IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 14, Issue 6 Ver. I (Jun. 2015), PP 15-17
www.iosrjournals.org

Serum Calcium and Urinary Hydroxyproline Levels in Postmenopausal Women: A Case Control Study in North Coastal Andhra Pradesh

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Abstract:
Background *: Bone resorption can be assessed by measuring plasma tartarate resistant acid phosphatase and urinary excretion of collagen degradation products: Hydroxyproline,Hydroxylysine glycosides,pyridinium crosslinks and associated peptides. We compared the excretion of Hydroxyproline in women of premenopausal age group to those of post menopausal age group and found a significant difference in the two age groups. Urinary Hydroxyproline was found to be significantly raised in postmenopausal women. This study was undertaken to diagnose at the earliest osteoporotic changes in the postmenopausal women by the easily available, reliable and cost effective colorimetric methods.*
Method : *A total of sixty (60) postmenopausal women were taken as cases. Forty (40) premenopausal women were taken as controls.*
Results: *Urinary Hydroxyproline levels were higher in postmenopausal women with 26.2 mg /24 hrs ± 4.67 mg/24hrs(MEAN ± SD) when compared to controls with 16.1mg/24 hrs ± 1.49 mg/24hrs(MEAN ±SD) with a statistically significant 'p' value of <0.01. Serum calcium levels were decreased in cases with 8.55 mg/dl ±1.02 mg/dl(MEAN±SD) compared to controls with 9.15 mg/dl ±0.642 mg/dl(MEAN±SD) with a statistically significant 'p' value of < 0.01.*
Conclusion: *Increased levels of Hydroxyproline can be used as a marker of bone turn over in postmenopausal women to assess risk of developing osteoporosis.*
Keywords*-Menopause, Hydroxyproline, Osteoporosis,Calcium.*

I. Introduction

Bone is a dynamic tissue that is being remodelled constantly throughout life. It is composed primarily of the inorganic minerals (calcium and phosphate) and type I collagen constitutes the organic matrix . There are two main types of bone cells i.e., osteoclasts and osteoblasts. Bone cells participate in the growth, modelling and remodeling of bone although they account for only a small fraction of bone volume[1].

Organic matrix consists principally of collagen (90%), other matrix proteins and proteoglycans. It is rapidly mineralized by osteoblasts in close apposition to and throughout the collagen fibrils[2]. Despite its seemingly static appearance, bone is remarkably a labile tissue. Rate of formation or degradation of the bone matrix can be assessed by measuring the enzymatic activity related to the bone forming or resorbing cells. Bone matrix components are released into the circulation, either by the osteoblasts or by the osteoclasts [3].

Bone formation is an orderly process in which inorganic mineral is deposited in relation to organic matrix. During bone resorption, first calcium and phosphorus are released into the extracellular fluid and organic matrix is then resorbed.. The concentrations of calcium, phosphate and magnesium in plasma are dependent on the net effect of bone mineral deposition and resorption , their intestinal absorption and renal excretion. Parathyroid hormone (PTH) and 1, 25-dihydroxycholecalciferol (calcitriol) are the principal hormones regulating these processes[2]. After 40-50 years of age, cortical bone is lost at a rate of about 0.3-0.5% per year in both the sexes. An accelerated loss of cortical bone is superimposed on age related loss around menopause[4].

Menopause is the consequence of the exhaustion of ovarian follicles which results in decreased production of oestradiol and other hormones. Osteoporosis is defined as a disease that cause a reduction in the mass of bone per unit volume and is one of the dreaded afflictions of aging[5]. There is a close relationship between oestrogen deprivation and development of osteoporosis. Several other factors like muscle bulk, body weight, malabsorption, smoking, alcohol and genetic factors also affect density of the bones[4]. Oestrogen plays an important role in the growth and maturation of bone as well as in the regulation of bone turnover in adult bone. During bone growth , oestrogen is needed for proper closure of epiphyseal growth plates both in females and in males. Also in young skeleton, oestrogen deficiency leads to increased osteoclast formation and enhanced bone resorption. In menopause, oestrogen deficiency induces cancellous as well as cortical bone loss. At cellular level, oestrogen inhibits differentiation of osteoclasts thus decreasing their number and reducing the amount of

active remodeling units. This effect is probably mediated through some cytokines, IL-1 and IL-6 being strongest. .Oestrogen deprivation is suggested by early development of osteoporosis in women who attained premature menopause either due to natural or surgical cause[5]. Decreased levels of oestrogen in post menopausal women prevents absorption and utilisation of bone calcium and hence there is development of osteoporosis in post menopausal women.

Most of the traditional and new markers of the bone resorption measure the collagen degradation products from osteoclast activity and these include urinary Hydroxyproline, Hydroxylysine and its glycosides, total or free pyridinoline crosslinks and crosslinked N or C telopeptides. Hydroxyproline is mainly found in collagen and accounts for 13% of total aminoacid content and derived from proline by post translational hydroxylation. It is used as a marker of osteoclastic bone resorption. Only about 10% Hydroxyproline released during collagen catabolism is excreted in urine. Significant amounts of Hydroxyproline are also found in c_1q fraction of complement. Hydroxyproline may be determined in timed fasting samples or 24 hours urine collection .It must be kept in mind that it is influenced by diet. Urinary Hydroxyproline was used as an indicator in assessment of postmenopausal women for risk of developing osteoporosis and fractures.

