow that manufactures blood cells [21].

1.3. Bone Homeostasis and Remodeling Cycle

Bone homeostasis is strongly regulated by remodeling [22], during which the skeleton demonstrated persistent reconstruction by a sequential remodeling process pattern (Figure1.1). In adults, remodeling is responsible for approximately 95% of bone turnover and about 10% of bone is replaced each year [23]. Osteoblasts and Osteoclasts cells are the main actors in the mechanism of bone reconstruction and repair process. Normally, there is an active balance between osteoblast and osteoclast activity [7].In general, bone homeostasis related to three categories of cells: osteoblasts, osteoclasts, and osteocytes [24]. Numerous growth factors controlled and organized the heterogeneous mix of cells, which is cytokines, hormones, and other signals [25].

Osteoclasts which are responsible for bone resorption, remove old or damaged bone by dissolving the mineral and breaking down the bone matrix [23]. In the end, the building of the mature bone occurs by calcifying in the form of osteoid which osteoclasts proceeds from the bone position go through resorption and exchanged by osteoblasts [26]. Osteocytes form a complex and organized network of interconnected cells throughout the mineralized bone matrix that supports bone structure and maintenance. Under normal conditions, the remodeling process of resorption followed by formation is closely coupled [24, 27]. This phase corresponds toward maintaining bone density and a balanced interaction throughout bone resorption and formation [28] and thus leading in no net change in bone mass [24].

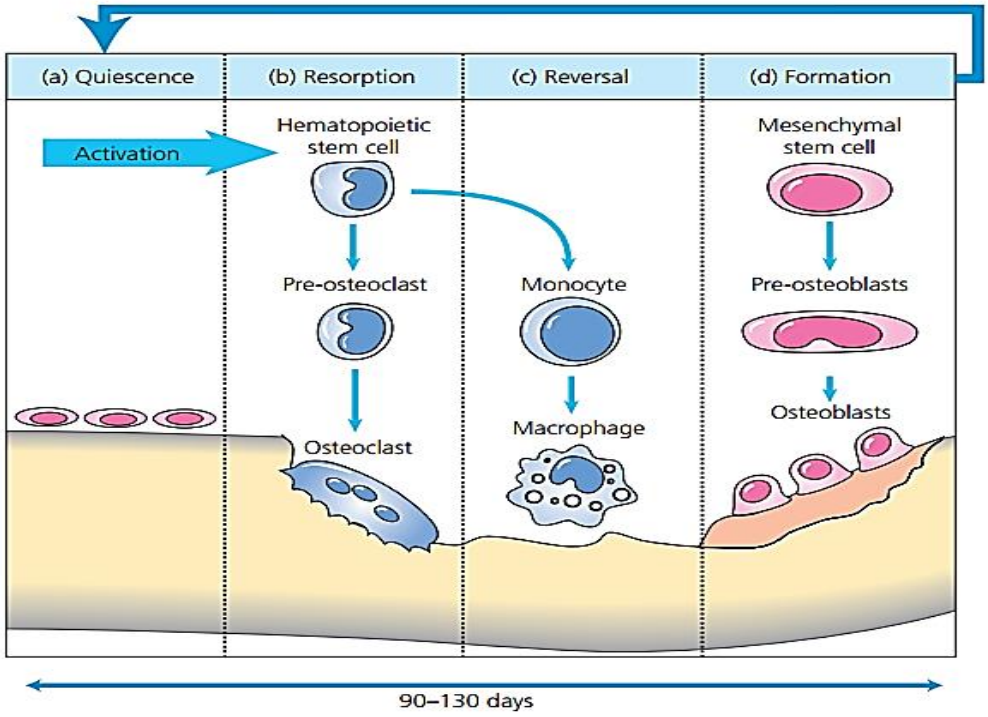

Figure 1.1. Remodeling Process of Bone

Osteoporosis results from an imbalance between osteoblastic and osteoclastic activity, in this case, bone resorption significantly exceeds bone formation [29, 30]. The body will start losing bone rapidly and renders the bone fragile and more sensitive to breaking [31].

1.4. Peak Bone Mass (PBM)

The amount of bony tissue present at the end of the maturation of the skeletal system is known as peak bone mass(PBM) [32]. PBM could be characterized as maximum bone mineral density, that peaks substantially to 80-90% in late adolescence, and later in life is a determinant factor of bone mass (Figure 1.2) [33], while the density of bone tissue is the variance between the quantity gained at adulthood and which loss with aging level throughout adult life [34].

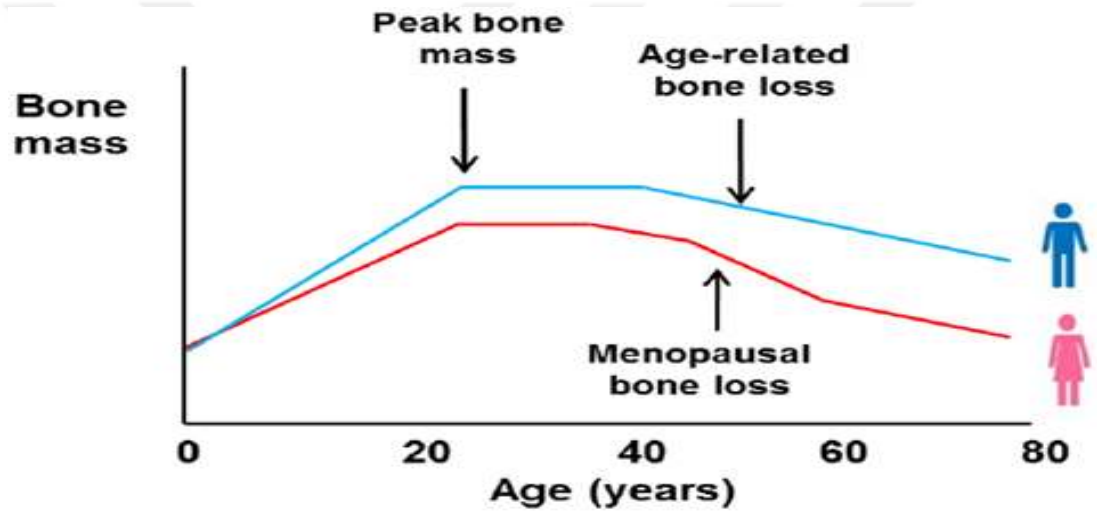

Figure 1.2. Alters in Bone Mass Across both Men and Women Related to Age.

1.5. Osteoporosis Overview

Osteoporosis (OP) recognizes by the world health organization (WHO) as A systematic skeletal dysfunction distinguished by low bone mass and micro-architectural deterioration of bone tissue, , with a subsequent increase in bone fragility and susceptibility to fracture [4]". (Figure 1.3) shows normal and osteoporotic bone.

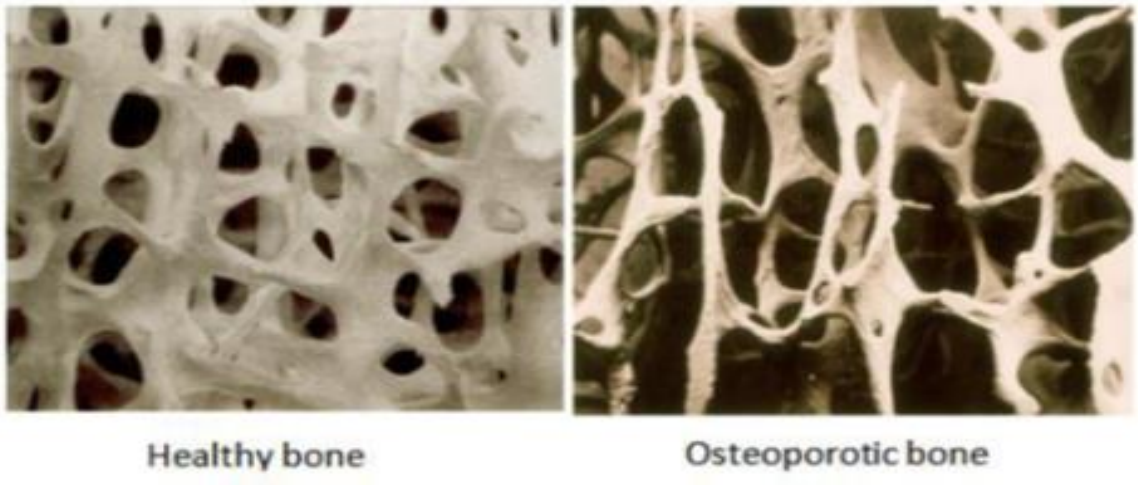

Figure 1.3. Healthy and Osteoporotic Bone

1.5.1. Osteoporosis Classification

Osteoporosis can be classified in to two type:

Primary Osteoporosis

The most popular type of this case is the primary osteoporosis, it does not connect to other conditions or complications, as contrasted to secondary osteoporosis [35], and include the juvenile and idiopathic osteoporosis. Osteoporosis of idiopathic may also be partitioned into (type I) postmenopausal and age-linked or senile (type II) osteoporosis [35, 36].

A- Juvenile Osteoporosis

This type of osteoporosis is rare and occur in children [36]

B- Idiopathic Osteoporosis

- Type 1 OP: Type 1 occurs in menopausal women, Therefore, this type is known as postmenopausal osteoporosis and takes place in 5% to 20% of women, going to affect menopausal women within 15-20 years [34].
- Type 2 OP: This type is known to as age-linked or senile OP and happens in men and women above 70 years old. This sort of osteoporosis is related to aging bone loss [37].

Secondary Osteoporosis

Secondary osteoporosis develops as a result of the major physiopathological effect of different illnesses and conditions of other organs and tissues in the body on the skeletal bones [4]. Secondary OP provides for maybe 40% of the entire percentage of fracture of osteoporosis by a specialist seen in various series of osteoporotic cases [34]. There are a number of diseases and

conditions associated with increased risk of secondary OP, including lack of balance in hormones (example; hypercortisolism (Cushing's syndrome)), several parts of myeloma in cancer, illnesses of the gastrointestinal tract (specifically malabsorption caused by inflammatory bowel sicknesses) and different other disorders. Moreover, the number of typical treatments are linked to increased secondary osteoporosis, involving glucocorticosteroids, anticonvulsants, gonadotrophin-releasing hormone agonists and estrogen antagonists(example; Tamoxifen) [38].

1.6. Osteoporosis Epidemiology and the Linked Fractures

It has been reported that 200 million women worldwide are affected by osteoporosis, with a third of these women aged 60 to 70 and the remaining over 70 [39]. The globally yearly prevalence of fracture in 1990 was measured at 1.29 million and is projected to increase to 2.6 million by 2025 and 4.5 million by 2050 [40]. In Northern Europe, Northern United States, Southeast Asia is documented for the maximum fracture frequencies with the minimum in African [41].

Approximately three-quarters of the world population reside in Asia [42]. In Asia, the frequency of breaks is expected to increase. It is projected the prevalence will hit 65000 by 2020, and approximately 175000 by 2050 [43]. Gorial, *et al*'s 2013[44] cross-sectional study in Baghdad city/Iraq to evaluate the frequency of osteoporosis in postmenopausal Iraqi women revealed a high prevalence of OP among the study population(22.8%) [44].

In the United States, approximately 44 million Americans are harmed by OP and low bone mass [45], and approximately 1.5 million breaks are due to OP yearly [46]. The estimation of the national institute for health and clinical excellence (NICE) is 2 million people in England and Wales have osteoporosis [47].

1.7. Risk Factors for Osteoporosis

Risk factors break into two major categories, modifiable, which are those that can be changed, and fixed or non-modifiable, those that cannot be changed. Though, there is no way to control fixed risk factors [5].

Unchangeable (unmodifiable)

Include race, sex, genetics, age, family history, body size, and a previous fracture [5].

Changeable (modifiable)

Involve chronic inactivity, low lifetime calcium ingestion, low body weight, drugs utilized, and oxidative stress-linked factors(such as smoking, alcohol intake, low antioxidant level, food deficiency, unnecessary sports activity and more than necessary caffeine intake) [5].

1.8. Oxidative Stress (OS)

Oxidative stress (OS) which presumably increases with age is a condition of excess formation of free radicals either by physiological or pathophysiological processes and states of insufficient antioxidant defense [48].

Oxidative stress occurs when the formation of free radicals and active intermediates are raised in a system that the ability of the system to neutralize and remove them [8, 49]. It is generally accepted that OS is an irreversible trait of life, induced by the reactive forms of oxygen produced during normal respiration by the oxidative explosion of macrophages in the answer of infection [50], trauma, inflammation and several exogenous agents like cigarette smoke or environmental pollution [51]. Figure 1.4 describes the role of OS in chronic disease growth.

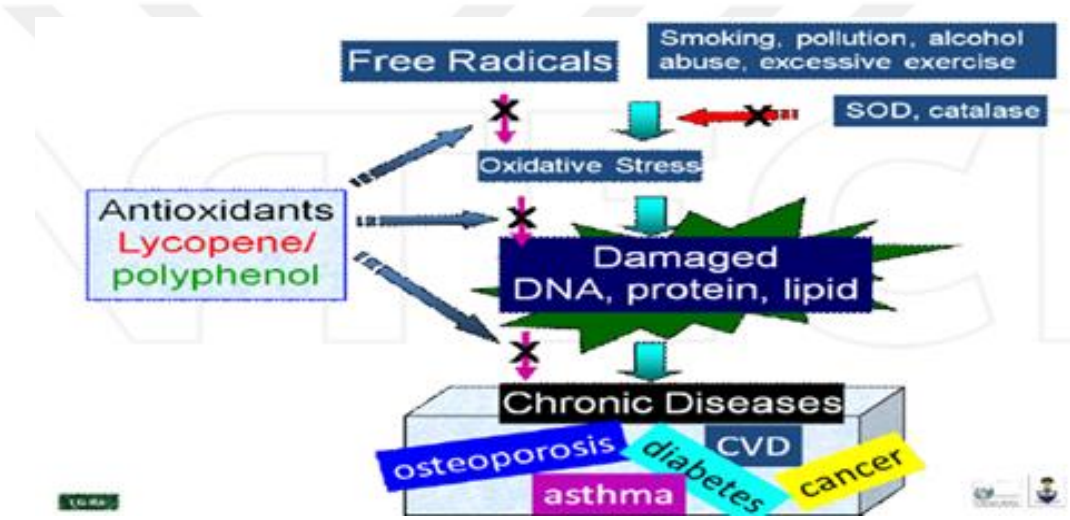

Figure 1.4. Oxidative stress/antioxidants and chronic diseases

1.8.1. Reactive Species in Biological Systems Overview

Free Radical can be characterize as reactive chemical species [52], which are capable of independent existence and contained one or more unpaired electrons [53]. Free radicals may be either oxygen derived (ROS , reactive oxygen species) or nitrogen derive (RNS , reactive nitrogen species) [54]. Both reactive species categories comprise unbound radicals which were intensely (atoms, molecules, and ions) considered as a reactive chemical species [55]. The free radicals oxidize and damage many biological structures, this is identified as oxidative damage, a potential cause of aging, cancer atherosclerosis, chronic processes of inflammation and cataracts [56]. Free radical isn't always harmful, nevertheless. They serve also a vital role in the human body [57]. The radicals of oxygen in the biological system are possibly combination needed in the cytology cycle

of purulence. White blood cells produce free radicals as part of a body protective mechanism towards disease to kill attacking pathogenic microbes. The total elimination of these radicals will thus not only be impossible but also hazardous [58].

Reactive Oxygen Species (ROS)

All molecules have this feature of reactive oxygen species (ROS), which are highly reactive and include oxygen. ROS is the consequence of normal cellular metabolism generated by living organisms [10], specifically in the Mitochondria [59] and involves unbound radicals such as superoxide anion ($\bullet O_2^-$), per hydroxyl radical ($HO_2\bullet$), hydroxyl radical ($\bullet OH$), nitric oxide (NO), and other species such as hydrogen peroxide (H_2O_2) [60], singlet oxygen ($^1O_2$), hypochlorous acid (HOC1), and peroxynitrite ($ONOO^-$) [59].

