Serum bone specific alkaline phosphatase and urinary deoxypyridinoline in postmenopausal osteoporosis

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Abstract

The objectives of this study were to: (i) evaluate the diagnostic sensitivity and specificity of the biochemical bone markers: serum total alkaline phosphatase (TALP), bone specific alkaline phosphatase (BSALP) and urinary deoxypyridinoline (Dpyr) in postmenopausal osteoporosis, (ii) compare the bone tumover of postmenopausal osteoporotic patients without and with hormone replacement therapy (HRT) against controls and (iii) identify the correlation between these bone markers and bone mineral density (BMD). We examined 42 postmenopausal women with BMD proven osteoporosis and 35 control subjects. Serum TALP, BSALP and urinary Dpyr were measured. All three biochemical bone markers showed comparable moderate diagnostic sensitivity but Dpyr had the highest diagnostic specificity. There were significantly higher serum TALP, BSALP and urinary Dpyr levels in non-HRT treated patients compared to controls ($p<0.005, <0.0001$ and <0.005 respectively). There were no significant differences in the levels of all three bone markers between HRT treated patients and control subjects. There was no significant correlation between TALP, BSALP or Dpyr and BMD in both controls and patients. In conclusion, the biochemical bone markers are not useful in diagnosis of postmenopausal osteoporosis but may have a role in monitoring progress and response to treatment. HRT treatment reduces bone tumover of postmenopausal osteoporosis.

Key words: Biochemical bone markers, bone specific alkaline phosphatase, deoxypyridinoline, postmenopausal osteoporosis.

INTRODUCTION

Osteoporosis is a common metabolic bone disease affecting postmenopausal women causing significant morbidity and mortality. The clinical usefulness of the newer biochemical bone markers in osteoporosis is still not well established. Serum total alkaline phosphatase (TALP) is the most commonly used marker of bone formation but it lacks sensitivity and specificity. Bone and liver alkaline phosphatase are the same gene product but differ in post-translational m cations. Bone specific alkaline phosphatase (BSALP) is a well established marker of bone formation even though its role in bone formation is uncertain.[1] BSALP is found in the plasma membrane of osteoblasts which is released into the circulation by an unknown mechanism.[1,2] It is a sensitive marker of increased bone turnover at the time of menopause with a mean increase that is significantly greater than that of TALP.[2] Urinary Dpyr, a marker of bone

resorption has been shown to increase by 50% to 100% at the time of menopause and revert to premenopausal levels under oestrogen therapy by three to six months.[2,3,4] Dpyr:BSALP ratio was shown to differentiate the untreated multiple myeloma subgroup from control subjects more significantly compared to Dpyr alone suggesting the uncoupling phenomenon in this condition.[5] Although the net increment in bone resorption in postmenopausal osteoporosis is well described, the bone resorption : bone formation indices in this condition is still not clear.

Oestrogen deficiency is a dominant factor in the pathogenesis of postmenopausal osteoporosis, hence, hormone replacement therapy (HRT) is effective in maintaining bone mass.[6] Oestrogen is believed to have a direct anti-resotptive effect on bone. The exact mechanism by which this occurs remains unclear. There is evidence that oestrogen receptors are present in osteoblast-like cells.[7] HRT may decrease bone loss to approximately 0.5% per year.[8] HRT may

decrease the fracture rate by 50 to 75% in postmenopausal women.[6] Bone mineral content is maintained when postmenopausal women receive HRT whereas it declines significantly in postmenopausal women who are not using HRT during the same period of time.[8]

Measurement of bone mineral density (BMD) is a useful tool in diagnosing osteoporosis and to predict fracture risk. A decrease in bone density by one standard deviation has been shown to increase the incidence of fracture by 50% to 60%. However, BMD is not able to detect change in bone turnover within a short period, expensive and not widely available. Currently there is no single biochemical bone marker in blood or urine available that can definitely establish the differential diagnosis of metabolic bone disease? The importance of the biochemical markers is associated with their ability to reflect small changes in bone turnover that is impossible to detect radiographically which takes months to visualise even with the best radiographic methods. The clear utility of the biochemical markers therefore lie in the ability to reflect very subtle but meaningful changes in bone turnover in a time frame not possible with radiographic methods.[9]

The objectives of this study were to: (i) evaluate the diagnostic sensitivity and specificity of the biochemical bone markers: serum total alkaline phosphatase (TALP), bone specific alkaline phosphatase (BSALP) and urinary deoxypyridinoline (Dpyr) in diagnosing postmenopausal osteoporosis, (ii) compare the bone turnover of postmenopausal osteoporotic patients without and with hormone replacement therapy (HRT) against controls and (iii) identify the correlation between these bone markers and bone mineral density (BMD).

