ORIGINAL ARTICLE

Bone Turnover Markers and Sphingosine-1-phosphate levels among the Chinese Community in Selangor, Malaysia and its Correlation with Bone Density

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ABSTRACT

Introduction: Prevention of osteoporotic fracture requires identification of individuals at high risk. Bone mineral density(BMD) is commonly used to estimate fracture probability despite inadequate predictive discrimination ability. Sphingosine-1-phosphate(S1P), a new marker of bone metabolism and bone turnover markers(BTM) such as procollagen-type-1 amino-terminal propeptide(P1NP) and C-terminal telopeptide of type I collagen(CTX) may complement current assessment. The study determined P1NP, CTX and S1P levels and their correlation with BMD, 25-hydroxyvitamin D (25(OH)D) and parathyroid hormone(PTH) in selected subjects. **Method:** A cross-sectional study involving Malaysian Chinese men and women aged 50-90 years old from Puchong and Kajang, Selangor. Each subject had BMD determined by dual-energy x-ray absorptiometry and blood samples taken for 25(OH)D, PTH, P1NP, CTX and S1P. **Results:** A total of 131 subjects [45(34.4%) males and 86(65.6%) post-menopausal women] with median age of 65(IQR=17) were recruited. P1NP and CTX were significantly higher in post-menopausal women (P1NP=61.71 ng/ml, CTX=0.489 ng/ml) compared to men (P1NP=46.94 ng/ml, CTX=0.381 ng/ml). P1NP and CTX differed significantly according to BMD categories with values highest in osteoporosis. S1P between men (2.12±0.75 µmol/L) and post-menopausal women (1.96±0.68 µmol/L) did not differ significantly and did not differ according to BMD categories. S1P did not correlate with BMD, P1NP, CTX and 25(OH)D. P1NP and CTX negatively correlated with BMD at all measured sites but not 25(OH)D. **Conclusion:** CTX and P1NP, but not S1P negatively correlated with BMD. CTX and P1NP were highest in those with osteoporosis. In this group of Malaysian Chinese subjects, CTX and P1NP rather than S1P reflects bone health.

Keywords: Sphingosine-1-phosphate, Procollagen-type-1 amino-terminal propeptide, C-terminal telopeptide of type I collagen, Osteoporosis, Bone mineral density

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INTRODUCTION

Osteoporosis-related fractures, mainly of the hip and vertebrae, negatively impact quality of life and increase risk of disability and mortality (1). A key point in osteoporosis management is recognising individuals at high risk of fracture (2). In Malaysia's multi-ethnic population, the Chinese have the highest incidence of hip fractures and osteoporosis as defined by bone mineral density (BMD) measurement, compared to other ethnicities (3). BMD provides information on the likelihood of fracture (4). However, approximately 30-50% of fragility fractures occur in those with normal or osteopenic BMD (4). The WHO fracture risk assessment tool (FRAX®) improves evaluation of fracture probability by combining clinical risk factors (CRFs) such as age, family or previous fracture history, and BMD [5]. Despite all this, the predictive discriminative ability to identify those at risk of fracture remains inadequate (5, 6). Inclusion of a single or several bone turnover markers (BTM), independently of BMD,

may improve fracture prediction. Serum procollagen type 1 amino-terminal propeptide (P1NP), a marker of bone formation and plasma C-terminal telopeptide of type I collagen (CTX) a marker of bone resorption, are the recommended BTM often used for monitoring responsiveness towards osteoporosis treatment (7). CTX are formed by degradation of type 1 bone collagen by the enzyme cathepsin K whilst P1NP are derived from post-translational cleavage of type I procollagen by proteases and secreted by osteoblasts (8). Increased P1NP and CTX levels when measured by standardised assays predict fracture risk in postmenopausal women (7). Sphingosine 1-phosphate (S1P), a metabolite of sphingolipid has recently been identified as a marker of bone remodelling, and its receptors (S1PR) are potential therapeutic targets in the treatment of bone diseases (9).

