

Urinary Crosslinked N-telopeptides of type I collagen (NTx) among Reproductive and Post-menopausal Thai women

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Purpose: To study the level of Urinary Crosslinked N-telopeptides of type I collagen (Urinary NTx) in reproductive and postmenopausal Thai women and their correlation with bone mineral density (BMD) and duration after menopause.

Methods: Second or third void morning urine samples were collected in 48 reproductive women and 42 postmenopausal women. Urinary NTx was measured by competitive-inhibition ELISA and corrected with spot urine creatinine. BMD was measured in all postmenopausal participants.

Results: The mean value of Urinary NTx in reproductive women was 36.19 nanomoles of bone collagen equivalents per millimole of Creatinine (nM BCE/mM Cr), standard deviation = 25.18 with 95% confidence interval = 28.88 to 43.50 nM BCE/mM Cr. The mean value of Urinary NTx in postmenopausal women was 58.89 nM BCE/mM Cr, standard deviation = 26.93 with 95% confidence interval = 50.50 to 67.28 nM BCE/mM Cr. Neither BMD nor duration after menopause is significantly correlated with Urinary NTx.

Conclusion: Urinary NTx increased with age in Thai women ($p < 0.0001$). A significant change was observed in postmenopausal women compared to reproductive women ($p < 0.0001$).

Keywords: Urinary NTx, bone turnover marker, Crosslinked N-telopeptides of type I collagen, menopause, osteoporosis

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As the elderly population increases worldwide, disorders related to the ageing process such as skeletal strength compromise become more emphasized. Several biochemical tests have been used to detect these changes through screening, diagnosis, follow-up, or prognostic evaluation in many bone diseases.

Measurement of biochemical changes in bone using bone turnover markers (BTMs) can demonstrate both the formation and resorption of bone. Abnormal amounts of these biomolecules demonstrate abnormal changes in bone, which are found in various pathological conditions which affect the bones. Evaluation of BTM levels is used in several research studies, including clinical trials and in terms of secondary outcomes of the effectiveness of new therapies.

To determine bone resorption, several biochemical tests can measure biomolecules from the decomposition of both collagen and non-

collagenous matrix proteins, such as Bone Sialoprotein⁽²⁾ or enzymes Tartrate-Resistant Acid Phosphatase (TRAP).⁽³⁻⁵⁾ However, most biochemical tests measure the degradation of collagen, including Hydroxyproline, Hydroxylysine-Glycosides, Hydroxypyridinium crosslinks of collagen, and crosslinked telopeptides of type I collagen.⁽⁶⁾

The crosslinked telopeptides of type I collagen are derived from specific regions of the collagen type I molecule, one end called carboxy-terminal telopeptide or C-telopeptide and the other end called amino terminal telopeptide or N-telopeptide. There is an area on the amino terminal that links to adjacent collagen fibrils called crosslinked N-telopeptides of type I collagen (NTx). Detection of NTx can be done by a biochemical measurement method, Enzyme-linked immunoabsorbent assay (ELISA), using antibodies to the area of surface antigen on the alpha-2 chain of type I collagen. The result of bone resorption, collagen breakdown products as crosslinked N-telopeptides of type I collagen, can be detected in blood and urine.⁽⁷⁻⁹⁾

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Currently, NTx excreted in the urine can be measured by clinical monitoring such as anti-bone degradation therapy with antiresorptive agents, which is more sensitive than measuring in serum.⁽¹⁰⁾ In addition, interpretation with bone formation markers or other monitoring tools such as measurement of bone density with a Dual Energy X-ray Absorptiometer (DEXA) enables a greater range of clinical benefits.^(1,3,11) However, clinical use requires a standard reference. Although there are many research studies of bone turnover markers in the Thai population,⁽¹²⁻¹⁶⁾ as well as a study in the amount of urine excretion of NTx in Japanese women,⁽¹⁷⁾ there is no reference range for Thai women of reproductive and post-menopausal ages.

This research therefore studies the amount of the NTx excreted in the urine in reproductive and post-menopausal Thai women, and the relationship of the quantity of NTx with bone mineral density (BMD).