MATERIALS AND METHODS

Patients
This was a cross-sectional case control study. Patients were postmenopausal women attending the menopausal clinics at a teaching hospital. Patients who fulfilled the set criteria were recruited and underwent a BMD measurement. Informed consent was obtained from all patients. Osteoporosis was defined according to the WHO criteria i.e. BMD of less than 2.5 standard deviation below the young adult mean reference value. Patients with osteoporosis were further divided into those without and with HRT. The inclusion criteria were (i) menopausal women who had cessation of menses for at least one year duration and (ii) age of ≥ 50 years. The exclusion criteria were (i) metabolic diseases eg. hyperparathyroidism, hypoparathyroidism, hyperthyroidism and diabetes mellitus. (ii) autoimmune diseases e.g. systemic lupus erythematosus and rheumatoid arthritis, (iii) surgery eg., gastrointestinal resection or malabsorption. (iv) liver or renal diseases, (v) drugs which may affect bone metabolism eg. glucocorticoids, aluminium containing antacids, frusemide, bisphosphanate, calcium, vitamin A, vitamin D, calcitonin. lithium, antiepileptics and anticoagulant. (vi) hypercalcaemia of malignancy, (vii) chronic granulomatous disease eg. sarcoidosis and tuberculosis, (vii) Paget's disease of bone, (viii) cigarette smoking (ix) alcohol abuse (>14 units/week) and (x) history of recent fractures (in the preceeding six months).

Controls
This group was recruited from postmenopausal women attending the menopausal clinic with normal BMD, defined as BMD of more than or equal to one standard deviation below that of young adults (T score ≤ -1.0). They were also matched for age, ethnicity, duration of menopause and body mass index (BMI). The inclusion and exclusion criteria were similar to that for patients. In addition, those on HRT were excluded from the control group.

A total of 77 postmenopausal women were recruited; 35 were control subjects and 42 were patients with osteoporosis. Of the 42 patients, 32 were not and 10 were on HRT. The height and weight were measured to determine the body mass index (BMI). BMI was calculated by dividing the weight in kilogram by the square of height in meter.

Samples
Serum and plasma
10ml non-fasting random venous samples were collected. Care was taken to avoid haemolysis. The blood was allowed to clot and serum was separated immediately. Serum aliquots were taken for renal profile, calcium, phosphate, BSALP and liver function tests which included TALP and albumin. The serum samples were stored frozen at -20°C and thawed prior to analyses. At the same time, 2ml of random venous samples were collected in fluoride-oxalate tubes, plasma was separated and analysed for random plasma glucose on the same day.

Urine

30 ml of non-fasting, early morning, second voided urine were collected and stored frozen at -20°C and thawed prior to analysis. The urine were analysed for free Dpyr cross-links and creatinine.

Bone mineral density

BMD was measured by a DEXA scan (Norland XR26). The areas measured for BMD were femoral neck (BMD-FN) and lumbar spine (BMD-LS). The results were expressed as BMD (g/cm2)

Serum TALP

The TALP method was performed according to the recommended reference method of the International Federation of Clinical Chemistry (IFCC). This method utilises 4-nitrophenyl phosphate (4-NPP) as substrate and 2-Amino-2-methyl-l-propanol (2A2M1P) as phosphate acceptor buffer. This assay was performed on a Cobas IntegraTM analyser (Roche Diagnostics). The inter and intra-assay coefficient variations (CV) were both 1.2 - 1.3%

Serum BSALP

The BSALP was measured by an enzyme immunoassay method (Alkphase-B, Metra Biosystems). This method utilises monoclonal anti-BSALP antibody coated on a microtitre plate to capture BSALP in the serum sample. The enzyme activity of the captured BSALP was detected with a p-nitrophenyl phosphate (PNPP) substrate and the optical density was read at 405nm. Each sample was assayed in duplicate. The intra-assay CVs were 5.0-5.4% and the inter-assay CVs were 1.1-1.8%.

