STUDY ON VITAMIN D STATUS AND DETERMINATION OF OPTIMUM VITAMIN D LEVEL BASED ON BONE TURNOVER MARKERS IN KOTA BHARU, KELANTAN

By

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND SYMBOLS

$1,25(OH)_2D$	: 1,25 dihydroxy vitamin D
7-DHC	: 7-dehydrocholesterol
25(OH)D	: 25 hydroxy vitamin D
BMD	: Bone mineral density
BTM	: Bone turnover marker
CTX	: C- terminal telopeptide of type 1 collagen
DEXA	: Dual energy x-ray absrptiometry
FGF 23	: Fibroblast growth factor 23
LOESS	: Locally weighted scatterplot smoothing
OC	: Osteocalcin
PTH	: Parathyroid hormone
P1NP	: Procollagen 1 intact N terminal
RANK	: Receptor activator of nuclear factor –κB
RANKL	: Receptor activator of nuclear factor –κB ligand
SLE	: Systemic lupus erythomatosus

ABSTRAK

Kajian status paras vitamin D dan penentuan tahap vitamin D yang optimum berdasarkan penanda aktiviti tulang di Kota Bharu, Kelantan

Latar Belakang: Masih tiada tahap optimum atau ambang yang jelas bagi menentukan sama ada terdapat bekalan yang mencukupi untuk vitamin D dalam badan untuk kesihatan tulang. Oleh itu, kajian kami adalah untuk menentukan tahap optimum vitamin D untuk penyelenggaraan tulang yang sihat berdasarkan proses pembentukan semula tulang.

Kaedah: Ini adalah satu kajian keratan rentas lintang yang melibatkan penduduk dewasa yang sihat di Kota Bharu, Malaysia, berumur 18-50 tahun. Kami mengukur serum vitamin D 25 (OH) D, serum hormon paratiroid (PTH), serum C-terminal telopeptide of type 1 collagen (*CTX*) dan jumlah Procollagen I Intact N-Terminal (P1NP) di kalangan 120 orang dewasa yang sihat dari 6 sub daerah yang telah dipilih secara persampelan berperingkat (64 lelaki, 56 perempuan).

Keputusan: Purata vitamin D dalam kajian ini adalah 23.50 nmol/L dan terdapat perbezaan yang signifikan pada tahap vitamin D antara jantina (26.81 ± 8.3 nmol/L)bagi lelaki dan(19.72 ± 7.68 nmol/L) bagi perempuan. Lebih daripada 50% daripada subjek perempuan mempunyai vitamin D kurang daripada 20 nmol/L, manakala hanya 20.3% daripada subjek lelaki mempunyai vitamin D di bawah 20 nmol/L. Berdasarkan loess plot, serum CTX tidak menunjukkan kenaikan di peringkat vitamin D pada tahap

35nmol/L. Jumlah Serum P1NP dilihat sebagai mendatar apabila vitamin D adalah sekitar 20 nmol/L. Begitu juga, pada 20 nmol/L terdapat penurunan tahap PTH.

Kesimpulan: Purata vitamin D di kalangan penduduk dewasa sihat di Kota Bharu adalah 23.50 nmol/L.Seterusnya, berdasarkanpenanda aktiviti tulang , kami membuat kesimpulan bahawa tahap vitamin D antara 20 nmol/L hingga 35 nmol/L dianggap mencukupi untuk mengekalkan tulang yang sihat.

ABSTRACT

Study on vitamin D status and determination of optimum vitamin D level based on bone turnover markers in Kota Bharu, Kelantan

Background: There is no optimum level or threshold that has been clearly established to define whether there is adequate store of vitamin D in the body for general bone health. Therefore, our study is to determine the optimum level of vitamin D for maintenance of healthy skeleton based on bone remodelling process.

Methods: This was a cross sectional study involving healthy adult population in Kota Bharu, Malaysia, aged 18-50 years. We measured serum 25(OH)D, serum parathyroid hormone (PTH), serum C-terminal telopeptide of type 1 collagen (*CTX*) and Procollagen 1 Intact N-Terminal (P1NP) in 120 healthy adult from 6 sub districts that had been selected by multi stage sampling (64 males, 56 females).

Results: The mean of vitamin D in this study was 23.50 (±8.74) nmol/L and there was significant difference of vitamin D between gender (26.81±8.3 nmol/L and 19.72±7.68 nmol/L males and females respectively), p value < 0.001. More than 50% of female subjects had vitamin D less than 20 nmol/L, while only 20.3% of male subjects had vitamin D below 20 nmol/L. Based on LOESS plot, the bone turnover markers showed plateau result at vitamin D level of 35 nmol/L for CTX and 20 nmol/L for P1NP. Then, at vitamin D level of 20 nmol/L there is a step decrease of PTH level.

Conclusion: The mean of vitamin D among general healthy population in Kota Bharu was 23.50nmol/L. Based on inspection of LOESS plot for CTX, P1NP and PTH versus

vitamin D level, we concluded that vitamin D level between 20 nmol/L to 35 nmol/L is considered sufficient to maintain healthy skeleton.

1. INTRODUCTION

Vitamin D deficiency is a global public health concern for most of the countries nowadays (Langlois *et al.*, 2010). New discoveries have proved that vitamin D deficiency is also related to higher risk of cardiovascular disease, diabetes mellitus, cancer and auto immune disease, apart from its key role in bone metabolism (Hullett *et al.*, 1998; Nagpal *et al.*, 2005; Martini and Wood, 2008).

