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Evaluation of Thyroid Stimulating Hormone, Thyroxine and Triiodothyronine in Post-menopausal Women with Osteoporosis

تقييم الهرمون المحفز للغدة الدرقية وهرمون الغده الدرقيه وثلاثي يودوثايرونين في النساء بعد سن اليأس المصابات بهشاشة العظام

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By

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الآية

قال تعالي:

{قَالَ رَبِّ إِنِّي وَهَنَ الْعَظْمُ مِنِّي وَاشْتَعَلَ الرَّأْسُ شَيْباً وَلَمْ أَكُن بِدُعَائِكَ رَبِّ شَقِيّاً}

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DEDICATION

In the name of Allah, Most Gracious, Most Merciful

All praise and glory to Almighty Allah who gave me the courage and patience to carry out this work.

Peace and blessing of Allah be upon last Prophet Muhammad (Peace Be upon Him).

This thesis is dedicated to my father who have faithfully Supported me throughout my entire life, but especially during my master's project.

Also to my friends and all women whom participate in this work.

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Abstract

Osteoporosis is a disease characterized by low bone mass and microarchitectural deterioration of bone tissue leading to enhanced bone fragility and a consequent increase in fracture incidence. The osteoporosis is becoming public health problem in Sudan and neighboring countries and worldwide.

Thyroid hormones and TSH are necessary to normal development and function of human skeleton, it increase bone turnover when bone resorption exceeding formation. The skeleton is exquisitely sensitive to thyroid hormones and TSH, which have profound effects on bone development, linear growth, and bone maintenance.

This is the cross sectional case control study carried out in a period from April to October (2018) in Khartoum, Sudan. The study was conduct on 100 post - menopausal women, 50 with osteoporosis as case, and 50 without osteoporosis as control. Women were enrolled in the study after taking their verbal consent, to evaluate thyroid hormones and thyroid stimulating hormone level.

Venous blood (3ml) was collected from the participants in Heparin containers and gently mixed. Thyroid hormones and TSH level were determined by Enzyme-Linked Immune Sorbent Assay technique.

The obtained data were analyzed by both Student's Independent samples T test and person correlation test using SPSS version 20 computer program.

By analysis of the result, noticed that the insignificant association (no differences) p. value more than 0.05 between case and control group regarding Thyroid stimulating hormone, Thyroxine, body mass index, age and Triiodothyronine

We also observed insignificant correlation between Body mass index & TSH, BMI & T3 and BMI and T4, in addition to insignificant correlation between TSH with T3 & T4.

In conclusion thyroid hormones and TSH is normal in studied groups.

مستخلص البحث

هشاشة العظام هو مرض يتميز بانخفاض كتلة العظام وتدهور النسيج العضلي العظمي مما يؤدي إلي زيادة هشاشة العظام وزيادة في حدوث الكسور. أصبح مرض هشاشة العظام مشكلة صحية عامة في السودان والدول المجاورة والعالم.

هرمونات الغدة الدرقية والهرمون المحفز للغدة الدرقية ضرورية للتطور الطبيعي ووظيفة الهيكل العظمي، تزيد من دوران العظام عندما يتجاوز إرتشاف العظم التكوين. الهيكل العظمي حساس بشكل رائع لهرمونات الغدة الدرقية والهرمون المحفز للغدة الدرقية والتي لها تأثيرات عميقة علي نمو العظام والنمو الخطي وصيانة العظام.

هذه دراسة تحليلية حالة وحالة ضابطة أجريت في الفترة من فبراير إلي أكتوبر 2018 في الخرطوم، السودان.

أجريت الدراسة علي 100 امرأه بعد سن اليأس، 50 امرأه مصابة بهشاشة العظام كحالة، و50 امرأه غير مصابة بهشاشة العظام كحالة ضابطة. تم تسجيل النساء في الدراسة بعد أخذ موافقتهن اللفظية، لتقييم مستوي هرمونات الغدة الدرقية والهرمون المحفز للغدة الدرقية. تم جمع الدم الوريدي 3 مل من المشاركين في حاويات الهيبارين وخلطها بلطف.

تم تحديد مستوي هرمونات الغدة الدرقية والهرمون المحفز للغدة الدرقية بواسطة تقنية مقايسة الإمتصاص المناعي المرتبط بالإنزيمات.

تم تحليل النتائج باستخدام الفرق بين المتوسطين غير المعتمدين في برنامج الحزم الإحصائية للعلوم الإجتماعية المحوسب.

من خلال تحليل النتيجة لاحظت ليس هناك اختلاف فيما يتعلق بالهرمون المحفز للغدة الدرقية ، الثايروكسين، كتلة الجسم، العمر و الثايرونين ثلاثي اليود بين الحالات والضوابط.

لاحظنا أيضا علاقة طفيفة بين مؤشر كتلة الجسم مع الهرمون المحفز للغدة الدرقية والثايروكسين والثايرونين ثلاثي اليود بالإضافة إلي إرتباط غير المهم بين الهرمون المحفز للغدة الدرقية مع الثايروكسين والثايرونين ثلاثي اليود.

يوصي بإجراء دراسات إضافية بحجم عينة اكبر بإستخدام تقنيات أكثر تقدما.

في الختام كانت هرمونات الغدة الدرقية والهرمون المحفز للغدة الدرقية طبيعية في المجموعات المدروسة.

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LIST OF ABBREVIATIONS

Abbreviation	Full text
TSH	Thyroid stimulating hormone
T3	Triiodothyronine
T4	Thyroxine
BMD	Bone mineral density
DEXA	Dual-energy X-ray absorptiometry
SD	Standard deviation
BMI	Body mass index
RANKL	Receptor activator of nuclear factor kappa B ligand
RANK	Receptor activator of nuclear factor kappa B
OPG	Osteoprotegerin
OP	Osteoporosis
WHO	World Health Organization
OF	Osteoporotic fracture
GH	Growth hormone
IGF-1	Insulin like growth factor-1
PC	Primary care
UK	United Kingdom
IJO	Idiopathic juvenile osteoporosis
GC	Glucocorticoid
SLE	Systemic lupus erythromatus
TBG	Thyroxine-binding globulin
CNS	Central nervous system
TRH	Thyroid releasing hormone
FT4I	Free thyroxine index
T3U	T3 uptake
RPM	Round per minute
ELISA	Enzyme-Linked Immune Sorbent Assay

SPSS	Statistical package for social science
TFT	Thyroid function test
Kg	Kilogram
m^2	Square meter
cm	Centimeter

CHAPTER ONE

Introduction

1.1 Introduction

Bone is a metabolically active tissue that experiences continuous remodeling via two reciprocal processes, bone formation and resorption. Respectively, osteoclasts, osteoblasts and osteocytes are responsible for bone resorption, formation and maintenance (Mohammadi *et al.*, 2014).

Osteoporosis is a chronic metabolic bone disorder characterized by low bone mineral density (BMD) and increased fracture risk. With the ageing world population, osteoporosis remains a significant public health problem today and for the future (Tosun and Press, 2018). The exact prevalence of osteoporosis remains unknown, but it may vary among countries and even among ethnic groups in the same country. Osteoporosis is known to be affected by numerous factors, including ethnic origin, socioeconomic status, diet, physical activity, lifestyle, alcohol and drug abuse, and insufficient exposure to sunlight (Tosun and Press, 2018). Moreover, postmenopausal osteoporosis is associated with adolescent pregnancy, number and frequency of parity, and duration and frequency of lactation (Tosun and Press, 2018). The mainstay diagnosis of osteoporosis is based on the assessment of BMD at the femoral neck and the anterior–posterior lumbar spine using dual-energy X-ray absorptiometry (DEXA) (Tosun and Press, 2018). A T-score #-2.5 standard deviation (SD) is considered osteoporosis (Tosun and Press, 2018).

Many environmental factors have been identified as risk factors of osteoporosis, including exercise and calcium intake (Zhao *et al.*, 2016). In addition, twin and family studies have shown that approximately 50–85% of heritability for BMD in the general population may be attributed to genetic factors (Zhao *et al.*, 2016). Genetic factors may also play a role in the development of osteoporosis (Zhao *et al.*, 2016). BMD prior or parental history of fracture, low body mass index (BMI), use of glucocorticoids, smoking, excessive alcohol consumption, untreated thyrotoxicosis, and other factors increase susceptibility to osteoporosis. Even subclinical hyperthyroidism, defined by a suppressed thyroid stimulating hormone (TSH) level in the presence of normal thyroid hormone concentrations, is associated with fracture (Murphy *et al.*, 2018).