II. Materials And Methods

The present study was conducted on sixty(60) outpatients from Department of Obstetrics and Gynaecology in King George hospital , Andhra medical college , Visakhapatnam, India. The mean age group was 46 to 75 years. Forty (40) healthy pre menopausal women were taken as controls. Their mean age group was 25 to 45 years. Most of cases and controls are vegetarians.

Inclusion Criteria

- Women who attained menopause.

Exclusion Criteria:

- Pregnant women.
- Women on Hormone replacement therapy(HRT).
- Other conditions which interfere with results like liver diseases, renal pathology , thyroid diseases, etc.

Method:

Estimation of urinary Hydroxy proline by modified Newmann and Logan method. The principle of this method includes: Oxidation of Hydroxyproline with hydrogen peroxide in presence of alkaline copper sulphate. Destruction of excess of peroxide by heat. Reaction of oxidation product with p- dimethyl amino benzaldehyde by heating in presence of dilute sulphuric acid to produce red colour and the absorbance is measured colorimetrically at 540 nm. Serum calcium was also measured by Arsenazo's method colorimetrically.

III. Results And Observations

In the present study , mean value of Hydroxyproline in cases is 26.2 mg/24 hrs ± 4.67 mg/24 hrs (MEAN ± SD) when compared to controls 16.1 mg/24 hrs ± 1.49 mg/24hrs (MEAN ± SD) with a statistically significant' p' value of < 0.01. Mean value of serum calcium among controls is 9.15 mg/dl ± 0.642 mg/dl (MEAN ± SD) when compared to cases 8.55 mg/dl ± 1.02 mg/dl (MEAN ± SD) with a statistically significant p value of < 0.01.

IV. Discussion

Majority of the controls (80%) and the cases (88%) were vegetarians. Diet has also been proven to be an independent risk factor for the development of osteoporosis. High protein diet (non-vegetarian diet) particularly leads to excessive acid formation which may contribute to "dissolution" of bones as the body tries to buffer the extra acid. Acidosis may also increase osteoclastic function directly.

A total of 100 subjects were included in the study. It included forty (40) premenopausal women (controls) and sixty (60) postmenopausal women (cases). The estimated mean serum calcium level in controls was in range of 9.15 mg/dl ± 0.642mg/dl (MEAN ± SD) and in postmenopausal women was in range of 8.55 mg/dl ± 1.02 mg/dl(MEAN ± SD) . It indicates that serum calcium level was significantly decreased in postmenopausal women than in controls with a statistically significant p value of < 0.01. The present study correlated with studies of Indumathi .v.vidya s patil[7] and steven[8]. In the Study of Vidya patil , total and ionized calcium were significantly decreased and urinary Hydroxyproline was significantly raised in postmenopausal women compared to premenopausal women.

The estimated mean value of urinary Hydroxyproline in controls is 16.1 mg/ 24 hrs ± 1.49mg/24hrs and in cases is 26.2 mg/ 24 hrs ± 4.67mg/24hrs. The result showed a significant increase in urinary Hydroxyproline value in cases compared to controls with a statistically significant p value of < 0.01. The results

correlated with studies of Indumathi.v[7], ashuma sachadeva, shashi seth[6] and Ren XH,XD[9]. Increased level of Hydroxyproline was due to excessive bone loss in postmenopausal women. Study of Ashuma Sachadeva , shashi seth was that urinary Hydroxyproline was significantly raised in postmenopausal women compared to women of reproductive age group. Study of Ren XH was that urinary Hydroxyproline was significantly raised in postmenopausal women compared to women of reproductive age group

Thus measure of urinary Hydroxyproline is an useful index of bone resorption in postmenopausal women . Hence it's measure will play an important role in early diagnosis of osteoporosis and will help in decreasing the incidence of fractures commonly observed in elderly women. Accordingly, we can supplement calcium or put the patient on HRT. Any single measurement of a single biochemical marker of bone turnover has limited utility in the individual person. Thus increase in urinary excretion of Hydroxyproline and decrease in serum calcium together were used and were found to be significant in postmenopausal women reflecting the increased bone activity (osteoclastic and osteoblastic) as compared to premenopausal women[10].

Much of the knowledge gained in the last decade on osteoporosis and other metabolic bone diseases has come from three different approaches: Bone density, Bone biopsy and Biochemical assays. Each approach has advantages and disadvantages. Bone density measurements are noninvasive, site specific and are sufficiently sensitive to measure changes in bone density. However they are expensive, have limited availability and are unable to identify early changes resulting from therapy[11]. Bone biopsy is an invasive procedure, hence biochemical assessment of skeletal metabolism holds great importance. These markers reflect alterations in bone remodeling much earlier than they are apparent radiographically. In addition ,these markers now make it possible to determine the efficacy of anti-resorptive drugs, and their optimum dosage in a time frame that is reasonably much less as compared to months before there is a radiographic evidence of a therapeutic response. They have untapped potential in the evaluation of patient at risk for accelerated bone loss e.g. in postmenopausal women[12].

V. Conclusion

Altered levels of serum calcium and urinary Hydroxyproline show that bone remodelling is accelerated at menopause . Increased levels of urinary Hydroxyproline can be used as a marker to assess bone turn over in post menopausal women. Thus assessing bone markers is useful in evaluation of osteoporosis.

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