S1P is a signalling molecule that acts as both an intracellular and extracellular messenger in many cells and is important in the regulation of numerous cell processes (10,11). In the circulation, S1P is bound to carrier proteins, namely albumin and high-density lipoproteins and higher concentration have been demonstrated in the circulation compared to normal tissues (10). In bone, it acts as a coupling factor between osteoblasts and osteoclasts (10,11). S1P stimulates proliferation, migration, and survival of osteoblasts, potentially leading to bone formation (10). The dominant S1P action however, is on bone resorption (12). This is mediated by augmentation of osteoclastogenesis by increasing receptor activator nuclear factor-κB ligand (RANKL) in osteoblasts (13). Osteoclast precursors move from the circulation to bone in the presence of substantial S1P gradient between the two sites (12).

In clinical studies, S1P is inversely correlated with bone mass, positively with bone resorption but not correlated with bone formation markers (14,15). In postmenopausal women, higher S1P levels were linked with higher risk of incident fractures, independently of BMD (15-17). Insufficient response to bisphosphonate therapy is also more commonly seen in postmenopausal women with higher S1P (16).

As a marker for bone health, S1P has so far been studied in a limited number of studies involving postmenopausal women. In addition, in Malaysia, there is little data looking at BTM in relation to bone health. This study aimed to determine the levels of BTM (P1NP and CTX) and S1P in a selected Malaysian population and to determine its correlation with BMD, and biochemical parameters [blood calcium, phosphate, 25-hydroxyvitamin D (25(OH)D), and parathyroid hormone (PTH)].

MATERIALS AND METHODS

Study Design

A cross-sectional study involving residents of Chinese ethnicity in selected residential areas in Puchong and Kajang in Selangor, Malaysia based on convenient sampling. The Chinese subjects were chosen as they had a significantly higher risk of osteoporotic fractures compared to other ethnicities in Malaysia (3). Data was collected from December 2015 to December 2017.

Subject recruitment

Research assistants disseminated brochures with details of the study from house to house in the selected areas. Inclusion criteria were Malaysian citizens aged 50-90 years old of Chinese ethnicity. The cut-off for the age was selected to include the middle-aged and elderly as they are at a higher risk for osteoporosis or osteoporotic fractures. The exclusion criteria were subjects already diagnosed with osteoporosis, subjects with renal impairment (eGFR <60 mls/min/1.73 m2), known to have or had metabolic bone disorders, malabsorption, thyroid disease, immobilisation or taking other drugs which affected bone homeostasis (e.g. corticosteroids, phenytoin, methotrexate, cyclosporine, oral contraceptive pill). Potential subjects were screened when they called for an appointment and eligible subjects were given a specific date for clinical assessment, blood sampling and BMD measurement. Respondents were asked on medication history as well as previous personal and parental fracture history. In those with history of fracture, the duration since the last fracture was noted. In women, additional information on the age of menopause was obtained. Height, weight and waist circumference were measured and body mass index (BMI) calculated. All subjects provided written informed consent. The study protocol was approved by the Ethics Committee of Universiti Putra Malaysia [FPSK(FR16)P002], and written informed consent was obtained from all subjects.

Sample size was estimated based on the correlation between S1P and lumbar spine BMD, using the following formula $N = \left(\frac{Z1\text{-}\sigma/2 + Z1\text{-}\beta}{C(r)} + 3\right)^{\wedge}2$, whereby σ is the estimated standard deviation; Z1-σ/2 = 1.96 (95% confidence level); Z1-β = 0.342 (80% power); C(r) = -0.355 (lumbar spine BMD in women) (14). The total sample size required was 131.

