

## Normative data of the urinary bone resorption biomarkers for osteoporosis DPD, CTx and NTx among females in Manipur and its correlation with bone mineral density measured by DEXA scan

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### Abstract

**Introduction:** Osteoporosis has become a major public health problem affecting more than 100 million people worldwide. Standard diagnosis depends on measuring bone mineral density (BMD) using a dual energy X-ray absorptiometry (DEXA). Bone turnover markers are metabolic products that mediate bone metabolism and can provide information regarding bone formation or resorption before the structural changes of bone occur. BMD and bone markers complement each other, and provide comprehensive information about a patient's bone status and bone activity.

**Aim:** To find the correlation between selected urinary bone turnover markers and bone mineral density measured by DEXA scan.

**Materials and Methods:** A cross sectional study was conducted among 2700 pre and post-menopausal women with intact ovaries aged between 21 to 70 years in three districts of Manipur. Performance of three urinary markers of bone turnover, i.e., Deoxypyridinoline (DPD), N-telopeptide (NTx) and C-telopeptide (CTx) were assessed and correlated with the bone mineral density (T-score) measured by DEXA scan.

**Results:** Significant correlation was found between DEXA T-score and NTx ( $r=0.070$ ,  $p<0.010$ ), but not for DPD and CTx.

**Conclusion:** It can be concluded that there is significant correlation between DEXA T-score and NTx, but not for DPD and CTx.

**Clinical Significance:** In the management of osteoporosis, bone markers can be used for determining the response to anti-resorptive treatment, amongst which NTx of collagen-I may be suggested to assess bone resorption for Manipuri women.

**Keywords:** Cross sectional study, DEXA, Deoxypyridinoline, C- telopeptide, N- telopeptide, Osteoporosis.

### Introduction

Osteoporosis has become a major public health problem of epidemic proportions affecting more than 100 million people worldwide. Women are most commonly affected i.e. one out of every three postmenopausal women are osteoporotic and a majority of elderly including men too are affected.<sup>1</sup>

Standard diagnosis usually depends on measuring the bone mineral density using a dual energy X-ray absorptiometry (DEXA).<sup>2</sup> Although DEXA is a useful and direct tool for measuring the bone mineral density (BMD), it does not provide information regarding bone formation or resorption, unless the change of bone mineral density is prominent. DEXA scan is also an expensive investigation with limited availability in many parts of India.

Bone turnover markers are the metabolic products of bone resorption or materials that mediate bone metabolism. These markers can provide information regarding bone formation or resorption before the structural changes of bone occur.<sup>3</sup> Of these, urinary Deoxypyridinoline (DPD), N-telopeptide (NTx) and C-telopeptide (CTx) appears to be the most promising bone resorption markers.<sup>4</sup>

Because BMD provides information about current bone status and bone markers provide information about current bone metabolism, both parameters

complement each other, and provide comprehensive information about a patient's bone status and bone activity. Thus, the wider availability of reliable, cost effective, sensitive, and specific assays for bone turnover markers would complement the measurement of BMD in the management of osteoporosis, especially in the follow-up of the patients who had been on anti-resorptive or bone formation therapies. Assays for several biochemical markers of bone turnover are commercially available, but their relative sensitivities are unclear.<sup>5</sup>

This study was done to find the correlation between selected urinary bone markers and bone mineral density (T-score measured by DEXA scan).

### Materials and Methods

A cross sectional study was conducted among 2700 pre and post-menopausal women with intact ovaries in the age range of 21 to 70 years in three districts of Manipur (Imphal west, Thoubal and Churachandpur districts representing urban, rural and hill regions based on the population census of the year 2001. All the wards were listed based on the recent electoral rolls along with their population. From the list, based on the population, wards were selected randomly to meet the required sample so as to have 550 women from 21 to 45 years age group and 350 women from the 46 to 70

years age group. Selection of the individual from the selected wards was done in a systematic random fashion.

For urinary DPD, a sample of 100 in each age group was considered sufficient for the given normal value with SD (taken from previous study) with 1% precision using formula for sample size used in the study.

Pregnant women, lactating women, on steroid or other hormone replacement therapy, chronic endocrinal disorders like thyroid diseases, parathyroid diseases, adrenal diseases' etc, on active medication for osteoporosis within 6 months, women on calcium channel blocker and women with severe systemic disease(s) were excluded from the study.

Study variables were age, urinary DPD, CTx, NTx levels and bone mineral density (T-scores of DXA scan). Bone mineral density was measured by using Lunar Prodigy DXA, GE Healthcare company.

For urinary DPD, CTx, NTx and creatinine estimation, early morning first voided urine sample from each participant was collected.

After being transported in cold box daily and stored at the fridge kept at -20°C, the samples were analysed within 2 days of its collection.