Materials and Methods

Ninety women were recruited from volunteers and those who obtained annual physical check-ups at Maharaj Nakorn Chiang Mai Hospital. Subjects were grouped into two groups using age of patient and menopausal status, 48 women in the reproductive group (age 20-49 years) and 42 women in the post-menopausal group (age 50 years or more). The participants received the information about this study and signed informed consent forms. Histories were taken to exclude medical condition affecting bone metabolism, including concurrent drugs and history of surgery. Patients known to have osteoporosis and menopause due to underlying disease or surgical menopause were excluded.

The second or third void of morning urine was collected. Urine samples were analyzed using enzyme-linked immunoabsorbent assay (Osteomark®, Inverness Medical Professional Diagnostics, Princeton, NJ, USA) to determine the level of NTx. The post-menopausal group was assigned for BMD measurement using DEXA.

The urinary NTx levels were corrected by calculation with urinary creatinine level for each sample. The data were analysed with STATA (version 10.0) software. Data means from the two groups were compared using Wilcoxon rank-sum (Mann – Whitney) test because distributions of the data were not normal. Correlations between age, BMD, and Urinary NTx were calculated with Spearman's correlation tests. According to the data from previous study⁽¹⁷⁾ and our presumptive setting, standard deviation (σ) was 20, difference of mean (Δ) was 15, type I error (α) was 0.05, and the power of study was 90 percent. The estimated sample size required 38 subjects in each group.

Results

The mean ages of women were 34 and 64 years old in the reproductive and post-menopausal groups respectively. The other demographic data from each group are shown in Table 1.

Table 1. Demographic data

	Reproductive (n = 48)	Post- menopausal (n = 42)	Total (n = 90)
Age (years)	34.04 ± 8.67	64.17 ± 9.50	48.10 ± 17.60
Height (cm)	158.52 ± 5.04	153.19 ± 5.93	156.03 ± 6.06
Weight (kg)	55.83 ± 9.78	55.36 ± 8.72	55.61 ± 9.25
BMI(kg/m²)	22.21 ± 3.77	23.56 ± 3.43	22.84 ± 3.66

BMI= Body Mass Index

Table 2. Urinary NTx level

Group	n	Mean	Std. Err.	Std. Dev.	[95% Conf. Interval]
Reproductive	48	36.19*	3.63	25.18	28.88 - 43.50
Post- menopausal	42	58.89*	4.16	26.93	50.50 - 67.28
Combined	90	46.78	2.98	28.26	40.87 - 52.70

*p<0.0001 Reproductive VS Post-menopausal

The urinary NTx in the reproductive group ranged from 4.51 to 125.78 nM BCE/mM creatinine. The mean value of this group was 36.19 nM BCE/mM creatinine with 95% confidence interval 28.88 to 43.50 nM BCE/mM creatinine. In the post-menopausal group, the urinary NTx ranged from 11.94 to 167.82 nM BCM/mM creatinine. The mean NTX level in this group was 58.89 nM BCM/mM creatinine with 95% confidence interval 50.50 to 67.28 nM BCM/mM creatinine. The results of urinary NTx were calculated and shown in Table 2. Data plotting found that urinary NTx level trended to increase along with increasing age to a statistically significant degree (p<0.0001). (Fig. 1)

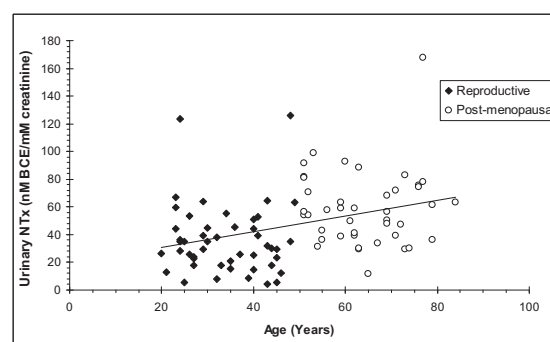


Fig. 1 Plot of urinary NTx against age in reproductive and post-menopausal women (Linear correlation of Urinary NTx VS age, p<0.0001)

Comparing urinary NTx levels between the reproductive and post-menopausal groups found that they were higher in post-menopausal group to a statistically significant degree as shown in Fig. 2.