Thyroid hormones have many important biological effect Thyroid hormones are known to (1) stimulate neural development and normal growth, (2) promote sexual maturation, (3) stimulate adrenergic activity with increased heart rate and myocardial contractility, (4) stimulate protein synthesis and carbohydrate metabolism, (5) increase the synthesis and degradation of cholesterol and triglycerides, (6) increase the requirement for vitamins, (7) increase the calcium and

phosphorus metabolism, and (8) enhance the sensitivity of adrenergic receptors to catecholamines. These effects are typically magnified in patients with either an overactive thyroid gland, such as in hyperthyroidism or reduced in patients with a sluggish thyroid such as in hypothyroidism (Carl A. Burtis, 2007).

Thyroid hormones exert widespread and complex actions in almost all tissues during development, throughout child-hood and in adults. The skeleton is an important triiodothyronine target (T3-target) tissue that exemplifies these processes, and yet understanding of the specific cellular and molecular mechanisms of T3 action in bone and cartilage remains incomplete (Williams, 2012).

Thyroid hormones are necessary for normal skeletal growth. However, their excess may lead to bone resorption. Ultimately hyperthyroidism is accompanied by osteoporosis (Simsek *et al.*, 2003). Although there is ample evidence for increased bone turnover in hyperthyroidism (Simsek *et al.*, 2003). Hormonal changes like hyperthyroidism—independent of etiology (Obermayer-Pietsch *et al.*, 2000) or treatment with excessively high doses of thyroid hormone (Obermayer-Pietsch *et al.*, 2000) can lead to high bone turnover and cause impressive and rapid bone loss in affected patients (Obermayer-Pietsch *et al.*, 2000). This effect surpasses the effects of menopause (Obermayer-Pietsch *et al.*, 2000) and may be further modulated by hereditary conditions of bone metabolism (Obermayer-Pietsch *et al.*, 2000).

Thyroid hormones affect bone remodeling in patients with thyroid disease by acting directly or indirectly on bone cells (Marwaha *et al.*, 2012). TSH may also affect bone health by interacting with TSH receptors expressed on osteoblasts and osteoclast precursors (Marwaha *et al.*, 2012). Studies in subjects with exogenous subclinical hyperthyroidism did not reveal any effect on BMD in men and premenopausal women, whereas the effect in postmenopausal women is equivocal (Marwaha *et al.*, 2012). The association of subclinical hypothyroidism and BMD are varied (Marwaha *et al.*, 2012). Many authors have studied relation of thyroid functions with BMD in euthyroid postmenopausal women, (Marwaha *et al.*, 2012) but there is limited information on correlation of thyroid function with BMD in euthyroid premenopausal women and males below 50 years of age (Marwaha *et al.*, 2012). Hence, we undertook this case-control study to evaluate the correlation of thyroid function in osteoporotic women.

Studies of the effects of thyroid hormones in cartilage and bone represent a novel and growing field in our understanding of the broader pathophysiological consequences of thyroid hormone

action. Although considerable advances have been made in recent years, there are important gaps in our knowledge and a number of specific areas require investigation (Williams, 2012).

The aim of the study is to evaluate thyroid hormones and TSH in post-menopausal women with osteoporosis.

1.2 Rationale

Osteoporosis is a non communicable global epidemic disease characterized by deterioration in microarchitecture of bone tissue that lead to increase bone frailty and susceptibility to fragility (low trauma) fracture. With aging societies and changing disease pattern worldwide, human, social and economic cost of osteoporosis will continue to rise. Globally approximately 200 million peoples are affected by osteoporosis, and in the developed countries one in three women and one in five men over the age of 50 years have a disease. Of particular concern are increasing number of hip fracture which is estimated to increase to 2.6 and 4.5 million by year 2025 and 2050.

Osteoporotic fracture are increase worldwide as the population age, with substantial human, economic and social costs. More than 2 million fracture each year in the US are attributed to low bone mass, including 300,000 hip fracture and 550,000 vertebral fracture. Fracture is the major cause of morbidity and disability in the elderly and may lead to premature death.

In the Middle East, information about osteoporosis prevalence and related fracture rate is sparse. In Iran, it was estimated that two million people at risk of osteoporosis related fracture. In Saudi Arabia, Ardawi et al. reported a prevalence of osteoporosis among Saudis aged 20 to 79 years to be 44.5 %.In Sudan many new cases have been diagnosed and there are no previous study about this disease, and there is no effective screening program for early detection of disease. Because of rising number of newly diagnosed osteoporosis cases it is necessary to perform an active investigation for all potential secondary conditions which can lead to reduced bone mineral density. It is very important because osteoporotic fractures are directly related with dropped quality of life, disability and mortality.

The study was conduct in Khartoum, (capital of Sudan) in a period from May to August 2018. In order to find a possible solution for osteoporosis and increase personnel awareness about the disease and its possible causes. Early detection of osteoporosis remain largely opportunistic, and its confirmation on clinical referral for objective assessment of bone health.

1.3 Objectives

1.3.1 General objective

The purpose is to assess the level of thyroid stimulating hormone, thyroxine and triiodothyronine in post-menopausal women with osteoporosis.

1.3.2 Specific objectives

1- To measure and compare TSH, T3 and T4 levels in post-menopausal women with osteoporosis and apparently healthy post-menopausal women.

2- To correlate between TSH, T3 and T4 in case group regarding to study variables.

Chapter Two

Literature Review

2. Literature Review

2.1 Bone

Bone is a specialized connective tissue consisting primarily of glycoproteins and proteoglycans. The fibers of bone are mostly composed of type-I collagen impregnated with mineral in the form of hydroxyapatite. The functional integrity and strength of the skeleton is maintained by this highly cross-linked structure. Several factors may be involved in determining bone quality, including bone density and qualitative determinants of bone strength such as the rate of bone turnover, the extent of trabecular connectivity, cortical and periosteal bone size and skeletal morphometry(Wheater *et al.*, 2013).

2.1.2 Bone remodeling

Bone is metabolically active and is constantly being repaired and remodeled throughout an individual's lifetime. Approximately twenty percent of bone tissue is replaced annually varying by site and type(Wheater *et al.*, 2013). Remodeling begins before birth and continues until death, it is a highly synchronized process contained within basic multicellular units. Recent research has demonstrated the role of receptor activator of nuclear factor kappa B ligand/ receptor activator of nuclear factor kappa B/ osteoprotegerin (RANKL/RANK/OPG) in regulating bone metabolism(Wheater *et al.*, 2013). Additionally, bone metabolism is now known to be at least partly regulated by osteocytes, the fully differentiated osteoblasts present in lacunae in the mineralized matrix and osteoid tissue of bone(Wheater *et al.*, 2013). Osteocytes detect mechanical loads and release signaling molecules which coordinate the recruitment and activity of osteoblasts and osteoclasts thereby controlling bone turnover(Wheater *et al.*, 2013).

Under normal conditions bone formation and resorption are tightly linked through a variety of regulatory signals. Osteoporosis occurs when bone resorption is the more active resulting in a low bone mass and micro architectural deterioration of bone tissue, leading to increased bone fragility and consequent increase in fracture risk (Wheater *et al.*, 2013).

2.1.3 Bone Remodeling Cycle

The skeleton undergoes continuous remodeling in response to mechanical stress and injury at multiple sites throughout the skeleton in order to maintain structural integrity and strength(Williams, 2012). The cyclical process of bone turnover and repair is initiated by osteocytes. These cells are embedded within calcified bone and communicate via an elaborate

network of dendritic processes. Osteocytes respond to changes in mechanical loading or micro-fracture by undergoing apoptosis with release of cytokines and growth factors that attract osteoclasts to sites of micro-damage. Osteoclasts resorb areas of dam- aged bone and communicate with osteoblasts, which are then attracted by various growth factors and by degraded matrix proteins released during bone resorption. Osteoblasts subsequently synthesize, secrete and mineralize osteoid to lay down new bone. Completion of the formation phase of the bone remodeling cycle by osteoblasts results in the repair of defective bone. Overall, the balanced coupling of bone resorption to bone formation is essential to maintain the architecture, mineralization and strength of bone(Williams, 2012).

