CORRELATION BETWEEN BONE MINERAL DENSITY AND VITAMIN D STATUS AMONG PATINETS WITH TYPE 2 DIABETES MELLITUS IN UNITED ARAB EMIRATES

HILDA ALLAM

UNIVERSITI SAINS MALAYSIA

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HILDA ALLAM

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AGEs	Glycation End Products
ALP	Alkaline Phosphatase
BTM	Bone Turnover Markers
BGLAP	Bone Gamma-Carboxy Glutamic Acid
BMD	Bone Mineral Density
BMI	Body Mass Index
BP	Blood Pressure
B-ALP	Bone Alkaline Phosphatase
βCTX	Beta Carboxy-Terminal Collagen Crosslinks
CRP	C-Reactive Protein
CTX	C-Terminal Telopeptide of Type I Collagen
DM	Diabetes Mellitus
DBP	Diastolic Blood Pressure
DXA	Dual-Energy X-Ray Absorptiometry
ECLIA	Electrochemiluminescence Immunoassay
ELISA	Enzyme-Linked Immunosorbent Assay
FFM	Fat Free Mass
FFAs	Free Fatty Acids
HDL-C	High Density Lipoprotein – Cholesterol
HC	Hip Circumference
HbA1c	Glycated Hemoglobin A1c
IGF	Insulin-Like Growth Factor
IL-6	Interleukin 6
IOF	International Osteoporosis Foundation

IOM	Institute of Medicine
LDL-C	Low Density Lipoprotein- Cholesterol
NTX	N-terminal Telopeptide of Type I Collagen
OPG mRNA	Osteoprotegerin
OC	Osteocalcin
PTH	Parathyroid Hormone
PCR	Polymerase Chain Reaction
QUS	Quantitative Ultrasound
RFLP	Restriction Fragment-Length Polymorphism
R. E	Restriction Enzyme
RANKL	Receptor Activator of Nuclear Factor Kappa-B Ligand
RANK	Receptor Activator of Nuclear Factor Kappa-B
RUNX2	Runt-Related Transcription Factor 2
SNPs	Single Nucleotide Polymorphisms
SBP	Systolic Blood Pressure
TG	Triglycerides
TGF	Transforming Growth Factor
T2DM	Type 2 Diabetes
TC	Total Cholesterol
UVB	Ultraviolet B Radiation
UAE	United Arab Emirates
VDREs	Vitamin D Response Elements
VDR	Vitamin D Receptor
VDBP	Vitamin D Binding Protein
WC	Waist Circumference
[25(OH)D]	25-hydroxy vitamin D

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KORELASI ANTARA KETUMPATAN MINERAL TULANG DAN STATUS VITAMIN D DALAM KALANGAN PESAKIT DIABETES MELITUS JENIS 2 DI EMIRIAH ARAB BERSATU

ABSTRAK

Pesakit diabetes jenis 2 (T2DM) mungkin mempunyai ketumpatan mineral tulang (BMD) yang normal atau tinggi dengan struktur tulang yang terjejas. Tahap vitamin D yang tidak mencukupi boleh menyebabkan pengurangan BMD dan menyumbang kepada kejadian diabetes. Penemuan ini masih dianggap kontroversi, dan mekanisma asas yang boleh menyumbang kepada patofisiologi BMD masih perlu difahami sepenuhnya. Umur, jantina, etnik, kawalan glisemik, dan tahap vitamin D menyumbang kepada keputusan yang berbeza. Kajian ini bertujuan untuk menilai skor BMD T dan mengaitkannya dengan paras serum vitamin D [(25OHD], penanda keradangan, dan penanda tulang dalam pesakit Emirati dengan T2DM. Satu kajian keratan rentas telah dijalankan di klinik diabetis pesakit luar di Hospital Universiti Sharjah dan Hospital Al Qassimi. Seramai 128 individu T2DM Emiriah berumur antara 25 hingga 65 tahun telah direkrut (lelaki: 46, perempuan: 82). Peserta menjalani pengukuran antropometri. Sampel darah diambil dan diuji untuk diabetes, perolehan tulang dan keradangan, selain tahap hormon parathyroid (PTH), kalsium dan vitamin D. Analisis polimorfisma gen VDR dan osteokalsin tidak menunjukkan perkaitan yang signifikan dengan BMD atau tahap vitamin D. Status BMD dinilai menggunakan imbasan ultrabunyi (QUS) pada tulang kanselus. Berdasarkan keputusan QUS, 69.5% peserta mempunyai BMD yang rendah, manakala 30.5% mempunyai BMD yang normal. Sebaliknya, 55.5% peserta mempunyai tahap vitamin D yang optimum, manakala 44.5% mempunyai tahap vitamin D yang rendah. BMD berkorelasi secara

signifikan dengan tahap vitamin D (r= 0.297, p < 0.001), manakala penanda tulang tidak menunjukkan perbezaan yang signifikan antara kumpulan BMD yang normal dan tidak normal. Sebanyak 83.6% peserta menunjukkan kawalan hemoglobin terglikosil (HbA1c ≥6.5) yang lemah, manakala 16.4% mempunyai kawalan glisemik yang baik. Tahap vitamin D yang optimum berkaitan dengan HbA1c yang lebih rendah (p = 0.030). Vitamin D dan jantina menunjukkan perkaitan positif yang signifikan dengan BMD (β= 0.012, p <0.001; β= 0.393, p = 0.017), manakala umur menunjukkan perkaitan negatif dengan BMD (β= -0.033, p < 0.001). Penemuan ini mencadangkan bahawa vitamin D, umur dan jantina secara kolektif menyumbang kepada peramalan BMD. Dalam analisis regresi logistik, tahap insulin yang tinggi berkait dengan skor BMD yang lebih baik (OR=0.961; 95%CI: 0.925-0.999, p = 0.042). Sementara, tahap IL-6 yang tinggi berkait dengan skor BMD yang rendah (OR=1.289; 95%CI: 1.071-1.551, p = 0.007). Tahap IL-6 boleh dianggap sebagai peramal terbaik untuk BMD. Kesimpulannya, BMD dipengaruhi oleh banyak faktor dalam T2DM. Oleh itu, penyedia penjagaan kesihatan perlu memberi perhatian kepada mereka yang mempunyai BMD yang rendah, memandangkan mereka mungkin berisiko tinggi untuk mengalami keretakan tulang.

