Reference Value for Urinary Deoxypyridinoline as a Specific Marker for Measuring Bone Resorption in Thais

PHANNEE PIDETCHA, M.Sc*,
KINGKAEW PATRAKARN, M.Sc.*,
SOMSAK FONGSUPA, B.Sc.**
SRISANIT INTRAMANEE, M.Sc.*,
PAIROJ LEELAHAKUL, M.D.*,

Abstract

In the next century, the increasing number of elderly and rising healthcare costs will bring with it metabolic bone problems, particularly osteoporosis. Deoxypyridinoline: Dpd in urine is a sensitive and specific marker for screening and monitoring of bone resorption in a variety of diseases affecting bone turnover and in risk groups especially in the postmenopause. The reference value among aging (21-60 years) of a healthy well defined group was studied by collecting the urine between 700-1000 hours and using the ELISA technique to determine the level of Dpd. The reference value of Dpd in 113 males and 298 females 1.3-6.5 and 1.5-6.9 nm Dpd/nm Creatinine respectively. The level of Dpd in females was significantly higher than in males at $p<0.028$. However, the average value of deoxypyridinoline in postmenopause was higher than premenopause but not different at $p = 0.05$. There are many factors which influence the results so the overall reference value is only a guideline for screening in bone resorption.

Key word : Deoxypyridinoline, Bone Resorption, Reference Value, Osteoporosis, Premenopause, Postmenopause

In the future will bring the elderly population in metabolic bone diseases, particularly osteoporosis and rising healthcare costs. The pathophysiology of bone loss leading to osteoporosis results from an imbalance in bone metabolism with the rate of bone resorption exceeding formation. Currently approved therapies to prevent bone loss or treat osteoporosis are antiresorptive agents. Thus, there is a significant need for accurate markers that reflect the bone resorption process.

Several new biochemical markers of bone resorption have been studied over recent years. Those include pyridinium cross-links of collagen[1]. The collagen crosslinks pyridinoline (Pyd) and deoxypyridinoline (Dpd) are found during maturation of extracellular collagen fibrils, and measure-

* Department of Clinical Chemistry, Faculty of Medical Technology, Mahidol University, Bangkok 10700,
** Laboratory Medicine, Thammasat Hospital, Thammasat University, Pathumthani, Thailand.

ment of these components in urine has been shown to provide valid clinical markers of bone collagen degradation[2-4]. Recent advances have resulted in the development of assays for new circulating and urinary markers that reflect specifically either bone formation or bone resorption. Among these newer markers, the urinary deoxypyridinoline crosslinks of collagen show particularly as specific and highly sensitive biochemical indices of bone resorption [5,6]. Elderly women studied immediately after hip fractures had positive correlation with urinary excretion of deoxypyridinoline compared with aged matched controls, suggesting that an increased bone resorption rate might be responsible for the low bone mass (osteoporosis) that characterized patients with hip fracture[7].

The primary aim of this study was to establish the reference value and effect of sex on the urinary excretion of deoxypyridinoline by the method using ELISA technique in well defined subjects.

MATERIAL AND METHOD

Morning urine specimens between 700-1000 hr were collected from 411 healthy women and men and were kept at 2-8°C for storage for less than 7 days or were frozen at 20°C for longer storage. The study group was screened for annual check up profile including chest X-rays. The subdivisions of total age ranging from 21-60 years of women (n = 298) and men (n = 113) respectively are shown in Fig. 1.

In the previous pilot study, morning urine was collected from 298 healthy women volunteers with known menstrual status. They were grouped as premenopausal (n = 218, aged 32-50 years), post-menopausal (n = 80, aged 42-60 years) subjects. For the postmenopause group there were twelve women using hormones as an antiresorptive drug (supplement group) by themselves to prevent bone loss.

Reliability of this method was evaluated in terms of accuracy and precision. Deoxypyridinoline (Dpd) was measured using the pyrilink-D immunoassay kit (Metra Biosystem Inc CA), using monoclonal antibody that preferencially recognizes the free Dpd.

All immunoassay data were corrected for urine creatinine concentration using the standard colorimetric method (Jaffe reaction).