2.2 Osteoporosis

2.2.1 Definition

The definition of osteoporosis (OP) by the World Health Organization (WHO) is densitometric and non-clinical and is based on the measurement of bone mass with the DEXA method in the spine or hip. It establishes four categories: normal, osteopenia, osteoporosis and established osteoporosis. The presence of pathological low bone mass, osteopenia or osteoporosis, is the best indicator of fracture risk for the region where the bone mass is measured, hence its interest, since bone loss is asymptomatic until it produces its natural consequence: the osteoporotic fracture (OF).

Osteoporosis is a skeletal disorder characterized by low bone mass and microarchitectural deterioration of the skeleton leading to bone fragility and a predisposition to fractures. Osteoporosis is a major cause of morbidity and mortality, particularly in post-menopausal women and older men. While the pathogenesis of the bone loss and skeletal fragility is not well understood, estrogen deficiency plays a role in both sexes. It is classified as primary osteoporosis when it occurs in post-menopausal women and in men in the absence of an underlying disease, and it is age-related(Mirza and Canalis, 2016).

2.2.2 Osteoporotic fracture and epidemiology

2.2.2.1 Fracture

OP is a health issue with important implications for individuals, families and the community. Untreated OP results in unnecessary pain, restriction of function (disability), decreased quality of

life, altered body image with low self-esteem, increased mortality and serious economic consequences(López *et al.*, 2011).

Annually, osteoporotic fractures cost €31 billion in Europe and $13 billion in the United States, and their prevalence will increase substantially as the population ages(Bassett *et al.*, 2018). The risk of osteoporotic fracture is determined by the acquisition of peak bone mass during growth and the rate of age-related bone loss there-after(Bassett *et al.*, 2018). Accrual of bone mineral is modified by endocrine status, and the risk of osteoporosis is influenced by fetal programming of vitamin D levels(Bassett *et al.*, 2018) and the set points of the gonadal steroid(Bassett *et al.*, 2018), corticosteroid(Bassett *et al.*, 2018), and growth hormone/insulin like growth factor-1 (GH/IGF-I)(Bassett *et al.*, 2018) axes. Even though thyroid hormones thyroxine (T4), T3 are essential for skeletal development and the maintenance of adult bone(Bassett *et al.*, 2018). Their role in the pathogenesis of osteoporosis has been largely overlooked (Bassett *et al.*, 2018).

The prevalence of thyroid disease increases with age: 3% of women over 50 receiveT4, and more than 20% are over treated. Subclinical hyperthyroidism affects a further 1.5% of women over 60, and its prevalence increases with age(Murphy *et al.*, 2018).

Osteoporotic fractures occur when a mechanical stress applied to the bone exceeds its strength. The most frequent fracture sites are the vertebral body, the proximal femur, the proximal humerus, and the distal radius. According to the WHO, fragility fractures result from low energy trauma due to mechanical forces equivalent to a fall from a standing height or less, which would not ordinarily cause a fracture(Tarantino *et al.*, 2017). It is now believed that skeletal fragility requires both decreased bone density and poor bone quality, defined as alterations in bone architecture, bone geometry, and the material properties of the microstructural constituents such as collagen and mineral, as well as the presence of microdamage(Tarantino *et al.*, 2017).

2.2.2.2 Epidemiology

Globally approximately 200 million peoples are affected by osteoporosis, and in the developed countries one in three women and one in five men over the age of 50 years have a disease. One in five women over the age of 50 has a spinal fracture and although some are asymptomatic and related mortality is low, some produce chronic pain, height loss, respiratory problems, constipation and abdominal pain, which limit the activity these symptoms(López *et al.*, 2011). The high prevalence and easy deployment of the available therapeutic arsenal and the characteristics of primary care (PC) relating to patient accessibility, early diagnosis and

treatment compliance make PC the optimum stage of care for the prevention, diagnosis and care of the osteoporotic patient, a view confirmed by organizations as prestigious as the National Osteoporosis Foundation(López *et al.*, 2011).

Approximately, one in three women and one in 12 men will suffer an osteoporotic fracture at some point in their lives(Practice, 2002), It has been estimated that 50 000 forearm fractures, 40 000 symptomatic vertebral fractures, and 60 000 hip fractures occur in the United Kingdom UK each year. These cause substantial morbidity, excess mortality, and health and social services expenditure. Up to 20% of all symptomatic vertebral fractures and 30% of hip fractures occur in men(Practice, 2002). Although, the incidence of forearm fracture is lower in males than females, men with forearm fractures are at increased risk of vertebral and hip fracture(Practice, 2002).

2.2.2.3 Clinical risk factor of fracture

(a) Low BMI. Low BMI is a significant risk factor for hip fracture, but the value of BMI in predicting other fractures is very much diminished when adjusted for BMD(Compston *et al.*, 2017).

(b) A history of a prior fracture at a site characteristic for osteoporosis is an important risk factor for further fracture. Fracture risk is approximately doubled in the presence of a prior fracture, including morphometric vertebral fractures. The increase in risk is even more marked for more than one vertebral fracture. The risks are in part independent of BMD(Compston *et al.*, 2017).

(c) A parental history of hip fracture is a significant risk factor that is largely independent of BMD(Compston *et al.*, 2017).

(d) Smoking is a risk factor that is in part dependent on BMD(Compston *et al.*, 2017).

(e) Glucocorticoids increase fracture risk in a dose- dependent manner. The fracture risk conferred by the use of glucocorticoids is, however, not solely dependent upon bone loss and BMD-independent risks have been identified(Compston *et al.*, 2017).

(f) Alcohol. The relationship between alcohol intake and fracture risk is dose-dependent. Where alcohol intake is on average 2 units or less daily, no increase in risk has been identified. Intakes of 3 or more units daily are associated with a dose-dependent increase in fracture risk(Compston *et al.*, 2017).

(g) Rheumatoid arthritis. There are many secondary causes of osteoporosis (e.g. inflammatory bowel disease, endocrine disorders), but in most instances, it is uncertain to what extent this is dependent on low BMD or other factors such as the use of glucocorticoids. By contrast,

rheumatoid arthritis increases fracture risk independently of BMD and the use of glucocorticoids(Compston *et al.*, 2017). Recent information suggests that diabetes (particularly type 2) may also exert BMD-independent effects on fracture risk(Compston *et al.*, 2017).

There are many additional risk factors for fracture that act solely by reducing BMD and others that have been less well validated or identify a risk that may not be amenable to particular treatments(Compston *et al.*, 2017).

2.2.3 Pathophysiology

Age-Related Bone Loss. It is convenient to divide the skeleton into two compartments consisting of cortical bone and trabecular bone. Cortical bone predominates in the shafts of long bones; trabecular bone is concentrated in the vertebrae, in the pelvis and other flat bones, and at the end of long bones. Trabecular bone is metabolically much more active than cortical bone, probably because of its greater surface area, and is more responsive to changes in mineral homeostasis(Riggs, 1991).

Three distinct phases of changes in bone mass over life can be recognized, two of which occur in both sexes and one third of which occurs only in women(Riggs, 1991), The first process leads to attainment of peak bone mass and represents the summation of growth (90% to 95%) and consolidation (5% to 10%)(Riggs, 1991). Peak bone mass is attained both by linear growth due to mineralization of the endochondrial growth plates and by radial growth due to a rate of periosteal apposition that exceeds that for endosteal resorption. After closure of the growth plate about age 20, the radial growth continues for another 10 to 15 years(Riggs, 1991). The second process consists of a slow, age-dependent phase of bone loss. This begins around age 40 for cortical bone and perhaps 5 to 10 years earlier for trabecular bone and continues into extreme old age(Riggs, 1991). The rate of slow bone loss due to this process probably is similar in men and women and results in losses of similar amounts of cortical bone and of trabecular bone(Riggs, 1991). In women, a third process, a transient accelerated postmenopausal phase of bone loss due to estrogen deficiency, is superimposed on the slow phase of bone loss and results in loss of disproportionately more trabecular bone than of cortical bone(Riggs, 1991), The amount of bone that is lost over life with each of these phases is not well defined. However, a reasonable estimate is that the slow phase produces a loss of about 25% from the cortical compartment and about 25% from the trabecular compartment in both sexes. During the accelerated phase, postmenopausal women lose an additional 10% from the cortical compartment and 25% from the

trabecular compartment. Thus, overall, women lose about 35% of cortical bone and 50% of trabecular bone during their lifetime, whereas men lose about two thirds of these amounts(Riggs,1991)

Age-Related Changes in Bone Remodeling. Bone formation and bone resorption occur at anatomically discrete foci called bone remodeling units(Riggs, 1991). At the beginning of each remodeling cycle, through a mechanism yet to be defined, flattened lining cells are activated and retract to expose the underlying bone(Riggs, 1991). Osteoclast precursor cells in marrow are recruited and migrate to the exposed area of bone. These fuse to form osteoclasts and, over a period of 1 to 3 weeks, the osteoclasts construct a tunnel in cortical bone or a lacuna on the surface of trabecular bone. The osteoclasts disappear during a reversal phase and are replaced by osteoblasts, which then fill in the resorption cavity over a period of 3 to 4 months to create a new structural unit of bone(Riggs, 1991).

