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Biochemical Markers in Pre and Post Menopausal Women

KEYWORDS: ALP, Osteocalcin, Urinary hydroxyproline, Acid Phosphatase.

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ABSTRACT *INTRODUCTION: -As part of the aging process, women above 45 years of age enter into the phase of menopause that leads to silent physical changes including osteoporosis and genito-urinary problems which are preventable to some extent. In the mean while, bones are constantly remodelled with old bone being replaced by new bone. However, the other main reason women experience rapid bone loss due to decline in estrogens during menopause. It is therefore, essential to identify these women at risk of developing osteoporosis.*

OBJECTIVE: - The study aimed at comparing the levels of biochemical markers such as bone formation and bone resorption in pre and post menopause women.

MATERIALS AND METHODS: - Study Design- Prospective and observational study. The study was carried out in the Department Of Biochemistry, MGM Medical College, and Navi-Mumbai. The study comprised of 50 pre-menopause women aged between 40-40 years were selected as control (group 1) with regular menses and no complications related to menstruation, whereas, 50 post-menopause women aged between 41-60 years were selected as (group2) with irregular menses and peri-menopause women (irregular menses that is before menopause) was also considered whereas, more than 1yr of menopause women were preferred more.

RESULT: - There was a significant decrease in Serum Total Calcium and Phosphorous in postmenopausal women ($p \leq 0.001$). Similarly it was observed that bone formation markers i.e. Serum ALP, Serum Osteocalcin levels and bone resorption markers Acid Phosphatase and Urinary excretion of Hydroxyproline were significantly increased in postmenopausal Women compared to premenopausal women ($p \leq 0.001$) and there was strong correlation between ALP and urinary hydroxyproline ($p \leq 0.001$).

CONCLUSION:-In our study after comparing the biochemical parameter in pre and post menopause women we conclude that osteocalcin is increased which is a bone-turnover marker provide clues for bone-turnover and bone loss. This bone loss is seen in post menopause women due to lack of estrogen production and bone remodelling.

INTRODUCTION

Over 75 million people worldwide suffer from osteoporosis of which 80% are postmenopausal women; (1) 44 million of these are in the United States. (2) The number of osteoporotic patients in India is approximately 26 million, with the numbers projected to increase to 36 million by 2013. (3)

Menopause is one of the most important physiological events: it is related to the cessation of ovarian function and the end of reproductive cycle. (4) The incidence of osteoporosis in postmenopausal women continues to increase with progressively aging populations. (2) Premenopause refers to a woman's reproductive or fertile life, from the first menstrual period to the last. Post-menopause is defined as the time after menopause, at least one year without a period. (4)

Early onset of menopause increases risk for many postmenopausal health complications, such as osteoporosis, ischemic disease, and ovarian cancer, whereas later menopause is a risk factor for endometrial and breast cancer. (4)

Osteoporosis is defined as impairment in bone strength due to an abnormal quantity and/or quality of bone. Quantity is evaluated by measuring BMD. Quality is affected by many factors, including the degree of mineralization, the rate of bone remodelling, the connectivity of the bony trabeculae, the quality of the collagen fibres, and the health of the bone cells. The 3 types of bone cells are osteoblast, osteoclasts, and osteocytes. Therefore, it is very important to know the osteoblast and osteoclasts production. (5)

In women, the two major causes of bone loss are estrogen deficiency after the menopause and age-related process. There is a direct relationship between the lack of estrogen after menopause and the development of osteoporosis. After menopause, bone resorption (breakdown) out spaces the building of new bone i.e. bone remodeling is imbalanced. (6)

Therefore, osteoporotic risk for women increases soon after menopause. Bone turnover markers are known to be associated with bone loss and fracture risk. (7)Hence it is significantly valuable to study different markers such as, osteocalcin, urinary hydroxyproline in detail and also the biochemical parameters such as vitamin C, calcium, phosphorous, alkaline phosphatase, and acid phosphatase.

