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Bone Resorption Marker Status of Pre and Postmenopausal Malay Women in Kelantan and Its Corresponding Risk Factors

(Status Petanda Resorpsi Tulang dalam Kalangan Wanita Melayu Pra dan Pascamenopaus di Kelantan dan Faktor Risiko yang Sepadan)

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ABSTRACT

*Menopause is the most prevalent cause of accelerated bone loss in women. Biochemical markers of bone resorption can be used clinically to predict future bone loss. This study aimed to determine the level of bone resorption markers in healthy pre and postmenopausal Malay women and determine their association with the risk. A total of 150 healthy women were recruited for this study (51 pre and 99 postmenopausal subjects). Data on socioeconomic, lifestyle habit and clinical were gained by personal interview. Fasting serum was collected to measure both C-telopeptide (CTx) and N-telopeptide (NTx) of type 1 collagen. Both markers were highly correlated with each other (*r=*0.568,* p<*0.001). Both intra- and inter-assay coefficient of variations (CV) of NTx were higher than those of CTx (8% and 12% vs 6% and 5%). The mean CTx values of pre and postmenopausal subjects were comparable with the expected values (0.2833 (0.1769) ng/mL and 0.4323 (1.851) ng/mL compared with 0.287 and 0.438 ng/mL, respectively). The NTx value for premenopausal subjects were higher than the expected values (15.2 (8.10) compared to 12.6 (3.20) nM BCE). The median was 19.929 nM BCE. The mean CTx and NTx levels of postmenopausal subjects were significantly lower than premenopausal subjects (*p<*0.05). The risk factors for bone resorption in this population were duration of menopause, marital status, body mass index (BMI), physical activity and education level. In conclusion, postmenopausal women showed a higher bone resorption, indicating higher bone loss. Increasing education and physical activity intervention might be effective to ensure better health in Malaysian older population.*

Keywords: Bone resorption marker (NTx, CTx); Malay; postmenopausal; premenopausal

ABSTRAK

*Menopaus adalah faktor utama yang mempercepatkan kadar hakisan tulang dalam kalangan wanita. Petanda biokimia bagi hakisan tulang boleh digunakan secara klinikal untuk meramal kehilangan jisim tulang. Objektif kajian ini adalah untuk mengenal pasti aras resorpsi tulang wanita pra dan pascamenopaus serta mengenal pasti faktor risiko resorpsi tulang dalam golongan ini. Seramai 150 subjek terlibat dalam kajian ini (51 pra dan 99 pascamenopaus). Data sosioekonomi, gaya hidup dan data klinikal diperoleh melalui temu bual secara individu. Serum subjek ketika berpuasa digunakan untuk menentukan petanda resorpsi tulang N- dan C- 'terminal telopeptides of type 1 collagen' (NTx dan CTx). Kedua-dua petanda resorpsi tulang ini mempunyai perkaitan yang tinggi (*r=*0.568,* p<*0.001). Kedua-dua 'intra' dan 'inter-assay' pekali ubah (CV) untuk NTx adalah lebih tinggi berbanding CTx (8% dan 12% vs 6% dan 5%). Nilai min CTx wanita pra- dan pascamenopaus dalam kajian ini sebanding dengan nilai jangkaan (0.2833 (0.1769) ng/mL dan 0.4323 (1.851) ng/mL berbanding 0.287 dan 0.438 ng/mL). Nilai NTx bagi wanita pramenopaus adalah lebih tinggi berbanding nilai jangkaan (15.2 (8.10) berbanding 12.6 (3.20) nM BCE). Nilai median adalah 19.929 nM BCE. Nilai min CTx dan NTx bagi subjek pascamenopaus adalah lebih tinggi berbanding subjek pramenopaus (*p<*0.05). Faktor risiko resorpsi tulang bagi populasi ini adalah tempoh pascamenopaus, status perkahwinan, indeks jisim badan (BMI), aktiviti fizikal dan tahap pendidikan (*p<*0.05). Kesimpulannya, wanita pascamenopaus mempunyai aras petanda resorpsi tulang yang menggambarkan kadar kehilangan tulang yang lebih tinggi. Meningkatkan tahap pendidikan dan intervensi aktiviti fizikal mungkin merupakan langkah yang berkesan bagi memastikan kesihatan tulang yang lebih baik dalam kalangan populasi warga tua di Malaysia.*

Kata kunci: Melayu; pascamenopaus; petanda resorpsi tulang (NTx, CTx); pramenopaus

INTRODUCTION

Bones are living connective tissues that makes up about 18% of the human body weight. It is metabolically actives and undergoes two distinct phase of continuous remodeling; the bone resorption and formation (Cassandra & Thomas 2001; Kraenzlin & Seibel 2006; Parakrama & Clive 1998). In recent years, cellular and extracellular components of bone matrix have been isolated and characterized, making

it useful as biochemical markers that reflect either bone formation or resorption (Caulfield & Reitz 2004). Good biochemical markers are chosen based on few criteria which includes; specific to the tissue, specific for either bone formation or resorption, the changes in the marker are not disease specific, can be easily measured either in serum or urine by specific and sensitive techniques and lastly, the factors that controlled its synthesis, metabolism, elimination and variation should be well known (Caulfield & Reitz 2004).

Bone-resorption marker is degradation products of osteoclast resorption activity on the matrix. It either relates to the dissolution of the mineralized matrix or the degradation of the type I collagen (Fox & Cole 2004). During collagen breakdown, the amino- (N) and carboxy- (C) terminal telopeptides are released into the circulation. These N- and C- terminal telopeptides are short non-helical peptides at both ends of the collagen molecule (Caulfield & Reitz 2004). The biochemical markers for bone resorption can either be urinary or serum-based. However, urine sample is subjected to variable ionic strength and day-to-day within-person variability. Besides that, as it needs to be corrected for creatinine excretion, it may leads to some variability in the results, making it less precise compared to serum-based sample (Garnero et al. 2001; Qvist et al. 2002). The resorption markers have a diurnal rhythm and as the marker level is highest in the morning, it is better to collect the sample in the morning, at approximately the same time (Fox & Cole 2004). Previous study also had shown that fasting reduces the amplitude of variation of the markers, hence increasing the sensitivity of the test (Qvist et al. 2002).