They act in physiological cell pathways at low to medium levels, however, at high concentrations they generate undesirable modifications to cell elements, such as lipids, proteins, and DNA [61]. These reactive molecules are the organisms many commonly researched, and play significant positions in many pathogens pathophysiology [49]. Throughout mammalian, there are the below biological origins of ROS; mitochondria [62], endoplasmic reticulum, peroxisomes, cytosol, plasma membrane, and extracellular space. Key ROS factors comprise the biological processes of cellular respiration [63].

Reactive Nitrogen Species (RNS)

Accumulative terms that belong to reactive nitrogen species (RNS) involving peroxynitrite($ONOO^-$), nitric oxide radical ($NO\bullet$), nitrogen dioxide radical ($NO_2\bullet$), and other oxides of nitrogen and consequences appearing when $NO\bullet$ reacts with $\bullet O^-_2$, $RO\bullet$, and $H\bullet NO\bullet$ [62].Overabundance manufacturing of reactive nitrogen species known as nitrosative stress. Nitrosative stress can come up with nitrosylation reactions which can alter the protein composition and consequently hinder its natural function [64].

1.9. Antioxidants

The compound that prevents oxidation of some chemicals called antioxidant. By neutralizing the dangerous outcome of free radicals preservation of cell components occur [54]. Antioxidants minimize oxidative damage to biological systems whether by avoiding ROS production or by quenching ROS before reacting with other biomolecules [60].

Oxidation is a chemical process that transfer electron to an oxidizing agent from a substance. Responses to oxidation can generate free radicals and harm the cell by producing series reactions. Antioxidants stope or terminate these series reactions by removing free radicals intermediate and preventing other oxidizing agents including thiols, ascorbic acid, or polyphenols [65]. They are

thus actually essential to maintaining optimum cellular and systemic health [66]. OP is mediated by induction of ROS, which are an important role in the promotion of this disease. Antioxidants that inhibit the production of ROS thus have a high clinical value in managing of OP [26].

1.9.1. Anti-oxidants Classification

Antioxidants can be either endogenous substances, created by the organism as a result of its ROS defense, or they could be exogenous compounds gained from the diet, according to the source [60]. The metabolism of the body produce some chemicals called endogenous antioxidants may be enzymatic or non-enzymatic [55, 67].

Enzymatic antioxidants that compromised in the ROS degradation are superoxide dismutase [52] catalase and glutathione peroxidase. Non-enzymatic antioxidants work by disrupting free reactions to the radical series. Vitamin C, vitamin E, plant polyphenol, carotenoids [68], metal-binding proteins (example; ceruloplasmin, glutathione, uric acid, melatonin, bilirubin, and polyamines are certain instances of the non-enzymatic [55].

1.10. Previous Studies Review of Oxidative Stress and Osteoporosis

Reports in vitro or animal experiments have shown which oxidative stress has a significant effect on the distinction and task of the osteoclast. Pathogenesis of bone loss can include ROS and antioxidant systems [11, 69, 70].

The enhanced development of reactive oxygen species as a source of superoxide is leading to osteoclastic activity in bone destruction and has related to the high production of reactive oxygen species accountable for higher serum malondialdehyde concentrations [30, 71]. The survey of Altindag, O., et al. [10] concluded that oxidative stress in postmenopausal osteoporosis increased osteoclastic interaction and a reduction in osteoblastic activity. Besides Sharma, T., et al., [72] checked for postmenopausal osteoporotic women, the connection between bone mineral density and oxidative stress. In postmenopausal women, which have osteoporosis researchers documented; serum glutathione peroxidase, superoxide dismutase, and catalase were lower than in the control population. Other surveys Maggio, D., et al., [73], women with osteoporosis osteoporotic women explored plasma antioxidants and indicated that antioxidant protections in osteoporotic females had reduced considerably. However, Wolf, R.L., et al., [74] observed that somehow the rate of serum antioxidants is not connected with mineral density in the bone.

1.11. Calcium Role

Calcium is considered an essential mineral for human health and is necessary for various metabolisms in the cells. Calcium is stated to be the critical component of the skeletal structure. In

particular, other body functions, such as natural cardiovascular function, neuromuscular movement, and blood coagulation, are also critical. Studies suggest that calcium is transported from bone to bloodstream under the effects of parathyroid hormone to accommodate for the reduced plasma level during life. Which is around 99% of body calcium is believed to be a solid mass deposited in bones and teeth, while the remaining mass is stored in plasma and extra cell fluid at around 1%. Moreover, the calcium present in plasma and extracellular fluid (ECF) is used for all physiological behavior of the body. To maintain a balance, the supplementation of daily calcium is necessary. Ultimately, the ingested calcium is absorbed by the action of vitamin D3 in the small intestines. On the other side, kidneys play a big role in excreting excess calcium [75].

Calcium is a crucial nutrient for the wellness of skeletal. Furthermore, bone loss and OP are not related to calcium deficiency unless in individuals suffering from serious malnutrition or in the very elderly. Very low calcium intake in growing children may prevent the accumulation of skeletal mass and disrupt the achievement of optimal bone mass which may expand the danger of OP in old age [76].

Bone functions like a calcium storing store, and if the blood concentration decreases beyond normal values [77], the controlling hormones of the body reply by extracting calcium from the bones to be used in other important functions of the body. But when this cycle will continue to occur over time, the bones become weaker because a calcium deficiency contributes to an incomplete calcification of the organic matrix produced [78] and therefore an increased risk of OP [79]. Information from cross-sectional research shows that bone loss after menopause happens progressively. In the 25 years after menopause, 12% of total bone calcium is lost, and up to 50% of this reduction arises in the first 10 years after menopause [80].

1.12. Vitamin D3 Role

Vitamin D3 is classified as lipid-soluble vitamins and plays a significant role in bone maintenance. Vitamin D is crucial for strong healthy bones and plays an important role in the elimination of osteoporosis [42]. The main job of vitamin D3 is to control intestinal calcium absorption and thus lower bone resorption [81]. The function is primarily to maintain healthy range levels of serum calcium and phosphorous, thus promoting bone mineralization [56, 78]. The principal sources of vitamin D3 are direct exposure to sunlight along with eating food rich in vitamin D3 and, as a supplement, With increasing age, there is a decrease in the ability of the skin to build precursors of the active vitamin D in the skin when exposed to sun [82]. At the same time, the potential of kidneys to activate 25(OH) D decreases [83]. As a response, deficient vitamin D may not just lead to the progressive loss of bone and enhanced delicacy even so also to neuromuscular weakness, which can enhance the danger of falling. Vitamin D deficiency has also been found to correspond with low BMD (Figure 1.5) [36]. Modern studies suggest that the

majority of the population (approximately 90%) aged between 50 and 70 years of age do not obtain adequate vitamin D3 and have vitamin D3 deficiencies [81].

The 7 dehydrocholesterol, which is typically present under the skin, is absorbed by the direct sunlight or ultraviolet B (UVB) radiation in terms of sunlight exposure. Provitamin D3 is produced appropriately. This material is unstable and is converted by heat into inactive vitamin D3 [84].

Therefore, after combining with vitamin D3 binding protein (DBP), inactive vitamin D3 was transported to extracellular space and then entered blood capillaries and traveled towards the liver. Therefore the first hydroxylation cycle to form [25(OH)D] 25-hydroxyvitamin D3 inside the liver Secind, it transfers from liver to kidney, particularly via vitamin D3 binding protein(DBP), to cells of the kidney tubules. As a contributor, the second hydroxylation pathway will result in the synthesis of [1,25(OH)2 D] 1,25-dihydroxy vitamin D whatever is biologically the energetic shape of vitamin D3 and takes part in a significant in calcium homeostasis [16].

Eventually, it should be mentioned that low exposure to sunlight can present a high endanger of vitamin D shortage because of vitamin D deficiency due to the synthesis for approximately 90% of vitamin D in the skin following exposure to sunlight. A limited amount of vitamin D can be processed by taking food [85]. In particular, the vitamin D mechanism can indirectly be associated with the elimination and osteoporosis treatment, probably due to its effects on calcium bioavailability [86].

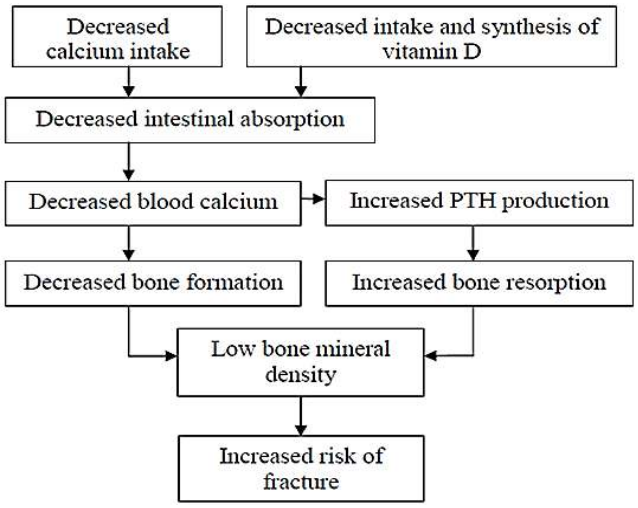

Figure 1.5. Flowchart of mechanisms leading to low bone mineral density and fractures

1.13. B-Vitamins and Bone Health

Bone loss and the risk of fracture caused by food deficiency, these are significant causes of the hip break in elderly [87-89]. Bone physiology crucial nutrients are calcium and vitamin D as reported by most researchers, other nutrients as documented in surveys can able has an important role in assist bone health [90, 91]. Bone health and avoidance of fractures are protected by vitamin B complex as researched. Energy making metabolic operation for carbohydrates, lipids, and proteins is enhanced by cofactors B vitamins. B vitamins have a significant role in retaining the nervous system functioning. B vitamins and bone health contribute in many papers that demonstrate the focus of B12 (cobalamin) and B9 (folate), these are considered as cofactors for the enzymes intimated in the re-metabolism of homocysteine [92-95]. Nevertheless, bone physiology functioned by these parts of (thiamine, riboflavin, niacin, pyridoxine, B9, and cobalamin) which are B vitamins.

Inadequate intake of vitamin B has been confirmed among patients with the break of the hip. In several observational surveys documented the connection between specific B vitamins (riboflavin, pyridoxine, folate, or cobalamin) and slighter danger of osteoporosis or hip fracture. Many other studies have been done among Caucasian populations, but results were contrary to between surveys or over various B complex vitamins. So, increasing the risk of the break was disturbed by homocysteine, however, it must be illustrated how these B vitamins change healthy bone terminated their bind to the metabolism of homocysteine. Until nowadays most papers on clinical surveys done do not demonstrate the use of administration of B vitamins extra element on the elimination of fracture and osteoporosis [96].

1.13.1. Vitamin B9 (Folic Acid) and Evidence from Observational Studies

Folate is the better name of vitamin B9,plays a central role in the one-carbon metabolism in the nucleotide synthesis, in the metabolism of homocysteine, as well as in the methylation of DNA, RNA, proteins and phospholipids [97, 98]. As with other B vitamins implicated in the homocysteine metabolism. In the methylation process, methionine synthesis relies on re-methylation from both folate and B12. Megaloblastic alters in the bone marrow and other tissues are symptoms of B vitamin inadequacy.

On the other hand, cysteine is produced by the homocysteine transsulfuration pathway, which is controlled by pyridoxine like a cofactor for the cystathionine-cysteine response of enzymes [97]. The United States recommended daily allowance for B9 is 400 μg/day for adults and a big dose of 600 μg/day for pregnant women are necessitate, in the elderly folate scarcity unusual [99]. *Rutaceae* family, deep green edibles natural products and beans are important sources of folate foods [97].

Dissimilar populations have non-identical results about the consequence of folate on the risk of break and bone-mineral density (BMD). A survey among Iranian women stipulated which 5-methyltetrahydrofolate(MTHF) red blood cells(RBC), which give back a lifelong status in the body, could be a good detector for BMD differentiate with short term detectors like 5-MTHF or serum B9 [100]. Ingestion per level of folate with enhanced BMD illustrated by some epidemiological searches [100-105] or decrease the chance of fracture [106, 107]; While others research in the Chineses Health Study Cohort in Singapore, in the Chinese Health Study Cohort in Singapore, invalid interrelations have been shown [108-110].

Inside these surveys, two possible types of research examine B vitamin food intake on BMD [105] and the risk of break [96, 105] [40,42]. Among perimenopausal women illustrated by the Danish Osteoporosis Prevention Research which B9 food supplementation was a great prognosticator for BMD evaluate at five years after baseline [105], no this survey or the Singapore Chinese Health Study findings have shown which dietary B9 was associated with the danger of fracture [96, 105].

1.13.2. Vitamin B12 (Cobalamin) and Evidence from Observational Studies

Cobalamin was discovered in 1855 with the introduction of this vitamin as the unique drug of harmful anemia and demyelinating injury of the central nervous system [97]. Cobalamin was originally first found to be associated accompanied by osteoporosis and fractures in pernicious anemia patients [111, 112]. Mutase L-methylmalonyl-coenzyme A(CoA), and synthase methionine, these two enzymes physiologically illustrated has a link with cobalamin [97]. Synthase methionine has participated in the metabolism of homocysteine. Because of gastrointestinal tract diseases in old age persons mild cobalamin deficiency 20% was documented [97]. Old age people with cobalamin deficiencies can too have impaired cognition [113]. The US RDA for adults 50 years or older is 2.4 μg per day [99]. The main sources of food involve meat, egg, milk products and B12 foods [97].

The connection of B complex vitamin with bone health studied by many researchers in divergent populations, and observed in Caucasian populations. Of the 19 studies we examined, 12 found no important association between B12 and BMD [16, 101-104, 114-116], biological markers of bone income [109, 115] or danger of break [105-107, 110, 116], no matter in serum samples if B12 was diagnosed [101-104, 106, 107, 109, 114] or from the evaluation of food ingestion [105, 110, 115, 116]. The remaining seven studies [108, 117-122], nevertheless, kept up the preservative role of B12 in protecting BMD [108, 117-119, 122] and reducing fracture risk [120, 121]. The role of cobalamin in the health of bone needs to be illuminated based on the conflicting findings of those studies. The BMD was suggested to explain at most only in part the link betwixt cobalamin and risk of break-in epidemiological researches [121]. B12 deficiency, for instance, may lead to

problems of neurology [97] to enhance the danger of falls in the old ages people. Many possible theories involve B12's consequence on BMD through its effect on the metabolism of homocysteine [108, 120], even so, this may not suffice to influence bone grade whether homocysteine is not accumulated in the bone tissue [123].

1.14. Effect of Menopause on osteoporosis

As a woman reaches menopause, the role of ovaries reduces the development of two hormones, estrogen, and progesterone. One role of estrogen is to maintain the normal bone remodeling rate [124]. Estrogen strengthens the bones and is deficient, increases bone resorption and can hinder the formation of compensatory bones [39] since the calcium loss in bone and other minerals much more quickly when estrogen levels decrease. Consequently, loss in the bone of about 2% per annum happens many years following menopause (Osteoporosis Australia, 2014).

Because of its high turnover rate, and since it is most vulnerable to estrogen deficiency, trabecular bone is most affected by menopause. As a result, the trabecular bone is fragile and eventually perforated or detached from the tissue around it. Over time, the trabecular bone weakens when enough of the bone has been removed. The loss of these trabecular connections is one of the reasons why bone becomes weak and breaks or fractures eventually [124].

2. MATERIAL AND METHOD

In this chapter, we explain the method of study also the material that I use in this study, also the instrument we used in this study we explain detail below, Also the software program we used to statistical parameter we explained.

2.1. Design of the Study

A cross-sectional design was utilized to realize the survey objectives.

2.2. Study setting and study period

The present study was conducted at the Rheumatic Disease of Duhok and Rehabilitation Center, from September 2019 until November 2019.

