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ORIGINAL ARTICLE

Effect of Antiresorptive Therapy on Urinary Hydroxyproline in Postmenopausal Osteoporosis

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Abstract Osteoporosis becomes a serious health threat for older postmenopausal women by predisposing them to an increased risk of fracture. Osteoporosis and associated fractures are an important cause of morbidity and mortality. Special attention is being paid to early detection, management, and treatment of postmenopausal osteoporosis in women. Biochemical markers can enable dynamic and rapid measurement of total body skeletal metabolism and will be clinically useful in the management of postmenopausal osteoporosis women (PMO) and also for assessing the effects of antiresorptive therapy. With this view, we planned to assess osteoclastic activity by determining urinary hydroxyproline in osteoporotic women. The aim of this study is to measure urinary hydroxyproline (expressed as mg of hydroxyproline/g of creatinine) and serum ascorbic acid in postmenopausal women with osteoporosis and without osteoporosis. These biochemical parameters were determined 3 months post antiresorptive therapy (alendronate + calcium + vitamin D) in postmenopausal osteoporosis patients. 60 postmenopausal women with osteoporosis in the age group 45–60 years and 60 healthy postmenopausal women (normal bone mineral density) in the same age group were included in the study. Urinary hydroxyproline levels were significantly increased ($P < 0.001$) in PMO at baseline level as compared to control group. These levels were decreased significantly ($P < 0.001$) post therapy in PMO patients. Serum vitamin C levels were significantly decreased ($P < 0.001$) in PMO patients at baseline level as compared to controls. No significant change occurred of serum vitamin C level post therapy. Raised excretion of hydroxyproline at the baseline level might be due to increased degradation of collagen type I from the bone matrix in osteoporosis. Breakdown of collagen seems to be lowered as reflected by lowering of hydroxyproline excretion post antiresorptive therapy. Alteration in the concentration of this marker can be very well utilized to monitor the effectiveness of therapy. Thus simple, direct urinary assay to measure bone resorption is very useful in monitoring the therapy in PMO and may become an integral part of the management of osteoporosis.

Keywords Postmenopausal osteoporosis women (PMO) · Bone resorption marker · Urinary hydroxyproline (OHPr) · Urinary creatinine · Serum ascorbic acid

Introduction

Osteoporosis is a disease that may have a tremendous impact on the lives of many postmenopausal women. Osteoporosis and it's potentially devastating sequelae of fracture are increasing as the population ages, hence assessment of skeletal health is an important component of a women's routine care [1].

This silently progressing metabolic bone disease is widely prevalent in India, and osteoporotic fractures are a common cause of morbidity and mortality in adult Indian men and women [2]. Expert groups peg the number of osteoporosis patients in India at approximately 26 million (2011 figures) with the numbers projected to increase to 36 million by 2013 [3]. One out of three females and one out of five males in India suffers from osteoporosis, making India one of the largest affected countries in the world [4].

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Thus, the occurrence of osteoporosis in postmenopausal women is very common problem especially in India who is exposed to many of the risk factors [5]. But there are very few Indian studies regarding the prevalence of osteoporosis in postmenopausal women and also regarding the biochemical markers which indicate bone turnover in our setup.

International consensus development conference has stated that osteoporosis is a progressive systemic skeletal disorder characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture [6]. The hallmark of menopause is a reduction in skeletal mass caused by an imbalance between bone resorption and formation due to loss of ovarian function. Hence, loss of ovarian function is the most important factor in the development of postmenopausal osteoporosis [7]. The pathogenesis of postmenopausal osteoporosis involves the interplay of many factors such as aging, hormonal, nutritional, environmental, and genetic and life style factors etc. [8].

Typically, the urinary markers of bone resorption include hydroxyproline, which serve as rapid predictors of changes in collagen metabolism [5]. Hydroxyproline is the major breakdown product of collagen, the main protein of the bone matrix. It is considered as clinical index of bone resorption and a major determinant of bone status [9]. In developed countries several studies are performed on the utility of urinary hydroxyproline as a bone resorption marker. Today's needs of research are studies on such markers of bone metabolism especially in Western Maharashtra, where such studies are not found to be performed.

Study of this bone resorption marker will throw light for better understanding the pathophysiology of disease, in addition to its aid in management of the osteoporotic patients receiving antiresorptive therapy. With this view, we planned to determine urinary hydroxyproline in postmenopausal osteoporotic patient's pre and post antiresorptive therapy.

Materials and Methods

Present study was conducted in the Department of Biochemistry, Government Medical College Miraj and P.V.P.Government Hospital; Sangli. Study group included 60 postmenopausal women in the age group 45–60 years and diagnosed as primary osteoporosis by clinicians. Diagnosis was based on clinical features, any fracture and radiological evidence of osteoporosis at one or more sites and lower BMD. Control group included 60 postmenopausal non osteoporotic women with normal bone density in the age group 45–60 years. Patients with secondary type of osteoporosis, liver disease, renal disease, metastatic bone disease, having a chronic debilitating illness (cancer, AIDS), patients taking hormone replacement therapy and anticonvulsants, were excluded from this study.