RESULTS

All data showed asymmetric distributions so the reference value was calculated at the 2.5th-97.5th percentile. Value of urinary deoxypyridinoline analysed by ELISA assay for the 411 healthy group representing the 2.5th-97.5th percentile of each group and the reference value for the healthy group aged 21-60 years was 1.4-6.8 and for each group according to the gender was 1.5-6.9 and 1.3-6.5 nmDpd/nM creatinine for women (n = 298) and men (n = 113) respectively and statistical evaluation showed that the urinary deoxypyridinoline in females was significantly higher than the male

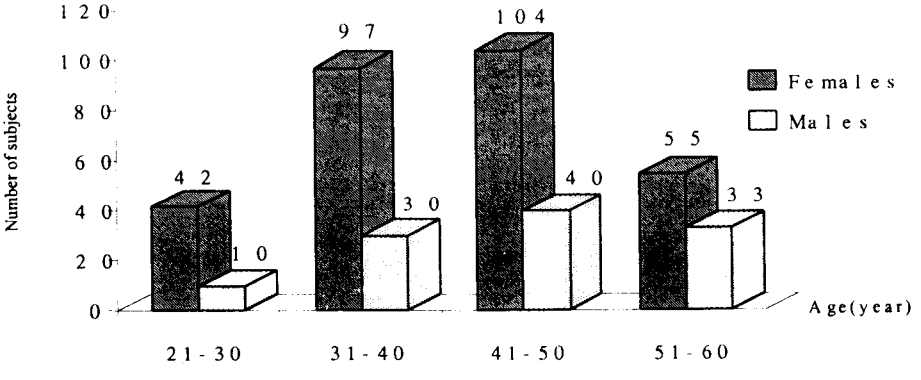

Fig. 1. Characteristics of 411 healthy volunteers for urinary deoxypyridinoline level.

group at p<0.028. From our previous pilot study of 298 healthy women volunteers with known menstrual status, the deoxypyridinoline excretion in the premenopausal (1.5-6.8) was statistically not significant from the post menopausal (1.6-7.0) at p = 0.05 and is shown in Fig. 2. The statistic parameter for this report for the marker of bone resorption (Dpd) from 411 healthy group was summarised and is shown in Table 1.

Results for within run and between run assay with the high and low level of deoxypyridinoline in urine are given in Table 2. The coefficient variation showed good precision and lower percentation of coeficient variation than that reported by Robin PS. in 1994.

The recovery linearity was determined by serially diluting samples and comparing observed values with the expected values. Typical results are shown in Table 3.

DISCUSSION

Study of urinary deoxypyridinoline levels in 411 healthy group of 298 women and 113 men have shown that the sex appears to influence the excretion of deoxypyridinoline with the significant level at p < 0.028. The finding correlates with the

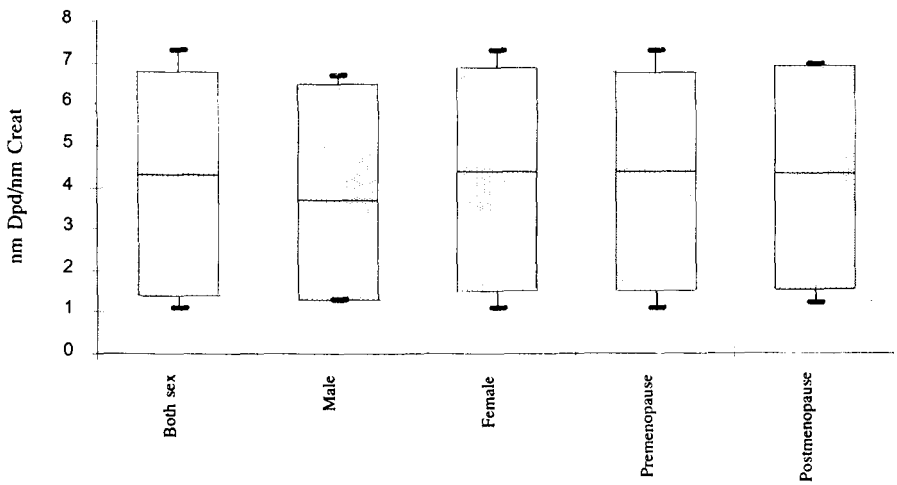

Fig. 2. Urinary deoxypyridinoline level in healthy volunteer.

Table 1. Summary of data in healthy 411 volunteers.

	n	Mean	SD	2.5th Percentile	97.5th Percentile
Both sex	411	4.23	1.45	1.40	6.80
Female	298	4.36	1.43	1.50	6.90
Male	113	3.89	1.45	1.30	6.50
Premenopause	218	4.35	1.44	1.50	6.80
Postmenopause (all)	80	4.42	1.41	1.60	7.00
Postmenopause (1 year)	32	4.29	1.53	1.30	7.00
Postmenopause (1-3 years)	23	4.44	1.33	1.20	6.90
Postmenopause (3-5 years)	16	4.63	1.22	2.00	6.40
Postmenopause (>5 years)	9	4.41	1.68	1.80	6.90
Postmenopause with Hormone	12	5.08	1.09	3.70	7.00
Postmenopause without Hormone	68	4.30	1.44	1.30	6.90

Table 2. Studies for precision of deoxypyridinoline in urine.