The main determinant of the rate of bone turnover is the frequency of activation of new bone remodeling units(Riggs, 1991). In normal young adults, the processes of bone resorption and bone formation are tightly coupled so that bone balance is maintained. However, during age-related bone loss, there is a remodeling imbalance with a relative or absolute increase in resorption over formation(Riggs, 1991). Because of this imbalance at each bone remodeling unit, an increase in bone turnover (i.e., an increase in the number of bone remodeling units) leads to increased bone loss(Riggs, 1991).

The slow and accelerated phases of bone loss are associated with two different abnormalities of bone remodeling. In the slow, age-dependent phase, the osteoclasts construct resorption cavities of normal depth, or even decreased depth, but the osteoblasts fail to refill them completely(Riggs, 1991). This leads to a gradual thinning of the trabeculae, but their connectivity is maintained(Riggs, 1991). In contrast, the accelerated, postmenopausal phase of bone loss is associated with a high rate of bone turnover; there is an increase in osteoclast number and the osteoclasts create a resorption cavity of increased depth. These processes lead to trabecular perforation and loss of structural trabeculae and trabecular connectivity(Riggs, 1991).

2.2.4 Diagnosis of osteoporosis

The major end point of osteoporosis is fracture, especially distal forearm, vertebral, and hip. Historically, the diagnosis was made on the basis of a low trauma fracture, defined as a fall from standing height or less. As there is an inverse relationship between BMD and fracture risk

methods have been developed to measure BMD using non-invasive techniques. The most widely used of these is dual energy x ray absorptiometry(Practice, 2002). The WHO has defined osteoporosis as a BMD 2.5 standard deviations or more below the mean value for young adults (T score<−2.5),whereas the term severe or established osteoporosis indicates that there has also been one or more fragility fractures(Practice, 2002). The WHO defines osteopenia as a BMD T score between −1.0 and −2.5. Although the WHO definition is useful for the diagnosis of osteoporosis, it does not necessarily represent a threshold for treatment. This is important as 70% of women above the age of 80 years have a T score of less than −2.5, but only a proportion of these will sustain an osteoporotic fracture(Practice, 2002).

The following four general descriptive categories are given below for adult men and women using measurements of DEXA at the femoral neck(Kanis *et al.*, 2008).

1. Normal: a value for BMD that is higher than 1 standard deviation below the young adult female reference mean (T-score greater than or equal to −1 SD).
2. Low bone mass (osteopenia): a value for BMD more than 1 standard deviation below the young female adult mean, but less than 2.5 SD below this value (T-score <−1 and >−2.5 SD).
3. Osteoporosis: a value for BMD 2.5 SD or more below the young female adult mean (T-score less than or equal to −2.5 SD).
4. Severe osteoporosis (established osteoporosis): a value for BMD 2.5 SD or more below the young female adult mean in the presence of 1 or more fragility fractures(Kanis *et al.*, 2008).

The original 1994 WHO criteria provided for diagnosis of osteoporosis at the hip, lumbar spine or forearm. Data arising with the development of new measurement techniques applied to many different skeletal sites indicate that the same T-score derived from different sites and techniques yield quite different information on fracture risk The inter- site correlations, though of statistical significance, are inadequate for predictive purposes. These considerations have led to the adoption of a reference site(Kanis *et al.*, 2008).

2.2.5 The main types of osteoporosis

Primary or idiopathic osteoporosis, which includes juvenile, postmenopausal, and senile osteoporosis, is the most common type of osteoporosis. Secondary osteoporosis may ensue from several diseases, such as endocrine (hypogonadism, hypocortisolism, hyperparathyroidism, acromegaly, diabetes mellitus), hematological (thalassemia, multiple myeloma), gastrointestinal (malabsorption, celiac disease), rheumatic (rheumatoid arthritis, systemic lupus erythematous,

ankylosing spondylitis, scleroderma), and kidney (renal failure, chronic tubular acidosis) disorders, or from medications such as glucocorticoids, anticoagulants, diuretics, and others(Tarantino *et al.*, 2017) . The characteristics of the main forms of osteoporosis will now be briefly described.

2.2.5.1 Idiopathic osteoporosis

Is the most common form of osteoporosis, secondary factors may contribute to the bone loss and increased fracture risk in patients presenting with fragility fractures or osteoporosis. Several medical conditions and medications significantly increase the risk for bone loss and skeletal fragility(Mirza and Canalis, 2016).

2.2.5.1.1 Juvenile osteoporosis

The term juvenile osteoporosis, or idiopathic juvenile osteoporosis (IJO), is used to indicate osteoporosis in children and adolescents, and usually does not refer to any specific type of osteoporosis in these age groups. Bone loss may occur from infancy to adolescence because of genetic mutations resulting in a reduced amount and impaired quality of the fibrous component of bone (e.g., leading to osteogenesis imperfecta), or may be secondary to a spectrum of other conditions, such as prolonged immobilization and chronic inflammatory diseases. Moreover, the use of anticonvulsants or steroids or the presence of life- threatening conditions such as leukemia may lead to fragility fractures, particularly at the spine. If an underlying cause cannot be identified, it is defined as IJO. This condition includes a group of heritable disorders characterized by low bone density and skeletal fragility, but without the extraskeletal findings reported in osteogenesis imperfecta. Skeletal involvement in patients with IJO is the result of impaired osteoblast activity and mainly affects cancellous bone(Tarantino *et al.*, 2017).

2.2.5.1.2 Postmenopausal osteoporosis

Postmenopausal osteoporosis is a type of primary osteoporosis where the pathogenesis is associated with estrogen depletion, which enhances the bone loss that occurs with aging. This condition is characterized by a specific skeletal disease pattern, including prevalent trabecular bone loss and perforation compared to cortical bone loss, leading to site- specific fracture risks at vertebral bodies and at the distal radius(Tarantino *et al.*, 2017). The rate of bone loss after menopause is a major factor in the development of postmenopausal osteoporosis. This is often characterized by high bone turnover, which is associated with a higher risk of trabecular perforation or intra- cortical porosity(Tarantino *et al.*, 2017). It is difficult to predict the clinical

outcome for each individual due to the variability in the rate of loss after menopause(Tarantino *et al.*, 2017). A low serum concentration of estrogen after menopause may lead to inhibited periosteal bone formation, as suggested by the results of a previous experimental study(Tarantino *et al.*, 2017).

2.2.5.2 Secondary osteoporosis

Secondary osteoporosis is an umbrella term for all clinical conditions where bone involvement is not the main pathological finding; rather, they are characterized (at least in part) by adverse consequences of the primary disease itself or resulting from related treatments, particularly glucocorticoid (GC) use. Bone remodeling and bone density are negatively affected by several diseases and treatments that are often associated with an increased risk of fall. Pathogenetic mechanisms of secondary osteoporosis are independent of estrogen deficiency. In fact, about two-thirds of men, > 50% of premenopausal women, but also 20% of postmenopausal women have secondary osteoporosis(Tarantino *et al.*, 2017).

Secondary osteoporosis is caused by readily identifiable conditions such as malignancy, endocrinopathies, systemic inflammatory diseases, the use of certain medications (e.g., GCs, aromatase inhibitors), as well as by other diseases that are more difficult to diagnose, such as hypovitaminosis D, hyperparathyroidism, or idiopathic hypercalciuria. Young individuals, premenopausal women, men under 65 years of age, all patients with accelerated bone loss, patients with severe osteoporosis, and patients receiving antiosteoporotic treatment who experience bone loss should be investigated for other underlying causes of osteoporosis. Biochemical evaluation has a sensitivity of 92% for the diagnosis of secondary causes of osteoporosis(Tarantino *et al.*, 2017).