Laboratory Analysis

Fasting blood samples were analysed for calcium, phosphate, 25(OH)D, PTH, P1NP, CTX and S1P. Samples for P1NP, CTX and S1P were centrifuged at 3000 RPM for 15 minutes and the serum was aliquoted into three aliquots (1 ml each) and stored at -80°C until batch analysis. Calcium and phosphate were measured on ADVIA 1200 Chemistry Analyser (Siemens Healthcare, Germany), PTH and 25(OH)D on ADVIA Centaur Immunoassay Analyser (Siemens Healthcare, Germany) whilst CTX and P1NP on Cobas e 411 Analyser (Hitachi Roche, Germany). S1P was assayed using a commercial S1P competitive ELISA kit (Bioassay Technology Laboratory). The assay was strictly performed following the

manufacturer's instructions. Each sample was assayed in duplicates, and the mean ± SD was calculated. The minimum detectable S1P is 0.01 µmol/L with intra- and interassay CV of <10% and <12%, respectively. All analyses were performed according to standard laboratory procedures.

Measurement of BMD

BMD (grams/centimetre2) was measured at the lumbar spine (L1-L4), left femoral neck and left total hip using HOLOGIC Discovery W densitometer (Hologic Corporation, Bedford, MA, USA). The instrument has a precision of ± 2%. The reference population used was the machine manufacturer's Asian population database. The procedure was performed by trained radiographers. BMD was classified into normal, osteopenia and osteoporosis based on T-scores using the WHO classification (18). A T-score greater than -1.0 was classified as normal, between -1 and -2.5 was classified as osteopenia and less than-2.5 was classified as osteoporosis.

Statistical Analysis

In descriptive analysis, mean and standard deviation (SD) were used to summarise normally distributed continuous variables, median [inter-quartile range (IQR)] for skewed distribution, and count (%) for categorical variables. Chi-square tests, independent t-test and Mann-Whitney U test were used to compare two groups. Pearson's or Spearman's correlation tests were used to determine various correlations. A p-value of less than 0.05 was considered to be statistically significant.

RESULTS

Table I and Table II display the characteristics and laboratory parameters of all the subjects and according to gender, respectively. A total of 131 subjects were recruited with the majority (65.6%) being females. All the women were postmenopausal. The median age was 65 years (IQR: 17; range 50-89 years old). More than half (54.2%) had normal BMI and 90.8 % had no history of hip fracture. There were 13.7% subjects with vitamin D deficiency defined as serum 25(OH)D <50 nmol/l. Osteopenia and osteoporosis were noted in 46.6% and 29.0% of the study subjects, respectively. No significant differences in the number of subjects in terms of age (both <60 and ≥ 60 years groups), BMI (underweight, normal and overweight), previous or parents history of fracture, vitamin D status and BMD characteristics between men and postmenopausal women were noted (Table II).

Table III shows the comparisons of the laboratory investigation results and BMD values between genders. There were significant differences in serum phosphate (p<0.001), 25(OH)D (p=0.045), P1NP (p=0.005) and CTX (p=0.029) concentrations between men and postmenopausal women. Men demonstrated higher

Table I : Sociodemographic factors, clinical characteristics and laboratory parameters of all the study subjects (N = 131)