The higher rate of bone resorption, compared with the rate of bone formation leads to the occurrence of osteoporosis. Osteoporosis is the most common form of diseases involving bones in adults, especially in the elderly. In Malaysia, the prevalence of osteoporosis is 24%, while for osteopenia is 51.6% (Khir et al. 2006). A study by Lim et al. (2005) showed that 42.1% postmenopausal women and 11.1% premenopausal women lived in urban area were osteoporotic. Like any other diseases, the prevalence of osteoporosis varies according to population. In Malaysia, the most common risk factors being investigated are age, gender, body mass index, ethnic groups, socio economic status, smoking, family history, usage of bone-depleting drugs, calcium and vitamin D intake and physical activities (Huguet et al. 2008; Khir et al. 2006; Lau et al. 2001; Lee & Khir 2007; Papp et al. 2007). Many studies had also shown the positive relationship between health and socio economic status. A significant increase in hip fractures was found with a decrease in income (Bacon & Hadden 2000). A study by Zingmond et al. (2006) estimated an incident rate ratio (IRR) of 0.79 in the highest group of income compared to the group with lowest income in the study. Apart from that, Guilley et al. (2011) showed that the medium income population has lower hip fracture incidence (OR 0.91) compared with the lowest income population.

The gold standard in determining bone health is by measuring bone mineral density using dual-energy x-ray absorptiometry (DEXA). However, this method is expensive as a screening tool and only measured specific sites. Hence, the usage of bone resorption markers is a good alternative. Bone resorption markers indicate the whole-body rates of bone resorption, relatively cheaper and have been shown to have positive correlation with bone density (Desai et al. 2007; Garnero et al. 2001; Heikkinen et al. 1997; Papp et al. 2007).

This study aimed to determine the risk factors related to the increase in bone resorption in the low socio economic status of pre and postmenopausal women in Kelantan. Identifying possible, modifiable risk factors is important as it can lead to earlier screening and detection of osteoporosis in the general populace. It can also help in planning healthy-lifestyles strategies to combat osteoporosis in a community.

Subjects and Methods

This cross-sectional study was conducted in three different districts in Kelantan. The study location comprises of Kedai Menanti in Pasir Puteh, Sering in Kota Bharu and Gunong Stong in Jeli. The participants were recruited using convenient-sampling. All subjects were recruited from lower income family, based on *Pendapatan Garis Kemiskinan (*PGK*)* 2009 by Economic Planning Unit, Prime Minister's Department Malaysia (total income RM770 in urban and RM740 in rural area). Premenopausal subjects were recruited among women aged 45 and above with regular menstrual cycle for the past six months of the study. Menopausal state was indicated by the absence of menstrual cycle for 12 months. Subjects were excluded if they took drugs known to affect bone metabolism (glucocorticoids, HRT, calcium and vitamin D supplement), had evidence of medical or surgical condition known to affect bone loss (hysterectomy, bilateral oophorectomy), currently known to be pregnant or breast feeding, or having critical illnesses or chronic condition that might affect bone resorption (osteoporosis, cancer, diabetes).

The research methodology was approved by the Human Ethics Committee of Universiti Sains Malaysia (USMKK/PPP/JEPeM(219.3.(05)). Participants were explained the purpose and procedure of the study and informed content were obtained from the participants.

Subjects were asked to fast overnight (no food or beverage but plain water after 12 am) and blood sample were collected between 8.00 a.m. to 11.00 a.m. Blood samples were stored in ice-pack filled ice-box upon collection (9-12°C). Serum was separated (3000 rpm, 10 min) (Hettich Zentrifugen Universal 32R, Beverly, USA) from the cells within 5 h after collection of blood. Upon centrifugation, the serum was kept under temperature of -18°C until further analysis. All samples and kits used were thawed at room temperature (18-26°C) 30 min before analysis.

The subjects were interviewed by trained panels to gain the socio economic, lifestyle habit and clinical data. Lifestyle questionnaire were derived from the iDecide physical activity score (with permission from Abbott Laboratory). Weight (weighing scale, SECA) and height (Bodymeter 208, SECA) were measured to the nearest 0.1 unit. Food frequency questionnaire (FFQ) and diet recall were taken by trained dietitians. Amount of calcium intake was calculated based on Nutrient Composition of Malaysian Foods (1997) and for commercialized food, the calcium content was calculated based on the nutrition information label on the packaging. The sufficiency of calcium intake was based on the recommended daily intake for Malaysian women, which are 800 mg/day for premenopausal group and 1000 mg/day for postmenopausal group (Chee et al. 2002; Khir et al. 2006).

The Serum crossLaps one step enzyme-linked immunoassay (ELISA) (Immunodiagnostic System Ltd) is used to quantify degradation products of C-terminal telopeptides of type 1 collagen of bone matrix in human serum. This sandwich assay used two monoclonal antibodies highly specific for the amino acid sequence of EKAHD-β-GGR. During the one step incubation process, two chains of EKAHD-β-GGR cross-linked and become a complex with biotinylated antibody and peroxidase conjugate antibody. This complex is then bound to the streptavidin coat at the wells via the biotinylated antibody. Chromogenic substance is used for colour reaction and absorbance is measured within 2 h at 450 nm with 650 nm as reference on ELISA reader (TECAN-GENious, Salzburg, Austria). Both inter and intra-assay imprecision are less than 10%. In this paper, the term CTx was used to represent C-terminal cross-linking telopeptides of type 1 collagen.

The Serum osteomark one step enzyme-linked immunoassay (ELISA) (Wampole Laboratories ®) is used to quantify degradation products of N-terminal telopeptides of type 1 collagen of bone matrix in human serum. It is a competitive-inhibition assay. Samples were added to NTx epitope-adsorbed microplates, followed by a horseradish peroxidase labeled monoclonal antibody. The samples NTx competes with NTx epitope for antibody binding sites. Following the washing step, the amount of labeled antibody bound is measured by colorimetric generation of peroxidase substrate. Absorbance is determined at 450 nm with 650 nm as reference on ELISA reader (TECAN-GENious, Salzburg, Austria). Assay values are reported in nanomoles bone collagen equivalents per liter (nM BCE). In this paper, the term NTx was used to represent N-terminal cross-linking telopeptides of type 1 collagen.