Urinary Dpyr

The Dpyr was measured by a competitive enzyme immunoassay (Pyrilinks-D, Metra Biosystems). This method utilises monoclonal anti-Dpyr antibody coated on a microtitre plate to capture Dpyr. The Dpyr in the urine sample competes with conjugated Dpyr-alkaline phosphatase for the antibody and the reaction is detected with pNPP substrate. The optical density was read at 405nm. Each sample was assayed in duplicate. The intra-assay CVs were 2-7% and the inter-assay CVs were 3-6%. The Dpyr levels (nmol/L) were corrected for urinary concentration by urinary creatinine measurement (mmol/L) and expressed as Dpyr/creatinine index (nmol/mmol).

Urinary creatinine

The urinary creatinine was determined by the buffered kinetic 'Jaffe' reaction without deproteinization method. Creatinine in the urine reacts with picric acid to form yellow-red complex. The rate of the dye formation is directly proportional to the creatinine concentration in the urine which was determined by measuring the increase in absorbance at 512nm. The inter-assay CVs were 1.0-1.5%.

Receiver-operating characteristic (ROC) curve

Diagnostic sensitivity is defined as the number of true positives in all individuals with disease and diagnostic specificity is defined as the number of true negatives in all individuals without disease. ROC curve was obtained by plotting diagnostic sensitivity versus specificity at various cut-off levels of each biochemical bone marker.

Statistics'

The differences between two populations were compared by unpaired t-test for variables with normal distribution and by Mann-Whitney U test for variables which were not distributed normally. The correlation between two variables was measured using the Pearson correlation coefficient for parametric variables and Spearman correlation coefficient for non-parametric variables or when the sample size was less than 30. The multivariate analyses were performed using linear regression. Probability values (p) of less than 0.05 were considered significant. All statistical analyses were performed using the SPSS™ statistical software on an IBM compatible computer.

RESULTS

1. Characteristics of study subjects

The distribution of age, ethnicity, BMI, duration of menopause and HRT treatment is shown in Table 1. There were no significant differences in mean age, BMI and duration of menopause between patients and controls.

2. Comparison of serum TALP, BSALP and urinary Dpyr in postmenopausal women with and without osteoporosis

The mean serum TALP, BSALP, urinary Dpyr levels and Dpyr:BSALP ratios in patients and controls are shown in Table 2. The distribution of serum TALP, BSALP and urinary Dpyr in all patients, non HRT treated patients, HRT treated patients and controls are shown in figures 1, 2 and 3.

TABLE 1: Clinical characteristics of patients and controls

	Controls	Patients - total	Patients - not on HRT	Patients - on HRT
n	35	42	32	10
Age (years)	57.5 ± 0.7	58.5 ± 0.8	59.6 ± 0.9	55.2 ± 1.0
BMI	26.9 ± 0.4	25.9 ± 0.5	26.2 ± 0.6	25.1 ± 0.8
Duration of menopause (years)	7.1 ± 1.2	8.7 ± 0.9	9.7 ± 1.2	5.5 ± 0.5
Duration of HRT (years)	_	_	_	3.6 ± 0.4
Ethnicity:				
Malays	27	34	31	3
Chinese	4	4	0	4
Indians	4	4	1	3

Data shows n and mean ± SEM

TABLE 2: The mean ± SEM for TALP, BSALP, Dpyr and **Dpyr:BSALP** ratio for each group

