



A COMPERATIVE STUDY ON RATIO OF CALCIUM AND PHOSPHORUS IN SERUM AND URINE AMONG PRE AND POSTMENOPAUSAL WOMEN.

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ABSTRACT

Osteoporosis is a bone disease resulting in reduction in bone mass and the bone become susceptible for fracture. The major factor involved in the development of osteoporosis includes, low calcium intake or decrease in Calcium absorption rate in aged population, bone strength in later life depends on development of bones earlier in life, adequate calcium intake during youth is essential to achieve peak bone mass. The Serum Calcium and Phosphorus, Urinary Calcium and Phosphorus were estimated by spectrophotometrically by O-cresolphthalein and Phosphomolybdate method respectively. Urinary creatinine was estimated by Spectrophotometric Jaffe's reaction. In our study, Urinary Calcium excretion was significantly increased with that of premenopausal woman of Indian sub population. Present study is an attempt to underscore urinary calcium quantification does increase diagnostic sensitivity by measuring it as derive parameter – CCR. It could as well be considered as an indicator for therapeutic intervention by studying large pre and postmenopausal woman population.

KEYWORDS : Menopause, Calcium, Phosphorus, Osteoporosis.

INTRODUCTION

Menopause, which is also referred as burning out of the ovaries, when estrogen production falls below a critical value, which can no longer inhibit the production of the gonadotropins such as FSH and LH. Instead, the gonadotropins FSH and LH (mainly FSH) are produced after menopause in large and continuous quantities, but as the remaining primordial follicles become atretic, the production of estrogen by the ovaries falls virtually to zero. Throughout a woman's reproductive life, about 400 of the primordial follicles grow into mature follicles and ovulate, and hundreds of thousands of ova degenerate. At about age 45 years, only a few primordial follicles remain to be stimulated by FSH and LH, and the production of estrogen by the ovaries decreases as the number of primordial follicles approaches zero. The loss of estrogen often causes marked physiological changes in the function of the body^[1]. Women are at greater risk of developing osteoporosis than men, particularly in menopausal state, as they need greater amounts of certain nutrients than men, particular iron and calcium. Menstruation, pregnancy, lactation and menopause are times of increased nutritional demand. The major factor involved in the development of osteoporosis includes, low calcium intake or decrease in Calcium absorption rate in aged population, bone strength in later life depends on development of bones earlier in life, adequate calcium intake during youth is essential to achieve peak bone mass^[2]. Development of peak bone mass and premenopausal bone loss is determined by the menstrual status of women^[3]. Decline in bone mass with age becomes accelerated during menopause. Menopausal bone loss refers to the accelerated bone loss that occurs during the premenopausal age and after the final menses^[4]. Bone as a dynamic tissue constantly undergoes formation and re-absorption and the process is balanced in healthy adults^[5]. Bone is a connective tissue that provides mechanical support to the body vital organs and act as reservoir of calcium and phosphate as 99% of calcium and 85% of phosphate are present in skeleton^[6]. Peak bone mass is achieved during the third decade of life which gradually declines leading to osteopenia (low bone mass) which predisposes to osteoporosis^[7]. Bone texture development and re-absorption is analyzed by measuring the concentration of bone turnover markers in blood or urine (or both). However bone re-absorption markers are considered as strong predictors of

bone loss as compared to bone formation markers^[8,9]. Bone mass can be determined by bone mineral density measurements for assessing fracture risk and diagnosing osteoporosis^[10]. Symptoms of postmenopausal syndrome includes, hot flashes characterized by extreme flushing of the, vasomotor instability, irregularity in blood pressure, fatigue, nervousness, emotional outburst like crying and anger, mental depression, insomnia, palpitation, vertigo, headache. The diminishing synthesis of estrogen leads to osteoporosis and atherosclerosis in woman.

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AIMS AND OBJECTIVE OF THE STUDY

The study will be conducted with the following objectives.

1. To assess the status of concurrent serum and urinary Calcium and phosphorous levels in pre and post-menopausal woman.
2. To correlate these bio-chemicals as an early marker of osteoporosis in postmenopausal women.

