
**UNIVERSITI PUTRA MALAYSIA**

# *BONE TURNOVER MARKERS (P1NP, CTX) AND SPHINGOSINE-1-PHOSPHATE (S1P) IN DETERMINING BONE HEALTH AMONG CHINESE ADULT RESIDENTS OF PUCHONG AND KAJANG IN SELANGOR, MALAYSIA*

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By

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**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

**BONE TURNOVER MARKERS (P1NP, CTX) AND SPHINGOSINE-1-PHOSPHATE (S1P) IN DETERMINING BONE HEALTH AMANG CHINESE ADULT RESIDENTS OF PUCHONG AND KAJANG IN SELANGOR, MALAYSIA**

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**April 2019**

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Prevention of osteoporotic fracture requires identification of individuals at high risk with WHO fracture risk assessment tool (FRAX®) commonly used to estimate fracture probability despite some inadequate predictive discrimination ability. Procollagen-type-1 amino-terminal propeptide (P1NP) and carboxy-terminal-collagen crosslinks (CTX), the current bone turnover markers (BTM) and sphingosine-1-phosphate (S1P), a novel marker of bone metabolism may complement the current model. This study aimed to determine the association of P1NP, CTX, and S1P with sociodemographic factors, clinical characteristics, bone mineral density (BMD) and blood tests for bone profile among Chinese adults in Puchong and Kajang, Malaysia. This was a cross-sectional study involving Chinese subjects aged between 50 to 90 years old in Puchong and Kajang who attended a health screening program in Puchong Specialist Centre from December 2015 to December 2017. Each subject had a BMD determined by a dual-energy x-ray absorptiometry (DXA) at lumbar spine, femoral neck, total hip and total body. 10 ml of fasting blood sample were taken and analysed for 25-hydroxyvitamin D (25(OH)D), parathyroid hormone (iPTH), calcium, phosphate, P1NP, CTX and S1P. Pearson's and Spearman's correlation tests were used to determine the associations between P1NP, CTX and S1P with sociodemographics, clinical characteristics, BMD and bone profile. All analyses were stratified by gender and BMD categories (normal BMD, osteopenia and osteoporosis). A total of 131 subjects [45 (34.4%) males and 86 (65.6%) post-menopausal women] with a median age of 65 (IQR=17) years old were recruited. Osteopenia and osteoporosis were diagnosed in 46.6% and 29.0% of subjects, respectively. P1NP and CTX were significantly higher in post-menopausal women (P1NP=61.71ng/ml, CTX=0.489 ng/ml) compared to men (P1NP=46.94 ng/ml, CTX=0.381 ng/ml). Both BTM differed significantly according to BMD categories ($p<0.001$) with values highest in osteoporosis and

lowest in normal BMD. However, there is no difference in S1P levels in men (2.12 ± 0.75 µmol/L) and post-menopausal women (1.96 ± 0.68 µmol/L) did not differ significantly (p=0.235) and did not differ according to BMD categories (p=0.457). S1P did not correlate with any individual BMD measurements nor with P1NP or CTX in both males and females. In post-menopausal women, CTX and P1NP significantly negatively correlated with BMD measurements at all sites except femoral neck. In men, CTX significantly negatively correlated with femoral neck (r=-0.360, p=0.015) and total hip (r=-0.456, p=0.002) whilst P1NP only with total body (r=-0.344, p=0.021). Osteoporosis was diagnosed in 29% of subjects. P1NP and CTX were significantly higher in post-menopausal women compared to men as well as significantly differed between osteoporosis, oeteopenia and normal BMD. In post-menopausal women, P1NP and CTX negatively correlated with all BMD sites measurements except for femoral neck. In men, P1NP only negatively correlated with total body whilst CTX only with femoral neck and total hip. In contrast, S1P did not differ according to BMD characteristics nor gender and not associated with any BMD sites measurement. Thus, in this group of Malaysian Chinese subjects, the current BTM reflects bone loss better than S1P, especially in females.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**PENANDA KITARAN TULANG (P1NP, CTX) DAN SPHINGOSINE-1-PHOSPHATE (S1P) DALAM PENENTUAN KESIHATAN TULANG DIKALANGAN PENDUDUK DEWASA BERBANGSA CINA DI PUCHONG DAN KAJANG, SELANGOR, MALAYSIA**

