Content available at: https://www.ipinnovative.com/open-access-journals

International Journal of Clinical Biochemistry and Research

Journal homepage: https://www.ijcbr.in/

Original Research Article

Study of bone turnover markers – Alkaline phosphatase and urinary hydroxyproline in postmenopausal women

Lakshmi D[1],*, Shivkumar Pujari[1]

[1]*Dept. of Biochemistry, Sri Siddhartha Medical College, Tumkur, Karnataka, India*

ARTICLE INFO

Article history:
Received 17-02-2021
Accepted 17-03-2021
Available online 30-04-2021

Keywords:
ALP
Bone turnover markers
Estrogen
Hydroxyproline
Osteoporosis
Post menopause

ABSTRACT

Introduction: Osteoporosis is a major cause for morbidity in elderly and is further accentuated by the low levels of estrogen in postmenopausal women. Recognition of bone changes early by estimating the levels of bone turnover markers in the post menopausal women in comparison with pre menopausal women would aidin decreasing the disability caused due to fracture.
Aim: To find the difference between the levels of ALP and urinary hydroxyproline in pre menopausal and postmenopausal women.
Materials and Methods: In our study 45 post menopausal women and 45 premenopausal women attending the OPD for different reasons were recruited and after obtaining informedconsent, blood sample and urine samples were collected. Alkaline phosphatase (ALP) was estimated inserum sample and Hydroxy proline levels were estimated in the urine sample.
Result: Both the serum ALP and urinary hydroxyproline levels were significantly ($p<0.001$) elevated in postmenopausal women.
Conclusion: Bone turnover markers play a role in assessing the bone health in post menopausal women and also aid in prognosis when an individual is on bone formation drugs.

1. Introduction

Bone is a dynamic tissue that undergoes remodelling constantly throughout life. It consists of two types of cells, Osteoblasts and Osteoclasts which aids in the formation and resorption of bone respectively.[1] Osteoclasts resorb bone by producing hydrogen ions to mobilize minerals and proteolytic enzyme to hydrolyze the organic matrix. Osteoblasts synthesize the organic matrix (Collagen and non collagenous Protein) and control mineralization of the newly synthesized matrix.[2]

Bone formation is an orderly process in which inorganic mineral is deposited in relation to organic matrix. Parathyroid hormone (PTH) and Vitamin D (Calcitriol) are the two principle hormones regulating the process of bone formation and resorption.[3]

In general the processes of bone formation and resorption are coupled, so that there is no net change in bone mass. In children and early adulthood, formation exceeds resorption so that bone density increases and then plateaus until the age of 30 to 40 yr. After 40 – 50 yrs of age, cortical bone is lost at a rate of about 0.3 – 0.5% per year in both the sexes.[4]

Menopause is the permanent cessation of menstruation due to loss of ovarian follicular function, which results in decreased production of estradiol and other hormones.[5] Decreased levels of Estrogen leads to increased osteoclast formation and hence enhanced bone resorption, which in turn leads to loss of bone density and destruction of local architecture resulting in micro fractures.[6] The loss of cortical bone is accentuated by age related loss around menopause.[7]

Osteoporosis, an important public health problem of elderly is the most common metabolic bone disorder.[8,9] It is characterized by a structural deterioration of bone tissue

* *Corresponding author*.
E-mail address: drlakshmimurthy@gmail.com (Lakshmi D).

https://doi.org/10.18231/j.ijcbr.2021.003

thus leading to an increased risk of fracture. It is estimated that 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 are at risk of osteoporosis.[10–12] Diagnosis of Osteoporosis is based on assessment of BMD by DEXA scanning.[13] It has its own limitations like being expensive and not being available in all parts of the country.

Bone turnover markers(BTMs) have shown to provide the valuable information in assessing the metabolic changes in the bone.[14] These include bone formation markers (Osteocalcin, Alkaline phosphatase, Procollagen / carboxy and N terminal extension peptides) and bone resorption markers (Type I collagen N and C telopeptide breakdown products, Urinary pyridinoline and Deoxy pyridinoline And Fasting Urinary calcium and Hydroxyproline). BTMs provide an index of the overall Bone Mineral Density(BMD) and would complement the findings obtained by DEXA scan(dual energy xray absorptiometry).[15]

ALP is a ubiquital enzyme that plays an important role in osteoid formation and mineralization.[16] Hydroxyproline is essential for the formation of cross links in the collagen, which gives the tensile strength to the fibres and has been considered as an index of bone resorption and a determinant of bone status.[17,18]

Hence, this study was conducted to find the difference between the levels of ALP and urinary hydroxyproline in pre menopausal and postmenopausal women, thus aiding in the diagnosis of early bone changes in menopausal women and hence prevent further damage to the bone by adopting few preventive measures. It also aids in the follow-up of the patients who are on antiresorptive or bone formation therapies.