	Controls (n = 35)	Patients - total (n = 42)	Patients - not on HRT (n = 32)	Patients - on HRT (n = 10)
TALP **(iu/L)**	52.8 ± 2.0	65.3 ± 3.4 [a]	65.9 ± 3.9 [a]	63.4 ± 7.4
BSALP **(iu/L)**	13.6 ± 0.8	19.1 ± 1.1[b]	19.8 ± 1.3[b]	16.9 ± 1.6
Dpyr (nmol/mmol)	5.7 ± 0.3	7.1 ± 0.3[a]	7.3 ± 0.4[a]	6.2 ± 0.6
Dpyr : BSALP ratio	0.44 ± 0.02	0.39 ± 0.02	0.39 ± 0.02	0.39 ± 0.03

[a] $p < 0.005$ vs. control
[b] $p < 0.0001$ vs. control

2.1 Comparison between all patients and controls.

The following biochemical bone markers were significantly higher in all patients compared to the controls (i) TALP $(p<0.005)$, (ii) BSALP $(p<0.0001)$ and (iii) Dpyr $(p<0.005)$. There was no significant difference in the Dpyr : BSALP ratio $(p>0.05)$ between the two groups.

2.2 Comparison between non-HRT treated patients and controls.

The levels of the following biochemical bone markers were significantly higher in non-HRT treated patients compared to the controls (i) TALP $(p<0.005)$, (ii) BSALP $(p<0.0001)$ and (iii) Dpyr $(p<0.005)$. There was no significant difference in the Dpyr : BSALP ratio $(p>0.05)$ between the two groups.

2.3 Comparison between the HRT treated patients and controls

There were no significant\differences in TALP $(p>0.05)$, BSALP $(p>0.05)$, Dpyr $(p>0.05)$ and **Dpyr:BSALP** ratio $(p>0.05)$ between the two groups.
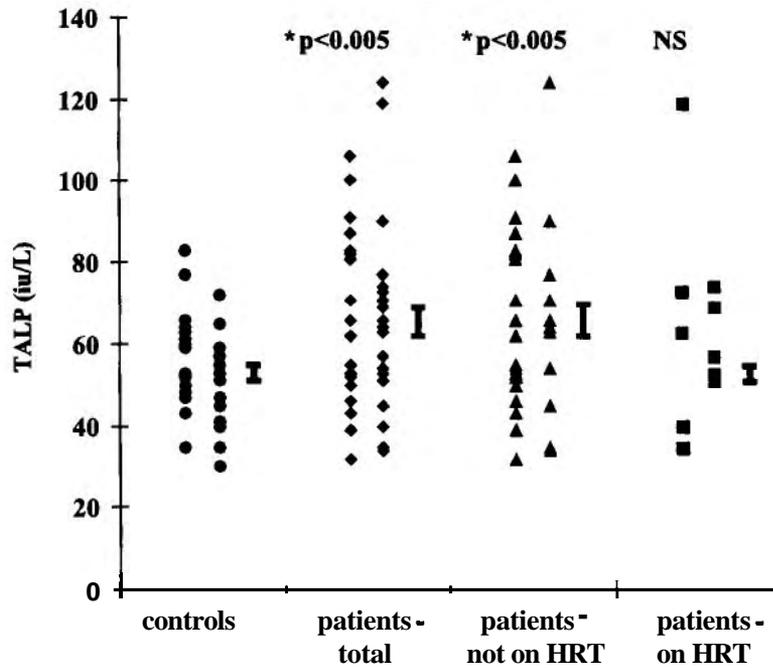
Fig. 1: Distribution of serum TALP levels in each group