Oleh

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Mengenal pasti individu berisiko tinggi untuk kepatahan tulang adalah penting dalam pengurusan pesakit osteoporosis. 'WHO fracture risk assessment*'* (FRAX®) menganggarkan keberangkalian kepatahan tulang, namun masih terdapat beberapa kelemahan. 'procollagen-type-1 amino-terminal propeptide' (P1NP) dan 'carboxy-terminal collagen crosslinks' (CTX) merupakan penanda kitaran tulang (BTM) semasa, manakala sphingosine-1-phosphate (S1P), penanda metabolisma tulang, berkemungkinan mengatasi kelemahan tersebut. Penyelidikan ini bertujuan menentukan kaitan di antara P1NP, CTX dan S1P dengan faktor-faktor sosiodemografi, ciri-ciri klinikal, kepadatan mineral tulang (BMD) dan ujian profil tulang dikalangan masyarakat dewasa berbangsa Cina di Puchong dan Kajang, Malaysia. Ini merupakan kajian keratan rentas melibatkan masyarakat Cina berusia 50 hingga 90 tahun di Puchong dan Kajang, yang menghadiri program pemeriksaan kesihatan di Pusat Pakar Puchong dari Disember 2015 hingga Disember 2017. Setiap individu menerima permeriksaan BMD melalui imbasan '*dual-energy x-ray absorptiometry'* (DXA) di tualng belakan lumbar, leher femoral, pinggul total dan keseluruhan badan. 10 ml sampel darah diambil dan dianalisa untuk 25-hydroxyvitamin D (25(OH)D), hormon paratiroid (iPTH), fosfat, kalsium, P1NP, CTX dan S1P. Ujian korelasi *Pearson* dan *Spearman* digunakan untuk menentukan hubungkait P1NP, CTX dan S1P dengan faktor sosiodemografik, klinikal karakteristik, BMD dan ujian profil tulang menggunakan perisian SPSS versi 25.0. Semua analisa dikelaskan mengikut jantina dan kategori BMD (normal, osteopenia dan osteoporosis). Kajian ini melibatkan 131 subjek [45 (34.4%) lelaki dan 86 (65.6%) wanita menopaus] dengan median umur 65 tahun (IQR=17). Seramai 46.6% telah dikategorikan sebagai osteopenia manakala 29.0% osteoporosis. P1NP dan CTX adalah lebih tinggi secara signifikan dikalangan wanita menopaus (P1NP=61.71ng/ml, CTX=0.489 ng/ml) berbanding lelaki (P1NP=46.94 ng/ml,

CTX=0.381 ng/ml). P1NP dan CTX juga berbeza secara signifikan mengikut kategori BMD ($p<0.001$) dengan paras paling tinggi dalam osteoporosis dan paling rendah dalam BMD normal. Tiada perbezaan statistik signifikan diantara paras S1P lelaki ($2.12 \pm 0.75$ µmol/L) dan wanita menopaus ($1.96 \pm 0.68$ µmol/L) ($p=0.235$) dan juga diantara kategori BMD ($p=0.457$). S1P tidak berkait secara signifikan dengan BMD dan juga dengan P1NP dan CTX. Di kalangan wanita menopaus, CTX dan P1NP berkait secara signifikan dengan kesemua BMD kecuali leher femoral. Di kalangan lelaki, CTX berkait dengan leher femoral ($r=-0.360$, $p=0.015$) dan pinggul total hip ($r=-0.456$, $p=0.002$) manakala P1NP hanya dengan keseluruhan badan ($r=-0.344$, $p=0.021$). Seramai 29% subjek mempunyai osteoporosis. Paras P1NP dan CTX berbeza mengikut kategori BMD dan lebih tinggi dikalangan wanita menopaus berbading lelaki. Di kalangan wanita menopaus, CTX dan P1NP berkait secara signifikan dengan kesemua BMD kecuali leher femoral. Manakala, tiada perbezaan paras S1P diantara lelaki dan wanita menopaus dan juga antara kategori BMD. Sehubungan itu, dikalangan warga Malaysia berbangsa Cina, BTM sedia ada memberi gambaran kerapuhan tulang lebih baik berbanding S1P.