Vitamin D, which is also called as sunshine vitamin is not a vitamin or nutrient by itself. In 1969, after the discovery of vitamin D receptor, it is recognized that the term vitamin D is inappropriate. Vitamin D is better to be defined as a complex endocrine system (Norman, 2008). The reasons are that, because of i) it can be endogenously synthesize, ii) presence of specific receptor , iii) actions in many organs and tissues, iv) induction of specific biologic response after interaction with its receptor. The actions of vitamin D are not limited to the target organs that are required for mineral ion homeostasis but its receptor is present in many more cells type and can elicit specific biological actions on such tissues (Gallieni *et al.*, 2009).

1.1 VITAMIN D – SOURCE AND PRODUCTION

Vitamin D has two forms, cholecalciferol (vitamin D_3) and ergocalciferol (vitamin D_2). Vitamin D_3 derives from dietary sources (salmon, mackerel, tuna, cod liver oil) but it can also be produced in the skin from 7-dehydrocholesterol (7-DHC) or provitamin D (Holick, 2007). During exposure to light, 7-DHC absorbs solar radiation which transforms it to previtamin D_3. After few hours, it undergoes isomerization to cholecalciferol. It is then transported from skin to circulation by binding to vitamin D binding protein. Factors that affect the vitamin D production by the skin include

latitude, time of day, season of the year, skin pigmentation, sunscreen usage, type of clothing and also age, as the old people have reduced 7-DHC in their skin (Marrone *et al.*, 2012).

Ergocalciferol (vitamin D_2) is a synthetic molecule produced from ergosterol which is a component of fungal cell membrane. This vitamin D_2 therefore can be found in fortified foods like milk, cheese, cereals and yogurt especially in the Western countries. This vitamin D_2 also can be administered as supplement either orally or parenterally. Table 1.1 shows list of food with vitamin D concentrations.

Food	IUs per serving*	Percent DV**
Cod liver oil, 1 tablespoon	1,360	340
Salmon (sockeye), cooked, 3 ounces	794	199
Mushrooms that have been exposed to ultraviolet light to increase vitamin D, 3 ounces (not yet commonly available)	400	100
Mackerel, cooked, 3 ounces	388	97
Tuna fish, canned in water, drained, 3 ounces	154	39
Milk, nonfat, reduced fat, and whole, vitamin D-fortified, 1 cup	115-124	29-31
Orange juice fortified with vitamin D, 1 cup (check product labels, as amount of added vitamin D varies)	100	25
Yogurt, fortified with 20% of the DV for vitamin D, 6 ounces (more heavily fortified yogurts provide more of the DV)	80	20
Margarine, fortified, 1 tablespoon	60	15
Sardines, canned in oil, drained, 2 sardines	46	12
Liver, beef, cooked, 3.5 ounces	46	12
Ready-to-eat cereal, fortified with 10% of the DV for vitamin D, 0.75-1 cup (more heavily fortified cereals might provide more of the DV)	40	10
Egg, 1 whole (vitamin D is found in yolk)	25	6
Cheese, Swiss, 1 ounce	6	2

Table 1.1 : Food sources of vitamin D IU: international unit; DV: daily value

Source : Adopted from USDA (United State Department of Agriculture)

1.2 VITAMIN D - METABOLISM

Vitamin D in circulation is bound to vitamin D binding protein and transported to the liver where it is converted to 25-hydroxy vitamin D [25(OH) D]. This is the major circulating form of vitamin D that is used to measure vitamin D status in a person (Jones, 2006). It is biologically inactive and must be converted to its active form, 1,25-dihydroxy vitamin D [$1,25(OH)_2$ D] or also called calcitriol. This step occurred in the kidney by the enzyme 25-hydroxyvitamin D-1 α hydroxylase.

The renal production of calcitriol is tightly controlled by many factors like serum calcium, phosphorus and parathyroid hormone (PTH) level (Prié *et al.*, 2005). This active form of vitamin D regulates the synthesis and secretion of the PTH and also increases intestinal calcium and phosphorus absorption together with renal calcium reabsorption (Tonelli *et al.*, 2005). Fibroblast growth factor 23 (FGF 23), which is released from the bone suppresses calcitriol synthesis as a negative feedback mechanism (Murer *et al.*, 2000).

Calcitriol induces its own destruction by rapidly inducing the 25-hydroxyvitamin D-24-hydroxylase, which leads to the multistep catabolism of both 25(OH)D and 1,25(OH)2D into biologically inactive, water-soluble metabolites including calcitroic acid and excreted in urine (Bosworth *et al.*, 2012).

