Urinary Deoxypyridinoline is a Useful Biochemical Bone Marker for the Management of Postmenopausal Osteoporosis

S L Yu,*MBBS, MRCOG (Lond), M Med (O & G), L M Ho,**BSc, B C Lim,***SRN, M L Sim,****SRN

Abstract

Most medical treatment of postmenopausal osteoporosis are inhibitors of bone resorption and urinary Deoxypyridinoline (D-Pyr) has been shown to be a reliable indicator of bone resorption. Fifty-one healthy women were divided into four groups. Group A: premenopausal women (n = 10), Group B: postmenopausal women, no osteoporosis, not on hormone replacement therapy (HRT) (n = 14), Group C: postmenopausal women, osteoporotic, not on HRT (n = 12), and Group D: postmenopausal women on HRT (n = 15). Fasting urine was collected and sampled for D-Pyr using Pyrilinks-D Kit (Metra Biosystems). Urinary D-Pyr was calculated in nM/creatinine mM. There was a significant difference between premenopausal and postmenopausal urinary D-Pyr showing a higher value of D-Pyr during menopause. Urinary D-Pyr results of postmenopausal women on HRT and premenopausal women showed no difference. Hence D-Pyr may be useful for the monitoring of hormonal treatment of postmenopausal bone loss.

***Ann Acad Med Singapore* 1998; 27:527-9**

Key words: Bone resorption, Hormonal replacement therapy, Immunoassay, Pyrilinks-D

Introduction

Postmenopausal osteoporosis is the most common cause of osteoporosis. It is heralded by the cessation of oestrogen production by the ovaries resulting in at least 10% to 15% bone loss over the next 10 to 15 years from menopause.[1] Oestrogens are known to decrease the rates of bone resorption with direct action on osteoblast and osteoclastic activity *in vitro*, probably via the action of cytokines.

Bone loss is characterised by an increase in bone turnover resulting in increased bone resorption. This leads to decreased bone mass and strength and eventually a fracture.[2]

Classical assessment of osteoporosis depends on the measurement of bone mineral mass on various bony sites usually the distal radius, lumber spine and hip. This can be used to predict the future risk of fractures.[3] As the bone mineral density change is small and the average postmenopausal bone loss over 1 year is only about 2%, slower rates of losses may not be detected.

Similarly, hormone replacement therapy (HRT) can reverse bone loss during postmenopause, but assessment of bone density changes needs be measured over a prolonged period of time of one to two years.[4]

Recently major interest has focused on various biochemical markers of bone formation resorption in order to assess more rapid changes in bone activity and rate of bone loss during menopause. The biochemical measurements of urinary hydroxyproline, plasma tartrate resistant acid phosphatase activity and the activity of bone isoenzyme of serum alkaline phosphatase have been shown to increase by 30% to 100% during menopause.[5]

Recent useful bone markers of resorption include urinary pyridinoline (Pyr, Collagen Crosslinks Kit, Metra Biosystems), free deoxypyridinoline (D-Pyr, Pyrilinks-D Kit, Metra Biosystems), type I collagen crosslinks N-telopeptides (Ntx, Osteomark Kit, Ostex™), C-telopeptides, (Ctx, Crosslaps, Osteometer).

Bone resorption has been assessed by measuring pyridium crosslinks of collagen in urine.6 These amino acids create covalent bonds between adjacent collagen chains and stabilize the extracellular matrix in bone and cartilage. Pyr is abundant in bone and cartilage and D-Pyr is found in significant amounts in type I collagen which represents 90% of the organic matrix of bone.7 In the process of bone degradation, Pyr and D-Pyr are released into the circulation and excreted in urine, in the free or peptide bound form and are not affected by diet. Thus the measurement of urinary D-Pyr has been used in this study instead of Pyr.

Classically, the measurement of D-Pyr has been measured by high performance liquid chromatography (HPLC)[6] but D-Pyr has been recently more easily measured by immunoassay (ELISA)[8] with a high correlation of r = 0.95.