MATERIALS

Patients who were attending at Padmashree Diagnostics, Department of Gynaecology, Vijayanagar, Main Road, Bengaluru, for elective diagnosis and treatment, who were belonging to pre and postmenopausal woman recruited for this study. After complete explanation of the study to the subjects, a written informed consent was obtained from subjects. A total of **seventy** pre and postmenopausal women (35 each) was recruited for the present study. Following criteria were used to include or exclude subjects for the study:

Table – I Inclusion and Exclusion criteria

Selection Criteria	Pre Menopausal	Post-Menopausal
Inclusion Criteria :		
Age in Years	25 – 45	48 – 67
Gender	Female	Female
Healthy Volunteers	Yes	No
Menopausal woman who had cessation of menses for at least one year duration	-	Yes

Woman who had menses at regular duration	Yes	-
Not on Hormone Replacement Therapy	Yes	Yes
Exclusion Criteria:		
Metabolic diseases like hypo/hyperparathyroidism, Hyperthyroidism and diabetes mellitus.	No	No
Auto immune diseases like SLE and rheumatoid arthritis	No	No
Surgery like gastrointestinal resection or malabsorption	No	No
Chronic liver or Renal diseases	No	No
Drugs which may affect bone metabolism like, Glucocorticoids, aluminium containing antacids, Frusemide, bisphosphonate, calcium, Vitamin A, Vitamin D, Calcitonin, lithium, antiepileptics and anticoagulant, hypercalcaemia of malignancy	No	No
Chronic granulomatous disease like sarcoidosis and tuberculosis	No	No
Paget's disease of bone	No	No
Cigarette smoking, Alcohol abuse	No	No
History of recent fractures (in the earlier six months)	No	No

Specimen handling and analysis

The blood and corresponding urine specimens received in the laboratory and the blood specimen was centrifuged (1800 x g /15mins) to separate the cellular components and the cell free serum processed for the analysis of routine biochemical parameters sought by the treating clinicians. Remaining specimens were aliquoted, labelled and stored at - 20°C till further analysis. Aliquots of specimens, once thawed were used for the analysis on the same day and not be subjected to repeat freezing and thawing to avoid any pre-analytical errors.

METHODS

Sampling method: Random Sampling

ANALYTICAL METHODS:

Serum Calcium and Phosphorus, Urinary Calcium and Phosphorus were estimated by spectrophotometrically by O-cresolphthalein and Phosphomolybdate method respectively ⁽¹²⁾. Urinary creatinine was estimated by Spectrophotometric Jaffe's reaction ⁽¹²⁾.

STATISTICAL ANALYSIS

SPSS Version 19.0 Data analysis package was used and applied to analyse the obtained data after discussion with the Biostatistician. All the values were expressed in mean ± SD. Statistical comparison was performed using student t test. The student's 't' test *p < 0.05; **p < 0.01; ***p < 0.001 was considered as significant.

ASSAY PROTOCOLS

The analytes of interest in serum and urine were assayed by the following methods. Details regarding the assay methods with respect to the precise volumes of specimens / reagents and assay conditions etc., is provided in Table – II.

Table – II: Summary of biochemical assay settings and performance characteristics

ASSAY PARAMETERS	CALCIUM	PHOSPHORUS	UR. CREATININE
Type of Specimens	Serum & Urine	Serum & Urine	Urine
Assay Method	End Point	End Point	End Point
Sample Volume (Normal) in µl	2 & 2	2 & 2	15
Sample Volume (Decrease) in µl	--	--	--
Sample Volume (Increase) in µl	--	--	--
Sample Dilution	--	--	Yes

Single Reagent Chemistry	Yes	Yes	Yes
Two Reagent Chemistry	--	--	--
R1 in µl	20	90	750
R2 in µl	20	38	--
Wavelength Primary (in nm)	552	340	505
Wavelength Secondary (in nm)	629	659	
Calibration Method	Single Point	Single Point	Single Point
Calibration Type	Linear	Linear	Linear
Calibrator (CRM) value	9.96	9.15	190
Traceability of CRM	GC-MS	GC-MS	GC-MS
Multi Point Method (I - VI Stds)	-	-	-
Std I	-	-	-
Std II	-	-	-
Std III	-	-	-
Std IV	-	-	-
SD limit	0.1	0.1	0.1
Duplicate limit	150	500	100
Sensitivity limit	2100	50	2000
Abs limit (inc/dec)	Inc	Inc	Inc
*Unit Expression	mg/dL	mg/dL	mg/dL
Specific Characteristics of Assay			
Detection limit	0.2-20	0.3 - 20	0.2 – 20
Reference Interval Serum	9.3 ± 1.3	3.6 ± 1.3	-
Reference interval Urine	14.3 ± 19.4	3.6 ± 1.3	95 ± 62.5
Precision: Mean/SD/ CV%			
Linearity: (Slope/ Correl/ Coeffi)	1.0260/0.9996	1.054/0.9998	1.024 / 0.998
Reagent Source	Commercial	Commercial	In-House prep
Instrument Used	COBAS INTEGRA 400 PLUS	COBAS INTEGRA 400/400 PLUS	Spectrophotometer