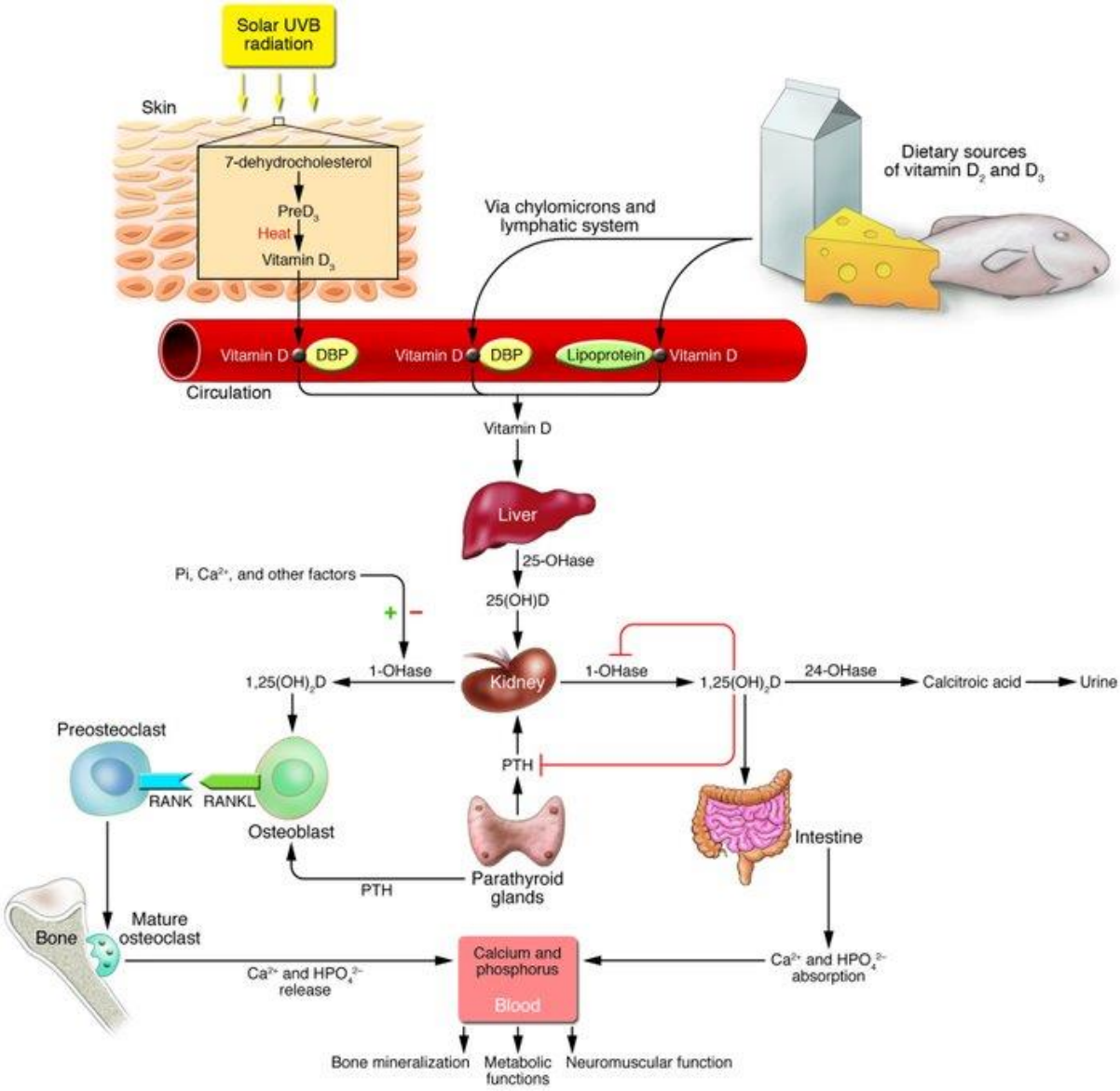

Figure 1.1: The photoproduction and metabolism of vitamin D and the various biologic effects of 1,25(OH)$_2$ D on calcium, phosphorus, and bone metabolism.

Source : Adopted from Holick, 2006

1.3 FUNCTION OF VITAMIN D

1.3.1 CLASSICAL ROLE

This vitamin D endocrine system is a foundation to maintain the extracellular calcium level within narrow limits for normal cellular function and skeletal integrity (Tsiaras and Weinstock, 2011). The target organs which are under influence of vitamin D are the intestine, bone, renal and parathyroid gland.

Intestine : Vitamin D enhances intestinal absorption of calcium and phosphorus by promoting active cellular calcium uptake and transport mechanism. It also amplifies active phosphate transport through stimulation of the expression of the sodium-phosphate cotransporter and changes in the composition of plasma membrane that augment phosphate uptake. Without vitamin D, about 10 to 15% of calcium and 60% phosphorus is absorbed. With the presence of vitamin D, the efficiency is elevated up to 30 to 40% for calcium absorption and phosphorus up to 80% (Lorenz-Depiereux *et al.*, 2006)

Renal : Calcitriol increases renal calcium and phosphate reabsorption. Apart from that, it strictly controls its own homeostasis through simultaneous suppression of 1α-hydroxylase and stimulation of 24-hydroxylase in the kidney (Gallieni *et al.*, 2009).

Bone : The vitamin D hormonal system plays an important role in allowing individuals to mobilize calcium from bone when it is absent from diet. The vitamin D hormonal system appears necessary for PTH-induced osteoclast production (Dusso *et al.*, 2005). Osteoblasts express a surface ligand, named as receptor activator of nuclear factor -κB ligand (RANKL), which can bind receptor activator of nuclear factor –κB (Tonelli *et al.*) (Tonelli *et al.*). The binding of RANKL to RANK generates a signalling cascade that results in differentiation and maturation of osteoclasts. Calcitriol, PTH and

prostaglandins stimulate RANKL expression, which causes an increase in osteoclastogenesis and osteoclast activity thus increased in bone resorption (Holick, 2007). Apart from that, vitamin D is also pivotal for development and maintenance of a mineralized skeleton along with adequate calcium level. It also serves a critical function for the normal bone turnover. In vitamin D deficiency, the bone turnover process becomes imbalance between the bone formation and bone resorption activity.

Parathyroid : Parathyroid cells expressed a great number of vitamin D receptor which respond to calcitriol binding by reducing the PTH synthesis and secretion. Vitamin D deficiency thus, will results in PTH hyperplasia, thereby increasing PTH synthesis and secretion. The PTH enhances the tubular reabsorption of calcium and stimulate the kidneys to synthesis calcitriol (Tonelli *et al.*, 2005). Apart from that, PTH also stimulates osteoblast, which augment the transformation of preosteoclast into mature osteoclast for bone resorption (Block *et al.*, 1998).

1.3.2 NON CLASSICAL ROLE

Apart from its classical function, this vitamin also has shown many other extra skeletal health benefits. Vitamin D receptor has been found in B and T lymphocytes in the immune system, hair follicle, muscle, adipose tissue, pancreatic beta-cell, bone marrow, and cancer cells (Holick, 2004).