CORRELATION BETWEEN BONE MINERAL DENSITY AND VITAMIN D STATUS AMONG PATIENTS WITH TYPE 2 DIABETES MELLITUS IN UNITED ARAB EMIRATES

ABSTRACT

Patients with type 2 diabetes (T2DM) may have a normal or high bone mineral density (BMD) with compromised bone structure. Inadequate vitamin D levels may reduce BMD and contribute to the development of diabetes. However, the results remain still controversial, and the underlying mechanism influencing BMD in T2DM are not fully understood. Factors such as age, sex, ethnicity, glycemic control, and vitamin D levels contribute to these variations. This study aimed to evaluate the BMD T score and correlate it with serum levels of vitamin D [25(OH)D], inflammatory, and bone markers in Emirati patients with T2DM. A cross-sectional study took place at the outpatient diabetic clinics of University Hospital Sharjah and Al Qassimi Hospital. A total of 128 Emirati individuals with T2DM aged 25 to 65 years were recruited (male: 46, female: 82). Participants underwent anthropometric measurements, and blood samples were collected to assess diabetic markers, bone and inflammatory markers, parathyroid hormone (PTH), calcium and vitamin D levels. Additionally, genetic polymorphisms in vitamin D receptor (VDR) and osteocalcin genes were analyzed for association with BMD and vitamin D. BMD was assessed using quantitative ultrasound scan (QUS) at the cancellous bone. According to QUS measurements, 69.5% of participants had low BMD, while 30.5% had normal BMD. Vitamin D levels were found to be low in 44.5% of participants, whereas 55.5% had optimal levels. BMD was significantly correlated with vitamin D levels ($r= 0.297$, $p < 0.001$), whereas bone markers did not significantly differ between normal and abnormal BMD groups.

The genetic analysis of VDR and osteocalcin gene polymorphisms showed no significant association with BMD or vitamin D levels. Poorly glycemic control (HbA1c ≥6.5) was observed in 83.6% of participants, while 16.4% had good glycemic control. Optimal vitamin D levels were associated with lower HbA1c ($p = 0.030$). Multivariate regression analysis revealed that vitamin D ($\beta= 0.012$, $p <0.001$) and sex ($\beta= 0.393$, $p =0.017$) had a significant positive association with BMD, whereas age showed a negative association with BMD ($\beta= -0.033$, $p < 0.001$). Logistic regression analysis further indicated that high insulin levels were linked with a better BMD score (OR=0.961; 95%CI: 0.925-0.999, $p = 0.042$), while high IL-6 levels were associated with low BMD scores (OR=1.289; 95%CI: 1.071-1.551, $p = 0.007$), suggesting that IL-6 is a strong predictor of BMD. In conclusion, BMD in T2DM is influenced by multiple factors, including vitamin D levels, age, sex, and inflammation. Given the high prevalence of low BMD, healthcare providers should monitor individuals at risk for fractures and consider intervention to improve health in patients with T2DM.

CHAPTER 1

INTRODUCTION

1.1 Background

Type 2 diabetes millets (T2DM) is the fastest-growing health problem on the planet; it is expected to rise from 2.8% in 2000 to 4.4% in 2030, bringing the number of affected adults from 171 million to 366 million (Espinoza *et al.*, 2022). Among the top five countries with the highest rate of T2DM is the United Arab Emirates (UAE), where 23% of the population is reported to have T2DM (Al Sabbah *et al.*, 2019). According to the World Health Organization (WHO) 2016, diabetes is globally ranked as the 7th leading cause of death (Al Awadi *et al.*, 2020). It has been documented in both developed and developing countries as a current and future disease burden with complications (Magliano *et al.*, 2020). This includes the UAE, one of the Gulf region's developing countries (Paulo *et al.*, 2019). The high incidence of T2DM among the UAE population is directly related to the country's economic growth. Economic development has led to significant lifestyle changes. These changes have increased obesity rates (Regmi *et al.*, 2020; Aljulifi, 2021).

Type 2 diabetes mellitus is characterized by hyperglycemia, associated with complications that are persistent even when hyperglycemia is under control. These complications have an impact on the quality of life of patients with diabetes and place a substantial extensive economic burden on the health care system.

One of the complications of diabetes is alteration in bone health. This condition is diabetic osteopathy (Asokan *et al.*, 2017). Bone mineral density has recently been found to be a significant predictor to future fracture risks. These fractures can lead to many secondary complications. Osteoporosis, a silent disease, is one of the

complications (Liu *et al.*, 2021). The WHO defined the architectural degeneration and bone loss that leads to bone fragility and increased risk of fractures as osteoporosis (LeBoff *et al.*, 2022). Long-term monitoring is necessary to minimize secondary complications related to diabetes.

Type 2 diabetes mellitus epidemiology is influenced by both genetics and lifestyle. Genome-wide association studies have revealed common glycemic genetic variations linked to T2DM. However, these common variations account for only 10% of the overall phenotypic variance. This indicates the presence of uncommon variants that need additional exploration. These variants play a crucial role in the development of T2DM (Galicia-Garcia *et al*., 2020). Poor diet and lack of physical activity contribute to obesity and insulin resistance, the primary causes of T2DM.