Each bar represents mean ± SEM
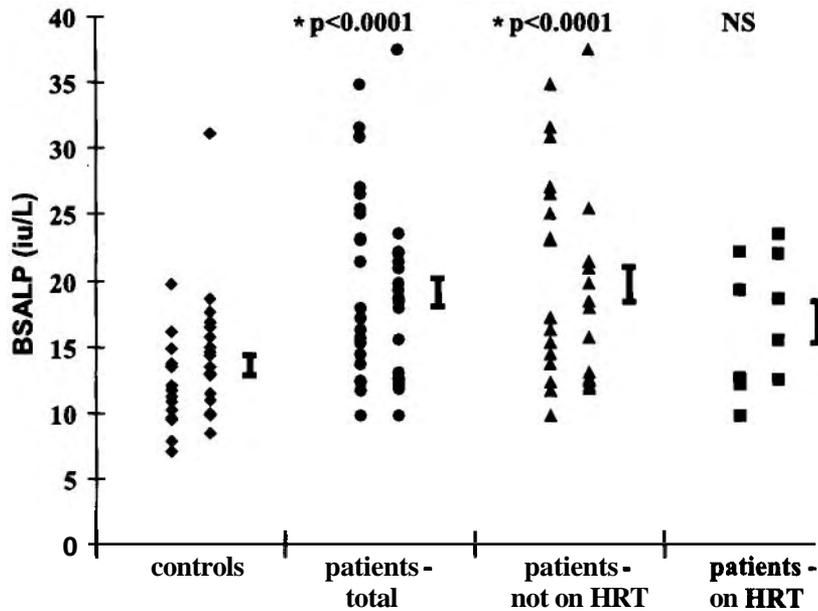*indicates level of significance compared to controls

Fig. 2: The distribution of serum BSALP levels in each group

Each bar represents mean ± SEM
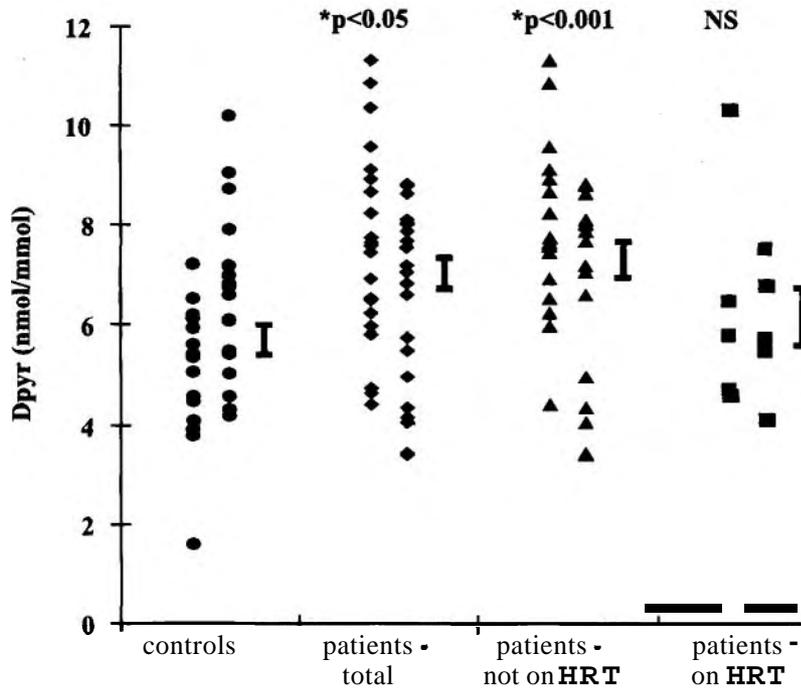*indicates level of significance compared to controls

Fig. 3: The distribution of urinary Dpyr levels in each group

Each bar represents mean ± SEM
*indicates level of significance compared to controls

3. Evaluation of the diagnostic sensitivity and specificity of serum TALP, BSALP and urinary Dpyr in diagnosing postmenopausal osteoporosis.

The diagnostic sensitivity versus specificity for each biochemical bone marker in diagnosing postmenopausal osteoporosis at various cut-off levels are shown figures 4, 5 and 6.

The receiver-operating characteristic (ROC) curve for TALP showed that the best diagnostic sensitivity and specificity for serum TALP was obtained at cut-off level of 55iu/L. The sensitivity and specificity of serum TALP at this level were 66% and 63% respectively (Figure 4).

The best diagnostic sensitivity and specificity for serum BSALP was obtained at cut-off level of 15iu/L. The sensitivity and specificity of serum BSALP at this level were 69% and 71% respectively (Figure 5).

The best diagnostic sensitivity and specificity for urinary Dpyr was obtained at cut-off level of 7nmol/mmol. The sensitivity and specificity of urinary Dpyr at this level were 66% and 80% respectively (figure 6).