An example of an autoimmune disease that can be modulated by the vitamin D system is type 1 diabetes mellitus. A study by Zella and Deluca (2003) have found that, 1) in rats, vitamin D deficiency caused an increase in incidence of diabetes; 2) large doses of calcitriol could suppress type 1 diabetes mellitus completely, preventing the destruction of islet cells. Similar results were gathered with models of other autoimmune diseases, such as systemic lupus, inflammatory bowel disease, and rheumatoid arthritis (Nagpal *et*

al., 2005). In parallel to that, the use of vitamin D receptor activators could be helpful in preventing transplant rejection (Hullett *et al.*, 1998).

Other studies found that several cancer cell lines proved to be respondent to calcitriol and other vitamin D analogs, such as prostate, breast, colon cancer cells as well as leukemic cells and squamous cell carcinoma (Nagpal *et al.*, 2005).

Cardiovascular effect is another important area of interest in vitamin D research, as epidemiological studies have shown an inverse correlation between UV light exposure and in general, an association between vitamin D status and blood pressure (Martini and Wood, 2008).

1.4 VITAMIN D AND BONE HEALTH

Vitamin D is paramount in the development and sustainment of a healthy skeleton from the intrauterine life until the old ages. Vitamin D deficiency in pregnant mothers was associated with a significant reduction in bone mineral acquisition in infants that persist after 9 years after birth (Mulligan *et al.*, 2010). In growing children, lack of vitamin D causes chondrocyte disorganization and hypertrophy at the mineralization front and defects in bone mineralization causing rickets. This disease is characterized by growth retardation, enlargement of the epiphyses of the long bones, deformities of the legs, bending of the spine, knobby projections of the ribcage, and weak and toneless muscles (Rajakumar, 2003).

In adults, vitamin D deficiency and hyperparathyroidism will result in low serum calcium and phosphorus predisposing them to osteomalacia, a defective mineralization of collagen matrix. This eventually will cause reduction of structural support and

associated with higher risk for fracture (Bergwitz *et al.*, 2006). Secondary hyperparathyroidism as a consequence of vitamin D deficiency is associated with osteoclastogenesis and an exaggerate bone resorption. Increased bone resorption precipitates and exacerbates osteopenia and osteoporosis in adults.

Vitamin D promotes bone health by keeping the PTH in healthy levels, boosting osteoblastic activity and fostering bone mineralization as well as reducing risk of falls thereby reducing risk of fracture.

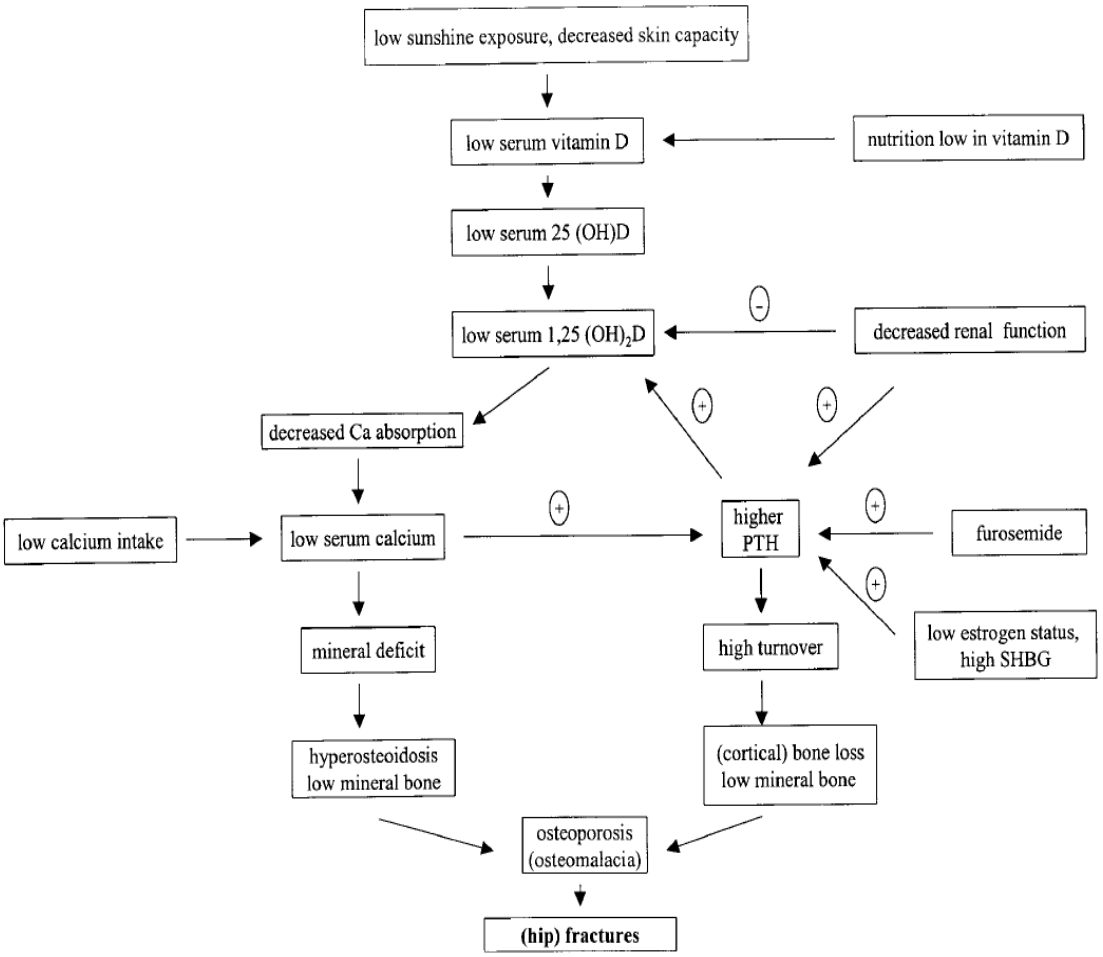

Figure 1.2: Schematic presentation of pathways from vitamin D deficiency and secondary hyperparathyroidism to osteoporotic fractures