Vitamin D, known as the sunshine vitamin (Nair and Maseeh, 2012), is essential for normal bone development and mineralization of a healthy skeleton. The non-classical consequences of vitamin D deficiency are acknowledged, and low levels of vitamin D are associated with many conditions, such as diabetes mellitus, hypertension, malignancies, autoimmune diseases, and increased overall mortality. Many factors contribute to vitamin D deficiency, like poor diet, compromised calcium absorption, genetics, and insufficient sun exposure (Keane *et al.*, 2017). On the other hand, many factors influence vitamin D activation in the skin. These include ultraviolet B (UVB) radiation, the duration and pattern of exposure, and skin pigmentation. Also, the area of skin exposed plays a significant role (Webb *et al.*, 2018). It is presumed that people who live in parts of the world where sunlight is abundant all year, like the Gulf region, would have adequate levels of vitamin D. But this is not the case due to the indoor lifestyle. Vitamin D deficiency has become a pandemic and worldwide health problem (Amrein *et al.*, 2020). Although the Middle East receives ample

sunshine, people in this region (15° to 36°N) have a high prevalence of hypovitaminosis D across all age groups (Sadiya *et al.*, 2014). Despite that, one recent study showed that exposing patients with T2DM to sunlight for a certain period had no impact on the co-existing medical conditions (Nasr *et al.*, 2022). Most public health guidelines on sun exposure emphasize sun protection without considering skin type, while cultural expectations may restrict sun exposure. Vitamin D deficiency was significantly higher among conservatively dressed women than less conservative counterparts (Al Zarooni *et al.*, 2022).

Findings from the UAE have revealed several risk factors for hypovitaminosis besides the poor testing rates of vitamin D levels, reluctance to vitamin D administration, and lack of knowledge on the impact of low vitamin D levels (Abboud *et al.*, 2020).

T2DM and osteoporosis are chronic diseases (Alfadhli *et al.*, 2022). In 2016, in the UAE, the rate of osteoporosis was 3.1% for Emiratis between the ages of 18 and 85 years old. Adequate levels of vitamin D can prevent osteoporotic fractures (Ismail *et al.*, 2020). Conversely, hypovitaminosis can cause secondary hyperparathyroidism, causing increased bone turnover and deterioration of bone (Ismail *et al.*, 2020). However, the results remain controversial. Some studies suggest that hypovitaminosis may not necessarily lead to hyperparathyroidism. Other studies have found a positive correlation between vitamin D and bone mineral density (BMD). However, some studies did not observe this correlation (Bischoff *et al.*, 2004; Kota *et al.*, 2013; Napoli *et al.*, 2014).

Therefore, understanding the underlying mechanisms affecting diabetic patients is essential. Various factors may affect BMD and can significantly impact the overall health of diabetic patients.

1.2 Problem statement

Diabetes mellitus is a pandemic and chronic metabolic disorder associated with substantial morbidity and mortality. Patients with T2DM exhibit a unique skeletal phenotype characterized by normal or increased BMD but with impaired structural and geometric properties, which impact bone health and subject them to fracture risk by 40 -70% (Picke *et al.*, 2019).

Despite the considerable efforts worldwide and the research done on diabetes mellitus and BMD, the effect of diabetes mellitus on BMD and bone metabolism is still controversial (Liu *et al.*, 2021). The differences could be due to ethnicity, age, or body regions studied. However, there are still conflicting results concerning the influence of diabetes on BMD in association with sex, glycemic control, BMI and vitamin D levels. Based on the background, studies highlighting vitamin D have shown that vitamin D contributes to diabetes and its complications (Ahmed *et al.*, 2020). In addition, the duration of diabetes increases bone fragility (Picke *et al.*, 2019).

Early identification of risk factors in Emirati patients with T2DM may prove helpful for preventing diabetic complications in the future.

This study aims to assess whether vitamin D levels impact bone heath in Emirati patients with T2DM in the UAE. Also, it seeks to explore the underlying mechanism that could affect BMD in individuals with diabetes.

1.3 Rationale and Significance of the Study

Changes in bone tissue microstructure induced by diabetes affect BMD and lead to major skeletal complications. However, research results remain controversial. Several factors contribute to these inconsistent findings. The heterogeneity of the study populations characterized by differences in age, sex, and diabetic duration. Different studies employ different methodologies. Also, diabetes, comorbidities and complications are additional factors that play a significant role. The interaction between diabetes and other bone-affecting factors further influences the outcomes (Picke *et al.*, 2019).

Vitamin D has a classical role in bone mineralization and calcium absorption. It also plays a critical role in increasing insulin sensitivity and reducing insulin resistance (Argano *et al.*, 2023). Despite that, evidence is still lacking regarding the specific relationship between levels of vitamin D and BMD in patients with diabetes (Gao *et al.*, 2022).

The UAE has seen a growing prevalence of T2DM. This condition brings several complications. Bone health can be one of the complications that could be significantly affected by diabetes. Understanding the specific factors contributing to bone health in individuals with diabetes is essential. This understanding requires investigating the association between these factors and exploring T2DM pathophysiology in depth.

This study analyzed a range of markers. It focused specifically on the correlation between BMD and vitamin D levels in Emirati patients with T2DM in the UAE. In addition, it investigated the association of relevant polymorphisms with BMD and vitamin D levels within the context of T2DM.

Limited studies in the UAE have examined the relationship between BMD and vitamin D levels in Emirati patients with T2DM. Finding the correlation between multiple parameters will help fill existing knowledge gaps (Gao *et al.*, 2022). And will help develop future prevention and management strategies.