* *Senior Consultant*
** *Laboratory Technician*
*** *Senior Staff Nurse*
**** *Staff Nurse*
Department of Obstetrics and Gynaecology, Singapore General Hospital
Address for Reprints: Dr Yu Su Ling, Department of Obstetrics and Gynaecology, Singapore General Hospital, 1 Hospital Drive, Singapore 169608.

Materials and Methods

Aim

The aim of this study was to assess the usefulness and reproducibility of an immunoassay for urinary D-Pyr (Metra Biosystems Mountain View, CA) in a cross-sectional group of Singapore postmenopausal women.

Subjects

A total of 51 healthy women between the ages of 27 and 63 years were recruited from the Department of Obstetrics and Gynaecology, Singapore General Hospital. There were 3 Indian women in the study while the rest were Chinese. Ten healthy premenopausal women having regular menstrual cycles between 27 and 50 years of age acted as controls (Group A). Of the 41 postmenopausal women, 14 were untreated with normal bone density (Group B), 12 were osteoporotic untreated women (Group C) and 15 women were on HRT for at least 3 months and beyond. Duration of menopause ranged from 6 months and 39 years amongst the untreated postmenopausal women.

Methods for Bone Resorption

First morning void fasting urine was collected from all the 51 women placed into a dark sterile plastic container and stored at <-20°C until the assay was done. The Pyrilinks-D Kit measures free D-Pyr crosslinks in urine using competitive immunoassay in a microtitre plate format, utilising a monoclonal anti-D-Pyr antibody coated on the plate to capture D-Pyr. The D-Pyr results were read at 405 nm using the Bio-rad Microplate reader Model 3550 and corrected for urinary concentration by creatinine measured using a standard colorimetric Jaffe method. The reference range for females was 2.0 to 6.0 nM D-Pyr / mM creatinine. Intra and inter assays coefficient of variability (CV) for D-Pyr were less than 10% and 15%, respectively and for creatinine were less than 3% and 7%, respectively.

Measurement of Bone Density

Bone mineral density (BMD) of postmenopausal untreated women was measured using the dual photon densitometer, DTX-100 (Hologic) at the ultradistal radius. Values of bone density that were less than 2.5 SD of the mean for normal premenopausal adult were used to classify women who were osteoporotic.

Statistical Analysis

The D-Pyr and BMD values were found to be normally distributed by the Levene test and assessed on Mann-Whitney U for non-parametric comparison (Table I).

Results

The mean age of premenopausal and postmenopausal women was 36.4 and 55 years, respectively. There was no difference in age between treated and untreated women.

TABLE I: MEAN URINARY D-PYR VALUES OF DIFFERENT GROUPS OF WOMEN

Description	Mean age (y)	Mean urinary D-Pyr values nM/mM creatine ± SD	Mean BMD values g/cm^2 ± SD
Premenopausal Women	36.4	5.37 ± 0.92	0.339 ± 0.04
Postmenopausal non-osteoporotic, untreated women	54.5	6.82 ± 1.51	0.326 ± 0.25
Postmenopausal osteoporotic, untreated women	56.6	6.94 ± 2.32	0.265 ± 0.34
Postmenopausal treated women	54.0	4.77 ± 1.77	0.323 ± 0.07

BMD: bone mineral density; SD: standard deviation

The BMD values of the osteoporotic untreated postmenopausal women (0.265 ± 0.03 g / cm^2) were significantly different from the premenopausal women (0.339 ± 0.04 g / cm^2) and the non-osteoporotic, untreated postmenopausal women (0.326 ± 0.02 g / cm^2) with P <0.01 (Table I). There was no significant difference in the BMD values between the premenopausal and HRT treated postmenopausal women.