Statistic parameter	Low concentration		High concentration (nm Dpd/nm creat)	
	within-day	between-day	within-day	between-day
mean	15.14	15.04	93.92	93.84
S.D	0.91	1.13	2.91	3.71
C.V. (%)	6.0	7.3	3.0	3.9

Table 3. The accuracy in term of recovery study in measurement of deoxypyridinoline value, expressed as nm Dpd/nm creat.

Dilution	Observed value	Expected value	Recovery (%)
1:4	36.7	34.8	95.3
1:2	52.6	54.5	96.0
1:3	67.1	67.6	99.3
1:4	75.6	74.2	101.8

report by Beardoworth et al[8] and Uebelhearot et al [9] in 1990. Several studies have reported significantly elevated concentrations of deoxypyridinoline excretion in untreated postmenopausal women compared with premenopausal women,[10] In this study, the treated postmenopausal women had a mean significantly higher than the untreated group (without hormone supplement) at $p = 0.0484$ but the mean value of deoxypyridinoline in postmenopausal women was not significantly higher than the premenopausal group at $p = 0.05$. So Asian people especially Thais have different data from the Western group, which may be caused by race, eating habits and difference in life style. However, in 1992 Schlemmer et al reported that the diurnal variation in postmenopausal women increased in crosslink excretion in the very early morning hours and lowest values were found at night[11]. Also Eastelll et al supported that between 0500 and 0800 hours urine Dpd level may be as little as 10 per cent higher or as much as 100 per cent compared with the nadir value at 1700 hours. Therefore, if spot urine samples are used as representative of crosslink urinary excretion, timing should be closely controlled.

From this study, bone markers such as urinary deoxypyridinoline level reflected the degree of collagen degradation in bone and the activity of bone resorption varied slightly with aging especially in postmenopause (aged over 50). In Table 1, the deoxypyridinoline level in hormone supplement group (postmenopause with hormone) showed much more resorption of bone than the untreated group (postmenopause without hormone). Further detailed study should be done on lifestyle, determination of hormone level, clinical investigations and also giving of hormone supplement which must be monitored by a physician.

SUMMARY

The ELISA technique for measurement of urinary deoxypyridinoline is sensitive and specific for index of bone resorption in a variety of diseases affecting bone turnover in older which shows the reliability test in terms of accuracy and precision. Reference values for 298 women and 113 men were 1.5-6.9 and 1.3-6.5 nmDpd/nm creatinine respectively.

The female group had a significantly higher urinary deoxypyridinoline than the male group at $p<0.028$. There are many factors that influence the reference value including diet, race, sex and lifestyle including diurnal variation, the timing of this study urine specimen was between 7.00-10.00 hours. So this reference value is only a guideline of screening.

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REFERENCES

1. Colwell A, Russell RGG, Eastell R. Factors affecting the assay of urinary 3- hydroxy pyridinium crosslinks of collagen as markers of bone resorption. Eur J Clin Invest 1993; 23: 341-9.
2. Editorial. Pyridinium crosslinks as markers of bone resorption, Lancet 1992; 340: 278-9.
3. Demers L. New biochemical marker for bone disease: Is it a breakthrough ? Clin Chem 1992; 38: 2169-70.
4. Delmas PD. Clinical use of biochemical markers of bone remodeling in osteoporosis Bone 1992; 13 (suppl 1): S17-S21.
5. Eyre DR. New biomarkers of bone resorption. J Clin Endocrinol Metb 1992; 74: 470-A-C.
6. Roux JP, Arlot ME, Gineyts E, et al. Automatic interactive measurement of resorption cavities in transiliac bone biopsies and correlation with deoxypyridinoline. Bone 1995; 17: 153-6.
7. Akesson K, Vergnaud P, Gineyts E, et al. Impairment of bone turnover in elderly woman with hip fracture. Calcif Tissue Int 1993; 53: 162-9.
8. Beardsworth LJ, Eyre DR, Dickson IR. Changes with age in the excretion of lyusyl and hydroxylsylpyridinoline: two new markers of bone collagen turnover. J Bone Miner Res 1990; 5: 671-6.
9. Uebelheardt D, Gineyts EC, Chapuy MC, Delmas PD. Urinary excretion of pyridinium crosslinks; a new marker of bone resorption in metabolic bone disease. Bone Miner 1990; 8: 87-96.
10. Uebelheardt D, Schlemmer A, Johansen JS, et al. Effect of menopause and hormone replacement therapy on the urinary excretion of pyridinium crosslinks. J Clin Endocrinol Metab 1991; 12: 367-73.
11. Eastell R, Calvo MS, Burritt MF, Offord KP, Russell RGG, Riggs BL. Abnormalities in circadian patterns of bone resorptions and renal calcium conservation in type I osteoporosis. J Clin Endocrinol Metab 1992; 74: 487-94.
12. Schlemmer A, Hassager C, Tensen SB, Christiansen C. Marked diurnal variation in urinary excretion of pyridinoline cross-links in premenopausal women. J Clin Endocrinol Metab 1992; 74: 476-80.
13. Robin PS, Woitge H, Hesley R, et al. Direct enzyme-linked immunoassay for urinary deoxypyridinoline as a specific marker for measuring bone resorption. J Bone Miner Res 1994; 9: 1643-9.