The 25(OH)-Vitamin D direct ELISA (Immundiagnostik AG, Stubenwald-Allee, Bensheim, Germany) is a competitive ELISA technique which use a selected monoclonal antibody that detect 25(OH)-vitamin D. The serum samples were pre-incubated with releasing agent to release the 25(OH)-vitamin D from its 25(OH)-vitamin D-DBP complex. The samples were then transferred to 25(OH)-vitamin D coated microplate and antibody was added. Samples were incubated for 18 to 22 h in ice-box filled with ice-packs to maintain the temperature at 8 to 10°C. During the overnight incubation process, 25(OH)-vitamin D in the samples and the one bound on the well compete for the binding with antibody. Peroxidase-conjugate antibody was added and a complex of 25(OH)-vitamin D-anti-25(OH)-vitamin D-peroxidase conjugate was formed. Acidic solution was used to stop the reaction and the intensity of colour reaction was inversely proportional to the concentration of 25(OH)-vitamin D. The absorbance was measured after 15 min at 405 nm with 690 nm as reference on ELISA reader (TECAN-GENious, Salzburg, Austria). Both the intra-assay and inter-assay precision (CV) were 7% (n=20).

For this paper, vitamin D severe Hypovitaminosis D is defined as serum 25(OH)D lower than 25 nmol/L, lowered serum 25-OH vitamin D level, or Hypovitaminosis D level is 25-50 nmol/mL and sufficient serum vitamin D level is >50 nmol/mL. These cut-off points were recalculated from the nmol/L unit from previous suggestions by the Institute of Medicine and comprehensive review on global Vitamin D status by Mithal et al. (2009).

The data was analyzed using the statistical package for social sciences version 18 (SPSS, Chicago, IL, USA). Statistical significance was set at the 5% level. Results were expressed in mean (standard deviation). One-way analyses of variance (ANOVA), t-test, Pearson's correlation, Tukey's test, Spearman correlation and multivariate linear regression were used whenever appropriate to determine the correlation, predictors and significant differences.

Results

A total of 51 healthy premenopausal women and 99 healthy postmenopausal women were recruited. The postmenopausal group was further divided into two groups; postmenopausal A group consists of 65 subjects who had been in menopausal state for less than 10 years and postmenopausal B groups consists of 34 subjects who had been in menopausal state for more than 10 years. As shown in Table 1, the mean age for all subjects was 55.8 (7.11) years. All subjects come from low income family, with the average monthly income of RM452.00 (231.45). There was no significant difference between all characteristics of both groups, except for the mean income; where the mean income for postmenopausal group (B) was significantly lower from the other groups (p<0.05).

The mean BMI of all subjects was 25.8 (4.83) (Table 2). Most of the subjects fall under the normal and overweight category (43.2% and 33.8%, respectively). There was no significant difference between the height, weight and BMI among the two groups.

The mean calcium intake of the subjects was 492.9 (316.51) mg/day. Based on these, 86.3% of the premenopausal and 91.9% of the postmenopausal groups have insufficient intake of calcium. The mean serum vitamin D for the subjects is 43.4 (7.01) nmol/L. 16.4% of

TABLE 1. Socio economic characteristics of subjects (n=150) (presented as mean (SD) or %)

Variables	Premenopause (n=51)	Postmenopause A (n=65)	Postmenopause B (n=34)
Number of children	7 (3)	6 (3)	6 (2)
Marital Status			
Married	84.3%	67.7%	61.8%
Divorced	9.8%	4.6%	8.8%
Widowed	5.9%	27.7%	29.4%
Number of children	7 (3)	6 (3)	6 (2)
Work			
Working	51.0%	46.2%	47.1%
Housewife	43.1%	43.1%	50.0%
Pensioner	5.9%	9.2%	2.9%
Pensioner and working	.0%	1.5%	.0%
Monthly income (RM)	510.2 (262.36)	440.3 (221.51)	386.4 (179.96)*
Source of income			
Pension	.0%	6.2%	2.9%
Salary	35.3%	35.4%	38.2%
Children	25.5%	41.5%	38.2%
Charity	2.0%	.0%	.0%
Others	37.3%	16.9%	20.6%
Duration of breast feeding			
< 1 year	.0%	1.5%	5.9%
1-2 years	94.1%	87.7%	76.5%
> 2 years	5.9%	10.8%	17.6%
Education level			
No education	21.6%	29.2%	44.1%
Primary School	29.4%	43.1%	52.9%
Secondary School	49.0%	26.2%	2.9%
Religious School	0%	1.5%	.0%
Family history of osteoporosis			
Yes	5.9%	10.8%	20.6%
No	94.1%	89.2%	79.4%
Smoking status			
Smoker	7.8%	7.7%	8.8%
Ex-smoker	.0%	6.2%	5.9%
Non-smoker	92.2%	86.2%	85.3%
Physical activity			
Sedentary	7.8%	16.9%	55.9%
Modest activity	76.5%	60.0%	26.5%
Active lifestyle	15.7%	21.5%	17.6%
Very active lifestyle	.0%	1.5%	.0%

Mean (SD)
* mean difference is significant at $p<0.05$ by one-way ANOVA, Tukey's test

the subjects have sufficient serum vitamin D level, while 82.9% were classified under Hypovitaminosis D category and another 0.7% have deficient serum vitamin D level or severe Hypovitaminosis D. There was no significant difference noted in the calcium and serum vitamin D level among all groups (Table 3).

Both of the CTx and NTx bone resorptions results were skewed toward the lower end of the range. The CTx marker produced normal distribution after logarithmic transformations. Both markers were highly correlated with each other ($r = 0.568, p<0.001$). The mean for both CTx and NTx level was significantly different ($p<0.05$) between pre and postmenopausal groups. The mean serum CTx of the subjects were 0.2833 (0.1769) ng/mL for premenopausal and 0.423 (0.2529) ng/mL and 0.510 (0.241) ng/mL for postmenopausal groups (A and B), respectively. The NTx value for premenopausal group was 15.203 (15.2025) nM BCE and for postmenopausal groups (A and B) were 17.900 (7.7959) and 19.351 (7.3775) nM BCE respectively, with the median of 19.929 nM BCE (Figures 1 and 2).