Source : Adopted from Lips 2001

1.5 VITAMIN D STATUS AND REQUIRED LEVEL OF VITAMIN D FOR BONE HEALTH

Vitamin D deficiency is now recognized as a global pandemic. A systematic review of vitamin D status in populations worldwide done in 2013 showed that 88.1% had mean vitamin D below 75 nmol/L, 37.3% had vitamin D level below 50 nmol/L and 6.7 % had values below 25 nmol/L (Hilger *et al.*, 2014). These showed that there was big variability of the vitamin D status across the studies. No significant age or sex related differences in 25(OH)D values were observed in the sample worldwide but there is difference noted if compared by region (Hilger *et al.*, 2014). Women tended to have lower vitamin D compared to men especially in the Asia and Middle East regions.

In Malaysia, a study done among adults in Kuala Lumpur revealed that approximately 41% of male and 87% of females had insufficient (< 50 nmol/L) level of vitamin D (Moy and Bulgiba, 2011). Another study conducted among primary school children in Kuala Lumpur revealed that 35.3% having vitamin D deficiency (<37.5 nmol/L) and 37.1 % having vitamin D insufficiency (37.5 - 50.0nmol/L) (Khor *et al.*, 2011).

Precisely defining vitamin D deficiency based on measurement of 25(OH)D concentration is still a matter of much debate. There is no optimum level or threshold that has been clearly establish to define whether there is adequate store of vitamin D in the body or not (Thacher and Clarke, 2011). A wide optimum range for 25(OH) D has been reported based on various studies which is between 30 nmol/L until 90 nmol/L for bone health.

Previous study showed a plateau in suppression of parathyroid hormone (PTH) when the 25(OH)D level reaches approximately 30 nmol/L which defines the optimum level (van Schoor and Lips, 2011). In addition, other study concluded that bone health in

older persons are likely to improve when serum 25(OH)D is raised over at least 50–60 nmol/L (Kuchuk *et al.*, 2009). There are also other studies by Vieth (2011) that mentioned, we should consider 25(OH)D value equal or more than 75nmol as an adequate or optimum level. The highest level of vitamin D for bone health is suggested by Krall (1989) which is up to 90 nmol/L.

1.6 BONE TURNOVER MARKERS

Bone is a metabolically active tissue that undergoes remodelling by two counteracting processes, which are bone formation and bone resorption. These processes depend on the activity of osteoblast (formation), osteoclast (resorption) and osteocyte (maintenance). These two processes are in balance under normal condition. The balance is regulated by various substances like vitamin D, parathyroid hormone and various cytokines (Seibel, 2005).

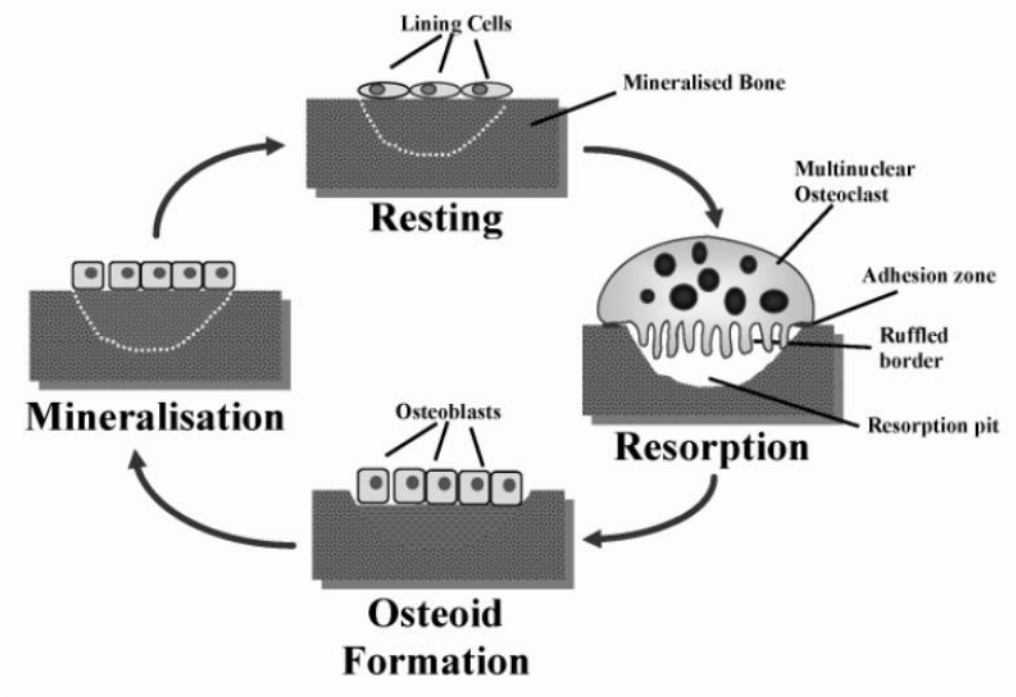

Figure 1.3: The bone remodelling cycle. Under normal conditions, the resorption (osteoclast) phase takes approximately 10 days, which is then followed by a formation (osteoblast) phase that can last for up to 3 months.

