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24 Hours Urinary Hydroxyproline - A Noninvasive, Cost-Effective and Early Biochemical Marker Which May be Used to Screen the Osteoporotic Lesion in Postmenopausal Women

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ABSTRACT

Introduction: Osteoporosis results due increased rate of bone turnover. It has multifactorial etiology and most common in women after menopause. **Objective:** The current study was aimed to assess the rate of bone turnover towards detection of osteoporotic changes by measuring 24 hours urinary hydroxyproline which is an early as well as non- invasive biochemical bone marker. **Methods:** Urinary hydroxyproline was measured in 40 postmenopausal women and compared with similar number of premenopausal women as controls. **Results:** 24 hours urinary hydroxyproline levels were significantly higher ($p < 0.001$) in postmenopausal women than premenopausal groups. This indicates a higher rate of bone turnover suggesting osteoporotic changes. 24 hour urinary hydroxyproline is also positively correlated ($r = 0.934$) with age. **Conclusion:** The present study suggests that measurement of 24 hours urinary hydroxyproline which is a cost-effective and non- invasive technique may be used for screening and early detection of osteoporotic changes in women of postmenopausal age group.

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INTRODUCTION

Metabolic bone disease refers to the diseases of bones that result from partial uncoupling or imbalance between bone resorption and bone formation. Decreased bone mass or osteopenia is more common than abnormal increases of bone mass. The most prevalent metabolic bone diseases are osteoporosis, osteomalacia, rickets and renal osteodystrophy.[1] Among these osteoporosis is the most common one. Osteoporosis can be defined as reduction in the quantity of bone or atrophy of skeletal tissue; an age-related disorder characterized by decreased bone mass and loss of normal skeletal microarchitecture, leading to increased susceptibility to fractures.[2] Osteoporosis is defined by the World Health Organization (WHO) as a bone mineral density that is 2.5 standard deviations or more below the mean peak bone mass (average of young, healthy adults) as measured by dual energy X-ray absorptiometry (DEXA).[3]

The disease may be classified as primary type 1, primary type 2, or secondary. The form of osteoporosis most common in women after menopause is referred to as primary type 1 or postmenopausal osteoporosis. Primary type 2 osteoporosis or senile osteoporosis occurs after age 75 and is seen in both females and males at a ratio of 2:1. Finally, secondary osteoporosis may arise at any age and affect men and women equally. This form of osteoporosis results from chronic predisposing medical problems or disease, or prolonged use of medications such as glucocorticoids, when the disease is called steroid- or glucocorticoid-induced osteoporosis.[4-6] Osteoporosis affects 55% of Americans aged 50 and above. Of these, approximately 80% are women. It is estimated that 1 in 3 women over the age of 50 worldwide have osteoporosis.[7]

Biochemical markers reflect alteration in bone remodeling much earlier than they are apparent radiographically.[8,9]

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It has been studied that, a period of 1 to 3 years must pass before measurement of bone mass (for example, dual energy X-Ray absorptiometry, (DEXA) can identify statistically significant changes. Biochemical markers of bone resorption like C and N telopeptides, pyridinoline, deoxypyridinoline, urinary hydroxyproline, tartarate resistant acid phosphatase, serum and urinary calcium and markers of bone formation like serum total alkaline phosphatase, bone specific alkaline phosphatase, osteocalcin, procollagen type 1 propeptide provide much earlier assessment of bone resorption and/or formation.[1] With the above background the aim of our study is to assess the rate of bone turnover in postmenopausal women by measuring 24 hours urinary hydroxyproline which is an early as well as non- invasive biochemical bone marker.

MATERIALS AND METHODS

This case control study was conducted in Burdwan Medical College and Hospital, a rural based medical college and hospital in West Bengal (India). Forty postmenopausal women as cases were selected for the study. The patients were selected with history of backache, knee pain and generalized body pain few years after menopause. Subjects with any chronic illness, cancer, hyperthyroidism and liver disease were not included in the study. Patients taking any medications like corticosteroids, methotrexate, hormone replacement therapy, selective estrogen receptor modulator and with history of smoking and alcoholism were also excluded from the study. Simultaneously, 40 premenopausal women of reproductive age group were selected as control. Fasting blood and 24 hrs urine sample were collected after obtaining the informed consent and maintaining the standard protocol for the biochemical tests. The study was pre-approved by institutional Ethics Committee. Urinary hydroxyproline level was measured following Modified Neuman and Logan method.[10-12] Three to four ml of urine sample was taken in a test tube. The sample was filtered through whattman filter paper twice. From it 1 ml of the sample was taken in a clean test-tube. To the sample, 1 ml of (0.01M) $CuSO_4$, 1 ml of (2.5 N) NaOH, 1ml of 6% H_2O_2, 4 ml of H_2SO_4, and 2 ml of Ehrlich's reagent (4 dimethyl aminobenzaldehyde) were added. Mixed well and waited for 5 minutes. Thereafter these were placed in water bath at 80˚C for 5 minutes. Then cooled, followed by keeping again in water bath at 70˚C for 15 minutes. The colour formed was read by spectrophotometer at 540 nm keeping distilled water as blank. The results were obtained from the standard curve and were expressed in mg/24 hours.