Therefore, laboratory assessment should be prescribed to investigate the main cause of bone loss, such as hyperthyroidism, hypercortisolism, multiple myeloma, or celiac dis- ease. It is advisable to perform a double tetracycline labeling transiliac bone biopsy to evaluate bone marrow disorders (e.g., no secretory multiple myeloma or mastocytosis) or defective mineralization in patients with fragility fractures and normal bone density, which are highly suggestive of secondary osteoporosis(Tarantino *et al.*, 2017).

2.2.5.3 Premenopausal osteoporosis

Osteoporosis is less common in premenopausal women than in postmenopausal women(Osteoporosis, 2018). Most premenopausal women with low trauma fracture(s) or low

BMD have a secondary cause of osteoporosis or bone loss. Women who present with unexplained fractures or low BMD should have a thorough clinical and laboratory evaluation to search for known causes of fractures and/or bone loss. Where possible, treatment of the underlying cause should be the focus of management(Osteoporosis, 2018).

Risk factors of premenopausal osteoporosis include the following: genetic influences, ethnicity, hormonal influences, nutritional factors, physical activity, disease factors, medications, and smoking(Cheng and Gupta, 2013). Bone loss can also occur due to prolonged amenorrhea and estrogen deficiency(Cheng and Gupta, 2013). Medications can also contribute to premenopausal osteoporosis(Cheng and Gupta, 2013).

Many secondary causes can be identified by a detailed history and physical examination. Medical history should include information on:

- Adult and childhood fractures.
- Adult and childhood illnesses and medication exposures.
- Menstrual history.
- Timing of recent pregnancy or lactation.
- Dieting and exercise behavior.
- Gastrointestinal symptoms.
- Nephrolithiasis.
- Family history of osteoporosis and/or nephrolithiasis.

Physical examination should seek signs of:

- Nutritional deficiency or eating disorder.
- Cushing syndrome.
- Thyroid hormone excess.
- Connective tissue disorders (e.g. osteogenesis imperfecta, Ehlers Danlos syndrome, Marfan syndrome),
- Inflammatory conditions (e.g. rheumatoid arthritis, systemic lupus erythromatus (SLE).

Laboratory evaluation may target hormonal, calcium metabolism, or gastrointestinal disorders(Osteoporosis, 2018).

2.3 Thyroid gland

2.3.1 Thyroid gland structure

Is a bilobed endocrine gland located in the lower part of the neck that is composed of groups of cells called follicles. This gland contains two cell types: Follicular cells produce the hormones thyroxine (T4) and triiodothyronine (T3), and parafollicular cells (lying adjacent to the follicles) produce the hormone calcitonin **(**Joel D. Hubbard, 2011).

2.3.2 Thyroid hormone

Thyroid hormones require iodine for their synthesis. The iodine combines with the protein thyroglobulin to form hormone precursors that in turn combine to form T3 and T4. The hormones are either stored within the follicle or released into the bloodstream. In the blood, most T4 eventually gives up an iodine molecule and forms T3. There is much more circulating T3 than T4. Approximately 98% of circulating T3 and T4 is bound to protein, including thyroxine-binding globulin (TBG) and thyroxine-binding albumin. Some hormone remains unbound or free, and this is the physiologically active fraction. Thyroid hormone function includes action at the cellular level to regulate carbohydrate, lipid, and protein metabolism. The hormones also act on the central nervous system (CNS), stimulate the heart, and have a role in physical growth and development **(**Joel D. Hubbard, 2011).

2.3.3 Regulation of T3 and T4

(1) Thyroid-releasing hormone (TRH) is released by the brain and stimulates the release of TSH (thyrotropin) from the pituitary gland.

(2) TSH stimulates iodine uptake by the thyroid gland and also causes the release of T3 and T4 from the thyroid gland.

(3) High serum levels of free T3 and T4 "shut off" the release of TSH from the pituitary gland, whereas decreased levels induce TSH release **(**Joel D. Hubbard, 2011).

2.3.4 Thyroid disorders

Are caused by increased or decreased levels of the circulating hormones T3 and T4. A wide variety of physical diseases can be traced back to a dysfunctional thyroid gland.

*Hypothyroidism:

Is a serum level of thyroid hormone that is insufficient to provide for the metabolic needs of cells. This disorder affects women four times more than men between the ages of 30 and 60 years. Hypothyroidism is usually referred to as primary, secondary, or tertiary, depending on the site of the dysfunction.

- Primary hypothyroidism:

Involves the inadequate secretion of thyroid hormones caused by a damaged or surgically removed thyroid gland. Congenital hypothyroidism is caused by the absence of the thyroid gland. Laboratory results indicate decreased T3, T4, free thyroxine index (FT4I), T3 uptake (T3U), and increased TSH.

- Secondary hypothyroidism:

Involves decreased production of TSH caused by pituitary disorder leading to low serum levels of the thyroid hormones. Laboratory results indicate all thyroid test values are decreased.

- Tertiary hypothyroidism:

Is caused by hypothalamic failure leading to a lack of TRH production.

In the laboratory evaluation of hypothyroidism, the earliest abnormality is increased TSH, followed by decreased serum levels of T4 and T3.

*Chronic immune thyroiditis (Hashimoto's disease):

Is caused by a genetic abnormality in the immune system and involves massive infiltration of the thyroid gland by lymphocytes. The symptoms match those of hypothyroidism.

*Hyperthyroidism:

Is caused by excessive thyroid hormone in the circulation. This causes cells to become overactive. The disorder is sometimes referred to as thyrotoxicosis. The causes of this disorder include pituitary tumors that cause excessive TSH secretion, thyroid carcinoma, or toxic multinodular goiter (gland produces excess hormones).

The laboratory evaluation of hyperthyroidism in the initial evaluation reveals elevated thyroid hormone serum levels and decreased serum TSH.

*Graves' disease:

Is an autoimmune disorder that occurs six times more frequently in women than in men. In this disorder, immunoglobulins stimulate the thyroid gland by binding to TSH receptors. Symptoms are similar to those of hyperthyroidism. Laboratory results indicate increased T3, T4, FT4I, and T3U, and decreased or normal TSH.

*Thyroiditis:

Is an inflammation of the thyroid gland caused by either bacterial or viral infection **(**Joel D. Hubbard, 2011).

2.3.5 Assays for thyroid function

Include testing for serum level of total (both bound and free) or free T3, total or free T4, TSH, and TBG. These tests are typically immunoassays. Other thyroid tests include the following:

a. T3 resin uptake analyzes the capacity of TBG to bind thyroid hormones. It is an indirect measurement of the number of free binding sites on the TBG molecule.

b. FT4I indirectly assesses the concentration of circulating free T4. It is calculated by multiplying the value of the total T4 by the percentage value of the T3 resin uptake.

c. Thyroid antibody screens assay for the presence of thyroid-stimulating immunoglobulins, such as those in Graves' disease and Hashimoto's thyroiditis.

d. TRH stimulation test measures pituitary TSH stores and is considered conclusive for hyperthyroidism, although it is not needed in most hyperthyroid patients. In patients with slightly elevated hormone levels (but other symptoms of hyperthyroidism), TRH is injected, and blood samples are assayed for TSH. TSH levels rise rapidly in a normal person but will not rise in a hyperthyroid patient **(**Joel D. Hubbard, 2011).

2.3.6 Thyroid hormone and bone

Thyroid hormone is essential for normal bone maturation in utero and in early life, as hypothyroid infants show features of delayed ossification at epiphyseal centres and children with hypothyroidism have stunted growth and short stature. In contrast, hypothyroidism have stunted growth and short stature. In contrast, hyperthyroidism in childhood may accelerate linear growth and bone maturation. In adults, recent evidence shows that an excess of thyroid hormones affects the remodeling system in cortical and trabecular bone and may contribute to the development of osteoporosis(Kung, 1994). Both hyperthyroidism and hypothyroidism have been associated with osteoporosis and increased risk of fractures(Mirza and Canalis, 2016).