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| | |
|---|---|
| ABCC1 | ATP-binding Cassette C1 |
| aBMD | areal Bone Mineral Density |
| ALP | Alkaline Phosphatase |
| BFMs | Bone Formation Markers |
| BMI | Body Mass Index |
| BM | Bone Mineral |
| BMD | Bone Mineral Density |
| BMMs | Co-cultured Bone Marrow-derived Macrophages |
| BMP | Bone Morphogenetic Protein |
| BRMs | Bone Resorption Markers |
| BSAP | Bone Specific Alkaline Phosphatase |
| BTM | Bone Turn over Markers |
| CRFs | Clinical Risk Factors |
| CTX | C-terminal telopeptide collagen type 1 |
| CXCL12 | C-X-C Motif Chemokine Ligand 12 |
| DM | Diabetes Mellitus |
| DPD | Deoxypyridinoline |
| DXA | Dual-energy x-ray absorptiometry |
| E2 | 17 □- estradiol |
| ECLIA | Electrochemiluminescence Immunoassay |
| eGFR | estimated Glomerular Filtration Rate |
| EPIDOS | Epidemiology of Osteoporosis |
| FBC | Full Blood Count |
| FRAX | Fracture Risk Assessment Tool |
| GPCRs | G Protein-Coupled Receptors |
| HDL | High-density lipoprotein |
| hMS | human Mesenchymal Stem |
| IOF | International Osteoporosis Federation |
| iPTH | Intact Parathyroid Hormone |
| LFT | Liver Function Tests |
| LRP5 | Low-density lipoprotein receptor-related protein 5 |
| MMP | matrix-metalloproteases |
| MOS | Malaysian Osteoporosis Society |
| NTX | N-terminal telopeptide collagen type 1 |
| OC | Osteocalcin |
| OFELY | Os des Femmes de Lyon |
| 25(OH)D | 25-hydroxyvitamin D |
| OPG | Osteoprotegerin |
| P1CP | Procollagen type I C-terminal Propeptide |
| PDGF | Platelet-derived growth factor |
| P1NP | Procollagen type I N-terminal Propeptide |
| PTX | Pertussis toxin |
| PYD | Pyridinoline |
| RANK | Receptor Activator of NF-kß |
| RANKL | Receptor Activator of NF-kß Ligand |
| Rac | a subfamily of the Rho family of GTPases |

| | |
|---|---|
| Rho | a family of small signaling G proteins |
| SD | Standard Deviation |
| SOST | Sclerostin |
| S1P | Sphingosine-1-Phosphate |
| SPHKs | Sphingosine kinases |
| Spns2 | S1P transporter spinster homolog2 |
| S1PRs | Sphingosine-1-Phosphate Receptors |
| TRACP 5b | Tartrate Resistant Acid Phosphatase 5b |
| VF | Vertebral Fracture |
| WHO | World Health Organisation |

# CHAPTER 1

# INTRODUCTION

## 1.1 Background

Osteoporosis subjects are at increased risk for fracture, which is associated with significant disability and increased mortality. Osteoporosis-related fractures result negatively on health and the economy. Increase in the population longevity worldwide makes osteoporosis-related fractures a growing health concern (Sattui & Saag, 2014). Thus, a major aim in osteoporosis patient's management is the prevention of fracture which requires identification of subjects at high fracture risk, so that specific pharmacological treatment can be administered to reduce their risk (Orimo et al., 2012; Peel, 2015; Watts et al., 2010). This imperative as by 2050, those aged over 50 years *i.e.* the at-risk population for osteoporosis, will compose of roughly a third of the overall population (Mithal, Ebeling, & Kyer, 2013). With the ever-increasing aging population in Malaysia and the projected burden of osteoporosis, there is a sense of urgency to identify those at risk of fragility fracture.

The current most widely used method for identification of individuals with osteoporosis is by means of bone mineral density (BMD) using dual-energy x-ray absorptiometry (DXA), which is used to predict fracture risk (Hans et al., 2006; Siris et al., 2004). A T-score value equal to or more than 2.5 SD below the mean for young healthy female (age 20-29 years) equates to osteoporosis (World Health Organization, 1994). Unfortunately, approximately 30-50% of fractures occur either in those within the osteopenic range (-1< T score > -2.5) or normal BMD (T score > -1.0) (Sanders et al., 2006; Schuit et al., 2004; Siris et al., 2004; Sornay-Rendu et al., 2005).

BMD provides information on bone mass. However, bone strength is a complex amalgamation involving micro-architecture, mineralisation, and bone remodelling (Orimo et al., 2012; Ott, Kilcoyne, & Chesnut, 1987; Touvier et al., 2015), of which BMD is unable to assess. Thus, WHO fracture risk assessment tool (FRAX®), was developed to evaluate the probability of fracture by combining clinical risk factors (CRFs) such as age, family history and previous fracture in addition to BMD. It provides a potential 10-year probability of hip and other major osteoporotic fracture *i.e.*, vertebra, proximal humerus and distal forearm fracture in untreated male and female subjects aged between 40 and 90 years (Bolland et al., 2011; Hillier et al., 2011; J. A. Kanis et al., 2007). There remains however, an inadequate predictive discrimination ability for fracture risk with area under the receiver operating characteristic curve (ROC) ranging between 0.60 - 0.63 (Bolland et al., 2011; Hillier et al., 2011), resulting in either overtreatment or under-treatment by 36% (Bolland et al., 2011) and 46%, respectively (Hillier et al., 2011). Furthermore, it does not

provide measurements of other skeletal determinants of bone strength (Díez-Pérez et al., 2007) such as bone microarchitecture; a significant factor of bone quality.