Source : Adopted from Seibel, 2005

If there is imbalance in these processes, it will lead to higher release of bone turnover markers, which are proteins originating from osteoclast and osteoblast activity or fragments released during the formation or degradation of type I collagen. In the event of vitamin D deficiency, there will be stimulation of secondary hyperparathyroidism due to hypocalcaemia. This hyperparathyroidism will caused negative balance in the bone metabolism by stimulating bone resorption and produces changes in bone structure, strength and mass. Changes in the bone structure and strength are difficult to measure in vivo and bone mass can be assessed by densitometric technique using dual

energy x-ray absorptiometry (DEXA) scan (Seibel, 2005). This scan produces a static measure and it can only detect clinical relevant changes of bone density after 2 years (Bonnick and Shulman, 2006). However the bone turnover markers are helpful tools to detect the dynamics of the metabolic imbalance in the bone (Seibel *et al.*, 2006). Markers like osteocalcin, N-terminal propeptide of type 1 collagen (P1NP) and bone specific alkaline phosphatase (BAP) are markers for bone formation and markers for bone resorption includes hydroxyproline, hydroxylysine- glycoside and carboxyterminal crosslinked telopeptide of type 1 collagen (CTX).

There were many studies showed that low serum 25(OH)D level are associated with an increased in bone turnover markers. Study done by Kuchuk *et al* ,2009 showed that parameters of bone turnover like osteocalcin and CTX were significantly lower in the higher vitamin D group. A study in German children also showed that increase in 25(OH)D was associated with a significant decrease in the bone resorption marker, CTX (Thiering *et al.*, 2015).

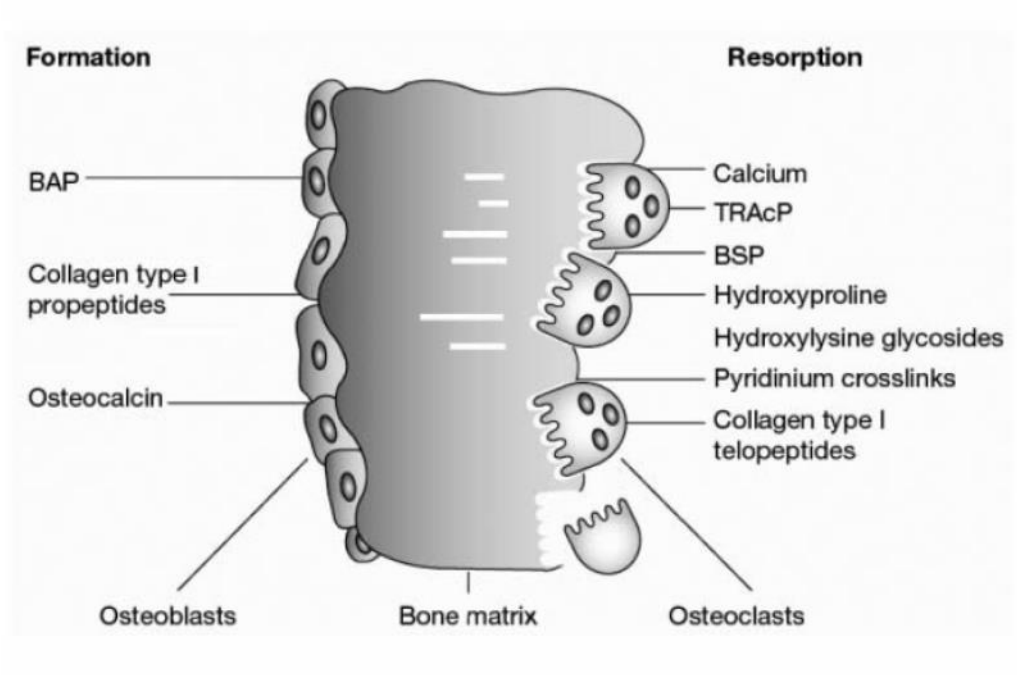

Figure 1.4 : Bone turnover markers

BAP : bone alkaline phosphatase ; BSP: bone sialoprotein, TRAcP : tartrate-resistant acid phosphatase

Source : Adopted from Seibel 2005

2. STUDY PROPOSAL

2.1 INTRODUCTION

Vitamin D, the sunshine vitamin, is a hormone which plays an important role in bone mineralisation and other metabolic processes in the body. This vitamin is important for optimum intestinal absorption of calcium and is a major physiologic reguIator of calcium absorption in the body. An altered plasma level of vitamin D is the most common mediator of increased or decreased calcium absorption in disease state. It is also needed for the release of calcium from bone in the regulation of plasma calcium and acts in concert with parathyroid hormone (PTH).

Vitamin D deficiency is now recognized as a global pandemic. A systematic review of vitamin D status in populations worldwide done in 2013 showed that, among the Asian Pacific countries, Thailand had mean vitamin D level of 67.6 nmol/L, followed by Malaysia with 44.4 nmol/L and Indonesia with 38.7 nmol/L (Hilger *et al*,2014). This figure is based only on one study done by Rahman *et al* (2004) among postmenopausal women. A study by Nurbazlin *et al* (2013), showed that Malaysian women had mean vitamin D level of 64.5 nmol/L with the urban group had mean of 31.9 nmol/L and rural group of 69.5 nmol/l. This study also concentrates on vitamin D level among women who are> 45years old. There is no data on vitamin D status in general population as previous study is done among specific group e.g postmenopausal women, metabolic syndrome, primary school children, SLE patient.

Vitamin D plays a crucial role in the development and maintenance of a healthy skeleton. It plays an important role in the calcium and phosphorus metabolism and helps to ensure adequate levels of these minerals for bone metabolism. Bone metabolism is comprised of two counteracting processes, namely bone formation and bone resorption.

Under normal condition, these two processes are tightly coupled to each other, so that the amount of bone resorbed is always equal to the amount of newly formed bone. This balance is achieved and regulated through the action of vitamin D, PTH and few others. In vitamin D deficiency, there will be imbalance between these two processes, thus will lead to bone mineral disease like osteoporosis, rickets and osteomalacia.