RESULTS AND DISCUSSION

The present study was carried out to assess the status of calcium and phosphorus in serum and urine, urinary creatinine and derived parameter, urinary calcium/creatinine ratio (CCR) and phosphorus/creatinine ratio (PCR). The patients were recruited for the study at the Padmashree Diagnostics, Department of Gynecology, Vijayanagar, Bangalore, The study initiation started after obtaining the written informed consent. The study population consisted of 70 participants of premenopausal (n=35) and postmenopausal women (n=35), mean age of (Fig: 1) 38.0 ± 4.0 and 54.4 ± 4.6 respectively.

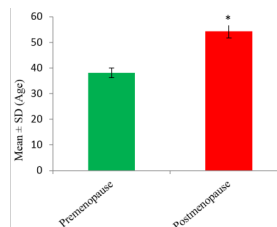


Fig: 1 Histogram represents the age distribution among the pre and post menopause women. Values expressed as Mean

± SD. Student's't test: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.*

It is apparent that Post-menopausal Osteoporosis becoming an important debilitating problem worldwide and it has increased the risk of sudden and unexpected fractures^[13]. Accordingly, with an emphasis on the required sensitivity of the assay methods, the present study was carried out on drug naive patient population suspected to be suffering from osteoporosis, by validating available analytical methods for serum and urinary parameters that are often used in laboratory medicine.

The biochemical investigations such as Serum calcium and phosphorus, Urinary calcium and phosphorus and creatinine were studied. The Functional role of Calcium in human body, has received substantial interest in both the medical literature and its role in osteoporosis and several other chronic diseases, including hypertension and colon cancer^[14, 20]. The amount of calcium needed is also affected by the decrease in intestinal absorption that occurs with age^[15].

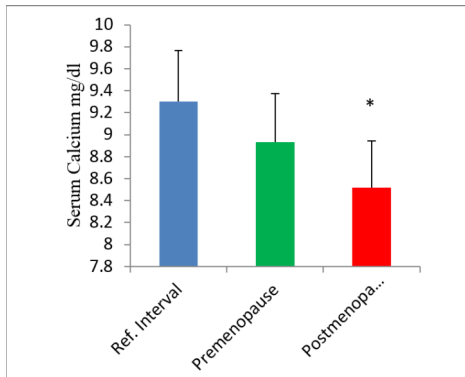


Fig: 2 Histogram represents the Serum Calcium among the pre and post menopause women. Values expressed as Mean ± SD. Student's't test: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

As a part of present study, the observed serum calcium was significantly lower ($*p < 0.05$) in postmenopausal woman compared to premenopausal woman. 8.9 ± 1.2 mg/dL and 8.5 ± 1.4 mg/dL respectively (Fig 2). However, the observed serum calcium level in both the group was within the reference interval.

Phosphorus and magnesium are among minerals that have been proposed as having an important role in bone metabolisms. Phosphorus, as phosphates combine with calcium ions to form Hydroxyapatite, the major inorganic molecule in teeth and bones^[16,17]. In present study, there was a significant ($*p > 0.05$) increase in serum phosphorus concentration in premenopausal women compared to postmenopausal women 3.9 ± 0.6 mg/dL & 3.7 ± 0.6 mg/dL respectively (Fig 3). Serum phosphorus showed a tendency to reduce might be due to early part of bone remodelling due to decrease in estrogen secretion. The outcome underscores that early medical intervention certainly minimizes the osteoporotic complications^[17,18].

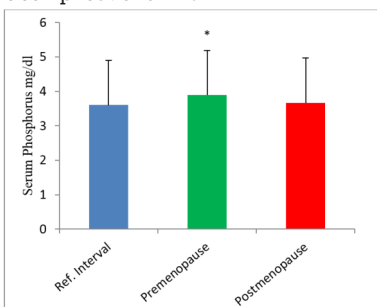


Fig: 3 Histogram represents the Serum Phosphorus among the

pre and post menopause women. Values expressed as Mean ± SD. Student's't test: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

It is important to monitor renal function to monitor various urinary biomarkers to rule out or to observe the manifestation by follow-up. By and large, biochemical investigations such as urea, creatinine, urinary creatinine clearance etc., explain the pathophysiology of the biochemical process^[18].