Thyroid hormones are necessary for normal skeletal growth. However, their excess may lead to bone resorption. Ultimately hyperthyroidism is accompained by osteoporosis(Simsek *et al.*, 2003). Although there is ample evidence for increased bone turnover in hyperthyroidism(Simsek *et al.*, 2003), the precise mechanisms for this action of thyroid hormone on bone remain unclear(Simsek *et al.*, 2003). But, Thyroid hormones affect bone remodeling in patients with thyroid disease by acting directly or indirectly on bone cells(Marwaha *et al.*, 2012). TSH may also affect bone health by interacting with TSH receptors expressed on osteoblasts and osteoclast precursors(Marwaha *et al.*, 2012). The relative contribution of thyroid hormone excess and TSH

deficiency in causation of bone loss remains unresolved. In experimental animals, reduced expression of TSH receptors leads to development of osteoporosis(Marwaha *et al.*, 2012).

Hormonal changes like hyperthyroidism—independent of etiology(Obermayer-Pietsch *et al.*, 2000)—or treatment with excessively high doses of thyroid hormone(Obermayer-Pietsch *et al.*, 2000) can lead to high bone turnover and cause impressive and rapid bone loss in affected patients(Obermayer-Pietsch *et al.*, 2000) This effect surpasses the effects of menopause(Obermayer-Pietsch *et al.*, 2000) and may be further modulated by hereditary conditions of bone metabolism(Obermayer-Pietsch *et al.*, 2000).

2.3.7 Cellular mechanism of thyroid hormone

Thyroid hormone also stimulates osteoblast activity(Kung, 1994). T3 receptor has been demonstrated in osteoblasts(Kung, 1994) but not osteoclasts, suggesting that increased osteoclast activity in bone cultures with T3 treatment is secondary to osteoblast activation(Kung, 1994).

Bone remodeling normally consists of cyclical erosion and repair of resorptive cavities on bone surface. The bone balance depends on the frequency with which the new cycles are initiated by the event of activation and the focal balance in each remodeling site. The latter depends on the depth of the resorption cavity and the thickness of new bone deposited within the cavity by the osteoblasts, or wall thickness of the bone structure units. In hyperthyroidism the activation frequency is increased and the mineralization time is shortened, resulting in uncoupled bone resorption and decreased mean wall thickness(Kung, 1994). Conversely, in hypothyroidism, activation frequencies are reduced and the phrase of the remodeling cycle are markedly prolonged. The final resorption depth is reduced whereas the mean wall thickness is increased. The final result is little change in the bone mass(Kung, 1994).

Chapter Three

Materials and Methods

Material and method

3.1 Study design

It is cross sectional case control, facility based study conducted in Khartoum State during (April to October 2018).

3.2 Study Area

The study was carried out in Khartoum (capital of Sudan).

3.3 Study population

For purpose of this study, 50 post-menopausal women with osteoporosis were selected as case and 50 healthy post-menopausal women as control in a period from April to October 2018.

3.4 Inclusion criteria

- Diagnosed post-menopausal women with osteoporosis.
- Healthy post-menopausal women as control group for comparison.

3.5 Exclusion criteria

Post-menopausal women with other types of bone diseases.

3.6 Ethical consideration

Ethical approval for conducting the research was obtained from the College of Medial Laboratory Science. The participants were provided with information about the study and assured that all the obtained information will be kept highly confidential and will not be used for any other purpose than for this study.

3.7 Data Collection

Collected after having verbal consent by a coded questionnaire**.**

3.8 Sample collection

Venous blood was collected using sterile disposable plastic syringes after cleaning the vein puncture area with 70% ethanol, the blood was add to the heparin container and mixed gently. Each sample was centrifuged at 4000 (rpm) for 5 min and the plasma was separated and stored at -20c until analysis.

3.9 Methodology

3.9.1 Test components of ELISA

1. Pre-coated, stabilized 96-well microtiter Plate.

2. Sample diluent.
3. Standards and control.
4. Detection antibodies.
5. Wash solution.
6. Buffers.
7. Substrate.
8. Stop solution.

3.9.2 Principles of TFT estimation by ELISA

- **For TSH estimation**

➢ Principle**:** Immunoenzymometric assay:

The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme conjugated and immobilized), with different and distinct epitope recognition, in excess**,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-TSH antibody. Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex (Appendix II).

- **For Total T3 estimation**

➢ principle: Competitive Enzyme Immunoassay:

The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen. Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme antigen conjugate for a limited number of insolubilized binding sites (Appendix III).

- **Total T4 estimation**

➢ principle Competitive Enzyme Immunoassay:

The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen. Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction

results between the native antigen and the enzyme antigen conjugate for a limited number of insolubilized binding sites (Appendix IV).

*Quality control

Each laboratory should assay controls at levels in the hypothyroid, euthyroid and hyperthyroid range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

- Quality control tests are a part of the good testing practice to confirm the expected result and validity of the assay and should be performed at regular intervals.
- The control test should be performed immediately after opening a new test lot to ensure the test performance is not altered.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.

3.10 Data analysis technique

Data were analyzed using Statistical Package for the Social Science program (SPSS program) version 20. 0.5 **%** was taken as cut off limit for 95 **%** statistical significance. Frequency and percentage testes were used and then the data were presented in tables. Pearson correlation coefficient and linear regression were used for quantitative variables, P value $\leq$ 0.05 was considered as the level of significance.

Chapter Four

Results

Results

In this study 100 blood sample have been collected, 50 from post-menopausal women with osteoporosis and 50 from healthy women. The age, weight and height of women have been presented. BMI was calculated from weight and height by using Kg/m^2 formula (weight in kilogram divided by square of height in meters).

TSH, T3 and T4 values were normal in 98%, 87.1% and 91% respectively of the studied population. T3 level was ranged from 0.1-7.0 ng/ml, while T4 ranged from 3.7-12.4 μg/dl and TSH ranged between 0.3-12.7 μIU/ml.

TSH level were increased in 2% of studied group, T4 is decreased in 8% and increased in 1%, while T3 decreased in 12.9 of studied group are explained in table 4.1.

The mean, standard deviation and p. value of age, BMI, TSH, T3 and T4 in case and control groups are explained in table 4.2.

Our study also showed insignificant correlation between BMI and TSH (p. value 0.11), BMI and T4 (p. value 0.63) and BMI and T3 (p. value 0.91), showed in figure 4.1, 4.2 and 4.3 respectively.

Table 4.1 Comparison between TSH, T3 and T4 levels in case and control group.

Parameter	**Normal**	**Increased**	**Decreased**
TSH (µIU/ml)	98.0%	2.00%	0.00%
T3 (ng/ml)	87.1%	0.00%	12.9%
T4 (µg/dl)	91.0%	8.00%	1.00%

Table 4.2: Mean ±standard deviation of BMI, TSH, T3 and T4 in case and control groups.

Parameters	**Mean ±SD of cases**	**Mean ±SD of control**	**P. value**
BMI (Kg/m^2)	29.5±6.08	27.6±6.02	0.76
Age (Year)	64.4±15.1	62.6±14.9	0.07
TSH (µIU/ml)	2.82±2.20	2.55±1.51	0.48
T3 (ng/ml)	0.73±0.30	0.80±0.91	0.59
T4 (µg/dl)	7.23±2.38	6.56±2.03	0.13

Independent Samples T test used for comparison, p. value < 0.05 considered significant.