Statistical analysis

Statistical analyses were carried using windows 7 excel and SPSS 17 software. Data were expressed as mean ± standard deviation with $p<0.001$ considered as significant.

RESULTS

Figure 1 shows the standard curve of urinary hydroxyproline. The clinical and biochemical parameters of the study subjects have been depicted in Table 1. 24 hours urinary hydroxyproline levels were found to be significantly higher in postmenopausal women. No significant difference between other parameters was observed.

DISCUSSION

Throughout life body keeps a balance between the loss of bone and the creation of new bone. The highest bone mass is at about age of 30 years. Then, sometime between age 30 and 35 years, body begins to lose bone faster than it can be replaced.[13] After that, resorption exceeds formation and bone density decreases through the rest of life, which in turn may lead to osteoporosis. Investigations like dual energy X-ray -absorptiometry (DEXA) may be used for screening women who would be more vulnerable to osteoporosis, but assessment of biochemical markers are easier and non- invasive way of detecting early osteoporotic changes in women, especially in places where the DEXA scan facilities are not so readily available.

A total of 80 women are included in this study out of which 40 are postmenopausal and 40 are premenopausal women according to their history.

In the present study hydroxyproline is measured in 24 hours urine. The cases were found to have significantly higher level ($p<0.001$) of urinary hydroxyproline when compared to controls (Table 1 and Figure 2). Rise in urinary hydroxyproline is due to increased collagen breakdown as a result of increased bone resorption in the postmenopausal women. Our results agree with the earlier observations.[14,15] Twenty-four hrs urinary hydroxyproline is significantly increased ($p<0.001$) with increase in age (Figure 2). These observations highlight that the rate of bone resorption increases with advancing age which has been also observed in earlier studies.[16,17] This change signifies that after

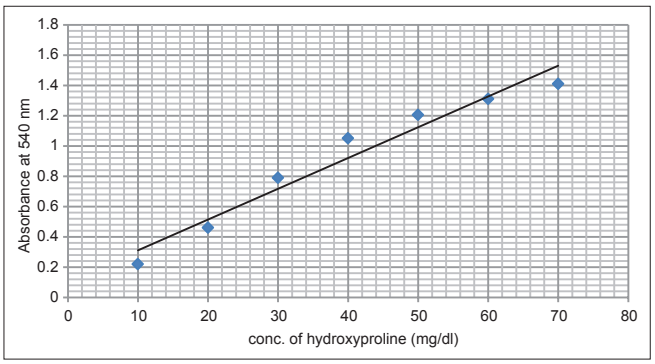

Figure 1: Standard Curve of Urinary Hydroxyproline

menopause the action of many hormones get altered like the levels of estrogen,[18,19] dehydroepiandrosterone, [20] testosterone, [21] and inhibin[22] get lowered and serum follicle stimulating hormone is increased[17] that ultimately leads to metabolic alterations. Previous study by Barbu C et al,[23] Charatcharoenwitthaya N et al,[24] Srivastava S et al[25] and Sachdeva A et al[10] also showed similar findings. The cellular and molecular mechanisms by which estrogen deficiency leads to bone loss are increasingly well understood. Estrogen deficiency increases receptor activator of nuclear factor kappa B ligand (RANKL) [26] leading to increased osteoclast recruitment and activation and decreased osteoclast apoptosis. RANKL is the final key molecule required for osteoclast development, and is normally expressed by bone marrow stromal/osteoblast precursor cells, T-lymphocytes, and B-lymphocytes.[27] RANKL binds to its receptor RANK on osteoclast lineage cells[28] and is neutralized in the bone microenvironment by its soluble decoy receptor osteoprotegerin (OPG), which is produced and secreted by osteoblast lineage cells.[29] Combined in vitro and in vivo studies have shown that estrogen normally suppresses RANKL production by osteoblastic cells and T- and B-lymphocytes and increases OPG production by osteoblastic cells so that estrogen deficiency leads to an increase in the RANKL/OPG ratio that favors bone resorption.[30] Estrogen is thought to suppress production of bone-resorbing cytokines such as interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-α, macrophage colony-stimulating factor (M-CSF), and prostaglandins by the appropriate cells. With estrogen deficiency, each cytokine likely accounts for cytokine-mediated age-related bone loss.[31] Further analysis of our results revealed that 24 hours urinary hydroxyproline has significant positive correlation (r= 0.934) with age (Figure 3).