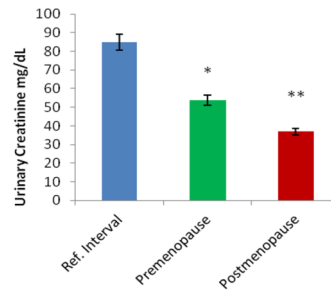


Fig: 4 Histogram represents the Urinary Creatinine among the pre and post menopause women. Values expressed as Mean ± SD. Student's't test: *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

It has also been considered as gold standard of practice for clinicians to normalize urinary excretion of many different biomarkers/abnormal metabolites to urine creatinine in chronic conditions such as microalbuminuria in diabetes mellitus and proteinuria in nephrotic syndromes. Lack of normalization may lead to falsely low biomarker/abnormal metabolites concentration interpretation in glomerular filtration rate. In fact, spot assessments of urinary biomarkers of chronic kidney diseases normalized to urine creatinine concentrations have succeeded to replace 24hrs urinary collections in many instances^[10,17].

Consequently, in the present study the pre and postmenopausal woman had significantly low creatinine excretion rate with that of normal reference interval 37.0 ± 27.3 and 53.7 ± 14.2 mg/dL respectively (Fig: 4). However, the used available reference interval value had 30 to 45% higher than the pre and postmenopausal woman group urinary creatinine, which is rather confounding. The observed decrease in urinary creatinine may be due to the ref interval for this parameter referred to in this study, which may not be the representative of the patient population studied or it is from normal general population.

The term of urinary calcium and phosphorus normally reported by relating to urinary creatinine excretion rate, Since, the relatively constant amount of creatinine excretion in urine proportional to the muscle mass of an individual^[19].

Hence, in present study the estimated pre and postmenopausal woman urinary calcium and phosphorus level are normalized to creatinine excretion rate and compared with respective normal reference interval.

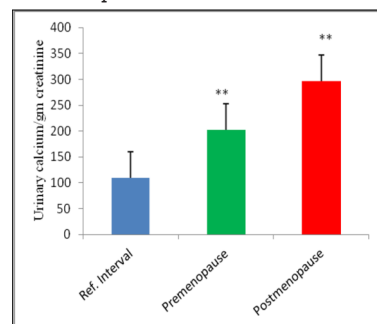


Fig: 5 Histogram represents the Urinary Calcium/creatinine

ratio among the pre and post menopause women. Values expressed as Mean ± SD. Student's't' test: *:p < 0.05; **:p < 0.01; ***:p < 0.001.

As shown in Fig 5 urinary excretion of calcium was significantly elevated from reference interval in pre and postmenopausal women. The concentration was 202.9 ± 155.6mg/g creatinine and 297.0 ± 378.4mg/gm creatinine respectively.

As shown in Fig 6 urinary excretion of phosphorus was significantly elevated from that of reference interval in pre and post menopausal woman population by (**p < 0.01) and (*p < 0.05) respectively. The concentration was 884.9 ± 907.3mg/g creatinine in premenopausal women and 657.1 ± 465mg/g creatinine in postmenopausal women.

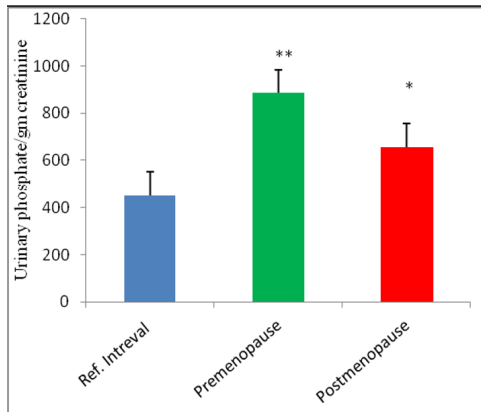


Fig: 6 Histogram represents the Urinary Phosphorus/creatinine ratio among the pre and post menopause women. Values expressed as Mean ± SD. Student's't' test: *:p < 0.05; **:p < 0.01; ***:p < 0.001.