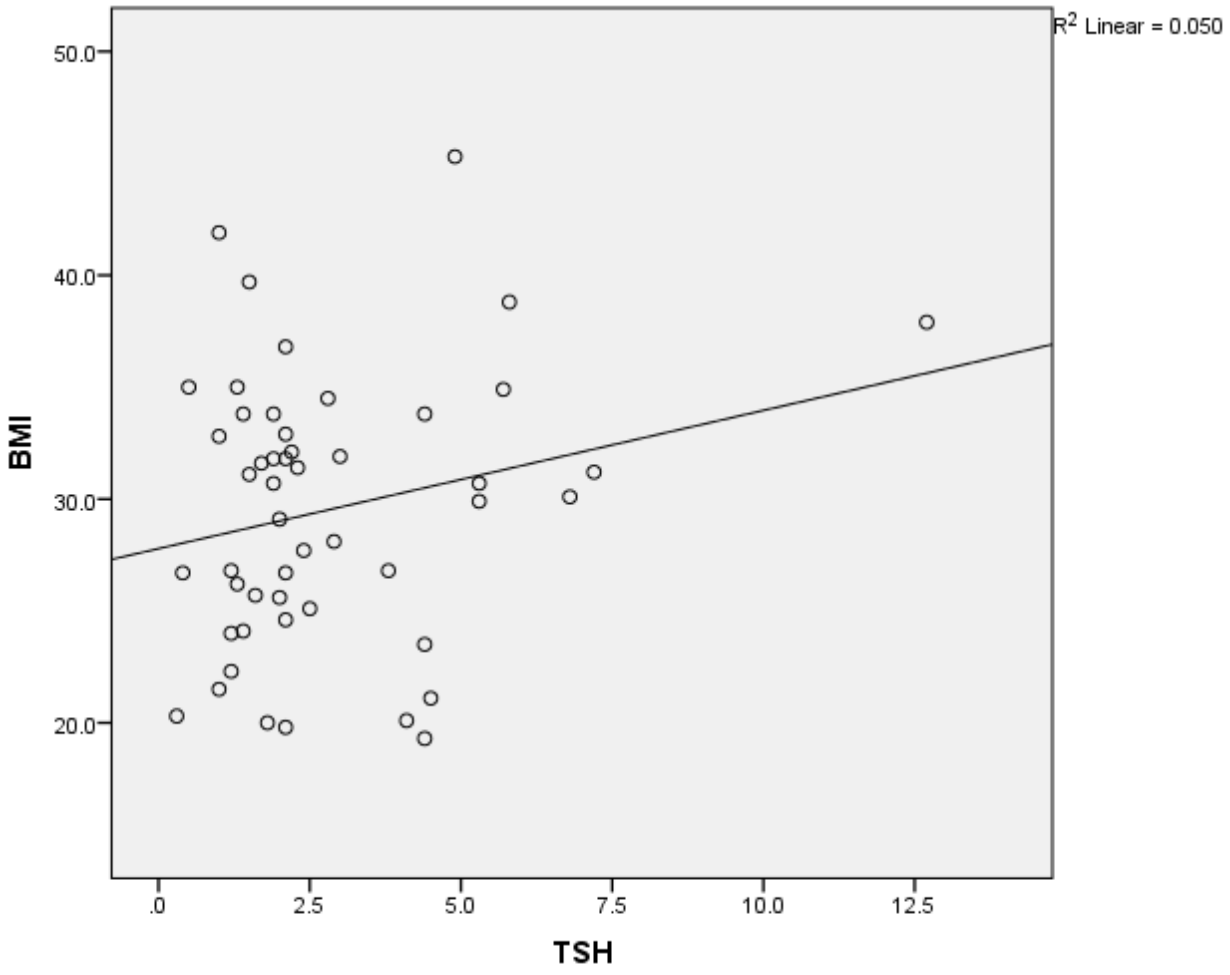

Fig. 4.1 Correlation between BMI and TSH in case group.

R=0.05 p. value 0.11

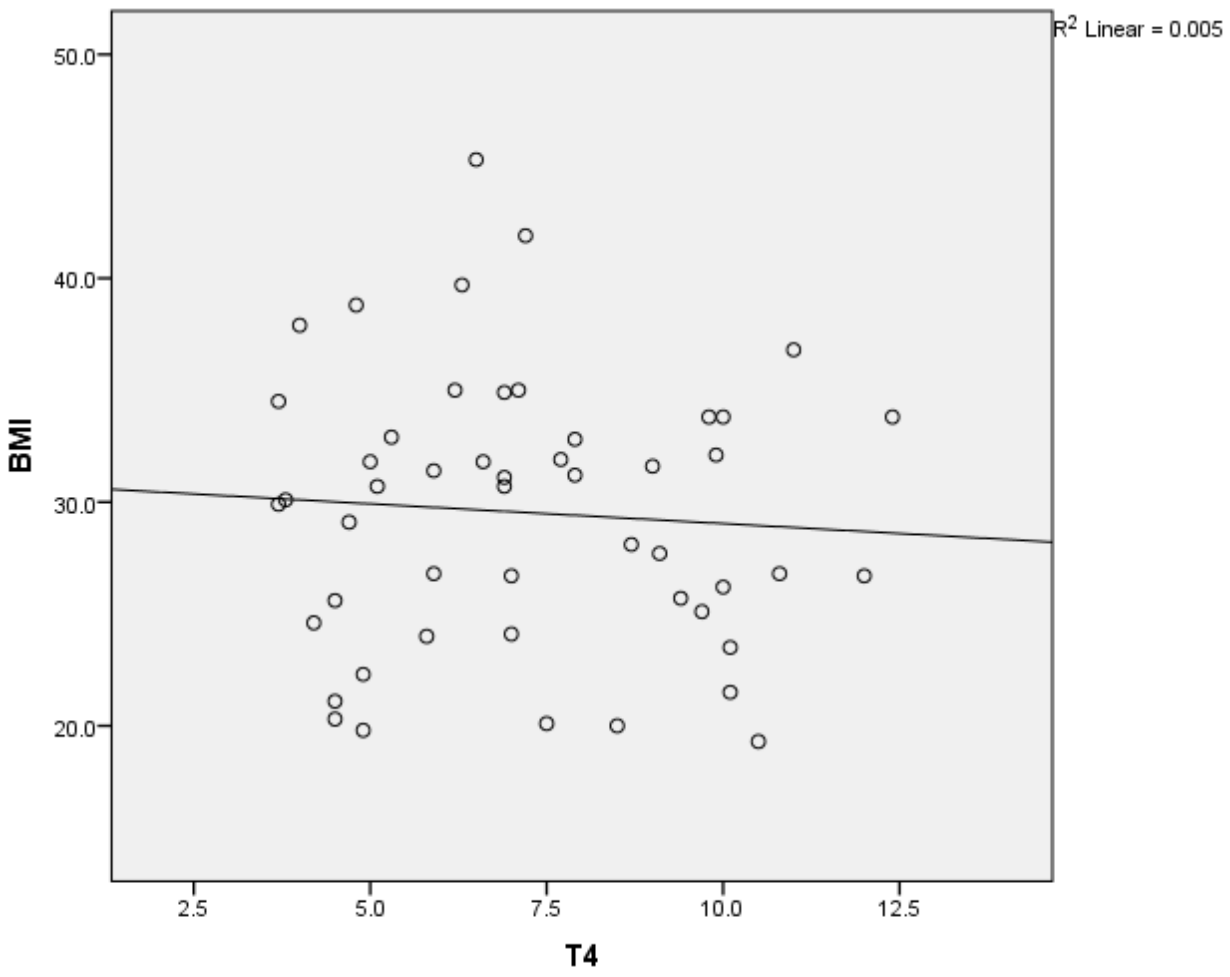

Fig. 4.2 Correlation between BMI and T4 in case group.