2. Materials and Methods

A cross-sectional study was conducted in Sri Siddhartha Medical College, Tumkur. A total number of 45 healthy premenopausal women volunteer (25 – 40 years) and 45 healthy postmenopausal women in the age group of 55 – 65 attending Obstetrics and Gynaecology OPD for different reasons were included in the study. Ethical Clearance was obtained from the Institutional ethical committee and Informed consent was obtained from patients and controls. Subjects with history of smoking and alcohol intake, surgically induced menopause and those on hormone replacement therapy (HRT) were excluded from the study.

Under aseptic conditions, 5ml of venous blood was collected from antecubital vein in plain tubes and serum was separated by centrifuging at 3000 rpm for 10 minutes. Serum levels of ALP were estimated on autoanalyser EM-200 by ALP-AMP method.

Subjects were instructed to collect 24 hours urine in a container with HCl as preservative. (Hcl 5 ml/l of Urine). Urinary hydroxyl proline was evaluated in this sample by Modified Neuman and Logan method.[19] Total volume was noted and calculation was done for 24 hours.

The data obtained was analyzed. The study variables were expressed in terms of mean ± standard deviation (S.D) and differences in the mean were compared using student's t-test The P value <0.001 was considered as significant. Statistical analysis was performed using software SPSS windows.

3. Results

In our study the mean age among pre menopausal women was 28 years and among postmenopausal women the mean age was 62 years. Table 1 shows the comparison of various biochemical parameters showing a significant increase in Alkaline Phosphatase and urinary hydroxyproline in the post menopausal women. ($p< 0.001$).

The increased excretion of hydroxyproline in the urine is due to increased resorption of bone leading to bone loss, a characteristic feature of the immediate postmenopausal period.

Table 1: Comparisons of biochemical parameters in serum and urine

Study variables	**Controls –** Premenopausal women	**Cases-** postmenopausal women	**P value**
Age	27.95± 6.33	62.43± 6.76	0.0001
Urinary HP(mg/day)	58.96±5.34	116.04±24.83	0.0001
Serum ALP (IU/L)	113.06±25.48	178.69±29.90	0.001

In our study the mean value of serum ALP in post menopausal women is 178.69±29.90 I.U/ L and in pre menopausal women is 113.06±25.48 IU/L respectively. The mean value of serum ALP in postmenopausal women is higher when compared to premenopausal women. The increase is found to be highly significant and isdue to the inhibitory effects of estrogen on bone turnover rate which is dependent on age and body mass index (BMI).

4. Discussion

Bone Turnover markers(BTMs) determine the dynamics of bone remodeling with respect to bone formation and resorption.

Our study shows that there is increase in the excretion of Hydroxy proline in post menopausal women and the findings are in accordance with the findings of Sachdeva et al.,[17] Indumati et al.[20] Deficiency of estrogen as observed in postmenopausal women, accelerates the process of bone resorption in them because of sub optimal functioning of estrogen receptors in the absence of the hormone.

During bone resorption, the collagen fibrils are broken down releasing hydroxyproline. The released

Hydroxyproline is excreted in the urine(OHPr) and the levels of which is considered as an index of bone resorption and a major determinant of bone status (Sachdeva et al., 2015). Thus monitoring the levels of Hydroxyproline in the urine plays a vital role in decreasing the incidence of fractures commonly observed in elderly women. The increased morbidity due to fracture in elderly postmenopausal women can be decreased by supplementing with calcium and Hormone replacement therapy(HRT).

Serum alkaline phosphatase(ALP) is the most commonly used marker of bone formation. ALP is a ubiquitous enzyme that plays an important role in osteoid formation and mineralization. In adults with normal liver function, approximately 50% of the total ALP activity in serum is derived from the liver, whereas 50% arises from bone. In our study the total ALP levels were significantly high in postmenopausal women in comparison to premenopausal women (Table 1). This shows that the bone mass continues to decline with age. The findings of our study are in accordance with the study conducted by Indumathi et al., 2007.[20]

Hence a direct, reliable, simple, cost-effective, sensitive, and specific assay to measure bone resorption has clinical applications as part of screening programs to assess the risk of osteoporotic fractures.[21] Monitoring bone status through urinary excretion of OHPr could serve as a surveillance measure in early intervention. These markers will also aid in the follow up of patients receiving bone formation treatment.

5. Source of Funding

None.

6. Conflicts of Interest

None.

7. Acknowledgement

We would like to acknowledge faculty of Department of OBG, Principal and my colleagues of Department of Biochemistry, SSMC for their continuous support.