This research was conducted at the University Hospital Sharjah (UHS) and Al Qassimi Hospital in collaboration with the University of Sharjah, Sharjah. The above two hospitals are based in the UAE and work to serve the community and provide the latest advanced healthcare services based on research evidence. This collaboration between clinical and academic institutes will enrich the community's knowledge about disease association and prevention.

1.4 Research Question(s)

i. What are the anthropometric measures, the BMD values, the levels of vitamin D, the Parathyroid Hormone (PTH) values, the bone markers, the inflammatory markers, the vitamin D Receptor (VDR) gene polymorphism, and the osteocalcin gene polymorphism categories in patients with T2DM?

ii. What is the relationship between the anthropometric measures, BMD, inflammatory markers, PTH, bone markers, VDR gene polymorphism, osteocalcin gene polymorphic categories and level of vitamin D in patients with T2DM?

iii. What are the relationships between inflammatory markers, PTH, bone markers, and BMD among patients with T2DM?

iv. What are the changes in levels of osteocalcin and BMD according to the osteocalcin gene polymorphism among patients with T2DM?

v. Are there differences in levels of inflammatory markers, PTH, and bone markers in the two VDR polymorphisms?

1.5 Objectives

1.5.1 General Objective

To evaluate the BMD T score using an ultrasound bone densitometer and correlate it with serum levels of vitamin D [25(OH)D], inflammatory markers, and bone markers in Emirati patients with T2DM.

1.5.2 Specific objectives

i. To determine the anthropometric measures, BMD T score, levels of vitamin D, PTH, bone markers, inflammatory markers, VDR gene polymorphisms, and osteocalcin gene polymorphism in Emirati patients with T2DM within a 12 month study period.

ii. To correlate anthropometric measures, BMD T score, inflammatory markers, PTH, and bone markers with levels of vitamin D [25(OH)D] in Emirati patients with T2DM. And to examine the relationship of vitamin D status among different vitamin D receptor gene polymorphisms and osteocalcin gene polymorphisms over a 12 month period.

iii. To correlate inflammatory markers, PTH, and bone markers with BMD T score in Emirati patients with T2DM over a 12 month period .

iv. To compare the serum levels of osteocalcin and BMD T score in osteocalcin gene polymorphisms within a 12 month study duration.

v. To compare serum levels of different inflammatory markers, PTH, and bone markers with VDR gene polymorphisms within a 12 month study duration.

1.6 Alternative Hypothesis

In Emirati patients with T2DM, there is a correlation between BMD, vitamin D levels, and bone markers.

1.7 Conceptual Framework

In the conceptual framework (Figure 1), BMD, levels of biochemical markers, and polymorphisms were assessed among Emirati patients with T2DM. The presence of obesity is frequently associated with decreased levels of vitamin D because of the accumulation of vitamin D in the adipose tissue. Obesity is related to a constant mild inflammation characterized by the release of pro-inflammatory cytokines from the fatty tissue, contributing to insulin resistance. Chronic inflammation affects the pathogenesis of T2DM. When the inflammation persists or is chronic for a long time, it can impact the bone remodelling process, leading to excessive bone resorption and decreased bone formation. This eventually results in reduced bone density and an increased risk of fractures.

Vitamin D has a possible anti-inflammatory effect, as it increases anti-inflammatory molecules in our body, thus potentially reducing inflammation.

Sufficient amounts of vitamin D can enhance insulin sensitivity, and low levels of vitamin D may contribute to insulin resistance, leading to hyperinsulinemia and the onset of T2DM over time. Poor diet and inadequate sun exposure may impact vitamin D levels.

Vitamin D affects bone health via many processes; it improves calcium absorption and other minerals from the intestine that are needed for bone mineralization and strength. This mechanism takes place with the regulation of the PTH. Vitamin D further affects the functions of osteoblasts and osteoclasts.

Vitamin D exerts its action via VDR, a protein that is vital in facilitating the physiological impacts of vitamin D within the body. Vitamin D binds to the receptor, forming a vitamin D receptor complex. The complex structure then binds to DNA sequences known as vitamin D response elements (VDREs) within the genes, activating several bone metabolism and health functions.

Genetic variations in the osteocalcin gene can impact the amount of osteocalcin expression. Specific mutations can result in either an increase or a decrease in the synthesis of this protein, known as a bone formation marker.