2.3. Study population

A total of 195 women who visited the Osteoporosis unite in Duhok Rheumatic disease and Rehabilitation center were registered in this study and they were living in areas around and inside Duhok province. Out of 195 patients, only (n=106) were enrolled in this study, while the others were not located under the standards of inclusion criteria for the current study (i.e. they were excluded).

2.4. Assessment of study variables

The samples were collected according to inclusion criteria only 106 samples were selected from patients and asked to participate in this study project.

The study population was grouped into 7 different classes based on Age, WC (waist circumferences, BMI (body mass index), serum Vitamin D3, Vitamin B12, Folic Acid and quartiles of T-score.

1. The participants were classified according to their ages into three groups with ten years' interval between each class starting from (40 – 50), (51 – 60) and (>=61).
2. Women with normal waist circumferences (< 88 cm) and high Waist Circumference (> 88 cm)
3. The study population accompanied by normal weight or healthy (BMI >= 25 Kg/m^2), overweight (BMI 25 - 29.99 Kg/m^2) and obese (BMI =< 30 Kg/m^2).

4. The study population was classified into three groups depending on the Vitamin D3 levels starting from Deficient (< 9.99 ng / ml) (n=28), Insufficient (10 to 29.99 ng per ml) (n equal to 61), adequate (more than 30 ng per ml) (n equal to 17).
5. The participants were grouped depending on the Vitamin B12 levels into two groups. Low vitamin B12 (<200 pg / ml) (n=26), Normal Vitamin B12 (>200 pg / ml) (n=80).
6. women were classified into two groups based on folic acid levels. The first group were low (< 5 ng / ml) (n=40), while the second one was with normal folate levels (>=5 ng / ml) (n=66).
7. The enrolled population was classified into two groups depending on the different quartiles of T-Score: T-score ranging (>= -1.00) and (less than -2.5) represented (n=24 normal) and (n=82 osteoporotic women) respectively.

2.5. Data Collection (Questionnaire)

A previously prepared questioner form was designed for patients who participated in this study. The following information was included

1. Personal information for instance patient name, years since menopause and address.
2. Physical examination such as height, weight (using an ordinary metric scale) and waist circumference measurement (using an ordinary tape measure).
3. Patient's medication for example folic acid, vitamin B12, and vitamin D3.
4. Chronic diseases such as liver disease, kidney disease, parathyroid disease, cancer and diabetes mellitus.
5. Antioxidant supplements.
6. Others such as previous fractures, smoking, and physical activities.

2.6. Study Variables

The evaluation of osteoporosis level after taking DEXA (dual-energy x-ray absorptiometry) which is the criterion method for identification of osteoporosis in Rheumatoid Center in Duhok city which includes the following details:

BMI Kg per square meter Body Mass Index

It is called the relationship between weight and height calculated by a special formula. To obtain the BMI for each subject, the height and weight measurement were used as follows:

- Kilograms for the weight (kg) divided by height in square meter m^2.
- The subjects were considered normal when BMI is less than 25 whereas those with BMI 25-29.9 were regarded as overweight and the remaining patients with BMI more than or equal

30 were regarded as obese.

Waist Circumference (WC)

The measurement started at the tip around the abdomen crossing the umbilicus in centimeters using ordinary metric tape. According to WHO criteria, the normal waist circumference ranged less than 88 cm and more than 88 cm is regarded as abnormal.

2.7. Inclusion Criteria

- Women aged 40 and above
- Osteoporotic and normal women.

2.8. Exclusion Criteria

- Receiving of hormone replacement therapy by involved patients.
- Patients suffering from renal diseases
- Patients suffering from liver disorders
- Patients under the effect of chemotherapy
- Type I and type II diabetes mellitus belong to long-suffering people.
- Patients on medication particularly multivitamins and antioxidants

2.9. The Diagnosis of Osteoporosis

Osteoporosis diagnosis is performed along with calculating the total T-score from the DEXA report of patients involved in this study [125]. The score of -2.5 and less is considered as an osteoporotic woman. Two Sorts of Scores are utilized to Quantify Bone Mineral Density

1. **T – Score** is the value of patients' mean BMD measurement comparing to the value of standard deviation, and the results are either below or above the normal young mean bone mineral density [39].
2. **Z – Score** is the value of the patient's age – matched means BMD comparing to the value of standard deviation and the results are above or below [126].

2.10. Interpretation of BMD

Based on BMD, the World Health Organization (Table 2.1.) set the following guideline for assessing and diagnosing osteoporosis [79, 83, 85].

1- **Normal bone mass**: any value of T-score is recorded more than or equal (-1) is regarded as normal bone status (T-score ≥ -1).

2- **Osteopenia (low bone mass):** In this case, the T – score value is between (-1 and -2.4). (T-score < -1 and > -2.5).
3- **Osteoporosis** is the condition that T – score measured less than or equal -2.5 (T-score ≤ -2.5).
4- **Severe osteoporosis**: is the last stage of bone loss, which is considered as advanced osteoporosis with one or more bone fractures. (T-score ≤ -2.5 and the presence of one or more fragility fractures)

Table 2.1. Diagnosis of osteoporosis clinically according to the standard of WHO

BMD T-Score	Diagnosis
T-score more than or equal to -1	Normal
-1 more than T-score more than -2.5	Low bone mass
T-score less than or equal -2.5	Osteoporosis
T-score less than or equal -2.5 with an existing fracture	Severe osteoporosis

2.11. Statistical Analysis

Data were calculated by utilizing SPSS (statistical package for social sciences program) version 15. To differentiate between proportions T-test was utilized. Statistically significant value P is must be equal or less than 0.05. One Way ANOVA was utilized to compare between more than two groups. The Pearson correlation was utilized to dictate the correlation coefficient between the study parameter

2.12. Ethical considerations

The study protocols were reviewed and approved by the Committee of Ethical of General Directorate of Health in Duhok/Iraq.

2.13. Collection of Blood Samples

In this study, patients diagnosed with osteoporosis by DEXA report were asked to participate as a volunteer and taking the venous blood sample. All of the participants were fasting overnight as well as avoiding heavy physical activities before taking the samples. Around 4-5 ml of venous blood were collected between 9:00-12:30 A.M. in a vein located in antecubital fossa using vacutainer AYSET gel tube 5ml ordinary tubes. After 30-60 minutes from blood collection, the serum was separated by centrifugation at 2500 - 3000 rpm. After that, the harvested serum samples

were stored in deep freeze (-22) using a 2.5ml Eppendorf tube labeled digitally for further investigation.

Finally, the stored serum for each one was allowed to melt at room temperature, the prepared plasma used for detecting total serum antioxidants by colorimetric assay kit and vitamin D3, vitamin B12, calcium and folic acid by automatic clinical chemistry analyzer COBAS 6000.

2.14. Biochemical Analysis

Auto analyzer machine extended by Hitachi Japan's company based on spectrometer technology. The technique is used to measure various biochemical parameters such as lipids, enzymes, proteins, and electrolytes. To obtain accurate and sensitive results, this system is broadly used by many private and public hospitals and clinics for diagnosis and comprehensive health examinations.

Figure 2.1. Clinical chemistry (left) and immunoassay (right) Automated Analyzer (Cobas 6000).

Furthermore, in the 1980s, the system has been improved due to the high demand for evaluation of hormones and markers of cancer show in Figure 2.1. Clinical chemistry (left) and immunoassay (right). Thus, the immunoassay technique was developed because many of these biochemical substances are present in very low amount in the blood and any other body fluids.

2.14.1. Measurement of Total serum antioxidant (TSA)

Total antioxidant was determined by ABTS colorimetric method by a commercial kit (Elabscince E-BC-K219-M).

There are two kinds of the antioxidant system; one is the enzyme system of antioxidant, involving superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px). The other is antioxidant of non-enzymatic systems, involving uric acid, vitamin C, vitamin E,

glutathione, bilirubin, α-lipoic acid, a carotenoid. Antioxidant capacity is thought to be the cumulative effect of all antioxidants in blood and body fluids

The principle of the ABTS method for determining the T-AOC is as follows. ABTS is oxidized to green $ABTS_+$ by appropriate oxidant, which can be inhibited if there exist antioxidants (Finger 2.2.). The T-AOC of the sample can be dictated and analyzed by measuring the absorbance of $ABTS_+$ at 414 nm or 734 nm. Trolox is an analog of VE and has a similar antioxidant capacity to that of VE. Trolox is utilized as a source for another antioxidant. For example, the T-AOC of Trolox is 1, then the antioxidant capacity of the other substance with the same concentration is showed by the ratio of its antioxidant capacity to Trolox antioxidant capacity.

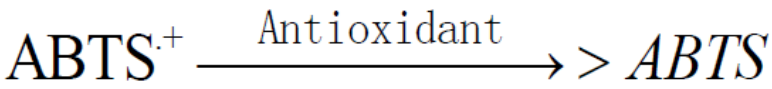

Figure 2.2. ABTS radical cation method equation.

2.14.2. Serum total folic acid (folate III)

In this case, serum total folic acid was detected using (folate III) (Roche) kit according to the standard procedure from company instructions using Cobas 6000 auto analyzer. Reference number: 07559992 190 and Standard Analytics E 170 Cobas e 411, Cobas e 601, and Cobas e 602.

2.14.3. Serum total vitamin B 12

Measurement of plasma total vitamin B12 is identified using a vitamin B12 III kit (Roche). Reference number 07212771 190 and Standard analytics E170 Cobas e 411, Cobas e 601, and Cobas e 602.

2.14.4. Serum Vitamin D3 (25-Hydroxy vitamin D3)

The detection and evaluation of plasma vitamin D3 are performed by using Vitamin D3 total 25-Hydroxyvitamin D3 kit (Roche) with reference number 05894913 190 and Standard analytics E170 Cobas e 411, Cobas e 601 and Cobas e 602.

2.14.5. Serum Calcium (S. Ca) level

Finally, the calcium (S. Ca) level is detected by using Calcium Gen.2 300 tests Roche/Hitachi Cobas c 311, Cobas c 501/502 with reference number 05061482 190.

2.15. DXA Scanning

The golden criterion for medical examination and following up of patients with osteoporosis is the DEXA scan technique.

2.15.1. DEXA Scan Principles

It is a particular sort of X-ray used to evaluate and measure bone mineral density. Nowadays it is commonly utilized in hospitals for it is relatively performed easily. On the other hand, it is noninvasive, rapid, and precise as well as it is characterized by a low amount of radiation exposure comparing to other types of X-rays [82]. The DEXA scanner show in **Figure 2.2.** is a device that makes two X-rays beams; each one has various energy concentrations. The first beam is characterized by high energy whereas the second one is characterized by low energy [22]. Finally, the quantity of X-rays that crosses via the bone is estimated for each beam hanging on the bone thickness.

Figure 2.3. Medilink DEXA Bone Densitometry

The result of the quantification will be differentiated to the bone density of young healthy men or women which is the symbol at as T-score or an adult of the same age and sex which is marked as Z-score. The method of scanning generally takes around twenty minutes hanging on the height of the patient, also it is non-invasive and without pain.

The measurement of bone mineral density is a useful tool for the classification of bone break and its risk as well as it is useful as a guide for therapy choice and response of patients to therapy [82]. Furthermore, DEXA scanning is contraindicated in pregnant women due to radiation. It has been recorded that there is a small possibility of cancer from excessive radiation exposure. Finally, it is recommended to avoid calcium intake as a supplement 24hr before the onset of scanning. Also, clothes with zipper and metallic accessories are avoided during the procedure of DEXA scanning**.**

3. RESULTS AND DISCUSSION

General characteristic of the study population was explained in this chapter and characterized as follows:

106 women were enrolled in this research. Participants were split into two groups, group 1 included 82 osteoporotic women who represented (77%) of the study population, group 2 which is healthy women were determined in 24 women who represented (23%) of the study population. In general, the percentage distribution of the Two groups is shown in Figure 3.1.

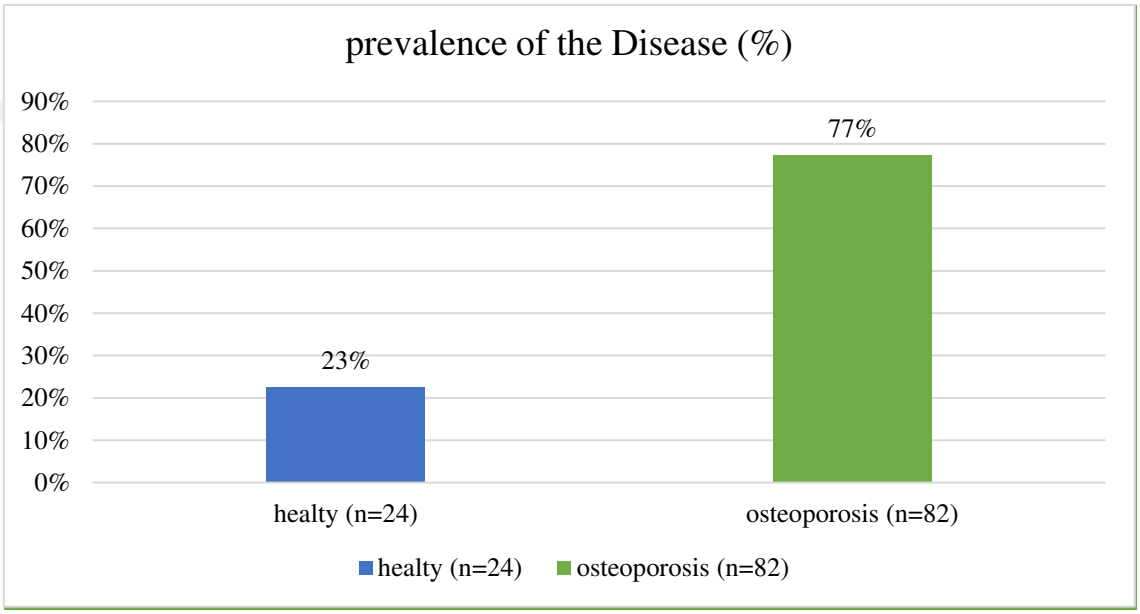

Figure 3.1. The percentage distribution of participants groups

General features of the survey population are summarized in Table 3.1; osteoporosis women introduced significantly higher mean values of age in comparison with control groups ($P < 0.001$). Osteoporotic women presented significantly higher BMD mean values and similarities T-score compared to the healthy group (P< 0.001). BMI (body mass index) was a significant mean value over the two groups (p <0.05), On the contrary, there was no significant difference in waist circumstance (WC) over the groups (P>0.05). While biochemical parameters show a significantly higher mean value of serum levels of TAS and Folic acid in women with osteoporosis differentiated to non-osteoporotic women (P<0.001). However, there were no significant variations found in serum levels of vitamin D, calcium, and Vit.B12 ($P > 0.05$) among the groups.

Table 3.1. Common Features of Study Population

Study Variable	**Healthy (n=24) Mean ± SD**	**Osteoporosis (n=82) Mean ± SD**	**P-value**
Age (year)	46.50±6.43	53.67±8.01	**<0.001**
BMI(Kg/m^2)	35.71±7.29	32.71±6.25	**<0.05**
WC(cm)	109.62±19.36	108.58±16.87	NS
T.scor	-0.93±0.07	-2.98±0.52	**<0.001**
BMD (g/cm^2)	0.911±0.205	0.629±0.112	**<0.001**
Vit.B12(pg/ml)	472.22±216.42	397.34±230.15	NS
Folic Acid (ng/ml)	12.59±4.83	6.33±4.12	**<0.001**
Vit. D(ng/ml)	22.19±14.38	18.91±14.99	NS
S.Ca (mg/dl)	9.46±0.44	9.28±0.57	NS
TAS (mmol/L)	3.01±0.18	2.73±0.16	**<0.001**

3.1. Age group

The participants were classified according to their ages into three groups with ten years' interval between each class starting from (40 – 50) (n =54), (51 – 60) (n=29) and (>=61) (n=23).