Variable	n (%)		
Gender			
Male	45 (34.4)		
Female	86 (65.6)		
Age (years)			
<60	48 (36.6)		
≥ 60	83 (63.4)		
BMI (kg/m²)*			
Underweight (<18.5)	15 (11.5)		
Normal (18.5-24.9)	71 (54.2)		
Overweight & Obese (≥25)	45 (34.4)		
Smoking status			
Yes	4 (3.1)		
No	127 (96.9)		
Alcohol status			
Yes	19 (14.5)		
No	112 (85.5)		
Previous fracture			
Yes	12 (9.2)		
No	119 (90.8)		
Parent's hip fracture			
Yes	7 (5.30)		
No	124 (94.7)		
Vitamin D status			
Deficiency (25(OH)D < 50 nmol/L)	18 (13.7)		
Sufficiency (25(OH)D ≥ 50 nmol/L)	113 (86.3)		
BMD categories⁻			
Normal	32 (24.4)		
Osteopenia	61 (46.6)		
Osteoporosis	38 (29.0)		
BMD (g/cm²)	**Mean (SD)**	**Min – Max**	
Lumbar spine	0.910 (0.187)	0.484 - 1.475	
Femoral neck	0.635 (0.149)	0.085 - 1.212	
Total hip	0.763 (0.151)	0.409 - 1.214	
Total body	0.971 (0.13)	0.684 - 1.302	
Bone profile			**Reference range**
Calcium (mmol/L)	2.39 (0.07)	2.19 - 2.58	2.12 – 2.52
Phosphate (mmol/ L)	1.16 (0.14)	0.77 - 1.60	0.80 – 1.60
25(OH)D (nmol/L)	72 (18)	29 - 118	≥ 50
PTH (pmol/L)	5.00 (3.30)[a]	1.20 - 16.20	1.48 – 7.63
P1NP (ng/ml)	58.03 (35.83)[a]	13.18 - 252.0	15 – 115
CTX (ng/ml)	0.464 (0.331)[a]	0.097 - 1.480	0.10 – 0.80
S1P (µmol/ L)	2.01 (0.70)	0.65 - 3.83	

[a] median (interquartile range)

*WHO Classification of adult underweight, overweight and obesity according to BMI.

⁻WHO definition of osteoporosis

25(OH)D, whilst P1NP and CTX were both higher in post-menopausal women. S1P concentration even though was higher in men was not significantly different (p=0.235) compared to postmenopausal women. Postmenopausal women had lower BMD at all sites in comparison to the men.

Table IV shows the laboratory investigation results and BMD values in groups of normal, osteopenia and osteoporosis. No significant differences in calcium, phosphate, 25(OH)D and PTH levels between the three BMD categories were demonstrated. In contrast, significant differences in P1NP and CTX levels were noted. Subsequently, pair-wise comparisons (not shown in table) were performed comparing two groups with p-value of 0.02 calculated as being significantly different. Those with osteoporosis had higher P1NP and CTX (p≤0.001) compared to normal BMD group. Those with osteopenia had higher P1NP compared to normal BMD subjects (p≤0.001) but no significant difference

Table II : Sociodemographic factors and clinical characteristics according to gender (men and post-menopausal women)

Variable	Men n=45 n (%)	Post-menopausal women n=86 n (%)	χ^2	p-value
Age [median (IQR)]	67 (20)	65 (16)		
< 60 years old	15 (33.3)	33 (38.4)	0.323	0.570
≥ 60 years old	30 (66.7)	53 (61.6)		
BMI				
Underweight	6 (13.3)	9 (10.5)	0.803	0.669
Normal	22 (48.9)	49 (57.0)		
Overweight & Obese	17 (37.8)	28 (32.6)		
Smoking status				
Yes	3 (6.7)	1 (1.2)	NA	0.117[a]
No	42 (93.3)	85 (98.8)		
Alcohol status				
Yes	9 (20.0)	10 (11.6)	1.670	0.196[a]
No	36 (80.0)	76 (88.4)		
Previous fracture				
Yes	7 (15.6)	5 (5.8)	NA	0.107
No	38 (89.4)	81 (94.2)		
Parent's hip fracture				
Yes	2 (4.4)	5 (5.8)	NA	1.000[a]
No	43 (95.6)	81 (94.2)		
Vitamin D status				
Deficiency	6 (13.3)	12 (14.0)	4.405	0.111
Insufficiency	12 (26.7)	38 (44.2)		
Sufficiency	27 (60.0)	36 (41.9)		
BMD characteristics				
Normal	15 (33.3)	17 (19.8)	3.380	0.185
Osteopenia	20 (44.4)	41 (47.7)		
Osteoporosis	10 (22.2)	28 (32.6)		