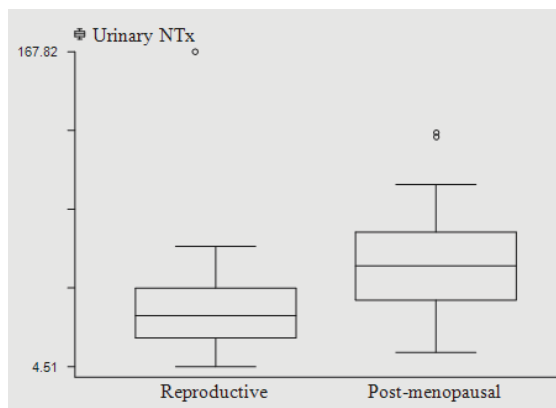


Fig. 2 Comparison of Urinary NTx level in reproductive and post-menopausal women

In the post-menopausal group we subdivided into two subgroups according to years since menopause, 20 years or less and more than 20 years. Comparing urinary NTx between post-menopausal subgroups found no statistically significant difference between them. After further study of the correlation between urinary NTx and the duration of menopause, we could not demonstrate association between them.

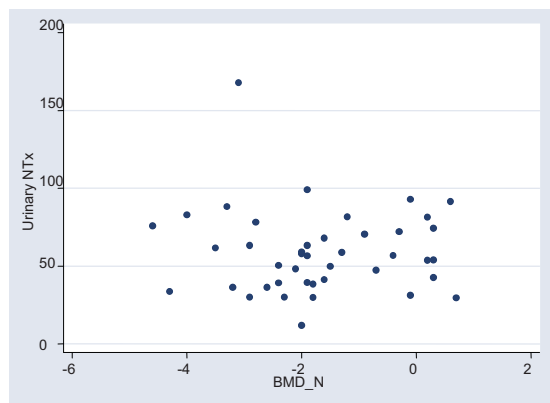
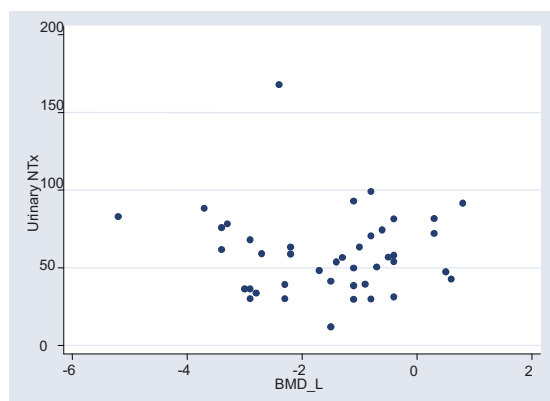


Fig. 3 Correlation between Urinary NTx and Bone Mineral Density (BMD) of Lumbar spine(BMD_L) and femoral neck (BMD_N)

Examining the urinary NTx and BMD of lumbar spines and femoral necks in the post-menopausal age group found no correlation between these them. (Fig. 3)

Discussion

Crosslinked N-telopeptides of type I collagen can be detected in both serum and urine. The measurement of urinary NTx is highly accurate, convenient, and free of pain from puncture. Like other bone turnover markers, urinary NTx is released in circadian rhythm and has diurnal variation, so we collected the second or third morning void of urine to control this variation.

The data collected from both the reproductive and post-menopausal age groups found that urinary NTx levels were higher in the post-menopausal group with statistical significance. The mean increase was about twofold. These results mean that bone is more rapidly degraded in the post-menopausal age group, which concurs with the study of the Japanese population by Sone T, et al.⁽¹⁷⁾

Our study showed that urinary NTx did not increase with the duration of menopause, unlike the study of Japanese women that found an association between urinary NTx and duration of menopause.⁽¹⁷⁾ This could be due to an inadequate sample size in this study to demonstrate statistical significance.