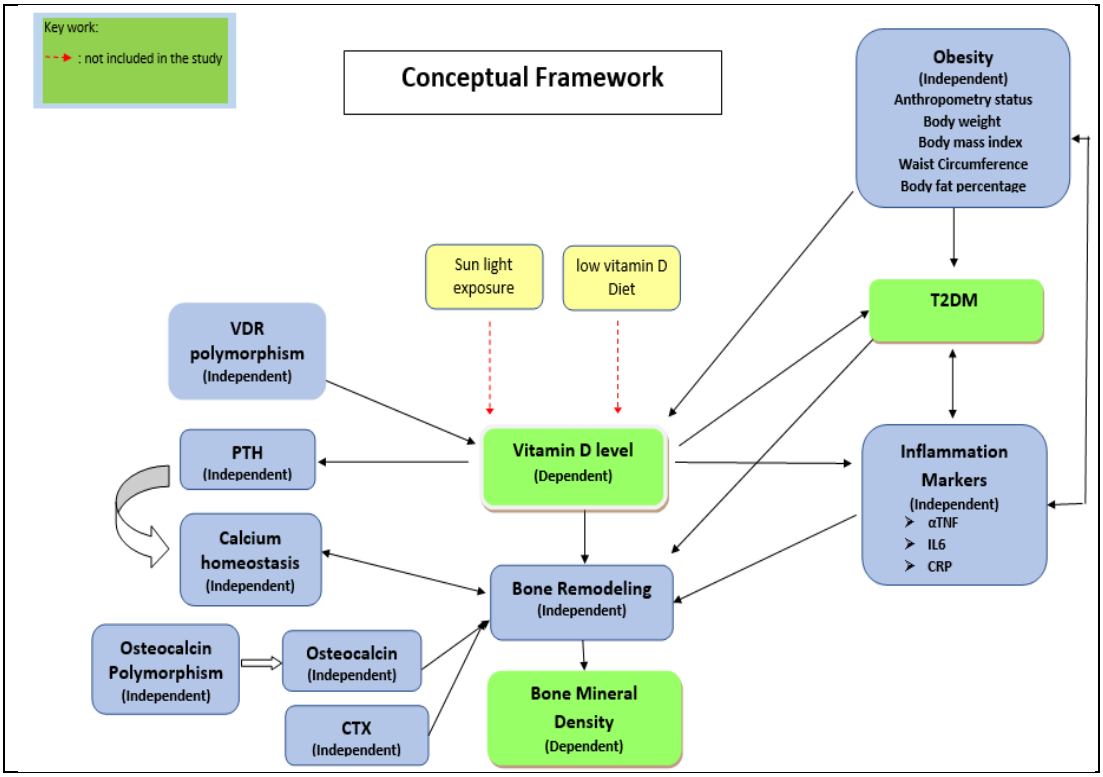

Figure 1.1 The role of the interconnected pathways in bone density

1.8 Operational definitions

i. **Bone Mineral Density –**the amount of minerals presents in bone tissue, which refers to the biomechanical properties of the bone. It provides a snapshot of bone strength. In this study, the BMD was determined by measuring the T-score.

ii. **T score –** refers to how much your bone mass differs from the bone mass of an average healthy 30-year-old adult. A T-score of -1.0 or above is normal bone density. A T-score between -1.0 and -2.5 means a low bone density. A T-score of -2.5 or below is a diagnosis of osteoporosis.

iii. **Bone remodelling –** is an essential process that creates new bone tissue (bone formation) and removes existing bone tissue (bone resorption).

iv. **T2DM –** is a chronic disease characterized by high levels of glucose in the blood, which occur when the body becomes resistant to insulin or does not make enough insulin.

v. **βCTX Cross Laps –** are known as carboxy-terminal collagen crosslinks. It is the degradation product of bone collagen that is released in the blood. It is used as a biomarker to measure the serum's bone resorption rate.

vi. **Osteocalcin –** is a non-collagenous bone matrix protein exclusively synthesized by osteoblast. It is used as a bone formation marker and, therefore, it is used in the assessment of bone turnover.

vii. **25 (OH) Vitamin D status –** the status of vitamin D is defined as deficient, sufficient, and insufficient according to the recommendations of the Institute of Medicine (IOM).

viii. **Serum 25 (OH)D –** serum level/concentration of vitamin D.

ix. **Inflammatory markers –** are a set of biomarkers used clinically to assess a patient for diagnosis and monitoring of inflammatory conditions.

x. **Polymorphisms** – the presence of two or more DNA sequence variants of genes.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of bone health

The relationship between vitamin D levels and BMD in patients with T2DM in the UAE poses significant health concerns. There is an association between low bone mineral density and the increased risk of fracture, which can be attributed to various factors, including alterations in bone metabolism. Patients with T2DM may also exhibit low vitamin D levels. It is well known that vitamin D plays a significant role in maintaining bone mineralization and bone health.

2.1.1 Bone structure

Bone is a mineralized porous structure composed of cells, vessels, and calcium compounds in the form of hydroxyapatite. The bone matrix is composed of collagenous and non-collagenous proteins. Collagen Type I, the predominant collagen protein, constitutes the organic framework that acts as a support structure for mineralization. The collagen matrix serves as the structural support for forming calcium phosphate crystals, specifically hydroxyapatite, which are responsible for the rigidity and durability of bones (Naomi *et al.*, 2021). At the same time, osteocalcin is the most abundant non-collagenous protein responsible for the organization of bone formation (Moriishi *et al.*, 2020).

There are two types of bones: cortical (also known as compact) and trabecular (spongy or cancellous) bones Figure 2.1. The cortical bone exhibits a cylindrical organization along its longitudinal axis and possesses a compact, calcified composition, including approximately 80 to 90% calcium by volume. Conversely,

trabecular bone is characterized by a complex, three-dimensional lattice structure consisting of thin, calcified, interconnected bone beams or trabeculae (Formosa *et al.*, 2024). The calcium content accounts for only 15 to 25% of the total volume. Trabecular bone is commonly located in the extremities of long bones, adjacent to joint surfaces, and flat bones. Cortical bone accounts for approximately 80% of the skeletal structure, while trabecular bone comprises the remaining 20%. The cortical bone essentially serves mechanical and defensive functions, while the trabecular bone predominantly involves metabolic processes (Schini *et al.*, 2022).

Bones are metabolically active connective tissues with several physiological functions. Bones provide structural stability and are essential in maintaining mineral homeostasis by releasing calcium into the bloodstream based on the body's metabolic needs. Additionally, it has been found that the skeletal system regulates energy metabolism through osteocalcin secretion (Chumachenko *et al.*, 2019).