R=0.005 p. value 0.63

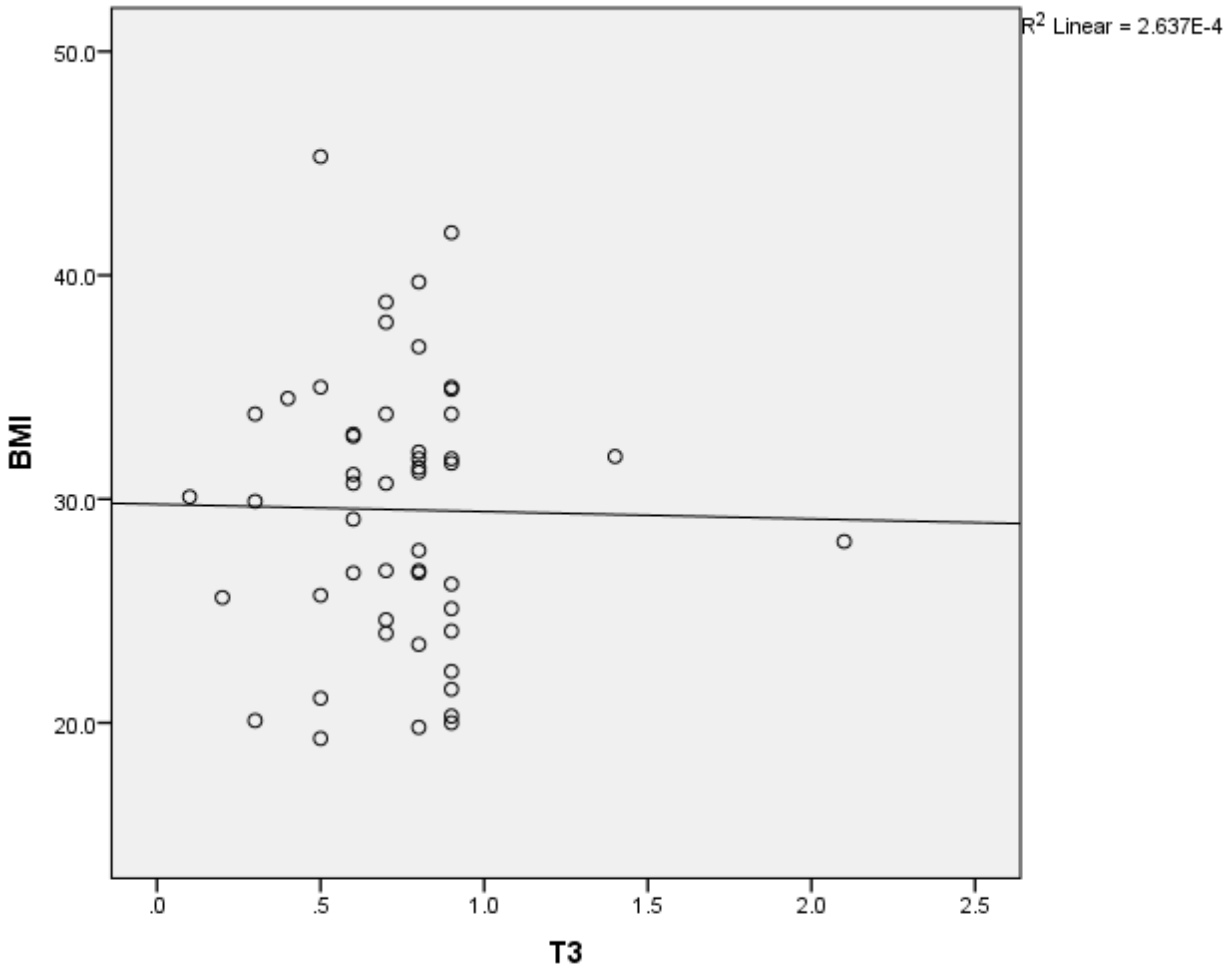

Fig. 4.3 Correlation between BMI and T3 in case group.

R=2.637 p. value 0.91

Chapter Five

Discussion, Conclusion and Recommendation

Discussion

Osteoporosis effects both bone quantity and quality with thinning in cortical bone and cavity expansion due to bone absorption at the endosteum surface as well as reduced bone density. It leads to enhanced bone fragility and an increase in fracture risk, the aim of this study was to evaluate thyroid hormone level in post-menopausal women with osteoporosis.

The 65 to 70 year-old age group was the most presented one with the frequency of 16.8%. In our study BMI did not very differ in case group compared to control group. *Kanis et al. (2008)* showed that a low BMI is a significant risk factor for hip fracture. Thus, the risk is nearly two-fold increased comparing individuals with a BMI of 25 kg/m2 and 20 kg/m2. It is important to note that the comparison of 25 versus 30 kg/m2 is not associated with a halving of risk, i.e. leanness is more of a risk factor rather than obesity being a protective factor. On the other hand *Sassi et al. (2015)* noteworthy that osteopenic and osteoporotic women had the lowest BMI. Also *López et al. (2011)*. showed that there are no statistically significant associations were found between TSH and the Body Mass Index (BMI), also *Obermayer-Pietsch et al.(2000)* BMI were not different in studied groups. *Yoshihara et al. (2016)* Saied that the osteoporosis group was significantly older ($p = 0.01$), was a greater number of years after menopause ($p = 0.01$), had significantly lower BMI values ($p = 0.0003$).

The result of the study showed that there are no different in TSH between case and control group and ranged in the normal value. Thyroid stimulating hormone may also have a direct effect on bone formation and bone resorption, mediated via the TSH receptor on osteoblast and osteoclast precursors, However *Wartofsky (1994)* approved that bone loss appeared independent of TSH levels in the experiments with mice lacking specific TR isoforms. Also he was investigated the impact of low serum TSH concentrations on fracture risk in a prospective cohort study of 686 white women over age 65 year followed for a mean of 3.7 years. Women with serum TSH concentrations of 0.1 mU/l or less at baseline were at increased risk for both hip and vertebral fracture (relative risk 3.6 and 4.5, respectively). Even euthyroid patients with lower TSH values have been shown to have a lower bone density than those with high normal TSH. *Article et al. (2016)* showed that low TSH levels was mostly present in women with osteopenia and osteoporosis.

Blum et al. (2016) also explained that risks for hip, any, and spine fracture were higher in participants with lower TSH levels, TSH levels of less than 0.10mIU/L were not associated with increased risk of non-spine fracture.

However *Murphy et al. (2018)* found that TSH was not associated with alterations in BMD, bone turnover markers, or other extraskeletal parameters. Also noticed that TSH concentration was unchanged with age. Some studies as in *Article et al. (2016)* focused on relationship between age and TFT he Saied "TSH levels in people without thyroid disease increases with age, and patient's age should be taken in account to interpret the results of thyroid function tests".

Indeed, there is little information about thyroid status in healthy euthyroid elderly individuals. Reduced synthesis and secretion of TRH and TSH appear to result in TSH levels in the low-normal range, and reduced thyroidal secretion of T3 and T4 is compensated by decreased clearance of thyroid hormones. *Tuchendler and Bolanowski (2014)* Saied that the receptors for TSH (TSHR) are located not only in thyroid follicular cells, but also in osteoblasts and osteoclasts. Data from scientific reports indicates that TSH is considered as a negative regulator of bone turn- over [4]. Its direct action on bone tissue cells leads to enhanced bone remodeling and osteoporosis(Tuchendler and Bolanowski, 2014).

To my knowledge, no studies have examined healthy euthyroid postmenopausal women specifically. Thus, the small increases in fT4 and fT3 with age along with maintenance of stable TSH concentrations may reflect the normal situation in healthy euthyroid postmenopausal women.

Bauer et al. 1997 measured TSH in 600 women more than 65 year of age from a population of 9704 that included subjects with thyroid disease and non thyroidal illness and individuals receiving drugs affecting bone metabolism. Women with suppressed TSH had an increased risk of hip and vertebral fracture during 3.7 year of follow-up.

In the study there are no association between BMI and TSH (insignificant correlation) p. value 0.11, the finding was relevant to finding of *López et al. 2011* who were found no statistically significant associations between TSH and the Body Mass Index (BMI), and the majority of patients had a normal BMI (up to 25).

In this study T3 appear to be different in case (mean 0.73 & SD 0.3) compared with control (mean 0.80 & SD 0.91). Also insignificant correlation between T3 with BMI and TSH (p. value 0.91 & 0.13 respectively) have been observed.

Furthermore thyroxine T4 did not different in case (mean 7.23, SD 2.38) and control (mean 6.56, SD 2.03). 91% ranged in normal scale while 1% with high level and 8% show low level of T4. There were insignificant correlation between T4 with TSH, BMI and T3 (p. value 0.12, 0.63 & 0.08 respectively).

Conclusion

Prevalence of osteoporosis is higher in post-menopausal women due to many factor that we mentioned previously. Our study focus on estimation of thyroid hormone level and TSH. Age and BMI have been recorded.

Studies of the effects of thyroid hormones in cartilage and bone represent a novel and growing field in our understanding of the broader pathophysiological consequences of thyroid hormone action. Although considerable advances have been made in recent years, there are important gaps in our knowledge and a number of specific areas require investigation.

I speculate from previous studies which mentioned that TSH may have permissive role in bone remodeling.

Recommendations

The increasing prevalence and awareness of osteoporosis, together with the development of treatments of proven efficacy, will increase the demand for the management of patients with osteoporosis. This in turn will require widespread facilities for the assessment of osteoporosis.

1. Osteoporosis awareness programs and preclinical assessment are recommended. Yearly assessment of all parameters that contribute to decrease bone mass is required.
2. Further studies with a large sample size using more advanced techniques are recommended.

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Appendix I

Questionnaire NO ◯

بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Evaluation of Thyroid Stimulating Hormone, Thyroxine and Triiodothyronine in Post-menopausal Women with Osteoporosis

(April, 2018)

Participant:

Name _____

Address _____

Phone number _____

Age _____

Weight _____

Height _____

Marital status:

Single ☐ Married ☐ Divorced ☐ Widowed ☐

Do you have thyroid problems?

Yes ☐ No ☐

Laboratory investigation:

Biochemical investigation	Result	Comment
Thyroid Stimulating hormone		
Thyroxine		
Triiodothyronine		