3.1.1. Prevalence of study population classified by age groups

Distribution of age groups is represented in Figure 3.2 which indicates that the lowest percentage (22%) of study population women was >= 61 years, while the highest percentage (51%) was at 40-50 years and (27%) represents the age between 51–60 years.

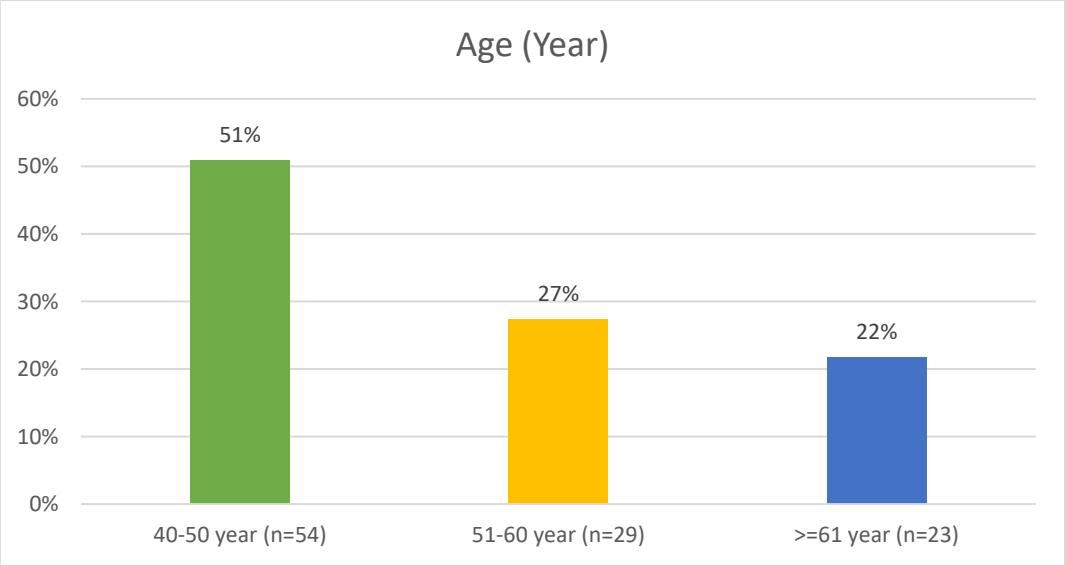

Figure 3.2. Prevalence of the whole study population classified by age groups

3.1.2. Arithmetic mean of BMD and TAS in study population women considering age groups

BMD and TAS classified by age groups in the whole study population women are illustrated in Table 2.2. A higher significance was found in the TAS among the age group ($p < 0.001$) and higher significance was detected in BMD ($P < 0.001$) among the age group.

Table 2.2 Arithmetic mean of BMD and TAS in whole study population women considering age groups

parameters	Mean± SD			*P*-value
	40-50 years (n=54)	51-60 years (n=29)	>= 61 years (n=23)	
BMD(g/cm^2)	0.752 ± 0.170	0.681 ± 0.181	0.569 ± 0.147	<0.001
TAS (mmol/L)	2.85 ± 0.19	2.83 ± 0.16	2.62 ± 0.20	<0.001

3.2. BMI group

The study population accompanied by normal weight or healthy (BMI equal or less than 25 Kg per square meter) (n equal 11), overweight (BMI between 25 to 29.99 Kg per square meter) (n equal 26) and obese (BMI equal to or more than 30 Kg per square meter) (n equal 69).

3.2.1. Prevalence of study population classified by BMI

Regarding body mass index in the whole study population (10%) were determined as normal individuals; while (25%) were determined as overweight individuals and (65%) were determined as obese individuals (Figure 3.3).

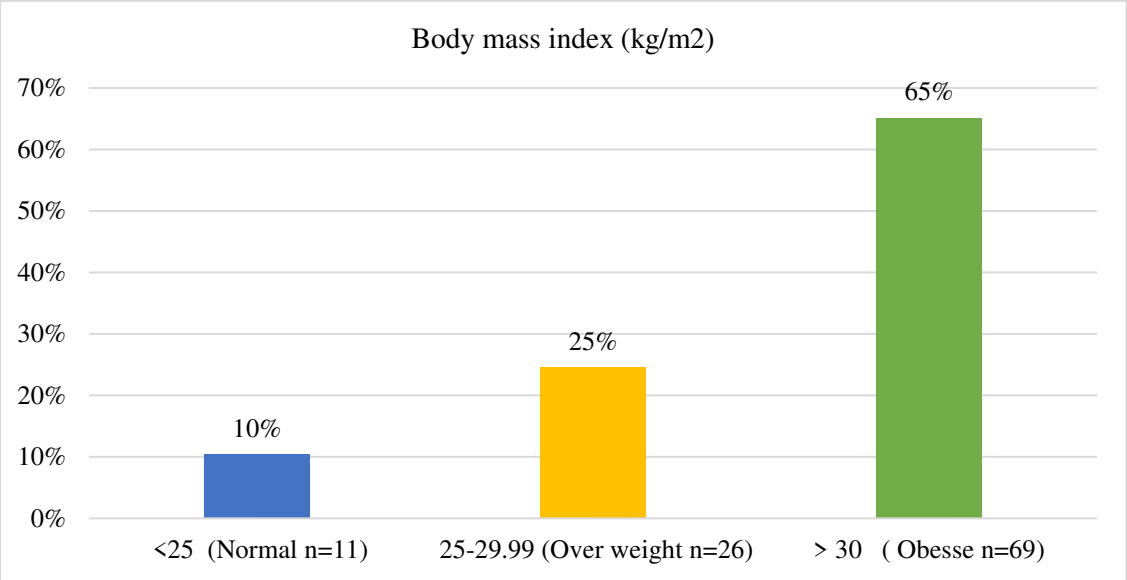

Figure 3.3. Prevalence of study population classified by body mass index (BMI)

3.2.2. Arithmetic mean of BMD and TAS study population women considering BMI groups

Table 3.3 demonstrate A significant was found in BMD among BMI groups ($p<0.01$). No significant was found between BMI group and TAS ($P>0.05$).

Table 3.3. Arithmetic mean of BMD and TAS study population women considering BMI groups

Parameters	**Mean± SD**			*P*-value
	<25Kg/m²(n=11)	**25-29.99Kg/m²(n=26)**	**> 30 Kg/m2 (n=69)**	
BMD(g/cm²)	0.584±0.094	0.613±0.113	0.740±0.19	<0.01
TAS (mmol/L)	2.81±0.18	2.78±0.14	2.79±0.23	NS

3.3. Vitamin B12 group

The participants were grouped depending on the Vitamin B12 levels into two groups. Low vitamin B12 (<200 pg / ml) (n=26), Normal Vitamin B12 (>200 pg / ml) (n=80).

3.3.1. Prevalence of study population classified by Vitamin B12

Distribution of Vit.B12 is represented in Figure3.4 which indicates that the lowest percentage (25%) of the study population women was (<200 pg/ml), while the highest percentage (75%) was at (>=200 pg/ml).

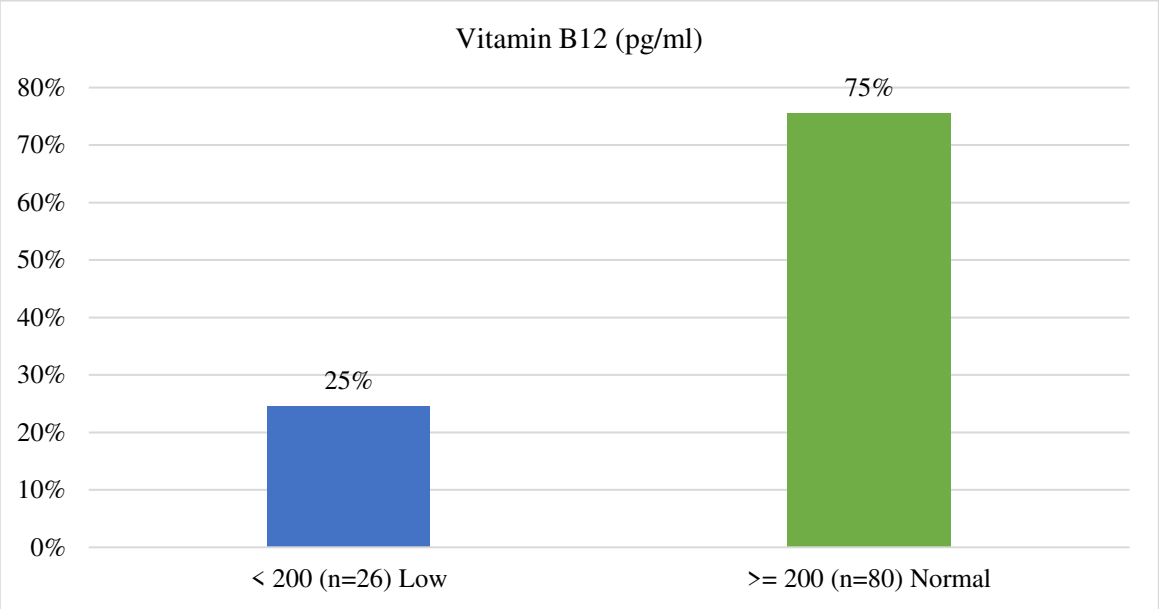

Figure 3.4. Prevalence of study population classified by Vitamin B12

3.3.2. Arithmetic mean of BMD and TAS in study population women considering Vitamin B12 groups

Table 3.4 demonstrate non-significant differences in BMD and serum level of TAS across the intervals of Vit.B12 in study population women ($P > 0.05$).

Table 3.4. Arithmetic mean of BMD and TAS study population women considering Vitamin B12 groups.

Parameters	Mean± SD		
	<200 pg/ml (n=26)	**>= pg/ml (n=80)**	***P*-value**
BMD(g/cm^2)	0.677±0.171	0.697±0.186	NS
TAS (mmol/L)	2.77±0.21	2.80±0.20	NS

3.4. Folic Acid group

Women were classified into two groups based on folic acid levels. The first group were low (< 5 ng / ml) (n=40), while the second one was with normal folate levels (>=5 ng / ml) (n=66).

3.4.1. Prevalence of study population classified by Folic Acid

Distribution of FA is represented in Figure 3.5 which indicates that the lowest percentage (38%) of study population women was (<5 ng/ml), while the highest percentage (62%) was at (>=5 ng/ml).

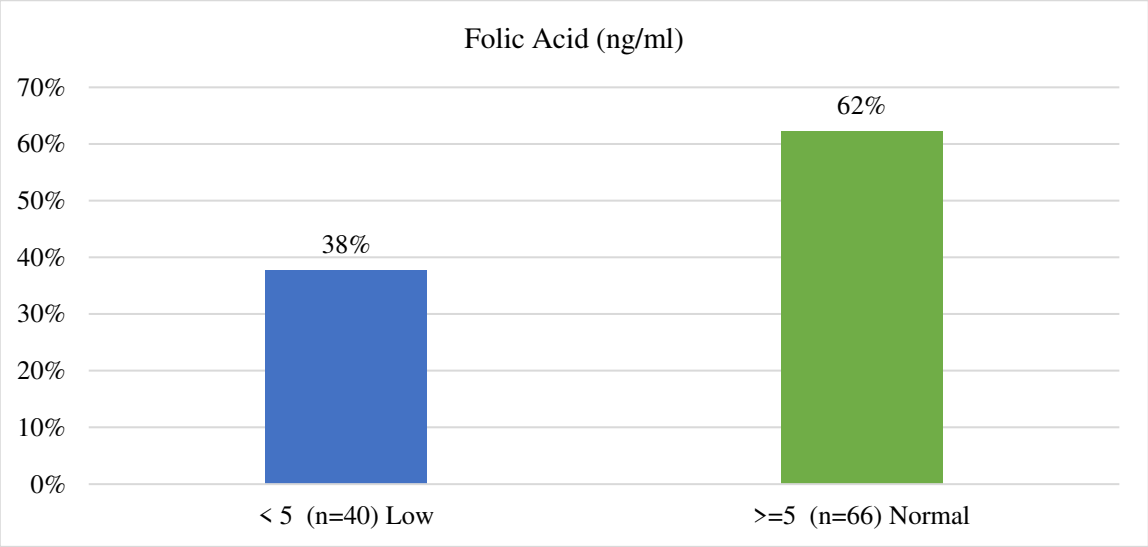

Figure 3.5. Prevalence of study population classified by Folic Acid.

3.4.2. Arithmetic mean of BMD and TAS in study population women considering Folic Acid groups

BMD and TAS classified by Folic Acid groups in the whole study population women are illustrated in Table 3.5. A significant was found in BMD among the Folic Acid group ($p<0.05$). No significant was found between the folic acid group and TAS ($P>0.05$).

Table 3.5. Arithmetic mean of BMD and TAS study population women considering Folic Acid groups.

parameters	Mean± SD		
	< 5 ng/ml (n=40)	**>=5 ng/ml (n=66)**	***P*-value**
BMD(g/cm^2)	0.642±0.146	0.723±0.195	<0.05
TAS (mmol/L)	2.77±0.17	2.81±0.22	NS

3.5. Vitamin D group

The study population was classified into three groups depending on the Vitamin D3 levels starting from Deficient (< 9.99 ng / ml) (n=28), Insufficient (10 to 29.99 ng per ml) (n equal to 61), adequate (more than 30 ng per ml) (n equal to 17).

3.5.1. Prevalence of study population classified by Vitamin D.

Results of serum vitamin D levels in study population show that 26% of women were vitamin D deficient and below (10 ng/ml), while some of the women showed as having vitamin D inadequate was 58% with vitamin D concentrations between (10-29 ng/ml). Eventually, women with vitamin D sufficient played 16% with vitamin D concentrations (equal or more than 30 ng/ml). Primarily, these results illustrate the high prevalence of vitamin D insufficient among the survey population as shown in figure 3.6.

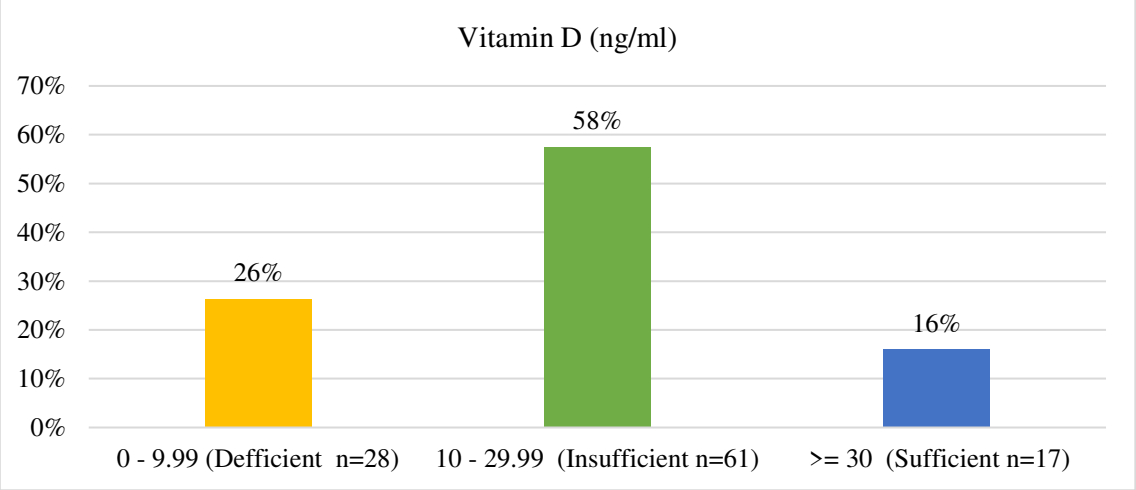

Figure 3.6. Prevalence of study population classified by Vitamin D

3.5.2. Arithmetic mean of BMD and TAS in study population women considering Vitamin D groups

Table 3.6 demonstrate non-significant differences in BMD and serum level of TAS across the intervals of Vit.D in study population women ($P > 0.05$).