χ^2 Chi-Square statistical test. [a] p-value for Fishers's exact test
p-value <0.05 is considered statistically significant. NA: not applicable

Table III : Bone Profile, P1NP, CTX, S1P and BMD values in men (n=45) and post-menopausal women (n=86)

Variable	Men	Post-meno-pausal women	Test- sta-tistics	p-value
	Mean (SD)	Mean (SD)		
Calcium (mmol/L)	2.33 (0.06)	2.33 (0.06)	0.116[c]	0.908
Phosphate (mmol/L)	1.08 (0.18)[a]	1.21 (0.12)	-5.091[b]	<0.001
25(OH)D (nmol/L)	77 (19.86)	70 (17.23)	2.023[c]	0.045
PTH (pmol/L)	4.90 (3.50)[a]	5.29 (2.47)	-1.137[b]	0.256
P1NP (ng/ml)	46.94 (31.22)[a]	61.71 (37.49)	-2.811[b]	0.005
CTX (ng/ml)	0.381 (0.291)[a]	0.489 (0.283)	-2.186[b]	0.029
S1P (µmol/ L)	2.12 (0.75)	1.96 (0.68)	1.193[c]	0.235
BMD (g/cm^2)				
Lumbar spine	0.994 (0.201)	0.865 (0.163)	3.957[c]	<0.001
Femoral neck	0.683 (0.167)	0.609 (0.133)	2.746[c]	0.007
Total hip	0.821 (0.163)	0.732 (0.135)	3.303[c]	0.001
Total body	1.038 (0.107)	0.910 (0.166)	-4.539[b]	<0.001

[a] median (interquartile range); b z-value (Mann-Whitney statistical test); c t-value (Independent-samples t-test).
p-value <0.05 is considered statistically significant.

was seen with CTX (p=0.056). When the osteopenia and osteoporosis groups were compared, no difference was noted in P1NP levels (p=0.216) but CTX was significantly higher in those with osteoporosis (p=0.006). The mean S1P was 2.08 ±0.67 µmol/L, 1.93-±0.76 µmol/L and 2.09±0.64 µmol/L in normal BMD, osteopenia and osteoporosis, respectively with no significant difference demonstrated between the three groups.

Table IV : Correlations between P1NP, CTX and S1P with age, BMI, bone profile and BMD in all subjects

	All subjects					
	S1P		P1NP		CTX	
Variable	r/r$_s$	p-value	r/r$_s$	p-value	r/r$_s$	p-value
Age (years)	0.069[a]	0.434	0.051	0.566	0.121	0.170
BMI (kg/m)	-0.117[a]	0.182	0.039	0.656	-0.038	0.665
Bone profile						
Calcium (mmol/L)	0.134[b]	0.127	-0.026	0.769	0.035	0.689
Phosphate (mmol/ L)	0.011[b]	0.904	0.217	0.013	0.167	0.057
25(OH)D (nmol/L)	0.013[b]	0.886	0.031	0.729	0.026	0.771
PTH (pmol/L)	-0.232[a]	0.008	0.161	0.067	0.206	0.018
BMD (g/cm^2)						
Lumbar spine	0.102[b]	0.246	-0.308	<0.001	-0.361	<0.001
Femoral neck	0.014[b]	0.871	-0.292	0.001	-0.290	0.001
Total hip	0.030[b]	0.735	-0.309	<0.001	-0.384	<0.001
Total body	0.050[a]	0.569	-0.384	<0.001	-0.335	<0.001

[a] Spearman correlation (r$_s$); [b] Pearson's correlation (r). Statistical significance at p <0.05.

Pearson product-moment correlations and Spearman's rank-order correlations were used to determine the relationship between age, BMI, biochemical bone parameters and BMD with S1P, P1NP and CTX (Table V). For S1P, there was a significant weak negative correlation with PTH (r=-0.232, p=0.008). S1P did not correlate with any of the BMD measurements. In contrast, P1NP and CTX negatively correlated with BMD measurements at all sites. For CTX, there was a significant weak positive correlation with PTH (r=0.206, p=0.018). After adjusting for age and BMI, all significant correlations of S1P, P1NP and CTX remained (r=0.204 to -0.391; p<0.05).