The mean urinary D-Pyr was 5.37 ± 0.92 nM / mM creatinine amongst the 10 premenopausal women who acted as controls. The mean urinary D-Pyr values in postmenopausal untreated women (6.82 ± 1.51 nM / mM creatinine) were significantly higher (27%) with P <0.01 than the control group (Fig. 1).

Urinary D-Pyr values of treated postmenopausal women (4.77 ± 1.77 nM / mM creatinine) were significantly lower (31%) with P <0.01 compared to the postmenopausal osteoporotic, untreated women (6.94 ± 2.32 nM / mM creatinine) (Fig. 2) and were not significantly different from the premenopausal control group.

However, there was no significant difference in urinary D-Pyr values between untreated non-osteoporotic (6.82 ± 1.51 nM / mM creatinine) and osteoporotic untreated postmenopausal women (6.94 ± 2.32 nM / mM creatinine).

Discussion

D-Pyr has been found to be a sensitive and specific marker of bone resorption. In a cross-sectional study by Ohishi et al[9] on Japanese women, D-Pyr values of the postmenopausal group were 37.5% higher (7.7 ± 2.4 nM / mM creatinine) than the premenopausal group (5.6 ± 2.2 nM / mM creatinine).

Another cross-sectional study by Uebelhart et al[10] on Caucasian women, showed that menopause induced an 82% increase in D-Pyr values (8.2 ± 3.4 vs 4.5 ± 1.4 pmol / umol creatinine) which returned to premenopausal lev-

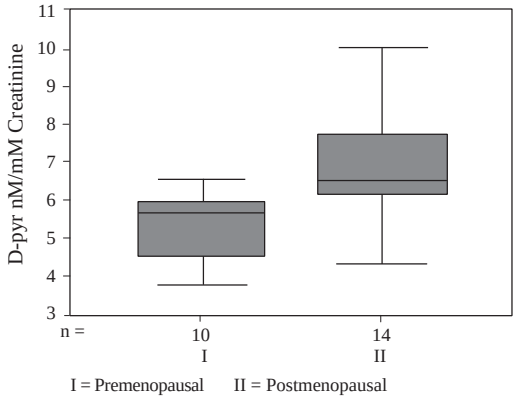

Fig. 1. Urinary D-Pyr: pre- versus postmenopausal women.

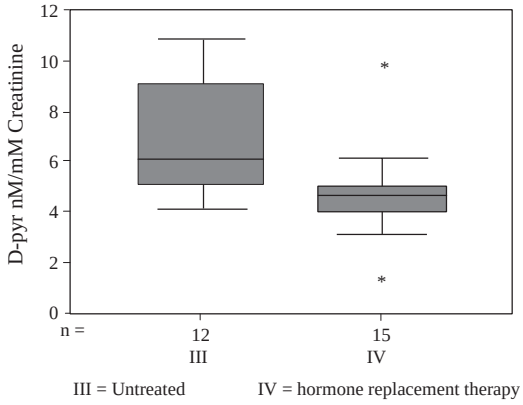

Fig. 2. Postmenopausal untreated women versus women on hormone replacement therapy.

els within 6 months after HRT.

A longitudinal study by Hassager et al[11] showed that D-Pyr values remain fairly constant in the years before menopause and start to increase about 6 months after the last menstrual bleeding. The mean postmenopausal values [8 ± 1 SEM (standard error of the mean) nM / mM creatinine] were 30% to 50% higher than the mean premenopause values [12 ± 1 (SEM) nM / mM creatinine] in the same subjects. Three months of postmenopausal HRT treatment reversed the D-Pyr values to premenopausal levels.

These findings are consistent with the findings of our cross-sectional study on Singaporean women although the percentage of significant difference of D-Pyr values between pre- and postmenopausal women was only 27%. Our study also showed that D-Pyr values between untreated and HRT-treated postmenopausals were significantly different by 31%. The D-Pyr values of HRT-treated postmenopausal women were not significantly different from the premenopausal values.