References

1. Simsek B, Karaca O, Karaca I. Urine products of bone breakdown as markers of bone resorption and clinical usefulness of urinary hydroxyproline: an overview. *Chin Med J*. 2004;117(2):291–5.
2. Florencio-Silva R, Sasso GRS, Sasso-Cerri E, Simões MJ, Cerri PS. Biology of Bone Tissue: Structure, Function, and Factors That Influence Bone Cells. *BioMed Res Int*. 2015;2015:1–17. doi:10.1155/2015/421746.
3. Calvo MS, Eyre DR, Gundberg CM. Molecular basis and clinical application of biological markers of bone turnover. *Endocr Rev*. 1996;17(4):333–68. doi:10.1210/edrv-17-4-333..
4. Puri V. Biochemical Markers: Diagnostic Considerations and Clinical Applications for Osteoporosis Assessment. *Bombay Hosp J*. 2003;45(2).
5. Finch CE. The menopause and aging, a comparative perspective. *J Steroid Biochem Mol Biol*. 2014;142:132–41. doi:10.1016/j.jsbmb.2013.03.010.
6. Manolagas SC. Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. *Endocr Rev*. 2000;21(2):115–37.
7. Seeman E. Age- and menopause-related bone loss compromise cortical and trabecular microstructure. *J Gerontol A Biol Sci Med Sci*. 2013;68(10):1218–25.
8. Nguyen VH. Osteoporosis prevention and osteoporosis exercise in community-based public health programs. *Osteoporos Sarcopenia*. 2016;3:18–31.
9. Durden E, Pinto L, Lopez-Gonzalez L, Juneau P, Barron R. Two-year persistence and compliance with osteoporosis therapies among postmenopausal women in a commercially insured population in the United States. *Arch Osteoporos*. 2017;12(1):22. doi:10.1007/s11657-017-0316-5.
10. Malhotra N, Mithal A. Osteoporosis in Indians. *Indian J Med Res*. 2008;127(3):263–8.
11. Shetty S, Kapoor N, Naik D, Asha HS, Prabu S, Thomas N. Osteoporosis in healthy South Indian males and the influence of life style factors and vitamin d status on bone mineral density. *Osteoporos*. 2014;doi:10.1155/2014/723238.
12. Meeta, Agarwal N, Digumarti L, Malik S, Shah R, Vaze N. Clinical practice guidelines on menopause: FNx01An executive summary and recommendations. *J Mid-life Health*. 2013;4:77–106. doi:10.4103/0976-7800.115290.
13. Genant HK, Engelke K, Fuerst T, Glüer CC, Grampp S, Harris ST, et al. Noninvasive assessment of bone mineral and structure: state of the art. *J Bone Miner Res*. 1996;11(6):707–30.
14. Paul TV, Shetty S, Kapoor N, Bondu JD, Thomas N. Bone turnover markers: Emerging tool in the management of osteoporosis. *Indian J Endocrinol Metab*. 2016;20(6):846–52. doi:10.4103/2230-8210.192914.
15. Delmas PD, Eastell R, Garnero P, Seibel MJ, Stepan J. The Use of Biochemical Markers of Bone Turnover in Osteoporosis. *Osteoporos Int*. 2000;11(0):S2–S17. doi:10.1007/s001980070002.
16. Kleerekoper M. Biochemical Markers of Bone Turnover: Why Theory, Research, and Clinical Practice Are Still in Conflict. *Clin Chem*. 2001;47(8):1347–9. doi:10.1093/clinchem/47.8.1347.
17. Sachdeva A, Seth S, Khosla AH, Sachdeva S. Study of some common Biochemical bone turnover markers in post menopausal women. *Ind J Clin Biochem*. 2015;20(1):131–4.
18. George BO. Urinary and Anthropometrical Indices of Bone Density in healthy Nigerian Adults. *J Appl Sci Environ Mgt*. 2013;7(1):19–23.
19. Leach AA. Notes on a modification of the Neuman and Logan method for the determination of the hydroxyproline. *Biochem J*. 1960;74(1):70–1.
20. Indumati V, Patil VS, Jailkhani R. Hospital based preliminary study on osteoporosis in postmenopausal women. *Indian J Clin Biochem*. 2007;22(2):96–100. doi:10.1007/bf02913323.
21. Nelson HD, Morris CD, Kraemer DF, Mahon S, Carney N, Nygren PM, et al.. Osteoporosis in postmenopausal women: Diagnosis and Monitoring. Summary, Evidence Report/ Technology Assessment: No.28. Agency for Healthcare Research and Quality Publication No. 01-E031, Feb 2001. Available from: http://www.ahrq.gov/clinic/epcsums/osteosum.htm.

Author biography

Lakshmi D, Assistant Professor

Shivkumar Pujari, Associate Professor

Cite this article: Lakshmi D, Pujari S. Study of bone turnover markers – Alkaline phosphatase and urinary hydroxyproline in postmenopausal women. *Int J Clin Biochem Res* 2021;8(1):12-14.