4. Correlation between the various biochemical bone markers and BMD

The correlations between TALP, BSALP, Dpyr, Dpyr:BSALP ratio and BMD in controls and patients are shown in Table 3.

4.1 Control group

There were significant positive correlation between (i) serum TALP and BSALP levels, (ii) serum BSALP and urinary Dpyr levels and (iii) urinary Dpyr levels and Dpyr:BSALP ratio but si cant negative correlation was seen between serum BSALP levels and Dpyr:BSALP ratio. There was no correlation between all three biochemical bone markers and the BMD.

4.2 All patients group

There were significant positive correlation between (i) serum TALP and BSALP levels, (ii) serum TALP and urinary Dpyr levels and (iii) serum BSALP and urinary Dpyr levels. Significant negative correlations were seen between (i) serum TALP levels and Dpyr:BSALP ratio and (ii) serum BSALP levels and Dpyr:BSALP ratio. There was no correlation between all three biochemical bone markers and the BMD.
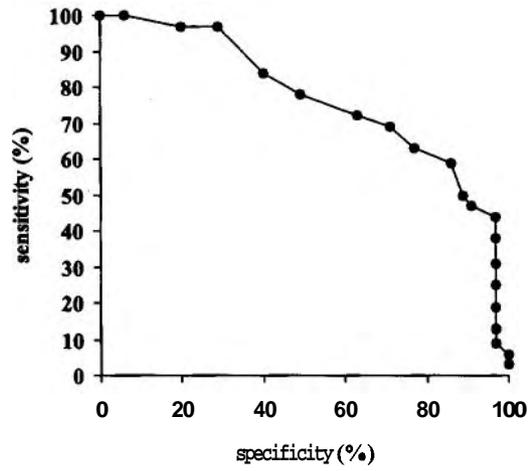
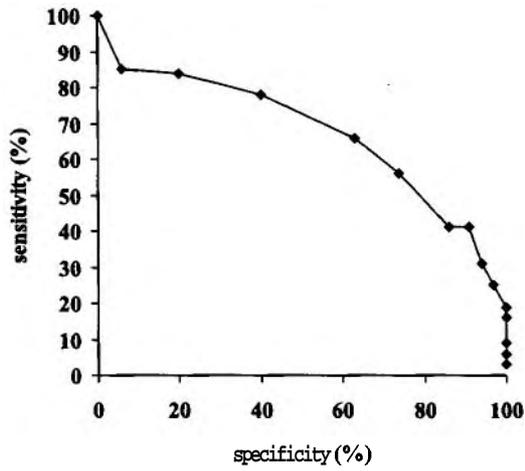**Fig. 4: The ROC curve for serum TALP in diagnosing postmenopausal osteoporosis**

Fig. 5: The ROC curve for serum BSALP in diagnosing postmenopausal osteoporosis
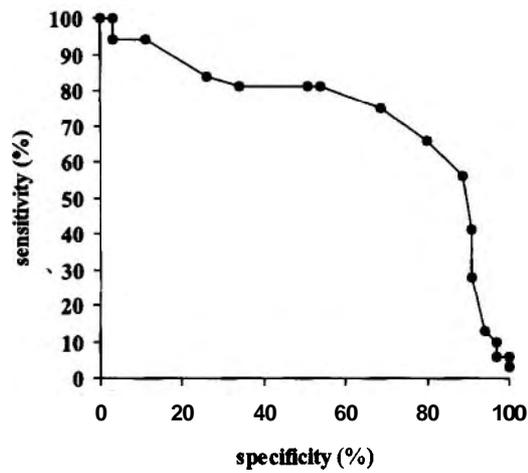

Fig. 6: The ROC curve for urinary Dpyr in diagnosing postmenopausal osteoporosis

TABLE 3: Correlation between TALP, BSALP, Dpyr, Dpyr:BSALP ratio and BMD in each group