Several researchers speculating that high dietary items are rich source of phosphorus content which is good enough to normal life when compared other elements. It is unlikely to find any phosphorus deficient in human population. However, the urinary excretion of phosphorus per gram creatinine does give guidance to monitor in various diseases progression like osteoporosis in post-menopausal woman [21]. As shown in Fig 6 urinary phosphorus excretion rate quite hilariously higher in premenopausal woman than postmenopausal woman it needs to be explained.

DISCUSSION

Details regarding the precise volumes of specimens/ reagents and assay conditions, detection range etc., is provided in (Table - II). The findings on descriptive statistics of various analysed and derived test parameters of serum and urine (Table - IIIB) in pre and postmenopausal woman of drug naive cases studied as two group (n=35 each) with respective of age differences (Table - IIIA) sub classified with respective reference intervals. In our current study, the distribution of data among pre and postmenopausal group (Table - IIIB) have shown significant difference between the groups and it underscores that studying serum and urinary calcium and phosphorus and its relevance to relate the finding with CCR and PCR is increased the diagnostic sensitivity to figure out the pathophysiology in postmenopausal osteoporosis population.

The present study involving the analysis of serum and urinary calcium and phosphorus comprising a pre and postmenopausal woman (n=35each). The finding was compared with available reference intervals on pre and postmenopausal women. Thus, the present randomized control, open-blinded and cross-sectional study was an

attempt to further validate the low cost test do has diagnostic sensitivity to identify the disease and to monitor the bone recovery process. Our study once again underscores the screening for urinary calcium and phosphorus in pre and postmenopausal women may help us in identifying the individuals who are at increased risk for frequent bone fracture. In present study the Serum calcium level of postmenopausal woman group had lower limit of normal when compared to premenopausal woman (Fig: 2). The urinary calcium: creatinine ratio has increased the diagnostic sensitivity (Fig: 5). The findings from this study between pre and postmenopausal woman serum phosphorus and urinary phosphorus: creatinine ratio surprisingly found to have very conspicuous, wherein premenopausal woman had suboptimal level of serum phosphorus than postmenopausal woman as compared to reference interval. Conversely, the derived parameter PCR increased than postmenopausal woman as compared to reference interval.

CONCLUSION

Osteoporotic fractures are one of the main causes of morbidity and mortality in India [22]. In our study, Urinary Calcium excretion was significantly increased with that of premenopausal woman of Indian sub population. Present study is an attempt to underscore urinary calcium quantification does increase diagnostic sensitivity by measuring it as derive parameter - CCR. It could as well be considered as an indicator for therapeutic intervention by studying large pre and postmenopausal woman population. Early detection and treatment of osteoporosis with available agents can significantly reduce the risk of fractures and associated morbidity and mortality.

SL No	Groups	Age in years		Unpaired t-test value
		Range	Mean ± SD	
1	Pre menopause	29-46	38.11 ± 4.30	15.27, p<0.05
2	Post menopause	47-67	54.40 ± 4.60	

Table - III A: Age Distribution Of Pre And Post Menopause Women.

Sl No	Parame ter	Premenopause		Postmenopause		Unpaired t test Value
		Range	Mean ± SD	Range	Mean ± SD	
1	S-CAL	6 - 11	8.93 ± 1.9	7 - 12	8.5 ± 1.4	<0.05
2	S-PHOS	3 - 5	3.89 ± 0.6	3 - 5	3.7 ± 0.62	<0.05
3	U- CAL	4 - 25	8.25 ± 4.5	3 - 22	8.6 ± 5.70	0.0002
4	U-PHOS	4 - 99	28.4 ± 18.6	6 - 92	24.0 ± 21.7	0.3E4
5	CCR	0 - 1	203 ± 156	0 - 3	297 ± 378	< 0.01
6	PCR	0 - 4	885 ± 907	0 - 4	657 ± 465	<0.01

Note: p < 0.05 significant at 5%level, p > 0.05 not significant at 5% level

Table - IIIB: Findings of Serum Calcium, Serum Phosphorus, Urinary Calcium, Urinary

SL No.	Parameters	Mean ± SD
1	Serum Calcium	9.3 ± 1.3
2	Urinary Calcium	14.3 ± 19.4
3	Serum Phosphorus	3.6 ± 1.3
4	Urinary Phosphorus	88.0 ± 67.9
5	Calcium/gm Creatinine	110 ± 155.6
6	Phosphorus/gm Creatinine	452 ± 557.2

Table - IIIC: Reference ranges of assay parameters [23, 24, 25]

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