



STUDY OF SERUM URIC ACID, CALCIUM AND ALP AND URINARY HYDROXYPROLINE CREATININE RATIO IN POSTMENOPAUSAL WOMEN

Biochemistry

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ABSTRACT

During menopause women succumb to physiological and biochemical changes along with middle age health hazards. In view of sudden fall in Estrogens during menopause Osteoporosis of aging is fortified by Estrogen deficiency. The present study was done to evaluate bone health through biochemical parameters in Guntur Urban area between September 2021 and January 2022. 50 Premenopausal women and 60 postmenopausal women were selected for the study after screening verbally. Serum Calcium, Phosphorus, Alk, Phosphatase and Uric acid along with 24Hrs urinary excretion of Creatinine, Calcium and Hydroxyproline were estimated in both groups. We could not find wide difference in the mean values of serum Ca, P, ALP & UA but Urine 24 Hrs Ca was lower in postmenopausal women (104.55 ± 23.86) compared to premenopausal group (124.42 ± 17.78). Urine Hydroxyproline (HOP) showed higher values in postmenopausal women (25.79 ± 1.44) than in Premenopausal women (18.00 ± 2.55). All the analytes showed statistically significant values between the groups and within the groups with P value value of <0.0001 . 24Hrs urine Hydroxyproline, which is collagen degradation product correlated with 24hrs urine Ca and Creatinine. With P value <0.0001 Serum ALP, Ca & Uric acid as shown by ANOVA, both within groups and between groups was statistically significant $P < 0.0001$. We conclude that collagen degradation product like HOP can be evaluated and considered as marker for assessment of bone health in postmenopausal women.

KEYWORDS

Menopause, Alkaline Phosphatase, Uric acid, Urine Hydroxyproline

BACKGROUND

Women attain menopause between 40yrs and 50yrs mean age being 49.66yrs (Agrawal et al, 2009). After menopause women have life expectancy of approximately 30-40yrs. Though we can not abbreviate aging, there is a constant search to predict forthcoming health issues following menopause and improve women's health in the postmenopausal period.

INTRODUCTION

Menopause is physiological cessation of menstrual cycles in women due to reduced secretion of Estrogens by ovaries. Nonavailability of Estrogens is basis for certain postmenopausal sequelae like Osteoporosis, Cardiovascular disease, Hypertension. Morbidity and mortality secondary to Osteoporosis was leading cause of concern in view of fractures following minimal trauma. FSH concentration increases after menopause. As menopause is attained in late middle age (around 5th decade) comorbid conditions like Diabetes mellitus, Hypertension, lipid abnormalities may also worsen the situation leading to more deleterious effects. Estrogens have uricosuric effect & hyperuricemia is detectable during menopause¹.

Osteoporosis is defined as reduced bone mass per unit volume. Osteoporosis (OP) is common after 50yrs in both men & women but more pronounced in postmenopausal women. Prevalance of OP is as high as 36 million in India (Indumati et al 2007^{2,3}). Bone mineralisation and Ca absorption from intestines decreases as age advances.⁴ Estrogens play a pivotal role in growth and maturation of osteoblasts as well as mineralisation. Estrogens deficiency of menopause increases production of cytokines (TNF α , IL -1, IL -6) in liver and adipose tissue. These cytokines increase growth and differentiation of osteoclasts.^{5,6} All these factors precipitate osteoporosis, bone remodelling activity increases. Evaluation of Bone Mineral Density (BMD) is a valuable tool to assess bone health, but instruments like Dual Emission X-ray Absorptiometry (DEXA) are not yet easily available in India. Hence we depend on biochemical markers of bone turnover to identify high risk group of OP.

Biochemical markers are classified as Bone formation markers & Bone resorption markers. Bone formation markers are Alkaline Phosphatase, Osteocalcin, Serum Calcium & resorption markers are Hydroxy proline in serum & Urine, urinary Calcium, Procollagen I N-terminal peptide (PINTP) are detectable in serum and urine. OP increases morbidity, mortality by aggravating risk of fractures (femur, hip).