The urinary DPD was measured by ELISA. DPD values were normalized to the urinary creatinine concentration and expressed as nmol DPD/nmol creatinine. Those with DPD level between 3.4 to 7.4 nmol/nmol creatinine were considered to have normal levels. C-telopeptide of collagen I (CTx) and N-telopeptide of collagen I (NTx) were measured in urine with an ELISA. Urinary C-telopeptide (CTx), 220 (SD 128) mg/mol creatinine and urinary N-telopeptide (NTx), 10-110 µmol BCE/mol creatinine were taken as representative reference intervals for CTx, NTx.

Descriptive statistics like mean, median, percentage and percentiles etc. were employed. ANOVA and post-hoc tests were used to determine significance of the findings. A probability value of less than 0.05 was considered significant.

## Result

Normative data is roughly equivalent to 2 standard deviation of the mean or 3<sup>rd</sup> and 97<sup>th</sup> centiles from the quartile distribution of the data. In the present study, the range between 3<sup>rd</sup> and 97<sup>th</sup> centiles of the quartile distribution of the data was referred to as the "Normative data".

**Table 1: Normative value of Urinary biomarkers DPD by age and districts**

Age groups	Imphal	Thoubal	Churachandpur	All combine
	Normative value DPD	Normative value DPD	Normative value DPD	Normative value DPD nmol/L)
<25	2.99-70.00	5.81-65.65	1.33-72.46	
25-39	1.30-76.67	2.01-81.85	1.54-72.55	
40-59	2.30-81.00	1.41-77.95	1.05-67.07	
>60	1.97-83.32	1.47-73.38	1.29-78.00	
Combine	1.80-79.00	2.10-77.00	1.40-71.97	

**Table 2: Normative value of Urinary biomarkers CTx by age and districts**

Age groups	Imphal	Thoubal	Churachandpur	All combine
	Normative value CTx	Normative value CTx	Normative value CTx	Normative value CTx (pg/ml)
<25	57.16-610.00	47.10-433.72	93.01-672.35	
25-39	30.16-743.40	24.31-580.52	26.00-618.25	
40-59	21.92-579.80	36.01-547.05	46.28-685.00	
>60	29.57-438.20	10.00-500.48	61.75-1154.50	
Combine	29.96-602.72	28.52-556.40	46.00-685.00	

**Table 3: Normative value of Urinary biomarkers NTx by age and districts**

Age groups	Imphal	Thoubal	Churachandpur	All combine
	Normative value NTx	Normative value NTx	Normative value NTx	Normative value NTx (nmol/L)
<25	18.68-164.88	13.03-72.41	17.28-235.08	
25-39	12.00-210.75	4.00-175.00	3.38-272.48	
40-59	11.00-219.10	2.00-193.76	2.00-260.00	
>60	9.62-189.25	7.76-196.62	1.00-252.59	
Combine	12.00-197.92	4.00-170.05	2.00-263.50	

Table 1-3 highlights that the normative value of Manipuri women for urinary DPD is 1.6 -77.0 nmol/L, CTx is 33.5 - 614.7 pg/ml and NTx is 4.5 -213.5 nmol/L respectively

**Table 4: Correlation between Urinary biomarkers and T-scores of DXA**

Imphal		Thoubal		Churachandpur		All Districts	
DPD vs DXA (T-score)		DPD vs DXA (T-score)		DPD vs DXA (T-score)		DPD vs DXA (T-score)	
- 0.120	0.000	0.051	0.129	0.017	0.619	- 0.026	0.172
CTx vs DXA (T-score)		CTx vs DXA (T-score)		CTx vs DXA (T-score)		CTx vs DEXA (T-score)	
0.029	0.553	0.034	0.439	- 0.138	0.006	- 0.032	0.246
NTx vs DXA (T-score)		NTx vs DXA (T-score)		NTx vs DXA (T-score)		NTx vs DXA (T-score)	
0.130	0.008	- 0.024	0.589	0.091	0.059	0.070	0.010

P value less than 0.05 is considered significant.

Table 4 showed that among the districts, significant correlation was found between DXA T-score and NTx ( $r=0.070$ ,  $p<0.010$ ), but not for DPD and CTX. Correlation is seen in the Imphal district alone ( $r=0.130$ ,  $p<0.008$ ). It may be inferred that among the three urinary biomarkers, only urinary value of NTx of collagen-I has correlation with T-score of DXA scan.

## Discussion

Osteoporosis increasingly is being recognized as an important cause of morbidity and mortality and it is major public health problem in Indian women. India is one of the leading countries affected by osteoporosis, with one out of two Indian women above the age of 50 years and one out of five Indian men above the age of 65 years at risk of osteoporosis.<sup>6</sup> Amongst the Manipuri women, prevalence of osteoporosis was found to be 21.3%.

To reduce accelerated bone loss in early postmenopause and to minimize the risk of fracture, a variety of antiresorptive treatments (e.g., calcium, calcitonin, estrogens, and bisphosphonates) have been used.<sup>5</sup> A positive response to therapy can be assessed by the measurement of bone mineral density (BMD), but a statistically significant change usually requires 1 or more years because of the imprecision of the BMD measurement.<sup>5</sup> This limitation may be avoided by use of biochemical markers of bone turnover in the serum or urine, which can show the effects of intervention sooner.