Biochemical markers of bone turnover like osteocalcin (OC), N-terminal propeptide of type 1 collagen (P1NP) and bone specific alkaline phosphatase can reflect bone formation activity. For bone resorption, markers like hydroxyproline, hydroxylysineglycoside and carboxyterminal crosslinked telopeptide of type 1 collagen (CTX) are used to assess bone resorption activity. There were studies showed that low serum 25(OH)D level are associated with an increased bone turnover.A study done by Natalia *et al* (2009) showed that serum osteocalcin (bone formation) and serum CTX (bone resorption), were significantly lower in the highest serum 25(OH)D group.

Our study is different from other studies as we use bone turnover markers (BTMs) instead of bone mineral density (BMD) measured by DEXA scan to estimate the optimum level of vitamin D. The BTM usage has been encouraged in clinical practice and clinical applications because of its usefulness. BTMs reflect the remodelling process of the bone but the BMD does not. The BTM will change earlier and in larger extent after starting of treatment in osteoporosis compared to changes in BMD (Yoshiki *et al*, 2004). It has been reported that a decrease in BTM in the early stage of treatment in osteoporosis may reflect a reduction in the long term risk of fracture (Eastell *et al*, 2003).

Until now, there is still no standard definition of optimal vitamin D status. The required serum 25(OH)D for adequate bone health has been debated. The optimum level of

serum 25(OH)D was estimated according to some studies to be at 30,50,70,75 and 80 nmol/L. Therefore, the aim of our study is to estimate the threshold of serum 25(OH)D with regard to PTH and bone turnover markers in a population of Kota Bharu, Kelantan. The reason why we choose to have this study done here is because Kota Bharu can represent both the urban and also the suburban population in Kelantan. Its population also has a heterogeneous background from government servants, odd job workers and etc.

2.2 JUSTIFICATION OF THE STUDY

There were very limited studies and published data on vitamin D status among general adult population in Malaysia. Most of the studies were done among specific group like postmenopausal women, metabolic syndrome, primary school children and among patient with diseases like SLE.

Study on optimum level of vitamin D that is needed for bone health is done mostly in Western countries only. The results of the studies showed that the optimum level of vitamin D varied between 30- 100 nmol/L. There is not much data about this in our Asian (Malaysian) population. We are expecting that the value would be different because of different in ethnicity, skeleton size, climate and also diet.

2.3 BENEFITS FROM THE STUDY

1. The result of this study can be used as a guide to determine the optimum level of vitamin D for maintaining good bone health in our own population.

2. The result from this study also can be a guide to the clinician at optimizing vitamin D level in patient who is vitamin D deficient and are at high risk to develop osteoporosis and fractures.

2.4 OBJECTIVES

General Objective

- To study on vitamin D status and its association with PTH and bone turnover markers

Specific Objectives

1. To determine the mean vitamin D level among Kota Bharu population

2. To determine the relationship of vitamin D level with PTH and serum bone turnover markers ,N-terminal propeptide of type 1 collagen (P1NP) and C- telopeptide(CTX).

3. To determine the threshold of optimum vitamin D level with regard to PTH and serum bone turnover markers, N-terminal propeptide of type 1 collagen (P1NP) and C- telopeptide(CTX).

2.5 RESEARCH QUESTIONS

1. What is the vitamin D status among Kota Bharu population?

2. Is there any relationship between vitamin D level with PTH and bone turnover markers, N-terminal propeptide of type 1 collagen (P1NP) and C- telopeptide(CTX)?

3. What is the optimum level of vitamin D for good bone health?

2.6 HYPOTHESIS

1. There is good relationship between vitamin D level with PTH and bone turnover markers, N-terminal propeptide of type 1 collagen (P1NP) and C- telopeptide(CTX)

2.7 METHODOLOGY*:*

Study design

Cross sectional study

Study duration

1 February 2015 till 31 January 2017

Study location

Kota Bharu, Kelantan

Reference population

Population in Kota Bharu aged 18- 50years

Source population

Residents in 6 subdistricts aged 18-50 years old

Sampling frame

Selected village of 6 subdistricts in Kota Bharu

Sampling methods

120 people will be chosen using multistage sampling. For the first stage, six out of 14 subdistricts in Kota Bharu will be selected. Secondly, a village from each 6 subdistricts will be randomly selected and later, all residents in the selected village who aged between 18-50 years old and fulfil the inclusion and exclusion criteria will be randomly chosen and offered to participate in the study.

They were given the information sheet and consent form. They should read and sign it before taking part in this study. 5 mls of blood then will be taken from each subject and put into bottle. It will then be analyzed for vitamin D, intact PTH, and bone turnover markers like P1NP and C- telopeptide(CTX).

Inclusion criteria

- Aged 18-50 years

Exclusion criteria

- People with established bone mineral disease e.g osteoporosis
- People on drugs that affect vitamin D and bone turnover; OCP,HRT, steroid
- People with history of severe chronic liver disease, diabetes mellitus type 1 and 2, chronic kidney disease stage 3 and thyroid disorders

- Those female who are pregnant and breastfeeding
- Those female who had menopause
- Those who take alcohol
- Those who had history of recent fracture (up to 1 year)
- Those who are on vitamin D supplement

2.8 SAMPLE SIZE DETERMINATION

Based on objective no.1,

Calculation done using single mean formula. Data taken from Moy and Bulgiba (2011), High prevalence of vitamin D deficiency.