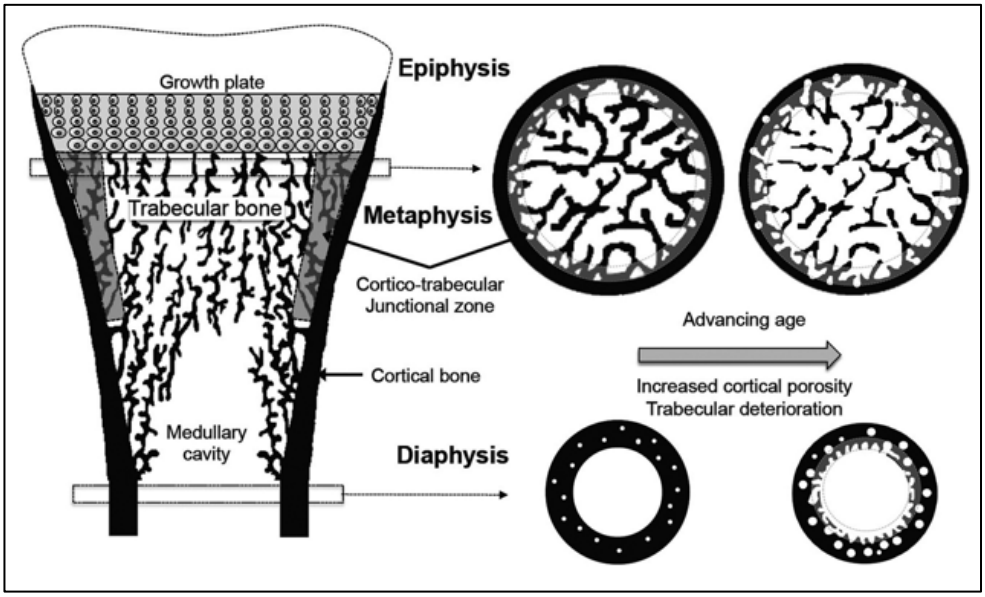

Figure 2.1 A cross section in a long bone

Source: Adopted from (Schini *et al.*, 2022)

2.1.2 Bone remodelling

Throughout the ageing process, bones undergo continual remodelling, a natural mechanism wherein old bone tissues are gradually replaced by new bones. This remodelling enables bones to adapt to mechanical stresses, accommodate alterations in the body, and uphold mineral strength and overall homeostasis (Bolamperti *et al.*, 2022). This mechanism inhibits the buildup of old bone, thus preserving its shape, quality, and size. Bone remodelling is faster in trabecular bones than in cortical bones (Schini *et al.*, 2022).

This process is facilitated by osteoclasts and osteoblasts. Osteoclasts break aged bone tissue; subsequently, mononuclear cells prime the bone surface for the deposition of newly generated material, and osteoblasts facilitate the formation of new bone by secreting type I collagen protein that serves as a bone matrix, followed by

mineralization with calcium and other minerals required for the bone formation process. An appropriate proportion of the organic matrix to the mineral is crucial to the flexibility and stiffness of the skeleton. Osteoblast cells become flatted cells (osteocytes) lining the bone surface (Rowe *et al.*, 2018) Figure 2.2. The role of cells is closely coupled, resorbing old bone damage and forming new bone simultaneously. Many biochemical and mechanical factors synchronize the coordination between different types of cells in this process.

Parathyroid hormone (PTH) stimulates bone remodelling by upregulating the RANKL mRNA and downregulating the OPG mRNA in osteoblasts, resulting in an elevated RANKL/OPG ratio that promotes osteoclastogenesis and bone resorption (Šromová *et al.*, 2023).

Moreover, PTH orchestrates the signalling network, which links bone resorption with bone formation, when it interacts with local osteotropic factors such as transforming growth factor (TGF) and insulin-like growth factor (IGF) (Schini *et al.*, 2022). The bone matrix contains abundant transforming growth factor (TGF), increasing PTH concentration. When bone resorption occurs, the active transforming growth factor TGF induces bone mesenchymal stem cells to migrate, coupling resorption with the formation of new bone (Šromová *et al.*, 2023).

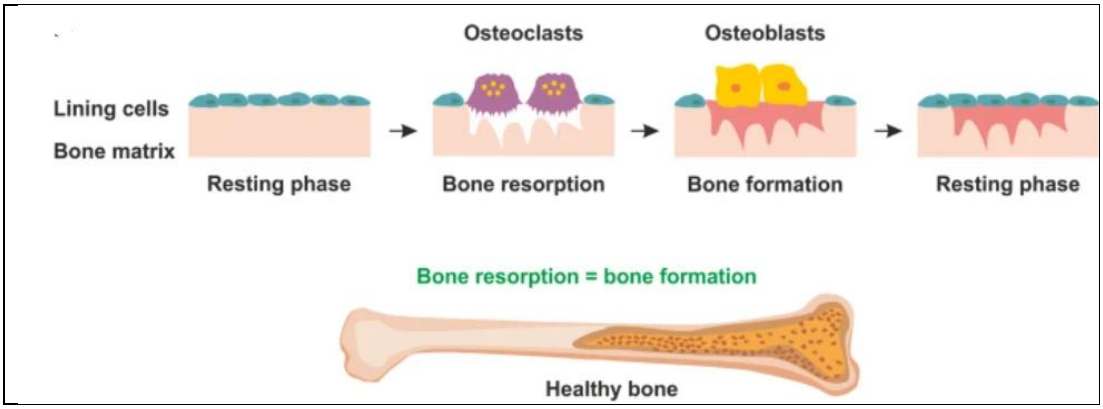

Figure 2.2 Bone remodelling cycle

Source: Adopted from (Bicer *et al.*, 2021)

The entire process results in the replacement of the old or damaged reabsorbed bone with new bone. It is worth mentioning that in physiological bone remodelling, the bone volume remains constant (Katsimbri, 2017). This tight coupling between bone resorption and formation is essential for the skeleton to remain intact between cycles of resorption and formation. Under ideal physiological conditions, bone resorption takes ten days while bone synthesis takes three months; thus, yearly remodelling can replace 20% of the skeleton (Shetty *et al.*, 2016).

However, an imbalance between bone resorption and creation can result in several bone diseases, such as osteoporosis, which is characterized by decreased bone density and increased risk of fractures (Sobh *et al.*, 2022).