In osteoporosis, as bone remodelling process accelerates, the concentration of bone markers in blood and urine are also increased. However, bone markers do not provide information about current bone mineral content which can be obtained through BMD measurements. As BMD provides clue about current bone status and bone markers provide about current bone metabolism, both parameters complement each other, and provide comprehensive information about a patient's bone status and bone activity. The suggested clinical applications for bone markers range from assessment of fracture risk and bone loss to diagnosing

bone diseases. Epidemiologic and cohort studies showed that bone markers do have the potential to be used to assess risk for fracture or rate of bone loss.<sup>3</sup> The most acknowledged application of bone markers is in determining the response to antiresorptive treatment.<sup>7</sup>

In the present study, normative value of urinary biomarkers DPD, CTx and NTx are 1.60-77.00 nmol/L, 33.53-614.70 pg/ml and 4.53-213.47nmol/L respectively. Significant correlation was found between DEXA T-score and NTx ( $r=0.070$ ,  $p<0.000$ ) but not for DPD and CTX. Therefore, NTx of collagen-I could be used to monitor biologic efficacy or adherence to anti-resorptive treatment although this of course implies that the bone marker be determined before initiation of treatment and during subsequent follow-up.

In previous studies like the multinational prospective, open-label, cluster-randomized study of postmenopausal women (IMPACT study), uNTX and sCTX levels were assessed in 2302 women treated with risedronate 5 mg/day and it was found that incidence of non- vertebral fractures was about 50% lower in patients with reductions of uNTX of 30% or more at 22weeks (1.6%) than in those with < 30% reduction (3.2%,  $P = 0.015$ ).<sup>8</sup>

In another study where one hundred and twenty women (mean age, 70 yr) were randomized to alendronate or placebo in a double blind, placebo controlled clinical trial for 2.5 yr, it was found that early changes in serum NTx and CTx, markers of bone resorption, predict long-term changes in vertebral BMD in elderly women receiving alendronate therapy and provide a useful tool to assess skeletal health.<sup>9</sup>

The strong association of bone markers with fracture risk reduction in various studies on osteoporosis treatment complements the use of these markers along with the assessment of BMD in the management of osteoporosis, amongst which NTx of collagen-I may be suggested to assess bone resorption for Manipuri women.

## Conclusion

From this study, it can be concluded that there is significant correlation between DXA T-score and NTx,

but not for DPD and CTX and so NTx of collagen-I may be suggested to assess bone resorption for Manipuri women.

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### References

1. Bonner FJ, Graham P, Adler RA. The Prevention and Treatment of Osteoporosis. In: Delisha JA, Gans BM, Walsh NE, Bockerhek WL, Frontera WR, Geiringer SR, Editors. Physical Medicine and Rehabilitation: Principles and Practice. 5th ed. Vol. I. Philadelphia: Lippincott Williams and Wilkins; 2010. p. 979-1015.
2. El Maghraoui A, Mouinga Abayi DA, Ghozlan I, Mounach A, Nouijai A, Ghazi M, et al. “Urinary markers of Bone turnover in healthy children and adolescents: Age-related changes and effect of puberty.” *Calcif Tissue Int.* 1998;63:369–374.
3. Garnero P. “Biomarkers for osteoporosis management.” *Mol Diag Ther.* 2008;12:157–170.
4. Iba K, Takada J, Hatakeyama N, Ozasa Y, Wada T, Yamashita T. “Changes in urinary NTX levels in patients with primary osteoporosis undergoing long-term bisphosphonate treatment.” *J Orthop Sci.* 2008;13:438–441.
5. Chailurkit L, Ongphiphadhanakul B, Piaseu N, Saetung S, Rajatanavin R. “Biochemical Markers of Bone Turnover and Response of Bone Mineral Density to Intervention in Early Postmenopausal Women: An Experience in a Clinical Laboratory.” *Clinical Chemistry.* 2001;47:1083-1088.
6. Malhotra N, Mithal A. “Osteoporosis in Indians” *Indian J Med Res.* 2008;127:263.
8. Delmas PD, Recker RR, Chesnut CH III, Skag A, Stakkestad JA, Emkey R, et al. “Daily and intermittent oral ibandronate normalize bone turnover and provide significant reduction in vertebral fracture risk: Results from the BONE study.” *Osteopor Int.* 2004;15:792-798.
9. Eastell R, Vrijens B, Cahall DL, Ringe JD, Garnero P, Watts NB. “Bone turnover markers and bone mineral density response with risedronate therapy: Relationship with fracture risk and patient adherence. *J Bone Miner Res.* 2011;6:662-1669.
10. Chen P, Satterwhite JH, Licata AA, Lewiecki EM, Sipos AA, Misurski DM, et al. “Early changes in biochemical markers of bone formation predict BMD response to teriparatide in postmenopausal women with osteoporosis.” *J Bone Miner Res.* 2005;20,962-70.

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