(a) Controls (n=35)						
	TALP (iu/L)	BSALP (iu/L)	Dpyr (nmol/mmol)	Dpyr: BSALP ratio	BMD-FN (g/cm²)	BMD-LS (g/cm²)
TALP (iu/L)						
BSALP (iu/L)	r=0.352 p<0.05					
Dpyr (nmol/mmol)	NS	r=0.464 p=0.005				
Dpyr: BSALP ratio	NS	r=-0.453 p<0.01	r=0.536 p=0.001			
BMD - FN (g/cm²)	NS	NS	NS	NS		
BMD - LS (g/cm²)	NS	NS	NS	NS	r=0.536 p=0.001	
(b) All patients - total (n=42)						
	TALP (iu/L)	BSALP (iu/L)	Dpyr (nmol/mmol)	Dpyr: BSALP ratio	BMD-FN (g/cm²)	BMD-LS (g/cm²)
TALP (iu/L)						
BSALP (iu/L)	r=0.786 p<0.0001					
Dpyr (nmol/mmol)	r=0.565 p<0.0001	r=0.667 p<0.0001				
Dpyr: BSALP ratio	I=-0.413 p<0.005	r=-0.557 p<0.0001	NS			
BMD - FN (g/cm²)	NS	NS	NS	NS		
BMD - LS (g/cm²)	NS	NS	NS	NS	r=0.534 p<0.0001	

NS - indicates no significant correlation (p>0.05)

5. Multivariate analyses

5.1 Control group.

When age, duration of menopause and BMI were considered simultaneously in a multiple regression model, the independent predictor for urinary Dpyr was age. None of the variables were independent predictor for serum TALP and BSALP. Neither serum TALP, BSALP or urinary Dpyr was an independent predictor for BMD.

5.2 Non HRT treated osteoporotic patients

Neither age, duration of menopause or BMI was an independent predictor of serum TALP, BSALP or urinary Dpyr. None of the three biochemical bone markers were independent predictors of BMD.

5.3 HRT treated osteoporotic patients

Neither age, duration of menopause or BMI was an independent predictor of serum TALP,

BSALP or urinary Dpyr. None of the three biochemical bone markers were independent predictors of BMD.

DISCUSSION

The diagnostic sensitivity and specificity of serum TALP, BSALP and urinary Dpyr were generally low and comparable to each other in diagnosing osteoporosis in postmenopausal women. However, amongst the three biochemical bone markers studied, urinary Dpyr had the highest specificity but all three biochemical bone markers showed moderate, comparable sensitivity in diagnosing postmenopausal osteoporosis. From the distribution pattern of all three biochemical bone markers in each subject group (Figures 1, 2, 3), there was a considerable overlap in the levels of each bone marker between the patients and controls. This has definitely affected the sensitivity and specificity of these biochemical bone markers in diagnosing postmenopausal osteoporosis. From this study, none of the three biochemical bone markers can be used singly or in combination to diagnose postmenopausal osteoporosis. Furthermore the sensitivity of **66%** and specificity of **63%** achieved by serum TALP at cut-off level of 55 **iu/L** is absolutely not useful in diagnosing postmenopausal osteoporosis as serum TALP of 55 **iu/L** is within the reference range. This study confirms that in postmenopausal osteoporosis serum TALP levels are within reference range, thus it is not useful in diagnosing postmenopausal osteoporosis. This is in keeping with other studies which showed that patients with vertebral osteoporosis, serum TALP levels are either normal or slightly elevated and correlated **poorly** with bone formation determined by iliac crest histomorphometry.[2] Postmenopausal osteoporosis is osteoclast-mediated and is characterised by rapid bone loss.' Although there is a concomitant increment in TALP and BSALP in postmenopausal osteoporosis as a secondary response to elevated bone resorption, it is insufficient for that degree of bone resorption. This results in an imbalance between bone resorption and bone formation resulting in the disease state. Although this study had shown strong positive correlation between serum BASLP and urinary Dpyr, and absence of the uncoupling phenomenon, the Concomitant increment in serum BSALP levels was probably still insufficient for the degree of enhanced bone resorption. In addition, the urinary Dpyr concentrations measured in this study was the free cross-links form. Even though the free form correlates highly with total cross-links measurements in normal **adults,**[1,2] this relationship is still unclear in osteoporosis.