Serum uric acid increases in elderly females due to increased cell turnover & reduced excretion by the kidneys.⁷

Aim :

To study relationship between Serum Uric acid, Ca⁺⁺, P, ALP and urinary Hydroxyproline/creatinine ratio (HCR), Urine HOP Ca⁺⁺ ratio in postmenopausal women.

Objectives :

1. To study correlation of Serum Ca⁺⁺ with Serum Phosphorus (P), Uric acid(UA) & ALP in premenopausal and postmenopausal women
2. To assess correlation of HOP/ Creatinine ratio in premenopausal women with postmenopausal women
3. To assess correlation of HOP / Ca ratio in premenopausal & postmenopausal women

MATERIAL AND METHODS :

This was a randomised controlled study conducted in the urban population of Guntur city, Andhra Pradesh, India. 50 Premenopausal women and 60 postmenopausal women in the age group of 40-60yrs were included in the study after taking their informed consent. Women who were not menstruating for more than 1 Yr were considered postmenopausal and included in the study. Individuals with Diabetes Mellitus, Hypertension, diseases of reproductive system (Ca Cx, DUB), Cardiovascular disease, Skin rashes, disease of bone /joints and hormonal disorders / therapy were ruled out by verbal interview.

Approximately 5ml of venous blood sample was collected under strict aseptic conditions and centrifuged at 3,000rpm for 5 minutes. The individuals were asked to collect 24 hrs urine in container with preservatives. The blood samples were analysed for Ca⁺⁺, Phosphorus, Uric acid, ALP, Total Proteins & Albumin in Johnson & Johnson Vitros 250. Urinary hydroxyproline (HOP) estimated by modified Neuman and Logan method, Creatinine by Modified Jaffe's method and urinary Ca⁺⁺ estimated by colorimetric method using Beacon calcium test kit.

Urine Ca⁺⁺ Estimation :

1ml urine sample is diluted to 10ml with Normal saline. (Principle) Calcium in an alkaline medium combines with O- Cresolphthalein Complexone to form a purple coloured complex. Intensity of the colour formed is measured at 540nm and is directly proportional to the amount of calcium in urine.

Determination of Urinary Hydroxyproline : (Modified Neuman and Logan Method)

Reagents	Test	Standard	Blank
Urine	1ml		
Standard		1ml	
DW			1 ml
.CuSO ₄ (0.01M)	1 ml	1 ml	1 ml
NaOH (2.5N)	1 ml	1 ml	1 ml
6% H ₂ O ₂	1 ml	1 ml	1 ml
H ₂ SO ₄ (3N)	4ml	4ml	4ml
p-dimethyl aminobenzaldehyde	2ml	2ml	2ml

Mixed and kept at RT for 5 minutes. Placed in a water bath at 80°C for 5 minutes. Cooled in ice followed by keeping in water bath at 70°C for 16 minutes. Read the colour at 540 nm. Intensity of colour is directly proportional to the con. of HOP. Ratios of urine HOP/Ca++ & HOP/Creatinine and Ca++/Creatinine are calculated.

RESULTS:

The study group consisted of 50 pre-menopausal and 60 post-menopausal women. Analysis of biochemical values expressed as mean $\pm$ SD (Table 1). Statistical comparison of values amongst the analytes was performed using Student's 't' test and ANOVA. P value of <0.05 with confidence interval of 95% was considered as statistically significant.

In the premenopausal group values of analytes were within normal range and in the postmenopausal women Serum Ca++, ALP and Uric acid were elevated. 24 Hrs Urine Ca++, HOP, & Creatinine were within normal range in both groups. The ratios of 24Hrs Urine Ca++ and Hydroxyproline with respect to creatinine were considered in this study as creatinine is excreted as phosphate and relatively constant. Ratio of urinary Ca++/Creatinine and HOP/Creatinine were within normal limits in both groups, though Ca / Creat ratio was marginally elevated in postmenopausal women. The ratio of HOP/ Creat is much lowered in both groups and the same is more pronounced in postmenopausal group.