The biochemical marker which was studied in pre and post-menopausal women are osteocalcin a bone formation marker and Urinary hydroxyproline a born resorption marker.

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Osteocalcin (OC) is useful as a biochemical indicator of bone turnover. Osteocalcin is found exclusively in bone tissue. Osteocalcin levels can also be elevated in post-menopausal osteoporosis due to increased or decreased bone turnover. Depressed levels of osteocalcin have been reported in hypo-parathyroidism and during long-corticosteroid therapy. Advantage of using osteocalcin as a clinical index of bone turnover is its specificity, its wide availability. (8)

Most of the traditional and new markers of the bone resorption measure the collagen degradation products from osteoclasts activity and these include urinary hydroxyproline, hydroxylysine and its glycosides, total or free pyridinoline cross links and cross linked N or C telopeptides.(9)

Calcium is one of the main bone forming minerals and 99% of the body's calcium resides in the skeleton. Whereas, phosphorous is an essential bone-forming element because it is required for the appropriate mineralization of the skeleton. Hence the ratio of phosphorous to calcium intake seems to be more important in bone health than absolute intake of phosphorous. (10)

Vitamin C is a cofactor in the hydroxylation of lysine and proline and therefore is required in the cross-linking of collagen fibrils in bone. Vitamin C stimulates alkaline phosphatase activity, a marker for osteoblast formation (10) and deficiency can lead to thinning of the cortices and loss of trabeculae architecture. (11)

Other markers of bone metabolism include enzyme levels in the serum that arise from osteoblast or osteoclasts activity; namely bone specific alkaline phosphatase (ALP) (osteoblast) and tartrate resistant acid phosphatase (osteoclasts). The present study is done to evaluate the levels of biochemical markers in pre and post menopause women. Study of these predicting factors as biochemical markers will help to study bone metabolism in pre and postmenopausal women.

AIM: - The present study was undertaken to evaluate the levels of biochemical markers in pre and post menopause women.

OBJECTIVES:-To evaluate the following parameters in pre and post-menopause women**.**

Bone formation markers :-
1) Serum osteocalcin

2) Estimation of serum calcium

3) Estimation of serum inorganic phosphorous

4) Estimation of serum alkaline phosphatase

5) Estimation of serum vitamin C

Bone resorption markers :-
1) Urinary Hydroxyproline

2) Serum Tartarate resistance acid phosphatase

MATERIALS AND METHODS
Necessary approval from the Institutional Ethics Committee was obtained before initiating the study.

STUDY DESIGN: - Prospective and observational study.

STUDY SITE: - The present study was carried out from April 2014 to April 2015 in the Department Of Biochemistry, MGM Medical College, and Navi-Mumbai.

SELECTION CRITERIA:-The study comprises of healthy women from college staff and other attending hospital as relatives of patient.

The patients were grouped in the following groups:-

Group I: - 50 patients with pre-menopause women- age 30 to 40 years

Group II: -50 patients with post-menopause women- age 41 to60years Written consent was taken from healthy individual.

Inclusion criteria: - for pre menopause women

Pre-menopause women (aged 30-40 Years) Regular menses.

No complications related to menstruation

Inclusion criteria: - for post menopause women Post-menopause women (aged 41-60 Years)

Peri-menopause woman is considered (irregular menses that is before menopause) and more than 1yr of menopause women is preferred more.

Exclusion criteria: - for both
Women with no fractures, any disease related bone, treatment with calcitonin vitamin-D, calcium supplementation.

Corticosteroids treatment within 6 months, diuretic or other medications that might interfere with water-mineral or lipid metabolism.

Women treatment with estrogen or progesterone, irregular menses and women with history of hysterectomy and myomectomy will be excluded.