DISCUSSION

The prevalence of osteoporosis (29%) and osteopenia (46.6%) as observed in the present study were comparable to a prior study in Malaysia, which reported a prevalence of osteoporosis and osteopenia of 24% and 51.6%, respectively (19). The median age was 65 years with more than 60% being above 60 years old, which is a good representation of those at risk of osteoporosis fracture as well as being an age recommended for BMD measurement in Malaysia (20). Less than 15% of the subjects had vitamin D deficiency, which is lower than previously reported in Malaysia. In 2011, 41% males and 87% females of Malay ethnicity were reported to be vitamin D deficient (21). Chinese ethnicity (compared to Malays and Indians) and lower BMI were associated with higher serum 25(OH)D (22). The difference in ethnic groups studied and BMI most likely contributed to the difference in the prevalence. In another study, the median 25(OH)D of postmenopausal Chinese was 68.8 nmol/L, similar to the mean obtained in our study (70.0 nmol/L) (23).

It is known that women have a higher fracture risk compared to men, contributed partially by the

dissimilarities in BMD, size and strength of bone (24). This was reflected by the significant differences in BMD measurements and BTM (P1NP and CTX) between genders in this study. P1NP and CTX were significantly higher in postmenopausal women (P1NP 61.71 ng/ml; CTX 0.489 ng/ml) compared to men (P1NP 46.94 ng/ml; CTX 0.381 ng/ml). The median P1NP and CTX obtained were also higher compared to other studies in both postmenopausal women and men. The Camargo Cohort study involving healthy postmenopausal women in Spain reported a mean P1NP of 47.7 ± 19.9 ng/ml and CTX of 0.387 ± 0.197 ng/ml, with both BTM higher in those with osteoporosis (25), similarly demonstrated in this study. Age and gender related changes in P1NP and CTX have been reported (26). In men, the mean P1NP and CTX increased significantly after 59 years old whilst in women, the mean P1NP and CTX rapidly increased after the age of 50 (26). In this study, the majority (63.4%) of subjects were elderly (≥ 60 years), which may explain the higher BTM (P1NP and CTX) obtained in this study.

Increased BTM reflects higher bone turnover, and predicts fracture risk complementary to BMD as the latter assesses bone mass whilst the former, bone microarchitecture (27). P1NP and CTX differed significantly between normal BMD, osteopenia and osteoporosis with values lowest in normal BMD, followed by osteopenia and osteoporosis. In a study involving postmenopausal women aged 50-89 years old, those with the highest BTM quartile had approximately two-fold increased fracture risk (28). There were no significant differences in other laboratory markers including S1P between normal BMD, osteopenia and osteoporosis groups.

In this group of healthy ambulant subjects, S1P did not differ between men and women. The values obtained in the postmenopausal women were similar to the S1P level of premenopausal women of previous studies (14,16). One study reported S1P levels in postmenopausal women as 5.0 ± 0.2 µmol/L, which was significantly higher compared to men (2.2 ± 0.5 µmol/L) or pre-menopausal women (2.7 ± 0.4 µmol/L) whilst no difference between men and pre-menopausal were found (14). Variations in ethinicity may have partially contributed to the lower S1P value obtained in this study as previously demonstrated for BTM (8). Interestingly however, the mean S1P values for men in our study subjects (2.12 ± 0.75 µmol/L) were very similar to those obtained by Lee et. al. 2012 (14).