Between the groups Ca++ , P, ALP and Uric acid were statistically significant (P value <0.0001) as shown in table 1. Urine parameters between the groups are statistically highly significant individually with a P value of <0.001. Whereas the ratios HCR & HOP/Ca were statistically significant (P value <0.0001; Table 1) These findings are in accordance with studies of Indumathi.v et al 2007, Ashuma Sachadeva, Shashi Seth⁸ and Ren XH,XD⁹

Total proteins and albumin were analysed to assess nutritional status of the subjects and found to be within normal range in both groups though they did not obey statistical significance (P value <0.589 & < 0.198 respectively)

Table 1: The values of analytes expressed as Mean $\pm$ SD in premenopausal and postmenopausal women and P value of analytes paired 't' test at 95% CI & α <0.05

SERUM PARAMETERS				
Parameter	Normal values	Premenopausal N=50	Postmenopausal N = 60	P Value
Ca (mg/dl)	9-11	9.18 $\pm$ 0.72	8.06 $\pm$ 0.64	<0.0001
Total protein (G/dl)	6.0 – 8.0	6.4 $\pm$ 3.41	6.0 $\pm$ 4.2	<0.589
Albumin G/dl)	3.5 – 5.5	3.8 1 $\pm$ 2.56	3.08 $\pm$ 3.23	<0.198
Phosphorus (mg/dl)	2.8 – 4.5	2.08 $\pm$ 0.89	2.78 $\pm$ 0.55	<0.0001
ALP (IU/l)	23 - 92	105.8 $\pm$ 46.6	129.2 $\pm$ 62.6	<0.031
Uric acid (mg/dl)	3.0 – 5.0	6.6 $\pm$ 1.79	8.9 $\pm$ 2.99	<0.0001
URINE PARAMETERS				
Ca (mgs / 24 Hrs) (Normal 100-300)	124.42 $\pm$ 17.78	104.55 $\pm$ 23.86		<0.0001
Hydroxy proline mgs/24hrs (Normal 7 – 49) REF 5)	18.00 $\pm$ 2.55	25.79 $\pm$ 1.44		<0.0001

Creatinine mgs/24Hrs (1000 – 2000)	1250 $\pm$ 179.37	980.2 $\pm$ 130.13	<0.0001
HOP /Ca Ratio (Normal 0.7 - 2.3)	0.144 $\pm$ 0.14	0.246 $\pm$ 0.06	<0.0001
Ca / Creat Ratio (Normal <0.14))	0.099 $\pm$ 0.09	0.11 $\pm$ 0.18	<0.6952
HOP / Creat Ratio (HCR) (Normal 0.003 – 0.015)	0.014 $\pm$ 0.014	0.026 $\pm$ 0.011	<0.0001

N is number of subjects. CI is confidence interval

ANOVA of serum Ca , P, ALP, UA and urine Ca & HOP within premenopausal and postmenopausal women was statistically significant as shown in Table 2.(P value < 0.0001) Similarly between the groups also Analysis of Variance was statistically highly significant (P value <0.0001 ; Table 3) except for total proteins and albumin.

ANOVA of HOP corrected to Ca and Creatinine was statistically significant at 95%CI with P<0.0001.(Table 4). These results correlated with the findings of Deepa S. Sajjanar¹ and Sanjeev L et al^{10,11}

Table 2 One-way ANOVA of serum Ca, P, ALP, UA, URINE Ca, & HOP within Premenopausal and Post-menopausal women

GROUP	F - ratio	P value
Premenopausal	368.120	<0.0001
Postmenopausal	247.065	<0.0001

Table 3 : One-way ANOVA of analytes between premenopausal and post- menopausal women