Simultaneous multiple regression was conducted to investigate the best predictors of CTx and NTx bone

TABLE 2. Anthropometric characteristics of subjects (*n*=150) (presented as mean (SD) or %)

Variables	Premenopause (*n*=51)	Postmenopause A (*n*=65)	Postmenopause B (*n*=34)
Height (cm)	151.0 (6.59)	150.4 (4.51)	148.2 (5.04)
Weight (kg)	59.6 (11.32)	59.2 (11.72)	55.2 (13.24)
BMI (kg/m^2)	26.1 (4.11)	26.2 (5.12)	24.9 (5.31)
BMI category			
Underweight[1]	2.0%	4.8%	5.9%
Normal[2]	40.0%	39.7%	52.9%
Overweight[3]	42.0%	31.7%	26.5%
Obese[4]	16.0%	23.8%	14.7%

Mean (SD)
[1] defined as BMI < 18.5 kg/m^2
[2] defined as BMI 18.5–24.9 kg/m^2
[3] defined as BMI 25–29.9 kg/m^2
[4] defined as BMI >30 kg/m^2

TABLE 3. Calcium and Vitamin D status of subjects (*n*=150) (presented as mean (SD) or %)

Variables	Premenopause (*n*=51)	Postmenopause A (*n*=65)	Postmenopause B (*n*=34)
Mean calcium intake (mg/day)	524.1 (285.80)	526.6 (344.76)	381.3 (285.40)
Mean serum vitamin D (nmol/L)	42.4 (6.79)	43.5 (6.98)	44.6 (7.35)
Vitamin D Status (%)			
Severe Hypovitaminosis D (< 25 nmol/L)	2.2	-	-
Hypovitaminosis D (25-49.99 nmol/L)	86.7	82.5	78.1
Sufficient (> 50 nmol/L)	11.1	17.5	21.9

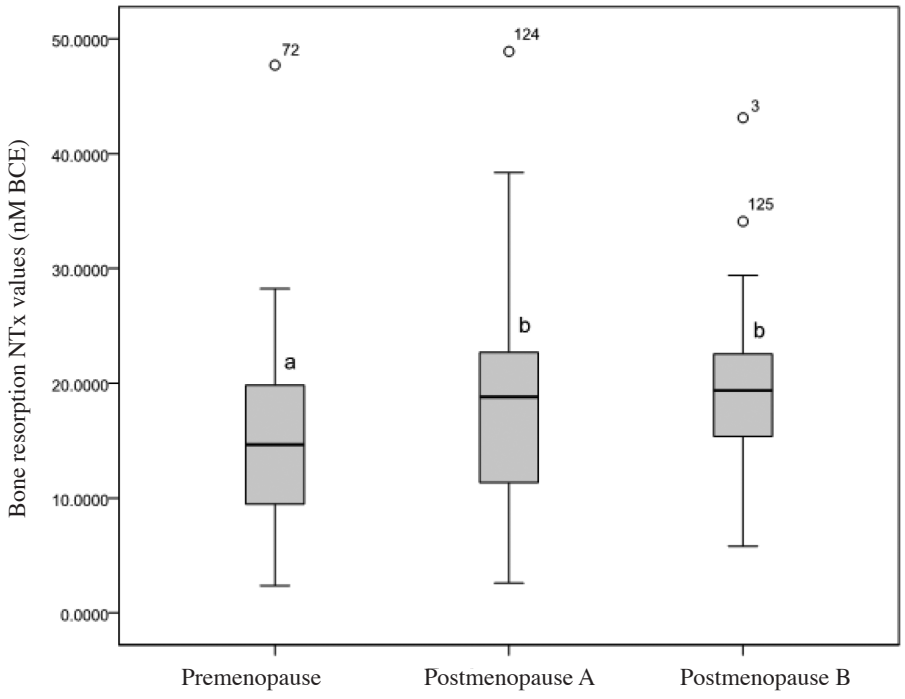

FIGURE 1. The differences between CTx bone resorption values for each groups (*n*=150)
a,b mean difference is significant at $p<0.05$ by one-way ANOVA, Tukey's test

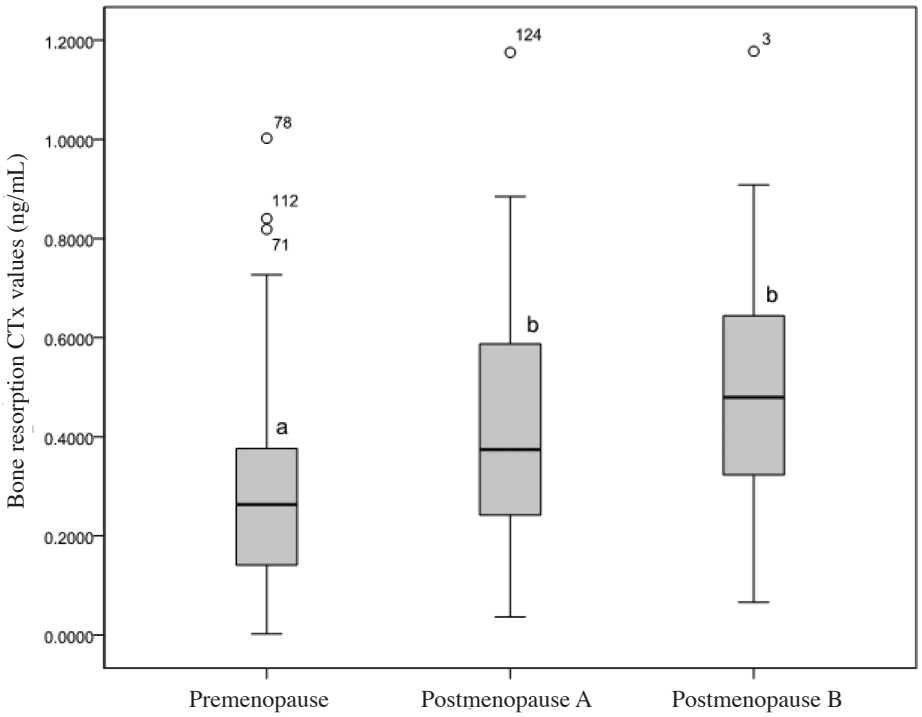

FIGURE 2. The differences between bone resorption NTx values for each groups (n=150)
a,b mean difference is significant at p<0.05 by one-way ANOVA, Dunnett C test

resorption markers in this population. Dummy variables were created for the categorical independent variables. The bone resorption markers were influenced by the duration of menopause, body mass index (BMI), physical activity, education level and marital status (Table 4).