In a longitudinal study, those in the lowest baseline S1P tertile (0.47 – 2.29 µmol/L) had the lowest risks of incident or prevalent fracture three years later compared to those in the highest tertile group (5.90–16.51 µmol/L) (16). S1P levels appeared to be stable in individuals during five years of follow up with the majority remained within the same quartile of plasma S1P at baseline (17). Those who sustained subsequent fracture had consistently high initial S1P and at several points of S1P measurement (17). This led to a suggestion that one measurement of S1P, may be adequate in identifying those at risk of fracture. The S1P level in our study for postmenopausal women were low, which if based on other studies may suggest that the risk of future osteoporosis is low (16, 17). Nevertheless, this could not be confirmed based on the current study design as none of our subjects had high S1P levels. This warrants further studies in a larger sample as well as in the other ethnic groups in Malaysia. Furthermore, in contrast to our findings, Bae et al, in 2016 demonstrated significant correlations between S1P with BMD, CTX and urinary NTX, another bone resorption marker, but not with bone formation markers, in their case bone-specific alkaline phosphatase and osteocalcin (16). However, we did not find significant correlations between S1P with CTX, P1NP and individual BMD values at all sites. The possible reason is the lower S1P values, which may have affected the outcome of the study. P1NP and CTX were however negatively correlated with BMD at all sites, similar to the other studies (16, 17).

This study is not without limitations. The main limitation is the cross-sectional design, which could not reveal whether these associations or lack of association will change over time. Secondly, the results may not represent S1P values in the other major races in Malaysian multi-ethnic community. The lifestyle of the subjects was also not assessed in these subjects and should be considered in future studies.

CONCLUSION

CTX and P1NP, but not S1P, levels negatively correlated with BMD. CTX and P1NP were highest in those with osteoporosis. In this group of Malaysian Chinese subjects, CTX and P1NP rather than S1P reflects bone health.

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REFERENCES

1. Sattui SE, Saag KG. Fracture Mortality: Associations with epidemiology and osteoporosis treatment. Nat Rev Endocrinol. 2014;10(10):592-602.
2. Orimo H, Nakamura T, Hosoi T, et al. Japanese 2011 guidelines for prevention and treatment of osteoporosis-executive summary. Arch Osteoporos. 2012;7(1-2):3-20.
3. Lee JK, Khir AS. The incidence of hip fracture in Malaysians above 50 years of age: variation