ค่าอ้างอิงของดีออกซีไพริดิโนลีนในปัสสาวะซึ่งเป็นดัชนีบ่งชี้ถึงการสลายตัวของกระดูกในกลุ่มคนไทย

พรรณี พิเดช, วท.ม., ศรีสนิท อินทรมณี, วท.ม.*,*
กิ่งแก้ว ภัทรกานต์, วท.ม., ไพโรจน์ ลีฬหกุล, พ.บ.*, สมศักดิ์ ฟองสุภา, วท.บ.***

ในอนาคตคาดว่า ค่ารักษาพยาบาลเกี่ยวกับโรคกระดูกโดยเฉพาะโรคกระดูกพรุน จะมีแนวโน้มมากขึ้นเพราะมีผู้สูงอายุมากขึ้น ดังนั้นการป้องกันโรคโดยการตรวจหาความเสี่ยงหรือดัชนีบ่งชี้ว่ามีภาวะการทำลายกระดูกจึงมีความสำคัญรวมทั้งเพื่อติดตามการรักษาด้วย การตรวจระดับของสารดีออกซีไพรดิโนลีน (deoxypyridinoline : Dpd) ในปัสสาวะเป็นดัชนีที่มีความไวและความจำเพาะสำหรับบ่งชี้ว่ามีภาวะการสลายตัวของกระดูกเนื่องมาจากหลายสาเหตุ เช่น ในหญิงเมื่อมีอายุมากขึ้นการทำลายหรือการสูญเสียเนื้อกระดูกอาจมีสาเหตุเนื่องมาจากการขาดสารเอสโตรเจนในวัยหมดประจำเดือน (postmenopause) การตรวจภาวะเสี่ยงจำเป็นจะต้องมีค่าระดับของสาร Dpd ของกลุ่มประชากรที่คาดว่ามีสุขภาพสมบูรณ์เพื่อใช้เป็นค่าอ้างอิงในการแปลผลและเป็นการสนับสนุนการวินิจฉัยการตรวจทางคลินิก และเพื่อวางแผนการรักษาติดตามผลการรักษาหรือดำเนินของโรค การวัดระดับ Dpd ในปัสสาวะใช้หลักการของวิธี ELISA พบว่าการเก็บปัสสาวะในตอนเช้าระหว่างเวลา 7.00–10.00 น. ของอาสาสมัครที่มีสุขภาพสมบูรณ์จำนวน เพศชาย 113 คน เพศหญิง 298 คน อายุระหว่าง 21 ถึง 60 ปี พบว่ามีค่าอ้างอิงปกติเท่ากับ 1.3–6.5 และ 1.5–6.9 nmDpd/nm Creatinine ตามลำดับ และพบว่าเพศชายมีค่า Dpd แตกต่างจากเพศหญิงอย่างมีนัยสำคัญทางสถิติ ที่ค่า $p < 0.028$ ส่วนเพศหญิงที่อยู่ในวัยหลังหมดประจำเดือนมีค่าเฉลี่ยสูงกว่าหญิงก่อนหมดประจำเดือน แต่ไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติที่ค่า $p = 0.05$

คำสำคัญ : ดีออกซีไพริดิโนลีน, การสลายตัวของกระดูก, ค่าอ้างอิง, กระดูกพรุน, ก่อนหมดประจำเดือน, หลังหมดประจำเดือน

* ภาควิชาเคมีคลินิก, คณะเทคนิคการแพทย์, มหาวิทยาลัยมหิดล, กรุงเทพ ฯ 10700
** ห้องปฏิบัติการเวชศาสตร์ชันสูตร, โรงพยาบาลธรรมศาสตร์เฉลิมพระเกียรติ, จ.ปทุมธานี