METHOD:-Total 5 ml of venous blood was collected under aseptic condition and was allowed to clot, centrifuged at 3000rpm. Serum was obtained and was collected in a clean and dry, plain bulb to study following parameters. Calcium, phosphorous and alkaline phosphatase (12) was given directly to analyse on Beckman coulter auto analyser AU480. The remaining serum samples were thawed at -20^{o}c and were used for the remaining test to be performed i.e. Osteocalcin (14), Tartarate resistance acid phosphatase (15), Vitamin C and Urinary Hydroxyproline was detected in the 24hrs.urine samples (collected in the morning) which were considered as a bone resorption marker. (9)

The data obtained was analyzed, and the differences in the mean of various parameters were compared using student's t-test. Statistical analysis was performed using software SPSS windows.

RESULTS
Table no 1 shows the comparison of all the biochemical parameters estimated and their mean values. There were significant decreases in Serum Total Calcium and Phosphorous in postmenopausal women ($p \leq 0.001$). Similarly it was observed that bone formation markers i.e. Serum ALP, Serum Osteocalcin levels and bone resorption markers Acid

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Phosphatase and Urinary excretion of Hydroxyproline were significantly increased in postmenopausal women when compared to premenopausal women (p≤0.001).

Graph 1 is depicting that there is significant correlation between levels of osteocalcin and urinary hydroxyproline (p≤0.05) whereas the study found that there is highly significant correlation between levels of ALP and urinary hydroxyproline as there (p≤0.0001) as seen in Graph 2.

Table 1:-Comparison of markers of bone turnover in pre- and post Menopausal women (values expressed as mean ± SD)

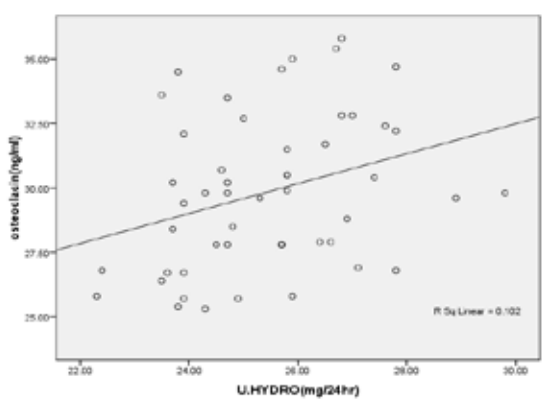
	Pre-menopause (n=50)	Post-menopause (n=50)
Age	36.49 ± 2.65	53.52 ± 5.30
Calcium (mg/dl)	10.064 ± 0.64	8.7496 ± 0.64**
P (mg/dl)	3.5296 ±0.58	2.065 ± 0.37**
ALP (U/L)	48.5 ± 4.82	101.7 ± 12.3**
Vit C (mg/dl)	1.08 ± 0.369	1.024± 0.335#
Osteocalcin (ng/ml)	15.686 ± 3.33	29.848 ± 2.98**
ACID.P (U/L)	6.762 ± 1.55	11.52 ± 1.17**
U.HYDRO (mg/24hr)	18.38 ± 1.33	25.44 ±1.64**

Indicates **highly significant (p≤ 0.001), # (p≥0.05) not significant.

Graph 1:- Showing Correlation Coefficient between Levels of Osteocalcin (Ng/Ml) Against Urinary Hydroxyproline (Mg/24hr) in Post-Menopause Women

	Post-menopause women
Pearson Correlation	0.319*
p-value	<0.05
N	50

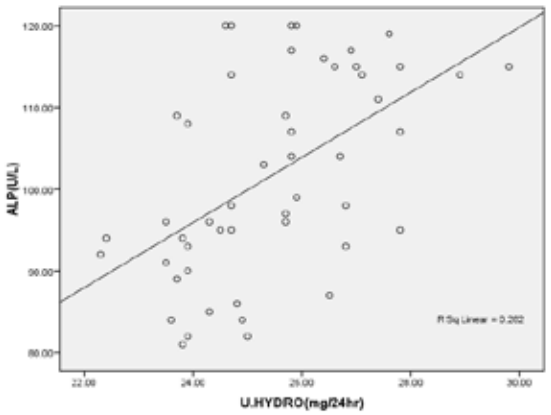
Indicates *significant p≤ 0.05



GRAPH 2:- Showing Correlation Coefficient Between Levels of Alp Against Urinary Hydroxyproline (Mg/24hr) in Post-Menopause Women