According to the data from both our study and the study in Japan, no association was found between urinary NTx and BMD of lumbar spines in the post-menopausal group. Even if the BMD results demonstrate bone strength and are a useful diagnostic tool, additional clinical risk factors are often used to evaluate fracture risks and to determine treatment.^(18,19) Although urinary NTx was not associated with BMD, it could demonstrate the status of bone turnover. Earlier detection of changing amounts of urinary NTx has more advantages than BMD in detecting response after treatment follow-up.⁽¹⁰⁾

Conclusion

Urinary NTx levels differed to a statistically significant degree between the reproductive and post-menopausal age groups. This study also determined the premenopausal range of urinary NTx in Thai women. These results can be used in clinical application for decisions and to follow up the treatment of osteoporosis. Decreasing urinary NTx levels to premenopausal status reflected positive response to antiresorptive therapy whether in terms of patient compliance, adherence,

or choice of antiresorptive agents. However, urinary NTx levels should be interpreted with other bone turnover markers as well as BMD in guiding therapeutic decisions in certain cases.⁽¹⁾

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การศึกษาระดับของครอสลิงก์เอ็นเทโลเปปไทด์ของคอลลาเจนชนิดที่หนึ่งในปัสสาวะ ของสตรีไทยวัยเจริญพันธุ์และวัยหมดระดู

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วัตถุประสงค์: เพื่อศึกษาระดับปริมาณของครอสลิงก์เอ็นเทโลเปปไทด์ของคอลลาเจนชนิดที่หนึ่งในปัสสาวะ (Urinary NTx) ในสตรีไทยวัยเจริญพันธุ์และวัยหมดระดู และความสัมพันธ์กับระยะเวลาที่หมดประจำเดือน และกับค่าความหนาแน่นกระดูก

วิธีการศึกษา: สตรีวัยเจริญพันธุ์จำนวน 48 คน และสตรีวัยหมดระดูจำนวน 42 คน ได้รับการเก็บปัสสาวะในตอนเช้าซึ่งเป็นปัสสาวะครั้งที่สองหรือสามของวันเพื่อมาตรวจวัดปริมาณของครอสลิงก์เอ็นเทโลเปปไทด์ของคอลลาเจนชนิดที่หนึ่งด้วยกรรมวิธี ELISA กลุ่มสตรีวัยหมดระดูจะได้รับการตรวจวัดความหนาแน่นของกระดูกร่วมด้วย

ผลการศึกษา: ค่าเฉลี่ยของระดับครอสลิงก์เอ็นเทโลเปปไทด์ของคอลลาเจนชนิดที่หนึ่งในปัสสาวะในกลุ่มสตรีวัยเจริญพันธุ์เท่ากับ 36.19 nM BCE/mM Cr, ค่าเบี่ยงเบนมาตรฐานเท่ากับ 25.18 ที่ระดับความเชื่อมั่น 95 เปอร์เซนต์ เท่ากับ 28.88 ถึง 43.50 nM BCE/mM Cr ค่าเฉลี่ยของระดับครอสลิงก์เอ็นเทโลเปปไทด์ของคอลลาเจนชนิดที่หนึ่งในปัสสาวะในกลุ่มสตรีวัยหมดระดูเท่ากับ 58.89 nM BCE/mM Cr ค่าเบี่ยงเบนมาตรฐานเท่ากับ 26.93 ที่ระดับความเชื่อมั่น 95 เปอร์เซนต์ เท่ากับ 50.50 ถึง 67.28 nM BCE/mM Cr ผลการศึกษาพบว่า ไม่มีความสัมพันธ์อย่างมีนัยสำคัญทางสถิติระหว่างระดับครอสลิงก์เอ็นเทโลเปปไทด์ของคอลลาเจนชนิดที่หนึ่งในปัสสาวะกับความหนาแน่นของกระดูก และกับระยะเวลาที่หมดประจำเดือน

สรุป: ระดับของครอสลิงก์เอ็นเทโลเปปไทด์ของคอลลาเจนชนิดที่หนึ่งในปัสสาวะเพิ่มขึ้นตามอายุอย่างมีนัยสำคัญ ($p < 0.0001$) และมีความแตกต่างอย่างมีนัยสำคัญทางสถิติระหว่างกลุ่มสตรีวัยเจริญพันธุ์และกลุ่มสตรีวัยหมดระดู ($p < 0.0001$)

คำสำคัญ: ครอสลิงก์เอ็นเทโลเปปไทด์ของคอลลาเจนชนิดที่หนึ่ง, เอ็นทีเอ็กซ์, วัยหมดระดู, กระดูกพรุน