Table 3.6. Arithmetic mean of BMD and TAS study population women considering Vitamin D groups.

parameters	Mean± SD			*P*-value
	0- 9.99ng/ml (n=28)	10 – 29.99 ng/ml (n=61)	> 30 ng/ml (n=17)	
BMD(g/cm^2)	0.658±0.190	0.714±0.177	0.673±0.184	NS
TAS (mmol/L)	2.77±0.19	2.81±0.20	2.76±0.22	NS

3.6. Correlation Analysis

According to the Pearson correlation coefficient (r), TAS represents a negative higher significant correlation with age, while it was positively high significant correlated with T. score and BMD. On the contrary, there was no significant correlation with Body mass index(BMI), Waist circumference(WC), Vit B12, folic acid, Vit.D and Calcium (Table3.7). BMD has a positive high significant correlation with BMI, WC, T-score, TAS and folic acid, while it was negative high significantly correlated with age (Table 3.8).

Table 3.7. Correlation between TAS and other parameters in the study population women.

Variables	(r)	P-value
Age (year)	**-0.435****	**<0.001**
BMI(Kg/m^2)	0.155	NS
WC(cm)	-0.011	NS
T.scor	**0.519****	**<0.001**
BMD(g/cm^2)	**0.457****	**<0.001**
Vit.B12 (pg/ml)	0.051	NS
Folic Acid (ng/ml)	0.141	NS
Vit. D (ng/ml)	0.016	NS
S.Ca (mg/dl)	0.096	NS

Table 3.8. Correlation between BMD and other parameters in the study population women.

Variables	(r)	P-value
Age (year)	**-0.440****	**<0.001**
BMI(kg/m^2)	**0.590****	**<0.001**
WC(cm)	**0.416****	**<0.001**
T.scor	**0.587****	**<0.001**
TAS (mmol/L)	**0.457****	**<0.001**
Vit.B12 (pg/ml)	0.094	NS
Folic Acid (ng/ml)	**0.359****	**<0.001**
Vitamin D (ng/ml)	0.004	NS
S.Ca (mg/dl)	0.177	NS

3.7. Discussions

Because of their role in pathogenesizing many diseases, including metabolic bone diseases, reactive oxygen species (ROS) and antioxidants have received much attention [127]. In vitro experiments or animal models showed that OS had an important effect on the differentiation and functions of osteoclast [128, 129]. There has also been evidence in a small number of human studies in which antioxidant and ROS processes can participate in the bone loss pathogenesis involved [130].

While OP is a common disease of the elderly, its key initiating factor is the decline in estrogen associated with menopause rather than the aging [27]. Many lines of evidence indicate that one of the strategies embraced by estrogens for shielding women from bone loss during reproductive age is to contrast ROS's supposed deleterious action against bone health [131]. Most of the data used to support the interplay between OS and OP were collected from in vitro and animal studies, which indicate a possible role for reactive species in uncoupling bone turnover [132, 133].

Nevertheless, the role of OS in the disturbance of bone homeostasis is not adequately confirmed. Accordingly, this research was conducted to investigate whether OS could play a role in this bone homeostasis disturbance and to assess the levels of TAS, vitamin D, vitamin B12, folic acid and serum calcium in women with osteoporosis compared to non-osteoporotic healthy women taking into account the effects of other clinical variables that could affect bone mass and focus osteoporosis.

The mean value of age in osteoporotic women was found to be (53.67 ± 8.01 years) which was relatively higher than the mean age of healthy women (46.50 ± 6.43 years) ($P < 0.001$) (Table 3.1). In the whole study population women, age indicated a turndown association with total BMD (r=-0.440, p=0.001) (Table 3.8). Naz *et al* (2015) [134] supported this finding and concluded which

with each 10 years rise in age, the danger of osteoporosis rise 2.18 fold. Many epidemiological studies concluded that increasing age, especially when women become postmenopausal is the main reason for primary OP in women [134, 135]. But this finding was not agreed with other studies (Maggio*, et al.* 2003, (Ozgocmen, *et al.* 2007), (Razmandeh, *et al.* 2014) and (Karakas, *et al.* 2016) [73, 130, 136, 137] that demonstrated statistically according to the age non-significant variation showed was P>0.05.

Future fractures reported as a danger factor related to the low body mass index, while an elevated BMI seems preservative [138]. Cho, *et al.* (2014) [139] found that osteoporosis patients had 0.760 times lower BMI than which of normal patients. Weight is closely related to bone mass. Consequently, bone mass is minor and bone loss in postmenopausal women with low BMI enhances, but BMD carries on with to be high in obese women [140].

In the present survey, there was a positive association between BMI and total BMD (r =0.590, $P = <0.001$) (Table 3.8) As well as, results proved the presence of a significant variation in mean values of BMI between groups (healthy group 35.71±7.29), (osteoporosis group 32.71±6.25),($P < 0.05$) (Table 3.1) and illustrated that women with low BMI are at enhancer danger of developing osteoporosis and the effect of higher BMI may compensate the negative influence of the hypoestrogenic state on BMD during menopauses. Several studies have found that greater weight of the body will support high bone density and lower fracture incidence (Laet, *et al.* 2005, Aggarwal, *et al.* 2011, Cho, *et al*. 2014, Heidari, *et al.* 2015, Hyassat, *et al.* 2017) [138, 139, 141-143].

Oxidative stress, sometimes triggered by an imbalance of oxidative of human and antioxidant level, have been correlated with age and a variety of illnesses in human, involving cancer, atherosclerosis, rheumatoid arthritis, osteoarthritis, fibromyalgia, osteoporosis [144-146]. In separate research, the correlation between oxidative stress and BMD has been searched. The oxidative stress consequence on the etiopathogenesis of the osteoporosis, however, is not obvious. Wolf, *et al.*, [74] illustrated the correlation between food ingestion, whole intake or serum antioxidant levels and BMD in postmenopausal women were examined. They documented in postmenopausal women no association between serum antioxidant level and BMD.

Studies have shown that free radicals derived from oxygen are associated with parathyroid hormone (PTH)-stimulated osteoclastic bone resorption, interleukin (IL)-1beta, tumor necrosis factor (TNF) alpha [10, 147].

Karatas et al. [148] have investigated rheumatoid arteries patients and have found a decrease in antioxidant status and an increase in oxidative stress, similarly, Bekpinar, S., et al., Basu, S., et al., [149, 150] have found that significantly increased plasma oxidants and decreased antioxidants in osteoporotic patients when compared to controls. Altindag et al. [151] have reported in knee osteoarthritis patients, there was an increase in oxidant parameters and a decrease in antioxidant

parameters. Ozgocmen, *et al.* [130] have found which oxidative stress criteria were enhanced and the level of antioxidant enzymes was decreased in patients with osteoporosis. Ozgocmen and colleagues [152] have been documented that oxidative stress plays an important role in the pathogenesis of PMO and drugs had effects on the antioxidant defense system. It was proposed that plasma ascorbic acid, alpha-tocopherol, complete thiol groups, and erythrocyte glutathione were reduced, and that lipid peroxidation in postmenopausal patients was decreased [153]. similarly, A clinical study done by Sanchez-Rodriguez et al.,2007 [153], showed a decrease in the concentration of TAS as well as the concentrations of the antioxidant enzymes glutathione peroxidase GPx, compared to that found in healthy control women, and were positively correlated with BMD. Deyhim et al. [154] have examined the role of antioxidant diet on bone strength; they have reported that drinking citrus juice for six days positively affects serum antioxidant capacity and bone mineralization. Maggio et al. 2003 [73], found that postmenopausal osteoporosis women had significantly lower serum levels of vitamins A, C and E in addition significantly lower GPx and SOD than controls.

In this study, we found lower serum TAS level in osteoporotic women compared with non-osteoporotic women (2.73±0.16, 3.01±0.18, P< 0.001) respectively (Table 3.1) .However, our study indicated that there is a highly significant positive correlation between serum TAS levels and total BMD (r=0.457, p=<0.001) (Table 3.7).we cannot find any relation between TAS and other biochemical parameters (vit.B12, folic acid, vit.D and calcium).

In postmenopausal osteoporosis, osteoclastic activity enhanced and decreased osteoblastic activity related to the imbalance between oxidant and antioxidant levels. Consequently, we conclude that maintaining the balance of oxidative stress is important in patients with PMO and supplementing the antioxidant-enriched food to the treatment may shed light on the making of new therapeutic approaches for osteoporosis.

Cofactor for methionine synthase is vitamin B12 in the remethylation of tHcy to methionine, which is a detector of the level. The groups did not differ in Vit.B12 (control group=472.22±216.42, patient group=397.34±230.15, $p > 0.05$) (Table 3.1), also in the present study we cannot find any correlation between Vit.B12 with BMD and TAS (r=0.094, P>0.05, Table (3.1), r=0.051, P>0.05, (Table 3.8) respectively. These results are in agreement with those of Cagnacci, et al. 2003 [101], Macdonald, et al. 2004 [115], Halıloglu, B., et al.,.2010 [109], Golbahar, J., et al.,2004 [102] and Baines, M., et al.2007 [103].While these five previous studies documented that there was no association between serum vitamin B12 levels and BMD in women with postmenopausal. In contrast ,Morris, *et al.* 2005 [108] illustrated from United States National and Nutrition Examination Survey which 1550 elderly Americans have more than 220pmol/L vitamin B12 was associated with BMD in a dosage connected, but that increases in serum vitamin B12 beyond 220 pmol/L were not so associated. Also Tucker, *et al*. 2005 [122] established which adults with

cobalamin levels below 148 pmol/l had lower mean BMD than those with higher levels, however with significant ranging from the site of BMD measurement (spine in women and men in hip positions). Stone, *et al.* 2004 [119] showed that even the yearly rate of decrease in total hip BMD, but not calcaneal BMD was higher for women with vitamin B12 concentrations below 207 pmol/L than for those with greater levels. The variance in results of serum cobalamin interaction with BMD could, therefore, be caused by population demographic differences and by the BMD site.

The detector of blood tHcy level is folate status. In present survey data (Table 3.1) approve an elevated positive significant association of folate with BMD ($r=0.359$, $p<0.0001$). The present result demonstrated a significantly high level of BMD with a high level of folic acid as shown in (Table 3.5). The mean value of Folic Acid in osteoporotic women was found to be (6.33±4.12 years) which was relatively lower than the mean value of healthy women (12.59±4.83 years) ($P < 0.001$) (Table 3.1). Even though low folate concentrations were illustrated as a risk factor for osteoporosis in Italian, Iranian and British women, Cagnacci, et al. 2003 [101] in the lumbar spine illustrated a significant connection between folate and BMD, and in comparison with non-osteoporotic controls plasma folate concentrations were decreased in the osteoporotic group. This corresponds with a survey of 271 Iranian women with postmenopausal by Golbahar,J., *et al*. 2004 in which BMD revealed a positive association with serum folate [102]. Also, several studies Ravaglia, G., et al,2005 [106], McLean, R.R, et al 2004 [155], Akpolat, V., et al, 2013 [156] and Baines, M., et al.2007 [103] have illustrated folate with BMD have a significant positive association.

In American and Turkish women documented by some researchers that no association between folate and BMD, although a current study of 1550 old American women subjects, that resulted that neither serum nor red blood cell folate was associated to BMD or osteoporosis, Morris, *et al.* 2005 [108], perhaps due to of an elevated average age and higher mean plasma folate levels. Bozkurt, *et al.* 2009 [118] documented plasma Hcy, nevertheless nor folate concentrations, were related to osteoporosis in comparison to young postmenopausal women of Turkey. It appears which food habits, race-related variation, the position of BMD computation, and the survey variation in a population (sex, age, et cetera), mat influence the study consequences examing the correlation between BMD and folate concentration as well as cobalamin. Proposing that folate may be free osteoporosis risk factor.

This research examined the status of vitamin D to survey the potential correlation between vitamin D and osteoporosis. Results showed that 58 percent of the women surveyed had insufficient vitamin D (figure 3.6), which are accurate with the results of the convergence survey in Kirkuk city/Iraq by Ali, N (2018) [157] who discovered that 59 percent of women had vitamin D insufficiency and had a mean value less than 20 ng/ml. Another survey performed in the Saudi Arabia Kingdom by Alkhenizan et al (2017) [158] confirmed a mild to intense vitamin D deficit in

61.5 percent of their patients with the mean serum value less than 20 ng/ml. The explanation for the decrease in vitamin D plasma concentration maybe because of decreasing women's outside activities and reduced or clothing at the study server coated sunlight exposure that averts the vitamin D production in the skin and consequently inhibits the conversion to the active shape [85] that is in charge of for maintaining the phosphorous and calcium concentrations in serum. The insufficiency in vitamin D encourages a compensatory rise in the levels of parathyroid hormones [159]. Consequently, loss of bone is increased and the risk of osteoporosis evolving increases [42].

Furthermore, this research failed to explain major dissimilarities between study groups in serum vitamin D concentrations due to frequent follow-ups of all of our studied patients and all patients administered with vitamin D and calcium with osteoporosis.

Mean values for subjects with low BMD were observed in this study (osteoporosis: 18.91±14.99 ng/ml) and subjects with normal BMD (22.19±14.38 ng/ml) ($p > 0.05$), (Table 3.1). In addition, we cannot find any relationship between serum vitamin D level and BMD and TAS (Table and Table 3.8). Similar findings were demonstrated by Hyassat et al (2017) [143] and Sahmani et al (2014) [135]. In contrast with these results, a review of the systematic of Gaugris et al (2005) [160] inadequate levels of vitamin D was observed to be a common risk factor for osteoporosis in postmenopausal women.

Calcium is one of the principal bone-forming minerals and an adequate supply of bone that is necessary at all stages of life [23]. However, the average value of serum calcium levels was within the reference range in postmenopausal women with osteoporosis (9.46±0.44 mg/dl), and in healthy women (9.46±0.44 mg/dl) and illustrated statistically non-significant changes between the groups (Table 3.1) we cannot find any correlation between serum calcium and BMD and TAS (Table3.7 and Table 3.8). Furthermore, so many studies suggest that the quantification of serum calcium statistically did not display any significant osteoporosis diagnostic values as their results were within a normal range (Sontakke and Tare, 2002 [127]; Hania, 2008 [36]; Razmandeh, *et al.* 2014 [136]; Sahmani, et al. 2014 [135]; Karakas, *et al*. 2016) [137]

4. CONCLUSION

We have found a signifiant decrease in total antioxidant status in patients with osteoporosis. Although there was no difference between vitamin D3, serum calcium, and vitamin B12 values. Folic acid values were significantly decreased in the patient group.

We also assumed that increased activity of osteoclastic and a decreased osteoblastic activity could be correlated to a mismatch from postmenopausal osteoporosis between oxidant and antioxidant. We therefore actually think that sustaining the oxidative stress balance in PMO patients is essential and supplementing the antioxidant-enriched food with therapy could shed light on the development of new osteoporosis pharmacological mechanisms.

It was found that BMI has a protective effect against osteoporosis and correlated with increased total BMD in OP.

Women with lower BMD were appeared older than women with normal BMD. Furthermore, age was negatively correlated with total BMD in osteoporotic women.

Finally, the present study concludes that total antioxidant status is negatively associated with age and positively associated with BMD.

REFERENCES

[1] Ethgen, Olivier, Mickaël Hiligsmann, Nansa Burlet, and J-Y. Reginster (2016). "Cost-effectiveness of personalized supplementation with vitamin D-rich dairy products in the prevention of osteoporotic fractures." *Osteoporosis international* 27, no. 1 : 301-308.

[2] Kanis, John A., L. Joseph Melton III, Claus Christiansen, Conrad C. Johnston, and Nikolai Khaltaev (1994). "The diagnosis of osteoporosis." *Journal of bone and mineral research* 9, no. 8 : 1137-1141.

[3] Samelson, Elizabeth J., and Marian T. Hannan (2006). "Epidemiology of osteoporosis." *Current rheumatology reports* 8, no. 1: 76-83.