2.1.3 Bone mineral density (BMD)

2.1.3(a) Definition of BMD

The amount of mineral per unit of bone is considered bone mineral density. Bone Mineral Density (BMD) is a crucial determinant of bone health and susceptibility to fractures, and it can be used as an indirect measure of bone strength to determine if a patient has osteopenia or osteoporosis (Haseltine *et al.*, 2021). The ability of the bone

to endure damage is affected by its geometry, microarchitecture, and size. A significant portion of the variation in bone strength, ranging from 75% to 90%, can be attributed to BMD, which is derived from the integration of bone density and quality (Pouresmaeili *et al.*, 2018).

BMD is measured and reported as T-score (Haseltine *et al.*, 2021). The World Health Organization (WHO) experts categorized BMD based on a specific threshold value for T-scores into three categories: a T-score above -1 SD indicates a normal BMD, a T-score between -1 and -2.5 SD suggests a condition of osteopenia, and a T-Score below -2.5 standard deviations (SD). Table 2.1. It is crucial to highlight that T-scores are not the only factors in evaluating fracture risk or overall bone health. However, fracture risk factors are independent of bone mineral density, so it would be more sensible to consider combining both instead of relying solely on the T-score to diagnose bone diseases (Ferrari *et al.*, 2019).

Table 2.1 WHO definitions of osteoporosis based on BMD

Classification	Bone Mineral Density	T Score
Normal	Within 1 SD of the mean level for a young adult reference population	T score at −1.0 and above
Low bone mass (Osteopenia)	Between 1 and 2.5 SD below that of the mean level for a young adult reference population	T score between −1.0 and −2.5
Osteoporosis	2.5 or more below the mean level for a young adult reference population	T score at or below −2.5
Severe or established osteoporosis	2.5 or more below the mean level for a young adult reference population with fractures	T score at or below −2.5 with one or more fractures

Adopted from (Sözen *et al.*, 2017).

2.1.3(b) Risk factors affecting BMD

Several risk factors have been identified that are linked to bone mineral density, such as genetic, endocrine, mechanical, dietary intake and lifestyle factors (smoking, alcohol use, and physical activity) (Yang *et al.*, 2023). Other factors have been found to affect BMD, such as the use of inhaled corticosteroids, history of fractures, diabetes, obesity, anaemia and metabolic syndromes (Heidari *et al.*, 2017). Low BMD is associated with an increased risk of fractures (Hsu *et al.*, 2021). The progression from low BMD to more severe disorders depends on the extent to which these risk factors are altered throughout life. Table 2.2 summarizes the modifiable and non-modifiable risk factors for osteoporosis.

Table 2.2 Risk factors for osteoporosis

Modifiable Risk Factors	**Non modifiable Risk Factors**
Dietary intake	Sex
Body mass index	Older age
Lifestyle	History of fracture
Cigarette Smoking	White Ethnic background
Alcohol consumption	Family history of osteoporosis
Stress	

Source: Adopted from (Pouresmaeili *et al.*, 2018)

i. Age

Bones show accelerated growth until the age of 30, following a plateau phase where bone mass becomes stable until 40 years, after which bone growth declines until 80 (Cossio-Bolanos *et al.*, 2022). In the age of menopause, which often begins at the age of 51 (de Villiers, 2024), due to an imbalance in the bone remodelling process, women typically undergo a rapid phase of bone loss that begins approximately 2 to 3 years before the cessation of menses and continues for up to 5 years post-menopause, where the quantity and quality of bone decline rapidly, resulting in a dramatic increase in the risk of fracture in postmenopausal women. Low bone density mainly occurs in post-menopausal women because of estrogen reduction and age (Qiu *et al.*, 2021).

ii. Sex

Bone density may differ between males and females due to bone structure and biological differences. Males and females vary in bone growth, size, and catabolism (De Martinis *et al.*, 2021b). Females are traditionally thought to be most affected by bone loss and fracture risk, but it has become widely acknowledged that males are also affected, especially as the population ages (Drake *et al.*, 2012). However, Yang et al. reported that male patients undergoing certain surgical procedurs may experience reduction in BMD at specific skeletal sites compared to females (Yang *et al.*, 2022).

Additionally, another study suggested that men exhibit a lower increase in bone formation than women. One of the key factor influencing BMD in both sexes is the role of sex hormones (Choi *et al.*, 2021).

iii. Genetics

Several studies have demonstrated a link between BMD among family members, suggesting that hereditary factors play a role in influencing BMD (Kranioti *et al.*, 2019; Hou *et al.*, 2020; Lv *et al.*, 2022). The frequency of having low bone mineral density in first-degree relatives of both sexes is significant; therefore, screening could be cost-effective and informative in identifying underlying genetic factors (Younes *et al.*, 2021)

Furthermore, variations in BMD have been attributed to many genetic factors, such as polymorphisms in genes implicated in bone formation, resorption, and mineralization. The genome-wide association studies have identified numerous susceptibility loci that have a potential impact on BMD; however, no specific genetic cause has been found (Piroska *et al.*, 2021). Genetic investigations are emphasized in another section of the literature.

iv. Anthropometric measurements

Anthropometric measures such as weight, body mass index (BMI), waist circumference (WC), hip circumference (HC) and waist-hip ratio (WHR) are found to be associated with bone mineral density (BMD) (Ma *et al.*, 2021). Anthropometric measurements may show mechanical strain on bones, affecting bone remodelling and mineralization and a higher body mass index (BMI) can impose a greater mechanical load on bones, resulting in increased bone remodelling and possibly increased BMD. In addition, increased waist circumference can place mechanical stress on the lumbar

spine and pelvis; body weight applies a mechanical load to weight-bearing bones, such as the spine and pelvis.