DISCUSSION

In this study, the mean serum CTx of the subjects were 0.2833 (0.1769) ng/mL for premenopausal and 0.423 (0.2529) ng/mL and 0.510 (0.241) ng/mL for postmenopausal groups (A and B) respectively. This value is comparable to the values reported by few other studies (Adami et al. 2008; Garnero et al. 2001; Kruger et al. 2011; La-or Chailurkit et al. 2001). Adami et al. (2008) produced a reference range for CTx for healthy premenopausal women as 0.26 (0.13) ng/mL, which is very similar to our result (Adami et al. 2008). Studies of South African women by Kruger et al. (2011) found the CTx value of 0.29-0.56 ng/mL for healthy women above 45 years old. Although there are many other studies that showed the value of CTx in pre and postmenopausal women, comparisons could not be made as different units for CTx were used (Heikkinen et al. 1997; Papp et al. 2007; Parker et al. 2009). Enzymatic degradation type 1 collagen generates fragments with different molecular sizes and the immunoassays detected any fragments which contained 8 amino acid sequences used to raise the antibodies. This makes it impossible to determine the specific molecular weight involved, hence, conversion of units cannot be calculated and the units used in two assays system in detecting CTx cannot be compared (Garnero et al. 2001). The NTx bone resorption markers may be detected in both urine and serum, with many studies used the urine NTx as their marker. However, it was shown that both urine and serum NTx results were comparable to each other (Clemens et al. 1997). NTx level of premenopausal group was significantly lower compared to postmenopausal groups. Our results were comparable to those of Mexican women (12.2 and 17.9 nmol BCE for pre and postmenopausal women, respectively) (Scarianoa et al. 2001). A study by Yoshimura et al. (2011) showed that Japanese women had lower NTx level (11.8 (2.5) nmol BCE for premenopausal and 16.6 (3.7) nmol BCE for postmenopausal women), while Baca et al. (1999) showed that premenopausal Nigerian women had much higher NTx level (21.7 nmol BCE). This variations showed that different population needs different cut-off points to defined bone metabolism. This study showed that CTx produced less variation in the results. This is expected as serum CTx had been proposed as one of the most sensitive marker of bone resorption, apart from being more convenient to used (Chopin et al. 2011; Guillemant et al. 2003). Qvist et al. (2002) also showed that both short-term and long-term within subject variability of serum CTx is twice lower than urinary NTx markers.

This study demonstrates the important risk factors associated with the increase of bone resorption in this population. Previous studies had shown that due to many reasons such as genetics, culture, lifestyle and nutrient intakes, risk factors for osteoporosis are different for different populations (Khir et al. 2006; Lee & Khir 2007). The bone resorption markers in this population were

TABLE 4. Simultaneous multiple regression analysis summary in predicting factors influencing CTx and NTx bone resorption marker (n=150)

Variable	CTx			NTx		
	B	SEB	β	B	SEB	β
Age	.000	.004	.013	.000	.004	.013
Duration of menopause	.007	.002	.265*	.013	.005	.211*
Marital status						
Divorce	.069	.050	.103	-.234	2.460	-.008
Widow	-.110	.032	-.256**	-.253	.045	-.192**
Amount of monthly income	.000	.000	.839	.000	.000	.082
Education						
Primary	.018	.031	.052	.729	1.533	.046
Secondary	.134	.034	.037**	-1.427	1.533	-.082
BMI	-.008	.003	-.230*	-.017	.008	-.188*
Physical activity						
Modest activity	.002	.040	.005	.729	1.533	.046
Active activity	.083	.033	.235*	2.998	1.170	.215*
Calorie Intake	.001	.000	-.098	.000	.000	-.180
Serum vitamin D level	-.002	.002	-.083	-.006	.006	-.089
Calorie intake	.000	.000	-.014	.000	.000	.180
Calcium intake	-.047	.030	-.161	.000	.000	-.076
Family history of osteoporosis	-.042	.041	-.076	-2.045	2.019	-.082
Smoking status	.024	.024	.076	-2.883	1.774	-.132

B = Unstandardized coefficients
SEB = Standard error of B
β = Standardized coefficients (correlation coefficients)
** p<0.01, * p<0.05

influenced by the duration of menopause, body mass index (BMI), physical activity, education level and marital status.

The duration of menopause was one of the main factors that contributed to the increase of bone resorption. This factor of accelerated bone loss in women can be explained by the declined ovarian function after menopause. This leads to the decrease of estrogen concentration, resulting in the reduction of the function of osteoblast (Parker et al. 2009). The rate of bone loss was shown to be at five to tenfold higher at all sides after menopause (Nilas & Christiansen 1988). Our results showed no significant difference between both postmenopausal groups (A and B) which contradicts to our earlier opinion that the rate of bone loss accelerates during the first few years of menopause, then declines due to the gradual declines in ovarian functions (Khir et al. 2006; Parker et al. 2009). On the contrary, Nilas and Christiansen (1988) showed slightly larger bone loss did occurred at trabecular bones before and immediately after postmenopause, however, much larger bone loss occurred throughout the skeleton after menopause in general and not necessarily immediately after.

Comparable to our observations, studies on bone fracture often indicate that body mass index (BMI) was a protective factor against fracture (Cummings et al. 1995; Huguet et al. 2008; Jokinen et al. 2010; Perez et al. 1993). Previous study conducted in Malaysia showed that the body weight of fractured group was significantly lower compared with control group (Lim et al. 2005). Similar to this, a study on Turkish and Spanish women showed the relative risk was reduced by half for those with BMI more than 25 compared with those who are underweight (Perez et al. 1993).