## 1.2 Problem Statement

The imperfect predictive abilities of BMD and FRAX® should be further enhanced, with an additional parameter(s) that can complement the current model. Such a parameter can be bone turnover markers (BTM) *i.e.* bone formation or resorption markers, which represent the activity of osteoblasts or osteoclasts, respectively. The evidence shows that high bone turnover, as is assessed by BTM, is associated with an increased risk of fracture. Despite the evidence, the independent use of BMD to predict fracture have yet to be put in place. BTM can be measured in either urine or blood. The established bone formation markers are carboxy and amino-terminal propeptides of type 1 collagen (P1CP, P1NP), osteocalcin (OC) and bone-specific alkaline phosphatase (BSAP), all of which reflects the synthesis rate of major bone constituents (Garnero, 2014). For bone resorption markers, the available assays include cathepsin K, pyridinoline, deoxypyridinoline and c-terminal telopeptide collagen type 1 (CTX), of which CTX is considered the marker of choice. The current bone markers are however, limited by:

i. non-specificity for bone,
ii. incapability to discriminate the metabolic activity of various skeletal compartments,
iii. only reflecting the function of either osteoblast and osteoclast rather than osteocytes which plays a pivotal role in skeletal integrity (Garnero, 2014).

Thus, a potential marker of bone metabolism that could overcome these limitations would be very useful with sphingosine-1-phosphate (S1P) being such a marker.

## 1.3 Significance of This Study

The risk of osteoporotic vertebral fracture (VF) increases with S1P in a dose-response manner, which remains after adjustment for lumbar spine BMD and possible confounders. (Kim et al., 2012). Plasma S1P was more than nine folds higher in individuals in the highest S1P quartile compared to those in the lowest S1P quartile irrespective of BMD (Kim et al., 2012). In a more recent study, Bae et al 2016 reported that the level of plasma S1P can be used as a likely predictor of fracture as well as a predictor of insufficient response to bisphosphonate therapy (Bae et al., 2016). Despite positive preliminary findings based on previous studies, it is nevertheless very limited and mainly done in a subset of populations mainly in Korea. In addition, no study on S1P has been done in the Malaysian population. With an estimated over 1 million people at risk of osteoporosis, the potential of osteoporosis-related fractures in Malaysia is a major concern (Malaysian Osteoporosis Society, 2012). In 1997, the direct hospitalisation cost for hip fractures in Malaysia was estimated at RM 22 million; a gross underestimation as the costs of rehabilitation and long-term nursing care was not taken into consideration (Lee & Khir, 2007). Therefore, in an aging population, the previously mentioned cost is

likely to increase substantially unless appropriate intervention is put in place (Malaysian Osteoporosis Society, 2012). Furthermore, there remain limited studies with regards to bone health and osteoporosis in Malaysia, in particular, relating to biomarkers and fracture data. This study will hence focus on S1P as a potential marker of bone metabolism and its association with sociodemographic factors and bone characteristics, which include BMD, and bone profile [blood investigations for calcium, phosphate, 25-hydroxyvitamin D (25(OH)D), parathyroid hormone (iPTH) and BTM (P1NP and CTX)].

## 1.4 Objectives

### 1.2.4 General Objective

To determine the association between P1NP, CTX and S1P with sociodemographic factors (age, gender, smoking, alcohol use), clinical characteristics [body mass index (BMI), previous history of personal and family history of fracture], BMD and bone profile [25(OH)D), iPTH, calcium and phosphate] among Chinese adult residents of Puchong and Kajang, Selangor, Malaysia.

### 1.4.2 Specific Objectives

1. To determine the sociodemographic factors, clinical characteristics, bone profile, P1NP, CTX and S1P levels among adult residents of Puchong and Kajang, Selangor, Malaysia.
2. To compare the sociodemographic factors, clinical characteristics, BMD, bone profile, P1NP, CTX and S1P levels between men and women.
3. To compare the sociodemographic factors, clinical characteristics, BMD, bone profile, P1NP, CTX and S1P levels in those with osteoporosis, osteopenia and normal BMD.
4. To determine the association of P1NP, CTX and S1P levels with
   i. sociodemographic factors (age, gender, smoking, alcohol intake)
   ii. clinical characteristics (BMI, previous history of personal and family history of fracture)
   iii. BMD;
   iv. bone profile [calcium, phosphate, 25(OH) D and iPTH].

## 1.5 Hypothesis

1. There are significant differences in sociodemographic factors, clinical characteristics, BMD, bone profile, P1NP, CTX and S1P levels between men and post-menopausal women.

2. There are significant differences in sociodemographic factors, clinical characteristics, BMD, bone profile, P1NP, CTX and S1P levels between those with osteoporosis, osteopenia and normal BMD.
3. There are significant associations between P1NP, CTX and S1P levels with
    i. sociodemographic factors (age, gender, smoking, alcohol intake)
    ii. clinical characteristics (BMI, previous history of personal and family history of fracture)
    iii. BMD;
    iv. bone profile [calcium, phosphate, 25(OH) D and iPTH].

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