in different ethnic groups. Int J Rheum Dis. 2007;10(4):300-5.
4. Sanders KM, Nicholson GC, Watts JJ, et al. Half the burden of fragility fractures in the community occur in women without osteoporosis. When is fracture prevention cost-effective? Bone. 2006;38(5):694-700.
5. Bolland MJ, Siu AT, Mason BH, et al. Evaluation of the FRAX and Garvan fracture risk calculators in older women. J Bone Miner Res. 2011;26(2):420-7.
6. Hillier TA, Cauley JA, Rizzo JH, et al. WHO absolute fracture risk models (FRAX): Do clinical risk factors improve fracture prediction in older women without osteoporosis? J Bone Miner Res. 2011;26(8):1774-82.
7. Vasikaran S, Eastell R, Bruyère O, et al. Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: A need for international reference standards. Osteoporos Int. 2011; 22:391–420.
8. Shetty S, Kapoor N, Bondu JD, Nihal T, Vizhalil PT. Bone turnover markers: Emerging tool in the management of osteoporosis. Indian J Endocrinol Metab. 2016 ; 20(6): 846-52.
9. Meshcheryakova A, Mechtcheriakova D, Pietschmann P. Sphingosine 1-phosphate signaling in bone remodeling: multifaceted roles and therapeutic potential. Expert Opin Ther Targets. 2017;21(7):725-37.
10. Rosen H, Stevens RC, Hanson M, et al. Sphingosine-1-phos¬phate and its receptors: structure, signaling, and influence. Annu Rev Biochem 2013;82:637-62.
11. Proia RL, Hla T. Emerging biology of sphingosine-1-phosphate: Its role in pathogenesis and therapy. J Clin Invest. 2015;125(4):1379-87.
12. Ishii M, Kikuta J. Sphingosine-1-phosphate signaling con¬trolling osteoclasts and bone homeostasis. Biochim Bio¬phys Acta 2013;1831:223-7.
13. Ryu J, Kim HJ, Chang EJ, et al. Sphingosine 1-phosphate as a regulator of osteoclast differentiation and osteoclast-osteoblast coupling. EMBO J 2006; 25:5840-51.
14. Lee SH, Lee SY, Lee YS, et al. Higher circulating sphingosine 1-phosphate levels are associated with lower bone mineral density and higher bone resorption marker in humans. J Clin Endocrinol Metab. 2012; 97(8):E1421-8.
15. Kim BJ, Koh JM, Lee SY, et al. Plasma sphingosine 1-phosphate levels and the risk of vertebral fracture in postmenopausal women. J Clin Endocrinol Metab. 2012; 97(10):3807-14.
16. Bae SJ, Lee SH, Ahn SH, Kim HM, Kim BJ, Koh JM. The circulating sphingosine-1-phosphate level predicts incident fracture in postmenopausal women: A 3.5-year follow-up observation study. Osteoporos Int. 2016;27(8):2533-41.
17. Ardawi MS, Rouzi AA, Al-Senani NS, Qari MH, Elsamanoudy AZ, Mousa SA. High plasma sphingosine 1-phosphate levels predict osteoporotic fractures in postmenopausal women: The center of excellence for osteoporosis research study. J Bone Metab. 2018;25(2):87-98.
18. World Health Organization. Assessment of Fracture Risk and Its Application to Screening for Postmenopausal Osteoporosis. WHO Technical Report Series, No.843. Geneva, WHO, 1994
19. Lim PS, Ong FB, Adeeb N, et al. Bone health in urban midlife Malaysian women: risk factors and prevention. Osteoporos Int. 2005; 16(12), 2069–79.
20. Yeap SS, Hew FL, Damodaran P, et al. A summary of the Malaysian Clinical Guidance on the management of postmenopausal and male osteoporosis. Osteoporosis and Sarcopenia. 2016; 2(1):1–12.
21. Moy FM, Bulgiba A. High prevalence of vitamin D insufficiency and its association with obesity and metabolic syndrome among Malay adults in Kuala Lumpur, Malaysia. BMC Public Health. 2011; 11(1):735.
22. Shafinaz IS, Moy FM. Vitamin D level and its association with adiposity among multi-ethnic adults in Kuala Lumpur, Malaysia: a cross sectional study. BMC Public Health. 2016; 16:232.
23. Rahman SA, Chee WS, Yassin Z, Chan, SP. Vitamin D status among postmenopausal Malaysian women. Asia Pac J Clin Nutr.2004; 13(3):255–60.
24. Cawthon P. Gender Differences in Osteoporosis and Fractures. Clin Orthop Relat Res. 2011 Jul; 469(7): 1900–1905.
25. Martinez J, Olmos JM, Hernandez JL, et al. Bone turnover markers in Spanish postmenopausal women. Clin Chim Acta. 2009: 409(1–2):70–4.
26. Hu W-W, Zhang Z, He JW, et al. Establishing reference rntervals for bone turnover markers in the healthy Shanghai population and the relationship with bone mineral density in postmenopausal women. Int J of Endocrinol. 2013; 2013: 513925.
27. Puntus T, Schneider B, Meran J, Peterlik M, & Kudlacek S. Influence of age and gender on associations of body mass index with bone mineral density, bone turnover markers and circulating calcium-regulating and bone-active sex hormones. Bone. 2011; 49(4):824–9.
28. Garnero P, Sornay-Rendu E, Claustrat B, Delmas PD. Biochemical markers of bone turnover, endogenous hormones and the risk of fractures in postmenopausal women: The OFELY study. J Bone Miner Res. 2000; 15:1526–36.