	Post Menopause group
Pearson Correlation	0.531***
p-value	<0.001
N	50

Indicates ***highly Significant (p<0.0001)



DISCUSSION

The hallmark of menopause is a reduction in skeletal mass caused by an imbalance between bone resorption (osteoclastic activity) and bone formation (osteoblastic activity) due to lack of estrogen. (16) It is therefore, essential to identify these women at risk of developing osteoporosis. The study was aimed at comparing the levels of biochemical markers such as bone formation and bone resorption in pre and post menopause women.

In osteoporotic women, deficiency of calcium may lead to lowering of formation of hydroxyapatite crystals. Thus, in the state of decreased rate of bone mineralization, free osteocalcin may be available for circulation in the blood. Related findings were in accordance with the studies of other investigation (**Storm *et al.*, 1998**). (17) This explains the increased concentration of osteocalcin in the serum and decreased levels of Ca and P in the osteoporotic postmenopausal women as showed in the table no.1.

The above data of this study indicated involvement of estrogen and formation in the pathogenesis of menopause women. Therefore, estrogen deficiency is one of the causes of menopausal progression. (18)

Fibrillar collagens are rich in the amino acid hydroxyproline, which is excreted in the urine after collagen degradation and are considered to be markers of bone resorption. The urinary excretion of Hydroxyproline is increased in states of physiologically high turnover, such as somatic growth, during menopause and high turnover osteopathy's. The urinary excretion of Hydroxyproline is increased in states of physiologically high turnover. Therefore, Serum total ALP and urinary hydroxyproline was significantly increased (p<0.001) and total calcium was significantly decreased (p<0.001) in cases compared to controls (table-no.1)

TRACP is increased significantly due to estrogen deficiency during menopause and increase in osteoclastic activity as there is complex interaction between hormonal effects and local regulation of bone formation and resorption at cellular level. Following menopause, osteoclastic activity leading to greater resorption of bone. (19)

Significant positive (p≤0.05) **correlation between osteocalcin and Urinary hydroxyproline were found in the study as** the osteocalcin increases during bone turnover so as same in the case of alkaline phosphatase an enzyme required for the formation during bone remodelling, as both are bone formation markers. They show positive correlation that means there is more osteoblast formation due to

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lack of estrogen during menopause for the need of bone remodelling. This clearly shows that in menopause women the bone turnover is high due to lack of estrogen.(graph 1)

Highly significant positive ($p \leq 0.0001$) **correlation between ALP and Urinary hydroxyproline** were observed in post-menopause women as showed in (graph 2). - The estrogen shortage at menopause raises the rate of the bone remodelling, which leads to an elevated bone turnover and the osteoblast receptors stop functioning efficiently due to the lack of hormones, which can be seen as a significant rise in the mean values of the markers of resorption from pre-menopause to post-menopause.

CONCLUSION

In our study after comparing the biochemical parameter in pre and post menopause women we conclude that osteocalcin is increased which is a bone-turnover marker provide clues for bone-turnover and bone loss. This bone loss is seen in post menopause women due to lack of estrogen production and bone remodelling. The incidence of osteoporosis in postmenopausal women continues to increase with age progression.

Our study could be beneficial for monitoring bone status using biochemical parameters and serve as a screening measure in early intervention against excessive bone loss. It is also essential to increase awareness of the implications of osteoporosis because available evidence

CONSENT & PROFORMA: Authors declare that written informed consents and proforma were obtained from the healthy individuals before their participation in the study.

ETHICAL APPROVAL: All authors hereby declare that approval from Institutional ethics review committee was obtained and study was carried out as per standards.

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