It is important to note that there is a large variability between analytical methods **and** standard from laboratory to **laboratory.**[10] High intraindividual and interindividual variability and large discrepancy in normal values make it difficult to identify individuals who may be at risk for **osteoporosis.**[10,11] Jensen et *al* concluded that large **biological** variability in the biochemical markers of bone turnover make them unsuitable for diagnosis of osteoporosis and for prediction of future bone loss in individual patients.''

Even though biochemical bone markers have limited utility in diagnosing osteoporosis, it has been used in estimating the rate of bone loss and response to anti-resorptive treatment. In addition, bone mineral density measurement does not provide information on the rate of bone loss over a short period of **time.**[12,13] Uebelhart et *al* has shown that the combination of a single measurement of serum osteocalcin, urinary hydroxyproline and Dpyr performed in early postmenopausal women was correlated to the rate of bone **loss.**[4]

This study confirms that biochemical bone markers are not useful in diagnosing postmenopausal osteoporosis. However, serial measurement of these biochemical bone markers may have potential use in monitoring disease progression, response to treatment, hence tailoring individual treatment. They may also have a role in predicting those at higher risk of developing subsequent postmenopausal osteoporosis.

The significantly higher levels of all three biochemical bone markers; serum TALP, BSALP and urinary Dpyr in the all patient group compared to controls indicated that there was an increased bone turnover in postmenopausal women with osteoporosis. The serum TALP, BSALP and urinary Dpyr levels were significantly higher in osteoporotic patients who were not on HRT compared to controls. In contrast, there were no **significant** difference in serum TALP, BSALP and **urinary** Dpyr between HRT treated patients compared to controls. These findings are in agreement with previous studies which showed that HRT reduces bone turnover and reverts the biochemical bone markers to premenopausal **levels.**[2,3,4] In addition, these findings suggested that oestrogen deficiency lead to the increased bone turnover in patients who were not on HRT. Although the means of all

three biochemical bone markers were higher in non-HRT treated than HRT treated patients, they did not reach statistical significance. This may in part be attributed to the small number of HRT treated osteoporotic patients recruited in this study. In addition, the wide SEM may have rendered the statistical insignificance.

The insignificant difference in the Dpyr:BSALP ratio between patients who were not on HRT and the control subjects indicated that there was absence of the uncoupling of the resorption : formation phenomenon in postmenopausal osteoporosis. This suggests that the increased bone resorption in untreated patients has resulted in a concomitant increment in bone formation. This relationship could also explain the positive significant correlation between serum BSALP and urinary Dpyr in the untreated patients.

Serum BSALP correlated positively and significantly with serum TALP in the untreated patients i.e. the increment in serum BSALP levels has resulted in a parallel increment of serum TALP. The contribution of liver alkaline phosphatase isoenzymes to significant increment of serum TALP was excluded by normal liver function tests. Therefore, in these circumstances the increment in serum BSALP is reflected by an elevation of TALP. The significant contribution of BSALP in the TALP explained the significant negative correlation between serum TALP levels and Dpyr:BSALP ratio.

There was poor correlation between serum TALP, BSALP or urinary Dpyr with the bone mineral density in the all patient group. The changes in bone mineral density is small (1% to 2% per year) and subtle and may occur over a longer period of time.[4] Appreciable and distinct changes in BMD using absorptiometry methods can be seen after one year of disease progression or therapy[9,13] but the activity or concentration of these three biochemical bone markers vary widely over a shorter time span especially in the presence of disease progression or interventive treatment.[2,4] However, Uebelhart *et al* found a significant correlation between BMD and fasting urinary Dpyr and Pyr measured once in early stage of menopause.[4]

In conclusion, the biochemical bone markers are not useful in diagnosing postmenopausal osteoporosis. All the three biochemical bone markers showed comparable moderate sensitivity in diagnosing postmenopausal osteoporosis but urinary Dpyr has the highest diagnostic specificity. There was an increased bone turnover but absence of the uncoupling bone resorption :

formation phenomenon in postmenopausal osteoporosis. HRT treatment reduces bone turnover of postmenopausal osteoporosis. The lack of correlation between the biochemical bone markers and BMD suggests the difference in the time frame during which changes were detected by each method.

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