$n = [(z (SD))/\Delta]^2$

$= [(1.96(13))/4]$

$= 40$

Based on objective no.2,

Calculation done using G power software 3.1.7 with effect size of 0.10 (small, Cohen, 1988)

α , probability of Type I error = 0.05, power $(1\beta) = 0.8$

Expected number of predictors = 2

Total sample size needed is 100

So, the biggest number of sample size is 100

Sample size, n = 100 + 20 % drop out = 100 + 20 workers

= 120

2.9 MEASUREMENT TOOLS / LABORATORY METHODS

Estimation of serum vitamin D, intact PTH and bone turnover markers (osteocalcin, P1NP and CTX) will be done by immunoassay technique using COBAS e 411.

2.10 STATISTICAL ANALYSIS

The data will be analyzed by using computer software SPSS version 20.

For objective 1, the numerical data will be expressed as mean (SD).

For objective 2, simple linear regression analysis will be performed to examine the correlation between vitamin D level with bone turnover markers.

For objective 3, LOESS plot will be performed by using Stata software. The optimum level of vitamin D will be determined by inspection of the LOESS plot.

3.0 MANUSCRIPT

STUDY ON VITAMIN D STATUS AND DETERMINATION OF OPTIMUM VITAMIN D LEVEL BASED ON BONE TURNOVER MARKERS IN KOTA BHARU, KELANTAN

3.1 Abstract

Background: There is no optimum level or threshold that has been clearly established to define whether there is adequate store of 25(OH)D in the body for general bone health. Therefore, our study was to determine the optimum level of 25(OH)D for maintenance of healthy skeleton based on bone remodelling process among healthy adult in Kota Bharu.

Methods: This was a cross sectional study involving healthy adult population in Kota Bharu, Malaysia, aged 18-50 years. We measured serum 25(OH)D, serum parathyroid hormone (PTH), serum C-terminal telopeptide of type 1 collagen (*CTX*) and Procollagen 1 Intact N-Terminal (P1NP) in 120 healthy adult from 6 sub districts that had been selected by multi stage sampling (64 males, 56 females).

Results: The mean level of 25(OH)D in this study was 23.50 (± 8.74) nmol/L and there was significant difference of vitamin D between gender (26.81±8.3nmol/L and 19.72±7.68 nmol/L males and females respectively) (p value < 0.001). More than 50% of female subjects had 25(OH)D less than 20nmol/L, while only 20.3% of male subjects had 25(OH)D below 20nmol/L. Based on LOESS plot, the bone turnover markers showed plateau result at 25(OH)D level of 35 nmol/L for CTX and 20 nmol/L for P1NP. Then, at 25(OH)D level of 20 nmol/L there is a step decrease of PTH level.

Conclusion: The mean level of 25(OH)D among general healthy population in Kota Bharu was 23.50nmol/L. Based on LOESS plot for CTX, P1NP and PTH versus vitamin D level, we concluded that vitamin D level between 20 nmol/L to 35 nmol/L is considered sufficient to maintain healthy skeleton.

Keywords: Vitamin D; Bone turnover markers; N-terminal propeptide of type 1 collagen (P1NP); C- telopeptide(CTX); Parathyroid hormones(PTH)

3.2 Introduction

Vitamin D plays a pivotal role in the development and maintenance of a healthy skeleton. It plays a critical function in the calcium and phosphorus metabolism and helps ensure adequate levels of these minerals for bone mineralization [1]. In adults, low vitamin D can results in osteomalacia which is a defective mineralization of the collagen matrix causing a reduction of structural support and being associated with an increased risk of fracture [2].

A low prevalence of vitamin D is expected in tropical countries which received abundant of sun exposure because the most important source of vitamin D is by cutaneous synthesis under the action of sunlight. The sunlight convert 7-dehydrocholesterol in the skin to vitamin D3, which will be transported to the liver and hydroxylated to 25-hydroxyvitamin D [(25-OH)D]. It will then be conveyed to the kidney and hydroxylated to 1,25-dihydroxyvitamin D[1,25$(OH)_2$D]. The primary circulating form of vitamin D is 25(OH)D and it has longer half life (2-3 weeks) compares to 4-6 hours for 1,25$(OH)_2$D, thus accepted as the determinant of vitamin D

status [3]. Having said that , the prevalence of vitamin D deficiency in countries like Hawaii, Iran, India, and Saudi Arabia which received sunlight throughout the year are high [4, 5, 6, 7].

Vitamin D deficiency is now recognized as a global pandemic. A systematic review of vitamin D status in populations worldwide done in 2013 showed that 37.3% of the sample had vitamin D level below 50 nmol/L and 6.7 % had values below 25 nmol/L [8]. In Malaysia, a study done among adults in Kuala Lumpur revealed that approximately 41% of male and 87% of females had insufficient (< 50 nmol/L) level of vitamin D [9]. Another study conducted among primary school children in Kuala Lumpur revealed that 35.3% having vitamin D deficiency (<37.5 nmol/L) and 37.1 % having vitamin D insufficiency (37.5 - 50.0nmol/L) [10]. Despite mounting evidence of vitamin D status globally, there are still lack of studies in our local setting, determining the level of vitamin D especially among general population.

Precisely defining vitamin D deficiency based on measurement of 25(OH)D concentration is still a matter of much debate. There is no optimum level or threshold that has been clearly established to define whether there is adequate store of vitamin D in the body or not [11]. Previous study showed a plateau in suppression of parathyroid hormone (PTH) when the 25(OH)D level reaches approximately 30 nmol/L which defines the optimum level [12]. In addition, other study concluded that bone health in older persons are likely to improve when serum 25(OH)D is raised over at least 50–60 nmol/L [13]. There are also other studies in which the researcher consider 25(OH)D value equal or more than 75nmol as an adequate or optimum level [14]. Therefore, this study aims to determine the optimum level of vitamin D based on bone remodelling process.