The study group was given alendronate 70 mg/week and Tablet containing calcium citrate 1200 mg (elemental calcium-253 mg) and calcitriol 0.25 μg was taken as once a day. Patients were instructed to take bisphosphonate on an empty stomach with a glass of plain water. Avoid lying down, stay fully upright (sitting, standing or walking) and other food, beverages or medication to be avoided for at least 30 min for better absorption and to avoid side effects (esophagitis).

The Institutional Ethical Committee approved the plan of study and informed consent was obtained from each participant in the study.

Fasting blood and fasting urine samples were collected from control group and from study group at baseline level under aseptic conditions. In the follow up study blood and urine samples were collected from study group after 3 months therapy. Serum was separated and analyzed for vitamin C by photometric method [10]. Urinary hydroxyproline was done by Bergman and Loxley method [11] and expressed as mg/g of creatinine. Creatinine in urine was determined by Jaffe's method [12].

The results were expressed as means ± SD. Statistical analysis was done by using z test and paired t test.

Results and Discussion

Biochemical parameters relevant to bone metabolism can give an idea as to the rates of bone formation and resorption. High rate of bone turnover correlates with a low bone mass. During bone loss, collagen fibrils are broken down and hydroxyproline is excreted in the urine. In this study urinary hydroxyproline is expressed as mg of hydroxyproline/g of creatinine, because creatinine is excreted in the urine in relatively constant amounts, thus serving as a reference standard [9, 13]. Significant increase in the levels of urinary hydroxyproline (mg/g Cr) was observed in PMO when compared with controls ($P < 0.001$, Table 1). Thus increased excretion of hydroxyproline indicates increased breakdown of collagen. Several factors might be responsible for excess bone resorption such as low estrogen level, calcium and vitamin D deficiency and age related reduced calcium absorption leading to excess parathyroid hormone.

During bone resorption, highly active osteoclasts may secrete factors into the space between the cell and bone surface such as acids, matrix metalloproteinases (MMPS) and cathepsin K in excess. These factors can degrade collagen type I into hydroxyproline. Our findings were also

Table 1 Biochemical parameters of bone turnover in control group and PMO women pre and post therapy

Sr. no	Biochemical parameters	Postmenopausal nonosteoporosis women	Postmenopausal osteoporosis women	
		Controls $n = 60$	Baseline $n = 60$	Post therapy $n = 60$
1	Urinary hydroxyproline (mg/g creatinine)	17.188 ± 5.110	34.751 ± 8.768***	24.958 ± 5.260***
2	Vitamin C (mg/dl)	2.063 ± 0.467	1.388 ± 0.519***	1.468 ± 0.495

The statistical method used to compare data was Z test and paired t test

*** $P < 0.001$ highly significant

supported by Indumati et al. [14] and Sachdeva et al. [5]. Similar observations were also reported by a number of other studies [9, 15, 16]. Table 1 shows that urinary hydroxyproline level was significantly decreased from baseline to post therapy of 3 months in PMO. The therapy contains a potent nitrogen containing drug i.e., alendronate which binds hydroxyapatite crystals of bone with high affinity and inhibit bone resorption by decreasing osteoclastic activity and its growth. After the inhibition of resorption, these agents become affixed to the bone matrix, where they reside until the remodeling begins again. Thus it has very long retention in the skeleton and may exert long term effects. Reduction in bone loss during antiresorptive treatment is demonstrated by decreased excretion of hydroxyproline post antiresorptive therapy. Our findings were also supported by Fardellone et al. [17], Kamel et al. [18], and Horowitz et al. [19] and will definitely aid the orthopaedicians of Western Maharashtra to monitor collagen and bone metabolism and better management of PMO.

Previous studies [20] have shown that lipid peroxidation was increased in osteoporosis due to oxidative stress. We were interested in measuring the status of antioxidant vitamin i.e. vitamin C. Reduction of the vitamin C level may impair hydroxylation of lysine and proline in protocollagen. Without this hydroxylation crosslinking of protocollagen into normal collagen may be affected. Thus fibrils or/and the structure may disintegrate rapidly. Our findings were also supported by Simon et al. [21], and Morton et al. [22]. Table 1 shows that after therapy, no significant change occurred in vitamin C level in PMO.

In this study, elevated osteoclastic (bone resorption) marker was found in PMO women. The result indicates that high bone turnover occurs in osteoporosis. Processes involved in bone formation are probably unable to keep pace with the rate of bone resorption. Rise in the excretion of hydroxyproline clearly shows degradation of collagen type I from the bone matrix in osteoporotic women. Our results demonstrate the impaired bone metabolism and reverse bone turnover in osteoporosis. Bone resorptive therapy contains alendronate + calcium + vitamin D is effective in optimizing the bone metabolism. The rate of osteoclastic activity and breakdown of collagen is lowered as reflected by lowering of hydroxyproline excretion after 3 months therapy. Thus we can conclude that control of osteoclastic activity was definitely initiated by the treatment. Prolonged treatment may be required to observe total normalization of bone metabolism. Thus simple, direct urinary assay to measure bone resorption are very useful in monitoring the therapy in PMO and may become an integral part of the management of osteoporosis.

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