PARAMETER	F Ratio	P - value
P	25.472	0.0001
Ca	75.54	0.0001
ALP	4.777	0.03
UA	22.764	0.0001
TP	0.293	0.59
ALB	1.676	0.198
URINE		
Ca	23.695	0.0001
HOP	405.344	0.0001
Creatinine	83.245	0.0001

Table 4 : One-way ANOVA of ratios of urinary analytes between the groups

	df	F-Ratio	P -value
Between	5	38.104	<0.0001
Within	324		
Total	329		

DISCUSSION:

Why we should worry about causes of Osteoporosis in post-menopausal women? Deleterious effects of middle age dietary and health problems add fuel to the fire. More than 46million women in India are estimated to be osteoporotic according to the studies conducted in small groups in South India in , reported in 2015^{12,13}. Fractures following minimal trauma (# of Femur, Hip, Spine) are commonly seen in elderly suggesting OP as the underlying factor. Low Ca intake , hypovitaminosis D and fibre rich diet are other additive factors (Storm et al., 1998)

In the present study we found reduced serum Ca and elevated Phosphorus levels in postmenopausal women (p value <0.0001). These results correlate with findings of Indumati et al., 2007; Riggs et al., 1969. Serum ALP in post-menopausal women was higher compared to premenopausal women ,P value <0.031 ,statistically significant and the findings correlate with Sachdeva et al., 2005 ; Agrawal et al., 2009; Brazier et al., 1995; Civitelli et al., 1988; Riggs et al., 1969; Reid et al., 2006; Suresh and Naidu, 2006

Hydroxyproline(HOP) is major component of protein collagen in the body ,approximately 13.5%¹⁴. As demineralisation progresses during menopause collagen matrix turnover increases & HOP levels increase both in serum and urine.(Sachdeva et al., 2005; Auth et al., 2008). In our study HCR and HOP/Ca ratio was increased in post-menopausal women compared to premenopausal group (P <0.0001) .Studies by Masse et al., 2005; Agrawal et al., 2009; Brazier et al., 1995; Riggs et al., 1969; Reid et al., 2006 showed similar results.

During menopause estrogen deficiency increases the risk of osteoporosis due to an imbalance between osteoclastic and osteoblastic activity. Estrogen deficiency leads to release of cytokines^{5,6}. This leads to elevated oxidative stress and Uric acid was found to be a better antioxidant than Vitamin C or E. Uric acid was found to have protective effect in diseases induced by oxidative stress. Hyperuricemia is risk factor for insulin resistance and Type 2 Diabetes independent of dyslipidemia¹⁵ & also increases bone fragility.¹⁶ In our study we found elevated SUA levels in postmenopausal women (8.9 ± 2.99) compared to premenopausal women (6.6 ± 1.79) and it was found to be statistically significant as shown by one-way ANOVA (P value <0.0001 Table 3). Hyperuricemia increases bone mineral density & the risk of fractures in postmenopausal women.¹⁷

Monitoring Urine HOP levels either in stat sample or 24 Hrs sample or HCR or both would be simpler measures than estimating Bone Mineral Density by DEXA. Sirekha P et al, Sajjanar D et al demonstrated a correlation between age and HOP, and we did not consider the same. We found statistically significant correlation between the groups for HCR and HOP /Ca ratio both in ANOVA & paired 't' test (Table 4, & 1; $P < 0.0001$)¹⁸

CONCLUSION

This is worthy study as we could correlate urine HOP with serum UA, ALP, Ca & Phosphorus positively as well as HCR is a useful predictor of OP. If we can add parameters which influence bone remodelling like serum Parathyroid hormone, Calcitonin, Vitamin D3, Vitamin C, HOP, Osteocalcin, Procollagen I N-Terminal peptide and Mg in the study, the knowledge on OP would improve.

Conflict of interest : None

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Abbreviations

OP = Osteoporosis ; **Ca++** = Calcium ; **P** = Phosphorus; **HOP** = Hydroxyproline **HCR** = Hydroxyproline/ Creatinine Ratio; **ALP** = Alkaline Phosphatase

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