Anthropometric measures can be a useful tool to predict BMD and indicate the risk of osteoporosis and fracture (Ma *et al.*, 2021). There is no consensus on which anthropometric measurements are most closely associated with osteoporosis. However, BMI is the most prevalent anthropometric measure that is linked to various diseases and mortalities (Murat *et al.*, 2021). The findings regarding the relationship between BMD T-scores at certain sites and anthropometric measurements are controversial. A previous study found weak positive correlations between weight, BMI, WC, body fat percentage and BMD (Murat *et al.*, 2021). However, other studies have shown no correlation (Cherif *et al.*, 2018).

v. Lifestyle and dietary intake

Lifestyle factors, such as physical exercise and nutrition, impact bone mineral accretion and peak bone mass development. Physical activity is the main lifestyle factor affecting peak bone mass. Endocrine factors directly influence bone synthesis or absorption, whereas genetic and lifestyle factors affect BMD via the endocrine system (Kralick and Zemel, 2020). Multiple studies have indicated the impact of physical exercise on bone mineral content, density, dimensions, and strength during childhood and adolescence, where muscular load creates mechanical forces that allow bone morphology to adapt to these forces (O'Leary *et al.*, 2019). Studies show that childhood physical activity increases bone mass and strength but may diminish if activity levels decline.

An average older woman would live 13 years longer without osteoporosis with a 10% peak bone mass gain (Rondanelli *et al.*, 2022). Rondanelli, in his study, cited a

systematic review that evaluated 18 observational studies on physical activity and found it positively associated with fracture reduction in adults. This finding demonstrates the importance of lifetime physical activity in bone health. Therefore, healthy eating and exercise can prevent osteoporosis. The mineral matrix of bones is composed of hydroxyapatite, a calcium-phosphate combination. Calcium, which is strictly regulated in the blood to support vital activities, is stored in bones. Vitamin D is essential for maintaining calcium balance. Calcium and vitamin D are crucial for maintaining bone mineral accumulation and optimizing the peak bone mass. Insufficient calcium and vitamin D intake can decrease bone mineralization and peak bone mass.

Instead, a diet rich in bone-building elements promotes bone health. Low calcium intake during childhood can have long-term effects, as women who consume less than one serving of milk per week have lower hip bone mineral density and higher fracture risk (Kralick and Zemel, 2020). In contrast, high sodium, caffeine, and alcohol intake and poor fruit and vegetable intake may lower bone density (Rondanelli *et al.*, 2022). In addition, the long-term consumption of trans-hydrogenated fats and fried meals may cause inflammation. In one study, long-term pro-inflammatory eaters had lower lumbar spine and total hip BMD than healthy eaters.

vi. Obesity

Obese or overweight individuals tend to have high BMD, which lowers the probability of developing fractures or osteoporosis. Obesity has a beneficial impact on BMD because of the mechanical loading effect of weight on the bones (Heidari *et al.*, 2017). Based on these findings, a moderate increase in BMI could be beneficial to bone health. However, given the inverted U-shaped association, excessively high BMI may have a harmful effect on bone health, particularly in women and black individuals (Li,

2022). The impact of obesity on skeleton is complex and influenced by factors, including mechanical loading, obesity type and distribution, bone site, age and cytokine secretion (Hou *et al.*, 2020).

vii. Smoking

The exact pathophysiological mechanisms by which smoking affects bone health are still unknown due to the lack of well-designed studies that elucidate these mechanisms and the presence of conflicting results (Al-Bashaireh *et al.*, 2018). However, a recent study has revealed in one of the studies that the adverse effects of smoking on bone health are mediated by two distinct mechanisms. Initially, it can directly exert a toxic effect on osteoblasts and blood flow, thereby potentially compromising bone health and increasing the susceptibility to fracture risk. Second, the adverse impact could be due to its frequent co-occurrence with other health-risk behaviours.

Smoking can disrupt the physiological inverse relationship between parathyroid hormone (PTH) and vitamin D, which indirect effects on bone health (Trevisan *et al.*, 2020). Smoking can influence bone metabolism through indirect mechanisms, including its impact on body weight, hormone concentrations, and oxidative stress levels (Weng *et al.*, 2022).

2.1.3(c) Osteoporosis and low BMD

BMD is a crucial tool for diagnosing osteoporosis and for forecasting fractures. In simpler terms, BMD is associated with fragility fractures, the primary severe outcome of osteoporosis (Li *et al.*, 2021).

Osteopenia and Osteoporosis defined by BMD, are bone diseases characterized by significant alterations in bone composition and subsequent disruption of the bone structure (Varacallo *et al.*, 2024). An increased fracture risk is one of the most apparent manifestations of this silent disease and is associated with significant morbidity and mortality (Al Anouti *et al.*, 2019).

Osteoporosis is characterized by a simultaneous disease in bone mineral density and bone matrix. As a result, the bone mass decreases, but its composition remains intact.

Osteoporosis has a significant impact on countries' economies. Consequently, osteoporosis is often diagnosed in patients only after a fracture has occurred. That is why some countries advise screening for osteoporosis irrespective of risk factors (Li *et al.*, 2022).

There are two types of osteoporosis: the primary type is related to age, gender, family history and decreased gonadal functions and the secondary type is associated with diseases such as type 2 diabetes, cardiovascular disease and vitamin D deficiency (Wu *et al.*, 2022a). It may be possible to alleviate fracture risk by treating the underlying cause and avoid unnecessary treatment with antiresorptive drugs if the underlying cause is correctly addressed. Fractures tend to occur in body areas that have a high amount of trabecular bone but may occur in other locations.

2.1.3(d) Measuring BMD

The bones could be examined and scanned using different x-ray modalities, one of them is the ultrasound waves at the peripheral skeletal sites as calcaneus bone in the heel area. Quantitative Ultrasound (QUS) is a safe, noninvasive technology that