Our results showed that subjects with active lifestyle have lower bone resorption values compared with those with modest and sedentary lifestyle. It has been well explained that under stress, more mineral salts will be deposited on bone as well as higher collagen fibers production by osteoblasts, thus making bone stronger. Hence, engaging oneself with weight-bearing activities such as walking will help retain and build bone mass, while lacking of physical activity leads to higher chances of developing osteoporosis (Tortora & Derrickson 2006). The Malaysian Dietary Guidelines (2010) stated that physical activity reduces the risk of osteoporosis by increasing the bone mineral growth during maturation, reducing the rate of bone mineral loss during aging, enhancing bone strength and reducing the risk of falling by improving muscle strength, flexibility, coordination and balance.

The guidelines also recommended 150 min of moderate-intensity or 75 min of vigorous intensity aerobic physical activity per week. Easy examples are 30 min brisk walk or raking leaves every day. Study by Loh et al. (2008) demonstrated that risk of fragility fracture is associated with those who does not engage in regular exercise of 3 times per week, with minimum of 30 min per session and Jokinen et al. (2010) reported that low physical activity is a significant predictor of bone fractures.

Our results suggested that there is a decrease of bone resorption values with an increase level of education. Similar relationship was observed in other study that relates a short period of education to the increase of hip fracture risk (Perez et al. 1993). A survey in American postmenopausal women also showed positive association between education and bone mineral density (Wang & Dixon 2006). Cutler and Lleras-Muney (2006) in their review on this topic had summed that the relationship between education and health is roughly linear after 10 years of school and an additional of one more year of education increases life expectancy by 0.18 years. This association might be explained by the differences in consumption of adequate nutrition and better medical service being sought by people with higher education level (Huguet et al. 2008; Xie et al. 2003).

We unexpectedly found a very significant correlation between marital status and the value of both bone resorption markers. Our results showed that widowed women have higher level of bone resorption compared with married and divorced subjects after adjustment of age. This finding is in line with a systematic review of the literature by Brennan et al. (2009) which showed a strong evidence for an association between being married or living with someone as being protective against osteoporotic fracture. Farahmand et al. (2000) also found marriage provides protective effect against hip fracture, but the reasons underlying this are still not clear. Avis et al. (1991) had listed few reasons relating the increased morbidity and mortality following widowhood which includes the development of unhealthy behaviour such as poor eating habits and leading sedentary lifestyle and higher rates of physiological symptoms due to the loss of an important source of social support.

The calcium intake for this population was low compared with Malaysian recommended nutrient intake (RNI), with a mean of 493 mg/day compared with the 1000 mg/day that was recommended (Ismail et al. 2005). This intake is similar to the intake of Chinese postmenopausal Malaysian women studied by Chee et al. (2002), which shows an intake of 447 (168) mg/day and 499 (211) mg/day from dietary records and FFQ, respectively. However, this is higher than the intake found by Suriah et al. (1996) on elderly population in peninsular Malaysia, which is extremely low, from a mean of 276.97 mg to 302.51 mg/day. None of the subjects have sufficient circulating level of 25(OH) D. Rahman et al. (2004) also found no sufficient vitamin D level among postmenopausal Malaysian women. The low calcium intake and vitamin D level in this population were expected to lead to higher bone resorption, however, we surprisingly did not find these variables to be the predictor of resorption markers. We do not have specific explanations for these findings. However, as our subjects hardly consumed enough calcium and none of them had sufficient level of serum vitamin D status, we could not test whether higher calcium intake and vitamin D level offer protection against bone resorption. There are quite a number of fairly large studies that found calcium and vitamin D provide no protective effect against fracture (Bischoff-Ferrari et al. 2007; Cauley et al. 2010; Cho et al. 2008; Lai et al. 2010). These conflicting results give rise to a need of more comprehensive studies to seek explanations for the association of calcium and vitamin D with bone resorption.

There are some limitations in the study. The sampling was done within 250 km radius from the university and did not include the outermost district in Kelantan. This is because the serums need to be processed on the same day and we did not have the facility to process it on-side. However, special considerations were taken in the sampling to include subjects from both rural and urban areas and subjects with various backgrounds to represent Kelantanese population. Apart from that, we only use 24 h diet recall to calculate the calcium intake, assuming the subjects have similar pattern of daily dietary intake. However, we used the food frequency questionnaire to validate the diet recall. Finally, due to cost constraint, we did not duplicate all of our samples for the bone resorption markers. Nevertheless, we make sure that both the intra-assay and inter-assay precision of the tests were below 10% for them to be valid.

Conclusion

This present study demonstrates that the mean for the resorption markers of premenopausal women was significantly lower than the postmenopausal groups. Comparing the CTx and NTx markers, CTx marker appears to be a more sensitive marker with lower variability. The markers were influenced by the duration of menopause, body mass index (BMI), physical activity, education level and marital status. Widowhood has an adverse effect on bone health. The reason underlying this factor is still unclear and further study is needed to investigate this matter. The modifiable risk factors are body mass index (BMI), physical activity and education level. Therefore, it is important to educate and increase cautiousness of the public towards maintaining a healthy body mass index and leading an active lifestyle to ensure good bone health status especially in older populations. Lifestyle intervention among this low income population might likely be an effective way to decrease health inequalities.

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REFERENCES

Adami, S., Bianchi, G. & Brandi, M.L. 2008. Bonturno study group determinants of bone turnover markers in healthy premenopausal women. *Calcif Tissue Int*. 82: 341-347.

Avis, N.E., Brambilla, J.D., Vass, K. & Mcinlay, J. 1991. The effect of widowhood on health: A prospective analysis from the Massachusetts women's health study. *Soc. Sci. Med*. 33: 1063-1070.

Baca, E.A., Ulibarri, V.A., Scariano, J.K., Ujah, I. & Bassi, A. 1999. Increased serum levels of N-telopeptides (NTx) of bone collagen in postmenopausal Nigerian women *Calcif Tissue Int*. 65: 125-128.

Bacon, W. & Hadden, W. 2000. Occurrence of hip fractures and socioeconomic position. *J. Aging Health* 12: 193-203.