[4] Bartl, Reiner, and Bertha Frisch (2009). *Osteoporosis: diagnosis, prevention, therapy*. Springer Science & Business Media.

[5] Rao, L. G. (2005) "Will tomatoes prevent osteoporosis." *Endocrinology Rounds* 5, no. 2: 118-135.

[6] Oveisi, Mohammad Reza, Naficeh Sadeghi, Behrooz Jannat, Mannan Hajimahmoodi, Molouk Hadjibabaie, and Abdolazim Behfar (2011). "Evaluation of antioxidants in bone mineral density of Iranian osteoporotic women." *Iranian Journal of Basic Medical Sciences* 14, no. 2 : 158-166.

[7] Deveci, Haci Ahmet, Gokhan Nur, Hulya Cicek, and Mahmut Karapehlivan(2017). "Evaluation of oxidative stress factors in patients with osteoporosis." *Med Sci* 6, no. 3 : 479-82.

[8] Baek, Ki Hyun, Ki Won Oh, Won Young Lee, Seong Su Lee, Mee Kyoung Kim, Hyuk Sang Kwon, Eun Jung Rhee (2010). "Association of oxidative stress with postmenopausal osteoporosis and the effects of hydrogen peroxide on osteoclast formation in human bone marrow cell cultures." *Calcified tissue international* 87, no. 3 : 226-235.

[9] Lee, Young Joo, Ji Yun Hong, Seung Chul Kim, Jong Kil Joo, Yong Jin Na, and Kyu Sup Lee(2015). "The association between oxidative stress and bone mineral density according to menopausal status of Korean women." *Obstetrics & gynecology science* 58, no. 1: 46-52.

[10] Altindag, Ozlem, Ozcan Erel, Neslihan Soran, Hakim Celik, and Sahabettin Selek(2008). "Total oxidative/anti-oxidative status and relation to bone mineral density in osteoporosis." *Rheumatology international* 28, no. 4 : 317-321.

[11] Sadeghi, Naficeh, Behrooz Jannat, Mohammad-Reza Oveisi, Mannan Hajimahmoodi, Ahmad-Reza Jamshidi, Golnaz Izadi Dolagh, Abdol-azim Behfar, Kheir-ollah Gholami, and Masoomeh Behzad (2010). "The erythrocyte catalase enzyme activity in Iranian osteoporotic women." *Iranian Journal of Pharmaceutical Sciences* 6, no. 1: 19-24.

[12] Kanis, John A., L. Joseph Melton III, Claus Christiansen, Conrad C. Johnston, and Nikolai Khaltaev(1994). "The diagnosis of osteoporosis." *Journal of bone and mineral research* 9, no. 8: 1137-1141.

[13] De Bree, A., N. M. J. van der Put, L. I. Mennen, W. M. M. Verschuren, H. J. Blom, P. Galan, C. J. Bates (2005). "Prevalences of hyperhomocysteinemia, unfavorable cholesterol profile and hypertension in European populations." *European journal of clinical nutrition* 59, no. 4: 480-488.

[14] Clarke, Robert(1998). "Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials." *British Medical Journal* 316, no. 7135: 894-899.

[15] Jacques, Paul F., Andrew G. Bostom, Peter WF Wilson, Sharron Rich, Irwin H. Rosenberg, and Jacob Selhub (2001). "Determinants of plasma total homocysteine concentration in the Framingham Offspring cohort." *The American journal of clinical nutrition* 73, no. 3: 613-621.

[16] Sandukji, A., H. Al-Sawaf, A. Mohamadin, Y. Alrashidi, and S. A. Sheweita (2011). "Oxidative stress and bone markers in plasma of patients with long-bone fixative surgery: role of antioxidants." *Human & experimental toxicology* 30, no. 6: 435-442.

[17] Compston, Juliet (2006). "The pathogenesis of osteoporosis." *Osteoporosis*.

[18] Bonjour, Jean-Philippe, and René Rizzoli (2003). "Peak bone mass acquisition." In *Osteoporosis*, pp. 61-81. Humana Press, Totowa, NJ.

[19] Yousef, F (2015). "MA. Peak Bone Mass and Prevention of Osteoporosis in Adolescence: Role of Vitamin D and Calcium." *World Journal of Medical Science* 12, no. 2: 170-182.

[20] Karimi, Winfred (2011). "Preventing osteoporosis in menopause: a literature review.".

[21] McLeod, Katherine M(2013).. "Osteoporosis Care Gap." PhD diss., Faculty of Graduate Studies and Research, University of Regina.

[22] Hania, Huda Mahmoud(2008). "Occurrence of Osteoporosis Among Menopausal Women in Gaza Strip." *Occurrence of Osteoporosis among Menopausal Women in Gaza Strip* (2008).

[23] Almalki, N., Fawwaz Algahtany, and Khaled Alswat(2016). "Osteoporosis knowledge assessment among medical interns." *Am J Res Commun* 4, no. 1: 1-1.

[24] Brown, Paul, Rob McNeill, Eman Radwan, and Jane Willingale(2007). "The burden of osteoporosis in New Zealand: 2007-2020." *Wellington: Osteoporosis New Zealand* .

[25] Frost, Steven Alfred(2013). "Defining the Contribution of Bone Loss to Fracture." PhD diss., University of New South Wales,.

[26] WHO Scientific Group on Prevention, Management of Osteoporosis, and World Health Organization (2003).. *Prevention and management of osteoporosis: report of a WHO scientific group*. No. 921. World Health Organization.

[27] Penrod, J., A. Smith, S. Terwilliger, and S. Gueldner (2008). "Demographic perspective: The magnitude of concern." *Osteoporosis: Clinical guidelines for prevention, diagnosis and management*: 9-18.

[28] Tsai, Midi. (2008)."The relationship between osteoporosis knowledge, beliefs and dietary calcium intake among South Asian women in Auckland: a thesis presented in partial fulfillment of the requirements for the degree of Master of Science in Human Nutrition at Massey University, Auckland, New Zealand." PhD diss., Massey University.

[29] Mohd-Tahir, N. A., and S. C. Li. (2017) "Economic burden of osteoporosis-related hip fracture in Asia: a systematic review." *Osteoporosis International* 28, no. 7: 2035-2044.

[30] Gorial, Faiq I., Nisreen D. Aubaese, and Nibrass H. Husaeen(2013). "Prevalence and associated factors of osteoporosis in post-menopausal Iraqi women: a cross-sectional two centers study." *Int. J. Modern Biol. Med* 3, no. 1: 41-49.

[31] Celenk, Cetin, and Peruze Celenk (2012). "Bone density measurement using computed tomography." *Computed tomography-clinical applications* : 123-136.

[32] Davies, Matt D., and Michael Bliziotes (1998). "Osteoporosis: Risk Factors, Diagnosis, and Therapy.": 418-421.

[33] Ferdous, HSa, Faria Afsana, Nazmul Kabir Qureshi, and Rushda Sharmin Binte Rouf (2015). "Osteoporosis: A review." *BIRDEM Medical Journal* 5, no. 1: 30-36.

[34] Östman, Bengt, Karl Michaëlsson, Johanna Helmersson, Liisa Byberg, Rolf Gedeborg, Håkan Melhus, and Samar Basu (2009). "Oxidative stress and bone mineral density in elderly men: antioxidant activity of alpha-tocopherol." *Free Radical Biology and Medicine* 47, no. 5: 668-673.

[35] Rahman, Taibur, Ismail Hosen, MM Towhidul Islam, and Hossain Uddin Shekhar (2012). "Oxidative stress and human health.".

[36] Basu, Tapan Kumar, Norman J. Temple, and Manohar L. Garg, eds(1999). *Antioxidants in human health and disease*. CABI Pub.

[37] Kim, Beom-Jun, Jung-Min Koh, Seong Hee Ahn, Seung Hun Lee, Eun Hee Kim, Sung Jin Bae, Hong-Kyu Kim, Jae Won Choe, and Ghi Su Kim(2013). "High serum total bilirubin as a protective factor against hip bone loss in healthy middle-aged men." *Calcified tissue international* 92, no. 6: 501-508.

[38] Bhattacharya, Susinjan. "Reactive oxygen species and cellular defense system(2015)." In *Free radicals in human health and disease*, pp. 17-29. Springer, New Delhi,.

[39] Halliwell, Barry (2001). "Free radical reactions in human disease." *Oxidative Stress and Disease* 7 (2001): 1-16.

[40] Shalaby, Emad A., and Sanaa MM Shanab(2013). "Antioxidant compounds, assays of determination and mode of action." *African journal of pharmacy and pharmacology* 7, no. 10: 528-539.

[41] Mirończuk-Chodakowska, Iwona, Anna Maria Witkowska, and Małgorzata Elżbieta Zujko. (2018)"Endogenous non-enzymatic antioxidants in the human body." *Advances in medical sciences* 63, no. 1: 68-78.

[42] Perez-Lopez, F. R., P. Chedraui, and J. L. Cuadros-Lopez (2010). "Bone mass gain during puberty and adolescence: deconstructing gender characteristics." *Current medicinal chemistry* 17, no. 5: 453-466.

[43] Bergamini, Carlo M., Stefania Gambetti, Alessia Dondi, and Carlo Cervellati(2004). "Oxygen, reactive oxygen species and tissue damage." *Current pharmaceutical design* 10, no. 14: 1611-1626.

[44] Kumar, Shiv (2011). "Free radicals and antioxidants: human and food system." *Adv Appl Sci Res* 2, no. 1: 129-135.

[45] Barrera, Giuseppina (2012). "Oxidative stress and lipid peroxidation products in cancer progression and therapy." *ISRN oncology* .

[46] Close, Dugald C., and Ann E. Hagerman (2006). "Chemistry of reactive oxygen species and antioxidants." In *Oxidative stress, Exercise and Aging*, pp. 1-8.

[47] Birben, Esra, Umit Murat Sahiner, Cansin Sackesen, Serpil Erzurum, and Omer Kalayci (2012). "Oxidative stress and antioxidant defense ." *World Allergy Organization Journal* 5, no. 1: 9-19.

[48] Dhawan, Veena. "Reactive oxygen and nitrogen species: general considerations (2014)." In *Studies on respiratory disorders*, pp. 27-47. Humana Press, New York, NY.

[49] Ozcan, Ayla, and Metin Ogun (2015). "Biochemistry of reactive oxygen and nitrogen species." *Basic principles and clinical significance of oxidative stress* 3: 37-58.

[50] Valko, Marian, Dieter Leibfritz, Jan Moncol, Mark TD Cronin, Milan Mazur, and Joshua Telser(2007). "Free radicals and antioxidants in normal physiological functions and human disease." *The international journal of biochemistry & cell biology* 39, no. 1: 44-84.

[51] Partap, Sangh. "A review on herbal antioxidants(2012)." *Journal of Pharmacognosy and Phytochemistry* 1, no. 4.

[52] Yadav, Anuj, Rewa Kumari, Ashwani Yadav, J. P. Mishra, Seweta Srivatva, and Shashi Prabha (2016). "Antioxidants and its functions in human body-A Review." *Res. Environ. Life Sci* 9, no. 11: 1328-1331.

[53] Pradedova, E. V., O. D. Isheeva, and R. K. Salyaev(2011). "Classification of the antioxidant defense system as the ground for reasonable organization of experimental studies of the oxidative stress in plants." *Russian Journal of plant physiology* 58, no. 2: 210-217.

[54] Nimse, Satish Balasaheb, and Dilipkumar Pal (2015). "Free radicals, natural antioxidants, and their reaction mechanisms." *Rsc Advances* 5, no. 35: 27986-28006.

[55] Hall, T. J., M. Schaeublin, H. Jeker, K. Fuller, and T. J. Chambers (1995). "The role of reactive oxygen intermediates in osteoclastic bone resorption." *Biochemical and biophysical research communications* 207, no. 1: 280-287.

[56] Manolagas, Stavros C. (2010). "From estrogen-centric to aging and oxidative stress: a revised perspective of the pathogenesis of osteoporosis." *Endocrine reviews* 31, no. 3: 266-300.

[57] Sendur, Omer Faruk, Yasemin Turan, Engin Tastaban, and Mukadder Serter. "Antioxidant status in patients with osteoporosis: a controlled study (2009)." *Joint Bone Spine* 76, no. 5: 514-518.

[58] Sharma, Tripti, Najmul Islam, Jamal Ahmad, Nishat Akhtar, and Mujahid Beg (2015). "Correlation between bone mineral density and oxidative stress in postmenopausal women." *Indian journal of endocrinology and metabolism* 19, no. 4: 491.

[59] Maggio, Dario, Mauro Barabani, Marco Pierandrei, M. Cristina Polidori, Marco Catani, Patrizia Mecocci, Umberto Senin, Roberto Pacifici, and Antonio Cherubini (2003). "Marked decrease in plasma antioxidants in aged osteoporotic women: results of a cross-sectional study." *The Journal of Clinical Endocrinology & Metabolism* 88, no. 4: 1523-1527.

[60] Wolf, Randi L., Jane A. Cauley, Mary Pettinger, Rebecca Jackson, Andrea Lacroix, Meryl S. Leboff, Cora E. Lewis (2005). "Lack of a relation between vitamin and mineral antioxidants and bone mineral density: results from the Women's Health Initiative–." *The American journal of clinical nutrition* 82, no. 3: 581-588.

[61] Bringhurst, F. Richard(2008). "Bone and mineral metabolism in health and disease." *Harrison's principles of internal medicine* : 2365-2377.

[62] McClung, Michael R(2003). "Prevention and management of osteoporosis." *Best Practice & Research Clinical Endocrinology & Metabolism* 17, no. 1: 53-71.

[63] Watson, Ronald Ross, and Daruka Mahadevan (2016), eds. *Handbook of nutrition and diet in therapy of bone diseases*. Vol. 13. Wageningen Academic Publishers,.

[64] Almeida Paz, I. C. L., and L. D. G. Bruno(2006). "Bone mineral density." *Brazilian Journal of Poultry Science* 8, no. 2: 69-73.

[65] Ringle, Kelly A. (2009) "An investigation of bone mineral density and bone mineral content among hispanic women by lifestyle factors." PhD diss., The Ohio State University.

[66] Winzenberg, Tania (2006). "Osteoporosis prevention in premenopausal women and children." PhD diss., University of Tasmania.

[67] Reid, Ian R. (2003) "Clinical aspects of the use of vitamin D and its metabolites in Osteoporosis." In *Osteoporosis*, pp. 293-307. Humana Press, Totowa, NJ.

[68] Mauck, Karen F., and Bart L. Clarke. (2006) "Diagnosis, screening, prevention, and treatment of osteoporosis." In *Mayo Clinic Proceedings*, vol. 81, no. 5, pp. 662-672. Elsevier.

[69] Salminen, Helena. (2007) *Osteoporosis in elderly women in primary health care*. Institutionen för neurobiologi, vårdvetenskap och samhälle/Department of Neurobiology, Care Sciences and Society,.

[70] MacLaughlin, Julia A., R. R. Anderson, and Michael F. Holick. (1982) "Spectral character of sunlight modulates photosynthesis of previtamin D3 and its photoisomers in human skin." *Science* 216, no. 4549: 1001-1003.

[71] Nguyen, Thi Thanh Huong. (2012) *Osteoporosis, a major health problem in Vietnam-Life style factors and determinants of bone mass*. Inst för kvinnors och barns hälsa/Dept of Women's and Children's Health,.

[72] Ferrari, Serge, René Rizzoli, and Jean-Philippe Bonjour. (2000) "Vitamin D receptor gene polymorphisms and bone mineral homeostasis." In *The genetics of osteoporosis and metabolic bone disease*, pp. 45-60. Humana Press, Totowa, NJ,.

[73] Bonjour, J-P., M-A. Schurch, and René Rizzoli. (1996) "Nutritional aspects of hip fractures." *Bone* 18, no. 3: S139-S144.

[74] Jordan, K. M., and C. Cooper. (2002)"Epidemiology of osteoporosis." *Best practice & research. Clinical rheumatology* 16, no. 5: 795-806.