Table 1: Clinical and Biochemical parameters of the study population

Parameters	Case Mean±SD N=40	Control Mean±SD N=40	p value
Age (yrs)	58.62±3.9	37.7±3.5	<0.001
BMI	21.4±2.0	22.1±1.8	0.076
Systolic pressure (mmHg)	122.87±11.28	121.39±9.31	0.532
Diastolic pressure (mmHg)	82.35±7.64	80.12±7.94	0.095
FBS (mg/dl)	94.83±18.74	93.65±14.70	0.761
TG (mg/dl)	125.8±42.8	118±38.6	0.476
LDL (mg/dl)	84.44±42.2	88.65±20.2	0.201
HDL (mg/dl)	40.34±30.1	44.65±24.6	0.457
Total cholesterol (mg/dl)	150.2±30.4	146.6±31.3	0.246
Total Bilirubin (mg/dl)	0.71±0.10	0.65±0.13	0.092
SGPT (IU/L)	20±1.6	19±1.8	0.072
SGOT (IU/L)	18±1.2	21±1.0	0.095
Urea (mg/dl)	24.87±7.56	26.65±10.12	0.189
Creatinine (mg/dl)	0.88±0.31	0.92±0.20	0.403
Urinary Hydroxyproline (mg/24 hrs urine)	32.64±2.03	16.75±1.29	<0.001

Despite the adverse effects of osteoporosis, it is a condition that is often overlooked and undertreated, because it is so often clinically silent before manifesting in the form of fracture. One in three women over age of 50 years will develop the disease during their lifetime. Osteoporotic fractures are a common cause of morbidity and mortality in postmenopausal women. This silently increasing metabolic bone disease is extensively prevalent in developing countries.

The biochemical markers of bone turnover like 24 hours urinary hydroxyproline has a great role in the assessment of rate of bone loss and towards early diagnosis of osteoporosis. This study substantiates the importance of regular screening of the women of postmenopausal age group attending the clinic with complaints of knee pain, generalized body ache etc. through clinical evaluation and measurement of 24 hours urinary hydroxyproline which is a very early and non- invasive marker of osteoporotic changes.

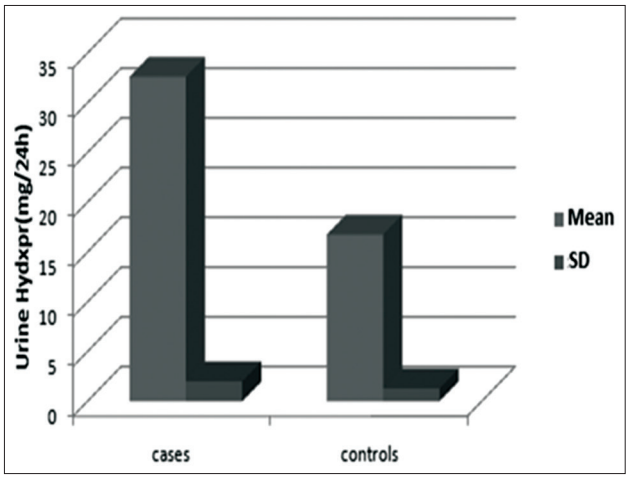

Figure 2: Showing the Mean and Standard SD of Urinary Hyrorxyproline in cases and controls

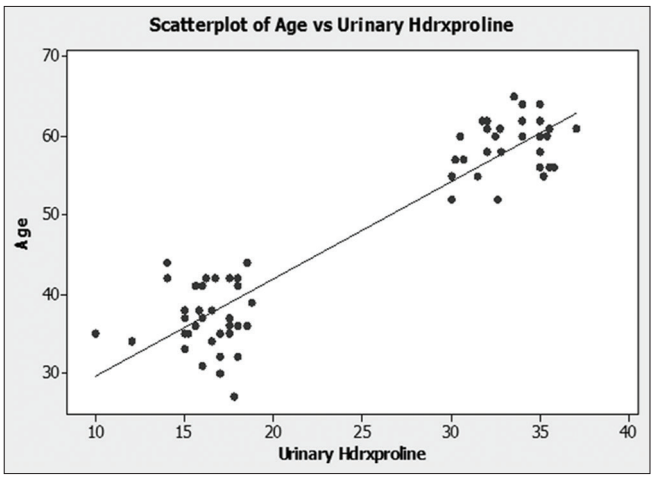

Figure 3: Figure showing Correlations of Age with Urinary Hydroxyproline

CONCLUSIONS

Although considering a small sample size we conclude that measurement of 24 hours urinary hydroxyproline which is a cost-effective and non- invasive technique may be used for screening and early detection of osteoporotic changes in women of postmenopausal age group.

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Authors Contribution:
MD – Designed the study, Data Acquisition, Data Analysis and Drafting of Manuscript. **UKB –** Data Analysis, drafting of Manuscript, Review of Manuscript. **AK –** Manuscript Preparation, Data Analysis, Review of Manuscript, Final Approval.

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