Bischoff-Ferrari, H.A., Dawson-Hughes, B., Baron, J.A., Burckhardt, P., Li, R., Spiegelman, D., Specker, B., Orav, J.E., Wong, J.B., Staehelin, H.B., O'Reilly, E., Kiel, D.P. & Willett, W.C. 2007. Calcium intake and hip fracture risk in men and women: A meta-analysis of prospective cohort studies and randomized controlled trials. *Am. J. Clin. Nutr*. 86: 1780-1790.

Brennan, S.L., Pasco, J.A., Urquhart, D.M., Oldenburg, B., Hanna, F. & Wluka, A.E. 2009. The association between socioeconomic status and osteoporotic fracture in population-based adults: A systematic review. *Osteoporosis International* 20: 1487-1497.

Cassandra, A. & Thomas, A. 2001. The bone organ system: Form and function. 2nd ed. In *Osteoporosis*, edited by Robert, M. F., David, F. & Jennifer, K. Elsevier Inc.

Cauley, J.A., Parimi, N., Ensrud, K.E., Bauer, D.C., Cawthon, P.M., Cummings, S.R., Hoffman, A.R., Shikany, J.M., Barrett-Connor, E. & Orwoll, E. 2010. Serum 25-hydroxyvitamin D and the risk of hip and nonspine fractures in older men. *Journal of Bone and Mineral Research* 25: 545-553.

Caulfield, M. & Reitz, R. 2004. Biochemical markers of bone turnover and their utility in osteoporosis. *Med. Lab. Obs*. Apr 36: 34-37.

Chee, W., Suriah, A., Zaitun, Y., Chan, S., Yap, S. & Chan, Y. 2002. Dietary calcium intake in postmenopausal Malaysian women: Comparison between the food frequency questionnaire and three-day food records. *Asia Pac. J. Clin. Nutr.* 11: 142-146.

Cho, K., Cederholm, T. & Lo¨kk, J. 2008. Calcium intake in elderly patients with hip fractures. *Food & Nutrition Research* DOI: 10.3402/fnr.v52i0.1654.

Chopin, F., Biver, E., Funck-Brentan, T., Bouvard, B., Coiffier, G., Garnero, P. & Thomas, T. 2011. Prognostic interest of bone turnover markers in the management of postmenopausal osteoporosis. *Joint Bone Spine* DOI: 10.1016/j.jbspin.2011.05.004.

Clemens, J.D., Herrick, M.V., Singer, F.R. & Eyre, D.R. 1997. Evidence that serum NTx (collagen-type I Ntelopeptides) can act as an immunochemical marker of bone resorption. *Clinical Chemistry* 43: 2058-2063.

Cummings, S.R., Nevitt, M.C., Browner, W.S., Stone, K., Fox, K.M., Ensrud, K.E., Cauley, J., Black, D. & Vogt, T.M. 1995. Risk factors for hip fracture in white women. *The New England Journal of Medicine* 332: 767-773.

Cutler, D.M. & Lleras-Muney, A. 2006. Education and health: Evaluating theories and evidence. *NBER working paper series*. National Bureau of Economic Research.

Desai, M., Bhanuprakash, K. & Khatkhatay, M.U.D. 2007. Age-related changes in bone turnover markers and ovarian hormones in premenopausal and postmenopausal Indian women. *J Clin. Lab. Anal*. 21: 55-60.

Farahmand, B.Y., Perrson, P.G., Michealsson, K., Baron, J.A., Parker, M.G. & Ljunghall, S. 2000. Socioeconomic status, marital status and hip fracture risk: A population-based case-control study. *Osteoporos Int*. 11: 803-808.

Fox, M. & Cole, N. 2004. Nutrition and health characteristics of low-income populations: volume I, food stamp program participants and nonparticipants. Contract No.: E-FAN No. (04014-1).

Garnero, P., Borel, O. & Delmas, P.D. 2001. Evaluation of a fully automated serum assay for C-terminal cross-linking telopeptide of type I collagen in osteoporosis. *Clinical Chemistry* 47: 694-702.

Guillemant, J.A., Accarie, C.M., Gueronniere, V.D.L. & Guillemant, S.E. 2003. Different acute responses of serum type I collagen telopeptides, CTX, NTX and ICTP, after repeated ingestion of calcium. *Clinica. Chimica. Acta* 337: 35-41.

Guilley, E., Herrmann, F., Rapin, C-H., Hoffmeyer, P., Rizzoli, R. & Chevalley, T. 2011. Socioeconomic and living conditions are determinants of hip fracture incidence and age occurrence among community-dwelling elderly. *Osteoporosis International* 22: 647-653.

Heikkinen, A., Parviainen, M., Niskanen, L., Komulainen, M., Tuppurainen, M.T. & Kröger, H.S.S. 1997. Biochemical bone markers and bone mineral density during postmenopausal hormone replacement therapy with and without vitamin D3: A prospective, controlled, randomized study. *J. Clin. Endocr. Soc*. 82: 2476-2482.

Huguet, N., Kaplan, M.S. & Feeny, D. 2008. Socioeconomic status and health-related quality of life among elderly people: Results from the Joint Canada/United States survey of health. *Soc. Sci. Med*. 66: 803-810.

Ismail, N.M. & Technical Working Group on Nutritional Guidelines. 2005. Recommended Nutrient Intakes for Malaysia. *National Coordinating Committee on Food and Nutrition (NCCFN)*. Ministry of Health. Putrajaya.

Jokinen, H., Pulkkinen, P., Korpelainen, J., Heikkinen, J. & Keinanen-Kiukaanniemi, S. 2010. Risk factors for cervical and trochanteric hip fractures in elderly women: A population-based 10-year follow-up study. *Calcified Tissue International* 87: 44-51.

Khir, A.S., Pheng, C.S., Abdullah, B.J., Tiong, C.W., Goh, E., Ismail, F., Lee, H.F., Kiong, L.J., Weng, L.T., Hing, L.H., Mumtaz, M., Yusof, M.D.C., Ismail, N.M.N., Parameshwaran, K., Damodaran, P., Nazaruddin, G.R., Srinivas, P., Chee, W., Sim, Y.S. & Ali, Z.B.M. 2006. Clinical practice guidelines on management of osteoporosis 2006. Malaysian Osteoporosis Society.