[75] Sambrook, Philip, and Cyrus Cooper. (2006)"Osteoporosis." *The Lancet* 367, no. 9527: 2010-2018.

[76] Tucker, Katherine L. (2009) "Osteoporosis prevention and nutrition." *Current Osteoporosis Reports* 7, no. 4: 111.

[77] Ahmadieh, Hala, and Asma Arabi. (2011) "Vitamins and bone health: beyond calcium and vitamin D." *Nutrition Reviews* 69, no. 10: 584-598.

[78] McLean, Robert R., and Marian T. Hannan. (2007)"B vitamins, homocysteine, and bone disease: epidemiology and pathophysiology." *Current osteoporosis reports* 5, no. 3: 112-119.

[79] Herrmann, Markus, Johannes Peter Schmidt, Natalia Umanskaya, Alexandra Wagner, Omid Taban-Shomal, Thomas Widmann, Graziana Colaianni, Britt Wildemann, and Wolfgang Herrmann. (2007)"The role of hyperhomocysteinemia as well as folate, vitamin B6 and B12 deficiencies in osteoporosis–a systematic review." *Clinical Chemistry and Laboratory Medicine (CCLM)* 45, no. 12: 1621-1632.

[80] Swart, Karin MA, Annelies C. Ham, Janneke P. van Wijngaarden, Anke W. Enneman, Suzanne C. van Dijk, Evelien Sohl, Elske M. Brouwer-Brolsma et al. (2016)"A randomized controlled trial to examine the effect of 2-year vitamin B12 and folic acid supplementation on physical performance, strength, and falling: additional findings from the B-PROOF study." *Calcified tissue international* 98, no. 1: 18-27.

[81] Clarke, M., M. Ward, J. J. Strain, Leane Hoey, W. Dickey, and H. McNulty. (2014) "B-vitamins and bone in health and disease: the current evidence." *Proceedings of the Nutrition Society* 73, no. 2: 330-339.

[82] Dai, Zhaoli, and Woon-Puay Koh. (2015) "B-vitamins and bone health–a review of the current evidence." *Nutrients* 7, no. 5: 3322-3346.

[83] Bowman, Barbara Ann Brown, and Robert M. Russell (2001). *Present knowledge in nutrition*. Pan American Health Org,.

[84] Fenech, Michael. (2012)"Folate (vitamin B9) and vitamin B12 and their function in the maintenance of nuclear and mitochondrial genome integrity." *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 733, no. 1-2: 21-33.

[85] Finglas, Paul M. (2000) "Dietary Reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin and choline.": 296-297.

[86] Golbahar, J., M. A. Aminzadeh, S. A. Hamidi, and G. R. Omrani. (2005) "Association of red blood cell 5-methyltetrahydrfoate folate with bone mineral density in postmenopausal Iranian women." *Osteoporosis international* 16, no. 12: 1894-1898.

[87] Cagnacci, Angelo, Francesco Baldassari, Giovanni Rivolta, Serenella Arangino, and Annibale Volpe. (2003) "Relation of homocysteine, folate, and vitamin B12 to bone mineral density of postmenopausal women." *Bone* 33, no. 6: 956-959.

[88] Golbahar, Jamal, Afshin Hamidi, Mohammad A. Aminzadeh, and Gholamhossein R. Omrani. (2004)"Association of plasma folate, plasma total homocysteine, but not methylenetetrahydrofolate reductase C667T polymorphism, with bone mineral density in postmenopausal Iranian women: a cross-sectional study." *Bone* 35, no. 3: 760-765.

[89] Baines, M., M-B. Kredan, J. Usher, A. Davison, G. Higgins, W. Taylor, C. West, W. D. Fraser, and L. R. Ranganath. (2007)"The association of homocysteine and its determinants MTHFR genotype, folate, vitamin B12 and vitamin B6 with bone mineral density in postmenopausal British women." *Bone* 40, no. 3: 730-736.

[90] Cagnacci, Angelo, Bruno Bagni, Annalisa Zini, Marianna Cannoletta, Matteo Generali, and Annibale Volpe. (2008)"Relation of folates, vitamin B12 and homocysteine to vertebral bone mineral density change in postmenopausal women. A five-year longitudinal evaluation." *Bone* 42, no. 2: 314-320.

[91] Rejnmark, Lars, Peter Vestergaard, A. P. Hermann, C. Brot, P. Eiken, and Leif Mosekilde. (2008)"Dietary intake of folate, but not vitamin B 2 or B 12, is associated with increased bone mineral density 5 years after the menopause: results from a 10-year follow-up study in early postmenopausal women." *Calcified tissue international* 82, no. 1: 1-11.

[92] Ravaglia, Giovanni, Paola Forti, Fabiola Maioli, Lucia Servadei, Mabel Martelli, Nicoletta Brunetti, Luciana Bastagli, Domenico Cucinotta, and Erminia Mariani. (2005)"Folate, but not homocysteine,

predicts the risk of fracture in elderly persons." *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* 60, no. 11: 1458-1462.

[93] Gjesdal, Clara Gram, Stein Emil Vollset, Per Magne Ueland, Helga Refsum, Haakon E. Meyer, and Grethe S. Tell. (2007) "Plasma homocysteine, folate, and vitamin B12 and the risk of hip fracture: the Hordaland Homocysteine Study." *Journal of Bone and Mineral Research* 22, no. 5: 747-756.

[94] Morris, Martha Savaria, Paul F. Jacques, and Jacob Selhub. (2005) "Relation between homocysteine and B-vitamin status indicators and bone mineral density in older Americans." *Bone* 37, no. 2: 234-242.

[95] Halıloglu, Berna, Fehime Benli Aksungar, Erdin Ilter, Hakan Peker, Figen Temelli Akın, and Umit Ozekıcı. (2010) "Relationship between bone mineral density, bone turnover markers and homocysteine, folate and vitamin B12 levels in postmenopausal women." *Archives of gynecology and obstetrics* 281, no. 4: 663-668.

[96] Dai, Z., R. Wang, L. W. Ang, J-M. Yuan, and W-P. Koh. (2013)"Dietary B vitamin intake and risk of hip fracture: the Singapore Chinese Health Study." *Osteoporosis International* 24, no. 7: 2049-2059.

[97] Goerss, Jean B., Chung H. Kim, Elizabeth J. Atkinson, Richard Eastell, W. Michael O'Fallon, and L. Joseph Melton III. (1992) "Risk of fractures in patients with pernicious anemia." *Journal of Bone and Mineral Research* 7, no. 5: 573-579.

[98] Melton, Mary E., and Michael L. Kochman. (1994)"Reversal of severe osteoporosis with vitamin B12 and etidronate therapy in a patient with pernicious anemia." *Metabolism* 43, no. 4: 468-469.

[99] Ryan-Harshman, Milly, and Walid Aldoori. (2008) "Vitamin B12 and health." *Canadian Family Physician* 54, no. 4: 536-541.

[100] Rumbak, Ivana, Vesna Žižić, Lea Sokolić, Selma Cvijetić, Romana Kajfež, and Irena Colić Barić. (2012)"Bone mineral density is not associated with homocysteine level, folate and vitamin B 12 status." *Archives of gynecology and obstetrics* 285, no. 4: 991-1000.

[101] MacDonald, Helen Margaret, F. E. McGuigan, W. D. Fraser, S. A. New, S. H. Ralston, and David M. Reid. (2004) "Methylenetetrahydrofolate reductase polymorphism interacts with riboflavin intake to influence bone mineral density." *Bone* 35, no. 4: 957-964.

[102] Yazdanpanah, Nahid, M. Carola Zillikens, Fernando Rivadeneira, Robert De Jong, Jan Lindemans, André G. Uitterlinden, Huibert AP Pols, and Joyce BJ Van Meurs. (2007) "Effect of dietary B vitamins on BMD and risk of fracture in elderly men and women: the Rotterdam study." *Bone* 41, no. 6: 987-994.

[103] Dhonukshe-Rutten, Rosalie AM, Martine Lips, Nynke de Jong, Marijke JM Chin A Paw, Gerrit J. Hiddink, Marijke van Dusseldorp, Lisette CPGM de Groot, and Wija A. van Staveren. (2003) "Vitamin B-12 status is associated with bone mineral content and bone mineral density in frail elderly women but not in men." *The Journal of nutrition* 133, no. 3: 801-807.

[104] Bozkurt, Nuray, Mehmet Erdem, Ercan Yılmaz, Ahmet Erdem, Aydan Biri, Ayca Kubatova, and Murat Bozkurt. (2009)"The relationship of homocyteine, B12 and folic acid with the bone mineral density of the femur and lumbar spine in Turkish postmenopausal women." *Archives of gynecology and obstetrics* 280, no. 3: 381-387.

[105] Stone, Katie L., Douglas C. Bauer, Deborah Sellmeyer, and Steven R. Cummings.(2004) "Low serum vitamin B-12 levels are associated with increased hip bone loss in older women: a prospective study." *The Journal of Clinical Endocrinology & Metabolism* 89, no. 3: 1217-1221.

[106] Dhonukshe-Rutten, R.A., et al., (2005) *Homocysteine and vitamin B12 status relate to bone turnover markers, broadband ultrasound attenuation, and fractures in healthy elderly people.* Journal of Bone and Mineral Research,. 20(6): p. 921-929.

[107] McLean, Robert R., Paul F. Jacques, Jacob Selhub, Lisa Fredman, Katherine L. Tucker, Elizabeth J. Samelson, Douglas P. Kiel, L. Adrienne Cupples, and Marian T. Hannan. (2008) "Plasma B vitamins, homocysteine, and their relation with bone loss and hip fracture in elderly men and women." *The Journal of Clinical Endocrinology & Metabolism* 93, no. 6: 2206-2212.

[108] Tucker, Katherine L., Marian T. Hannan, Ning Qiao, Paul F. Jacques, Jacob Selhub, L. Adrienne Cupples, and Douglas P. Kiel. (2005)"Low plasma vitamin B12 is associated with lower BMD: the Framingham Osteoporosis Study." *Journal of Bone and Mineral Research* 20, no. 1: 152-158.

[109] Herrmann, Markus, Britt Wildemann, Alexandra Wagner, Martin Wolny, Heike Schorr, Omid Taban-Shomal, Natalia Umanskaya et al. (2009)"Experimental folate and vitamin B12 deficiency does not alter bone quality in rats." *Journal of bone and mineral research* 24, no. 4: 589-596.

[110] Lane, Nancy E. (2001) *The Osteoporosis Book: A Guide for patients and their families*. Oxford University Press,.

[111] Bonura, Frank. (2009)"Prevention, screening, and management of osteoporosis: an overview of the current strategies." *Postgraduate medicine* 121, no. 4: 5-17.

[112] Agrawal, Tripti, and A. K. Verma. (2013)"Cross sectional study of osteoporosis among women." *medical journal armed forces india* 69, no. 2: 168-171.

[113] Sontakke, A. N., and Rahul S. Tare. (2002) "A duality in the roles of reactive oxygen species with respect to bone metabolism." *Clinica Chimica Acta* 318, no. 1-2: 145-148.

[114] Park, Byung Guk, Chong Il Yoo, Hui Taek Kim, Chae Hwa Kwon, and Yong Keun Kim. (2005) "Role of mitogen-activated protein kinases in hydrogen peroxide-induced cell death in osteoblastic cells." *Toxicology* 215, no. 1-2: 115-125.

[115] Lean, Jenny M., Chris J. Jagger, Barrie Kirstein, Karen Fuller, and Timothy J. Chambers. (2005) "Hydrogen peroxide is essential for estrogen-deficiency bone loss and osteoclast formation." *Endocrinology* 146, no. 2: 728-735.

[116] Ozgocmen, Salih, Huseyin Kaya, Ersin Fadillioglu, Rabia Aydogan, and Zumrut Yilmaz. (2007)"Role of antioxidant systems, lipid peroxidation, and nitric oxide in postmenopausal osteoporosis." *Molecular and cellular biochemistry* 295, no. 1-2: 45-52.

[117] Lean, Jenny M., Julie T. Davies, Karen Fuller, Christopher J. Jagger, Barrie Kirstein, Geoffrey A. Partington, Zoë L. Urry, and Timothy J. Chambers. (2003)"A crucial role for thiol antioxidants in estrogen-deficiency bone loss." *The Journal of clinical investigation* 112, no. 6: 915-923.

[118] Valacchi, Giuseppe, Claudia Sticozzi, Alessandra Pecorelli, Franco Cervellati, Carlo Cervellati, and Emanuela Maioli. (2012)"Cutaneous responses to environmental stressors." *Annals of the New York academy of sciences* 1271, no. 1: 75.

[119] Cervellati, Carlo, Gloria Bonaccorsi, Eleonora Cremonini, Arianna Romani, Enrica Fila, Maria Cristina Castaldini, Stefania Ferrazzini, Melchiorre Giganti, and Leo Massari. "Oxidative stress and bone resorption interplay as a possible trigger for postmenopausal osteoporosis." *BioMed research international* 2014 (2014).

[120] Naz, Marzieh Saei Ghare, Giti Ozgoli, Mir Amir Aghdashi, and Fatemeh Salmani. (2016) "Prevalence and risk factors of osteoporosis in women referring to the bone densitometry academic center in Urmia, Iran." *Global journal of health science* 8, no. 7: 135.

[121] Sahmani, Mehdi, Shideh Omidian, Amir Javadi, Majid Sirati Sabet, and Mahnaz Abbasi. (2014)."Association between the serum levels of zinc, copper and lipid profile with osteoporosis in Iranian postmenopausal women." *Biotechnology and Health Sciences*.

[122] Razmandeh, Rezvan, Ensieh Nasli-Esfahani, Reza Heydarpour, Farnoush Faridbod, Mohammad Reza Ganjali, Parviz Norouzi, Bagher Larijani, and Davood Khoda-Amorzideh. (2014) "Association of Zinc, Copper and Magnesium with bone mineral density in Iranian postmenopausal women–a case control study." *Journal of Diabetes & Metabolic Disorders* 13, no. 1: 43.

[123] Karakas, Emel Yigit, Alpaslan Yetisgin, Dursun Cadirci, Hatice Sezen, Rıza Altunbas, Fehmi Kas, Mehmet Demir, and Turgay Ulas. (2016)"Usefulness of ceruloplasmin testing as a screening methodology for geriatric patients with osteoporosis." *Journal of physical therapy science* 28, no. 1: 235-239.

[124] De Laet, C., J. A. Kanis, Anders Odén, H. Johanson, Olof Johnell, P. Delmas, J. A. Eisman et al. (2005)"Body mass index as a predictor of fracture risk: a meta-analysis." *Osteoporosis international* 16, no. 11: 1330-1338.

[125] Cho, Jae-Hwan, Min-Tae Kim, Hae-Kag Lee, In-Sik Hong, and Hyon-Chol Jang. (2014)"Factor analysis of biochemical markers associated with bone mineral density in adults." *Journal of Physical Therapy Science* 26, no. 8: 1225-1229.

[126] Pacifici, Roberto. (1996)"Estrogen, cytokines, and pathogenesis of postmenopausal osteoporosis." *Journal of Bone and Mineral Research* 11, no. 8: 1043-1051.

[127] Aggarwal, Neelam, Ainharan Raveendran, Niranjan Khandelwal, Ramesh Kumar Sen, J. S. Thakur, Lakhbir Kaur Dhaliwal, Veenu Singla, and Sakthivel Rajan Rajaram Manoharan. (2011)"Prevalence and related risk factors of osteoporosis in peri-and postmenopausal Indian women." *Journal of mid-Life health* 2, no. 2: 81.

[128] Heidari, Behzad, Reza Hosseini, Yahya Javadian, Ali Bijani, Mohammad Hassan Sateri, and Haj Ghorban Nouroddini. (2015) "Factors affecting bone mineral density in postmenopausal women." *Archives of osteoporosis* 10, no. 1: 15.