There were too few non-Chinese women in our study to demonstrate any possible ethnic differences in the urinary D-pyr values.

Even though the number of women in each group was small, the D-Pyr and BMD values were statistically significant and normally distributed by the Levene test. The study could have been more definitive if there were a larger number of subjects.

Our data showed that 2 of the HRT-treated postmenopausal women exhibits D-Pyr values which were out of range and may reflect lack of compliance or lack of response to treatment (Fig. 2).

The urinary D-Pyr is not sensitive enough to differentiate between postmenopausal women with normal and low bone mass. Perhaps these two indices, i.e, bone marker and bone density measurement are two separate independent factors for postmenopausal osteoporosis.

Previous assays for urinary D-Pyr has been done using high performance liquid chromatography (HPLC). It has been shown that the D-Pyr immunoassay is highly correlative to HPLC measurement.[8] The immunoassay is rapid and easy to use if care has been taken to prevent exposure of urine to prolonged sunlight. Only a small amount of urine, 50uL, needs to be used each time.

In conclusion, urinary D-Pyr is a sensitive indicator in differentiating between premenopausal and postmenopausal women as shown in this study. It is also a useful indicator for treatment response to HRT. Larger series and further longitudinal studies are needed to confirm its usefulness in clinical practice.

REFERENCES

1. Richelson L S, Wahner H W, Melton L J, Riggs B L. Relative contributions of aging and estrogen deficiency to postmenopausal bone loss. N Engl J Med 1984; 311:1273-5.
2. Parfitt A M. Bone remodelling: Relationship to the amount and structure of bone, and the pathogenesis and prevention of fractures. In: Riggs B L, Melton L J, editors. Osteoporosis: Etiology, Diagnosis and Management. New York: Raven Press, 1988:45-93.
3. Wasnich R D, Ross P D, Heilbrun L K, Vogel J M. Prediction of postmenopausal fracture risk with use of bone mineral measurements. Am J Obstet Gynecol 1985; 153:745-51.
4. Christiansen C, Christensen M S, Transbol I. Bone mass in postmenopausal women after withdrawal of estrogen / gestagen replacement therapy. Lancet 1981; 1(8218):459-61.
5. Stepan J J, Pospichal J, Presl J, Pacovsky V. Bone loss and biochemical indices of bone remodelling in surgically induced postmenopausal women. Bone 1987; 8:279-84.
6. Black D, Duncan A, Robins S P. Quantitative analysis of the pyridinoline crosslinks of collagen in urine using ion paired reversed - phase - high performance liquid chromatography. Anal Biochem 1988; 169:197-203.
7. Eyre D R, Dickson I R, Van Ness K P. Collagen crosslinking in human bone and articular cartilage. Age-related changes in the content of mature hydoxypyridinium residues. Biochem J 1988; 252:495-500.
8. Robins S P, Woitge H, Hesley R, Julie J, Seyedin S, Seibel M J. Direct, enzyme-linked immunoassay of urinary deoxypyridinoline on a specific marker for measuring bone resorption. J Bone Miner Res 1994; 9:1643-9.
9. Ohishi T, Takahashi M, Kawana K, Aoshima H, Hoshino H, Horiuchi K, et al. Age-related changes of urinary pyridinoline and deoxypyridinoline in Japanese subjects. Clin Invest Med 1993; 16:319-25.
10. Uebelhart D, Schlemmer A, Johansen J S, Gineyts E, Christiansen C, Delmas P. Effect of menopause and hormone replacement therapy on the urinary excretion of pyridinium crosslinks. J Clin Endocrinol Metab 1991; 72:367-73.
11. Hassager C, Colwell A, Assiri A M, Eastell R, Russell R G, Christiansen C. Effect of menopause and HRT on urinary excretion of pyridinium crosslinks: a longitudinal and cross-sectional study. Clin Endocrinol 1992; 37:45-50.