Kraenzlin, M. & Seibel, M. 2006. Measurement of biochemical markers of bone resorption, In *Dynamics in Bone and*

Cartilage Metabolism, edited by Seibel, M., Robins, S. & Bilezikian, J. San Diego, CA: Academic Press.

Kruger, M.C., Kruger, L.M., Wentzel-Viljoen, E. & Kruger, A. 2011. Urbanization of black South African women may increase risk of low bone mass due to low vitamin D status, low calcium intake, and high bone turnover. *Nutrition Research* 31: 748-758.

Lai, J.K.C., Lucas, R.M., Clements, M.S., Roddam, A.W. & Banks, E. 2010. Hip fracture risk in relation to vitamin D supplementation and serum 25-hydroxyvitamin D levels: A systematic review and meta-analysis of randomised controlled trials and observational studies. *BMC Public Health* 10: 331.

La-or Chailurkit, B.O., Piaseu, N., Saetung, S. & Rajatanavin, R. 2001. Biochemical markers of bone turnover and response of bone mineral density to intervention in early postmenopausal women: An experience in a clinical laboratory. *Clin. Chem.* 47: 1083-1088.

Lau, E.M.C., Lee, J.K., Suriwongpaisal, P., Saw, S.M., De, S.D., Khir, A. & Sambrook, P. 2001. The incidence of hip fracture in four asian countries: The asian osteoporosis study (AOS) *Osteoporos Int*. 12: 239-243.

Lee, J.K. & Khir, A.S.M. 2007. The incidence of hip fracture in Malaysians above 50 years of age: Variation in different ethnic groups. *APLAR Journal of Rheumatology* 10: 300-305.

Lim, P., Ong, F., Adeeb, N., Seri, S., Noor-Aini, M., Shamsuddin, K., Hapizah, N., Mohamed, A., Mokhtar, A. & Wan, H. 2005. Bone health in urban midlife Malaysian women: Risk factors and prevention. *Osteoporos. Int*. 16: 2069-2079.

Loh, K., Shong, K., Lan, S., Lo, W. & Woon, S. 2008. Risk factors for fragility fracture in Seremban district, Malaysia: A comparison of patients with fragility fracture in the orthopedic ward versus those in the outpatient department. *Asia Pac. J. Public Health* 20: 251-257.

Nilas, L. & Christiansen, C. 1988. Rates of bone loss in normal women: Evidence of accelerated trabecular bone loss after the menopause. *Eur. J. Clin. Invest*. 18: 529-534.

Malaysian Dietary Guidelines. 2010. *Technical Working Group on Nutritional Guidelines*. Ministry of Health. Putrajaya.

Mithal, A., Wahl, D.A., Bonjour, J.P., Burckhard, P., Dawson-Hughes, B., Eisman, J.A., El-Hajj Fuleihan, G., Josse, R.G., Lips, P. & Morales-Torres, J. IOF Committee of Scientific Advisors (CSA) Nutrition Working Group. 2009 Global vitamin D status and determinats of hypovitiminosis D. *Osteoporosis International* 20(11): 1807-1820.

Papp, A.E.D., Bone, H.G., Caulfield, M.P., Kagan, R., Buinewicz, A., Chen, E., Rosenberg, E. & Reitz, R.E. 2007. A cross-sectional study of bone turnover markers in healthy premenopausal women. *Bone* 40: 1222-1230.

Parakrama, C. & Clive, R. 1998. *Concise Pathology*. 3rd ed. Appleton & Lange.

Parker, N., Bugdayci, D., Tekdos, D. & Dere, C.B.K. 2009. Relationship between bone turnover and bone density at the proximal femur in stroke patients. *Journal of Stroke and Cerebrovascular Diseases* 18: 139-143.

Perez, R.C., Galan, F.G. & Dilsen, G. 1993. Risk factors for hip fracture in Spanish and Turkish women. *Bone* 14: 69-72.

Qvist, P., Christgau, S., Pedersen, B.J., Schlemmer, A. & Christiansen, C. 2002. Circadian variation in the serum concentration of C-terminal telopeptide of type I collagen (Serum CTx): Effects of gender, age, menopausal status, posture, daylight, serum cortisol, and fasting. *Bone* 31: 57-61.

Rahman, S.A., Chee, W., Yassin, Z. & Chan, S. 2004. Vitamin D status among postmenopausal Malaysian women. *Asia Pac. J. Clin. Nutr.* 13: 255-260.

Scarianoa, J.K., Garry, P.J., Montoya, G.D., Wilson, J.M. & Baumgartner, R.N. 2001. Critical differences in the serial measurement of three biochemical markers of bone turnover in the sera of pre- and postmenopausal women. *Clin. Biochem*. 34: 639-644.

Suriah, A., Zainorni, M., Shafawi, S., Suraya, S.M., Zarina, N., Zainuddin, W.W. & Zalifah, M. 1996. Nutrient intake among elderly in southern Peninsular Malaysia. *Mal. J. Nutr*. 2: 11-19.

Tortora, G. & Derrickson, B. 2006. *Principles of Anatomy and Physiology*. 11th ed. New Jersey: John Wiley & Sons, Inc.

Wang, M.C. & Dixon, L.B. 2006. Socioeconomic influences on bone health in postmenopausal women: Findings from NHANES III, 1988-1994. *Osteoporos Int*. 17: 91-98.

Xie, B., Gillilanda, F.D., Li, Y.F. & Rockett, H.R. 2003. Effects of ethnicity, family income, and education on dietary intake among adolescents. *Preventive Medicine* 36: 30-40.

Yoshimura, N., Muraki, S., Oka, H., Kawaguchi, H., Nakamura, K. & Akune, T. 2011. Changes in serum levels of biochemical markers of bone turnover during 10 years among Japanese men and women: Associated factors and birth-cohort effect. The Taiji Study. *The Japanese Society for Bone and Mineral Research* 29: 699-708.

Zingmond, D.S., Soohoo, N.F. & Silverman, S.L. 2006. The role of socioeconomic status on hip fracture. *Osteoporos Int*. 17: 1562-1568.

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