[129] Hyassat, Dana, Taghreed Alyan, Hashem Jaddou, and Kamel M. Ajlouni. (2017) "Prevalence and risk factors of osteoporosis among jordanian postmenopausal women attending the national center for diabetes, endocrinology and genetics in Jordan." *BioResearch open access* 6, no. 1: 85-93.

[130] Halliwell, Barry. (1994)"Free radicals, antioxidants, and human disease: curiosity, cause, or consequence?." *Lancet (British edition)* 344, no. 8924: 721-724.

[131] Bagis, Selda, Lulufer Tamer, Gunsah Sahin, Ramazan Bilgin, Hayal Guler, Bahadir Ercan, and Canan Erdogan. (2005) "Free radicals and antioxidants in primary fibromyalgia: an oxidative stress disorder?." *Rheumatology international* 25, no. 3: 188-190.

[132] Yalin, S., S. Bagis, G. Polat, N. Dogruer, S. Cenk Aksit, R. Hatungil, and C. Erdogan. (2005) "Is there a role of free oxygen radicals in primary male osteoporosis?." *Clinical and experimental rheumatology* 23, no. 5: 689.

[133] Bekpinar, S., N. Kilic, Y. Ünlüçerçi, A. Akdag-Köse, G. Azizlerli, and Z. Özbek-Kir. (2005) "Evaluation of nitrosative and oxidative stress in Behcet disease." *Journal of the European Academy of Dermatology and Venereology* 19, no. 2: 167-171.

[134] Karatas, F., I. Ozates, H. Canatan, I. Halifeoglu, M. Karatepe, and R. Colak. (2003) "Antioxidant status & lipid peroxidation in patients with rheumatoid arthritis." *Indian Journal of Medical Research* 118: 178-181.

[135] Bekpinar, S., N. Kilic, Y. Ünlüçerçi, A. Akdag-Köse, G. Azizlerli, and Z. Özbek-Kir. (2005) "Evaluation of nitrosative and oxidative stress in Behcet disease." *Journal of the European Academy of Dermatology and Venereology* 19, no. 2: 167-171.

[136] Basu, Samar, Karl Michaëlsson, Helena Olofsson, Sara Johansson, and Håkan Melhus. (2001) "Association between oxidative stress and bone mineral density." *Biochemical and biophysical research communications* 288, no. 1: 275-279.

[137] Altindag, Ozlem, Ozcan Erel, Nurten Aksoy, Sahabettin Selek, Hakim Celik, and Mustafa Karaoglanoglu. (2007)"Increased oxidative stress and its relation with collagen metabolism in knee osteoarthritis." *Rheumatology international* 27, no. 4: 339-344.

[138] Ozgocmen, Salih, Huseyin Kaya, Ersin Fadillioglu, and Zumrut Yilmaz. (2007)"Effects of calcitonin, risedronate, and raloxifene on erythrocyte antioxidant enzyme activity, lipid peroxidation, and nitric oxide in postmenopausal osteoporosis." *Archives of medical research* 38, no. 2: 196-205.

[139] Vural, Perin, Cemil Akgül, and Mukaddes Canbaz. (2005) "Effects of menopause and tibolone on antioxidants in postmenopausal women." *Annals of clinical biochemistry* 42, no. 3: 220-223.

[140] Deyhim, Farzad, Kristy Garica, Erica Lopez, Julia Gonzalez, Sumiyo Ino, Michelle Garcia, and Bhimanagouda S. Patil. (2006) "Citrus juice modulates bone strength in male senescent rat model of osteoporosis." *Nutrition* 22, no. 5: 559-563.

[141] McLean, Robert R., David Karasik, Jacob Selhub, Katherine L. Tucker, Jose M. Ordovas, Giuseppina T. Russo, L. Adrienne Cupples, Paul F. Jacques, and Douglas P. Kiel. (2004)"Association of a common polymorphism in the methylenetetrahydrofolate reductase (MTHFR) gene with bone phenotypes depends on plasma folate status." *Journal of Bone and Mineral Research* 19, no. 3: 410-418.

[142] Akpolat, Veysi, H. Murat Bilgin, M. Yusuf Celik, Mahmut Erdemoglu, and Birgul Isik. (2013) "An evaluation of nitric oxide, folate, homocysteine levels and lipid peroxidation in postmenopausal osteoporosis." *Advances in clinical and experimental medicine: official organ Wroclaw Medical University* 22, no. 3: 403.

[143] Ali, N. K. (2018)"Estimation of some mineral (calcium, phosphorous, vitamin 25 (OH) D and alkaline phosphatase) in osteoporosis patients in Kirkuk city." *J Osteopor Phys Act* 6, no. 2.

[144] Alkhenizan, Abdullah, Ahmed Mahmoud, Aneela Hussain, Alia Gabr, Suad Alsoghayer, and Abdelmoneim Eldali. (2017)"The relationship between 25 (OH) D levels (Vitamin D) and bone

mineral density (BMD) in a Saudi population in a community-based setting." *PLoS One* 12, no. 1: e0169122.

[145] Plehwe, Walter E. (2003) "Vitamin D deficiency in the 21st century: an unnecessary pandemic?." *Clinical endocrinology* 59, no. 1: 22-24.

[146] Gaugris, S., R. P. Heaney, Steven Boonen, H. Kurth, J. D. Bentkover, and Shuvayu S. Sen. (2005)"Vitamin D inadequacy among post-menopausal women: a systematic review." *Qjm* 98, no. 9: 667-676.

APPENDIX

APPENDIX (1): APPROVAL OF RESEARCH ETHICS COMMITTEE

Kurdistan Regional Government
Ministry of Health
Duhok Directorate General of Health
Department of Planning
Scientific Research Division

Form B: Approval of Research Ethics Committee

Date: 2nd July, 2019

Reference number:02072019-4

Research title: Evaluation of Total serum Antioxidant and some Biochemical Parameters in Women with osteoporosis.

Researcher name(s) : Kawar Farsat Ali.

Type of approval: Conditional, taking into consideration the following mandates:

1) Obtaining the approval ofCentral Laboratory in Duhok.
2) Filling the Consent Form (Form C) for all the participants in the study and returning them to Research Ethics Committee at Duhok Directorate General of Health after completion of the study.
3) Grantee of this letter is not given the right to publish the results of the study until returning Form C (Consent Form). Upon returning these forms, the researcher(s) will be provided by unconditional letter from Research Ethics Committee to publish the results.
4) The participants don't bear the responsibility for paying for any procedure.
5) The participants have the right to withdraw from the study.
6) Confidentiality of the data should be secured.
7) Notifying the participant about the results of the procedure performed.
8) Any change in the methods of the study needs prior approval of Research Ethics Committee.

Dr. Rajab Hassan Sanaan
Member

Dr. Wjam S. Al Hassan
Chairman

Dr. NazikAbdulRaheemAbdulKareem
Member

Dr DilovanAbdulMajed
Member

Dr. HashimDawood Musa
Member

Dr. KaramSaeedSulaiman
Member

DIRECTORATE GENERAL OF HEALTH - DUHOK
DGoH DUHOK
RESEARCH ETHICS

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Kurdistan Regional Government
Ministry of Health
Duhok Directorate General of Health
Directorate of Planning
Scientific Research Division

Form B: Approval of Research Ethics Committee

Date: 11 October, 2019

Reference number: 02072019-4.

Research title: Evaluation of Total serum Antioxidant and some Biochemical Parameters in Women with osteoporosis.

Researcher name(s): Kawar Farsat Ali

Type of approval: Conditional.

The forms submitted by the researcher meet the ethical requirements mandated by Research Ethics Committee. Grantee of this letter is given the right to publish the results of the study.

Dr. Nazik A. Abdulkarim
Research Ethics Committee
Duhok Directorate General of Health
Duhok, Iraq

DIRECTORATE GENERAL OF HEALTH - DUHOK
DGoH DUHOK
RESEARCH ETHICS COMMITTEE

Address: Room 1, Floor 2,
Duhok Directorate General of Health
Duhok, Kurdistan Region, Iraq
Postcode: 42001

APPENDIX (2): RESEARCH QUESTIONNAIRE

1. Patient's Name: ……
2. Age: …….
3. Job: ……..
4. Address: ……..
5. Tel.: …….

Diseases History

- Chronic kidney diseases: Yes No
- Chronic liver diseases: Yes No
- Blood pressure Yes No
- Diabetic mellitus Yes No
- General inflammation: Yes No
- Taking medications including:
  -Folic acid
  -Vitamin B12
  -Vitamin D
  -Calcium supplements
  -Antioxidants

Physical examinations

1. Width circumferences (WC)…… ( )
2. Body mass index (BMI)….. (kg/m^2)

Weight: …….. (Kg) Height: …….. (cm)

Chemical investigations

1. Total serum antioxidant:
2. Serum vitamin B12:
3. Serum folic acid:
4. Serum Vitamin D:
5. Serum Calcium:

APPENDIX (3): DEXA SCAN REPORT SAMPLE

DIRECTORATE GENERAL OF HEALTH DUHOK 1/3

SPECIALIZED CENTER OF RHEUMATIC DISEASES

IRAQ - KURDISTAN

DUHOK

Phone number : **E-mail :** **Fax :**

Patient :	**Sex :**
Patient's ID :	**Ethnic :**
Birth Date :	**Current Age :**

Whole Body

Bone

Lean

Composition

Pictures not for diagnostic use

Scan information :

Operator :
Prescribing doctor :
Physician :
Scan Date :
Analysis date :
Scan Age :
Menopause age :
Height : **Weight :**
BMI:
Site :
Mode scan:
Analysis :

	Bone data					Lean data				Fat data	
ROI	**BMD(g/cm²)**	**BMC(g)**	**Area(cm²)**	**T-score**	**Z-score**	**LBM(g/cm²)**	**Lean(g)**	**Area(cm²)**	**BFM(g/cm²)**	**Fat(g)**	**Area(cm²)**
Left Arm	0.569	96.26	169.16	-2.5 (71%)	-1.4 (82%)	4.318	2931.05	678.73	3.646	2474.93	678.73
Right Arm	0.615	89.97	146.34	-2.0 (77%)	-0.9 (88%)	4.112	2602.00	632.83	3.911	2474.87	632.83
T Spine	0.727	100.76	138.66	-1.7 (81%)	-0.6 (93%)	12.254	1804.33	147.25	6.207	913.93	147.25
L Spine	0.870	31.47	36.17	-2.4 (73%)	-1.3 (84%)	12.420	502.15	40.43	8.619	348.49	40.43
Pelvis	0.857	221.75	258.83	-2.5 (72%)	-1.4 (82%)	9.724	7079.29	728.04	8.096	5894.57	728.04
Left Leg	0.816	310.77	380.64	-1.8 (80%)	-0.7 (91%)	5.670	5734.36	1011.27	3.861	3904.37	1011.27
Right Leg	0.817	299.19	366.11	-1.8 (80%)	-0.7 (91%)	5.233	5856.43	1119.19	4.257	4764.11	1119.19
Total	**0.769**	**1150.16**	**1495.92**	-2.0 (77%)	-0.9 (88%)	**6.083**	**26509.61**	**4357.74**	**4.767**	**20775.27**	**4357.74**
Left Ribs	0.455	61.79	135.88	-5.1 (43%)	-4.0 (49%)	10.303	3984.45	386.71	6.103	2360.10	386.71
Right Ribs	0.481	62.84	130.70	-4.9 (45%)	-3.8 (52%)	10.909	4445.09	407.47	6.081	2477.64	407.47
Head	1.340	257.11	191.90	NC	NC	10.547	2315.32	219.52	2.731	599.50	219.52

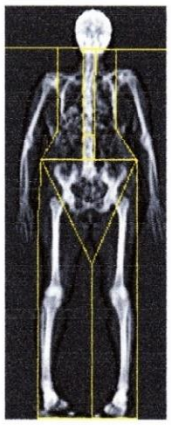
DUHOK CENTER OF RHEMATIC DISESES

OSTEOPOROSIS UNIT
IRAQ KURDISTAN
42001 DUHOK

Phone number : **E-mail :**

Patient :	**Sex :**
Patient's ID :	**Ethnic :**
Birth Date :	**Current Age :**

Whole Body



Bone

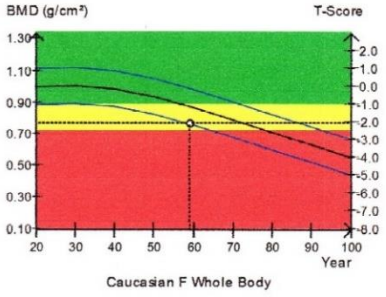
Scan information :

Operator :
Prescribing doctor :
Physician :
Scan Date :
Analysis date :
Scan Age :
Menopause age :
Height : **Weight :**
BMI:
Site :
Mode scan:
Analysis :

Image not for diagnostic use.

Bone data

ROI	BMD(g/cm²)	BMC(g)	Area(cm²)	T-score	Z-score
Left Arm	0.569	96.26	169.16	-2.5 (71%)	-1.4 (82%)
Right Arm	0.615	89.97	146.34	-2.0 (77%)	-0.9 (88%)
T Spine	0.727	100.76	138.66	-1.7 (81%)	-0.6 (93%)
L Spine	0.870	31.47	36.17	-2.4 (73%)	-1.3 (84%)
Pelvis	0.857	221.75	258.83	-2.5 (72%)	-1.4 (82%)
Left Leg	0.816	310.77	380.64	-1.8 (80%)	-0.7 (91%)
Right Leg	0.817	299.19	366.11	-1.8 (80%)	-0.7 (91%)
Total	**0.769**	**1150.16**	**1495.92**	**-2.0 (77%)**	**-0.9 (88%)**
Left Ribs	0.455	61.79	135.88	-5.1 (43%)	-4.0 (49%)
Right Ribs	0.481	62.84	130.70	-4.9 (45%)	-3.8 (52%)
Head	1.340	257.11	191.90	NC	NC



BMD from Whole Body mode not for diagnostic use.

DEXA Printing date/hour 22/11/2017 12:41:33 pm
Normality Curve : Caucasian F TotalBody from DMS normality curves, 2003/2004.
* Effective and input doses are measured for rachis and femur
in normal mode with normal patient (18<BMI<25). Stratos dosimetry (February 2009).

Ver.V3.0.8.3 13/01/2014 / H100 130 - SN: G14 015D 375/1.0

STRATOS

DUHOK CENTER OF RHEMATIC DISESES

OSTEOPOROSIS UNIT
IRAQ KURDISTAN
42001 DUHOK

Phone number : **E-mail :**

Dear Colleague,

Patient:

FirstName: **Height:**
LastName: **Weight:**
Birthdate: **Exam Date:**
Sex: **BMD System:**

Results:

Measure Type	Region	Exam Date	Age	BMD	T-Score	Z-Score
Whole Body	Total				**-2.0**	-0.9

REPORT Low Risk

Evaluation:

T-score definitions of World Health Organization :

Normal:	*T-score above -1.0*
Osteopenia:	*T-score less than or equal to -1.0 and greater than -2.5*
Osteoporotic:	*T-score less than or equal to -2.5*

CURRICULUM VITAE

Kawar Farsat Ali HUSSEIN

PERSONAL INFORMATIONS

Birth of Place : Duhok-Iraq
Birth of Date : 28-April-1987
Nationalty : Iraqi
Address : Emergency Teaching Hospital in Duhok/Iraq
E-mail : kzawity3@gmail.com
Languages : English-Arabic-Kurdish

EDUCATION

University of Duhok /College of Science /Chemistry Department

RESEARCH EXPERIENCES

- ✓ Main Laboratory Instruments I used in this research were Cobas 6000 Roche, ELISA and DEXA Scan.
- ✓ Computer Programming languages available (office, Photoshop)
- ✓ Computer programs you can use (office, Photoshop)

WORK EXPERIENCE

2012–2013: I worked in private water factory as a chemist.
2013–2018: I worked in private medical laboratory.
2013-2018: I worked in Emergency Teaching Hospital in Duhok.
2018-2020: I studied master degree.