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Original Research Article

Study of Serum Calcium, Microalbuminuria, Urinary Calcium/Creatinine Ratio in Postmenopausal Women

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Abstract
Deficiency of estrogen increases generation and deposition of extracellular protein which causes glomerular injury. Microalbuminuria is a reflection of vascular damage and marker of early arterial disease in normal healthy individuals without diabetes and hypertension. Calcium/creatinine ratio is a well defined marker indicating the rate of bone resorption and duration of menopause. Aim of the study is to find out the presence of microalbuminuria and estimation of serum calcium and calcium/creatinine ratio are compared to that of controls. The study was conducted by taking 50 no of cases and 50 no controls. Cases include non diabetes and non hypertensive post menopausal women of 45 to 60 yr age group without taking vit D, calcium and hormone replacement therapy. Controls were of normal premenopausal women of 35 to 45 yr age group. The study showed that urinary microalbumin and urinary calcium/ creatinine ratio was significantly increasd in cases as compared to that of controls showing $p<0.05$. serum calcium level was significantly decreased($p<0.005$) in cases which are found to be stastistically significant. Serum calcium level showed a negetive correlation with age and serum creatinine showed a negative correlation with urinary creatinine The study shows that microalbuminuria is an early indicator of subclinical organ damage. Serum calcium and ca/cr ratio can be used an early marker of bone resorption in postmenopausal women. So the above parameters can be used as screening method for detection of high risk groups who are prone to further cardiovascular disease and osteoporosis. So that early intervention can be done.
Keywords: *Microalbumin (MA), Calcium/Creatinine ratio(ca/cr).*

Introduction

Menopause occurs because of the natural or surgical cessation of estradiol and progesterone production by the ovaries, which are a part of the body's endocrine system of hormone production, which make reproduction possible and influence sexual behavior. After menopause, estrogen continues to be produced in other tissues, not only in the ovaries, but also in bone, blood vessels and even in the brain. The increasing longevity has resulted in an increasing number of senior citizens globally and life expectancy at present is 67 years in India and is expected to increase to 77 years by 2050 (1) thus increasing longevity and a greater proportion of the Indian population over the age of 50 years are likely to result in an increased number of people affected by osteoporosis. The data on prevalence of osteoporosis among women

in India come from studies conducted in small groups spread across the country, estimates suggests that of the 230 million Indians expected to be over the age of 50 years in 2015,20% i.e~ 46 millions are women suffer from osteoporosis. Thus osteoporosis is a major public health problem in Indian women.

The most dramatic endocrine alteration of perimenopause involves the decline in the circulating level and production rate of estradiol. The average menopausal level of circulating estradiol is less than 20 pg/mL. Estrogen being important for the formation and growth of bone in women decreased period of exposure to estrogen during a womens lifetime may contribute in a major way to the development of osteoporosis. [(2)]. Numerous studies have reported increasing prevalence of osteoporosis with advancing age, and this trend had been observed to an great extent among Indian women than men.[(3,4,5)]

Thus early screening is important to take action about early prevention and treatment of the osteoporosis. Radiological examination to assess BMD with dual X-ray method absorbtiometry (DEXA) is the gold standard for diagnosing osteoporosis[(6)] However, the radiological examination needs high technology and cost. Therefore, it cannot be applied in all health care centers. Whereas, the biopsy examination is too invasive and the results on one part of skeleton cannot be applied to the other skeleton. [(7,8)] In order to obtain an effective and efficient method, several biochemical markers reflecting a bone state or bone remodeling process have been developed for diagnosing osteoporosis.

Estrogens exert a major effect in women on bone re-modelling by inhibiting interleukin (IL)-6 productions that reduces bone resorption and also controls the timing of osteoclast apoptosis. Estrogens deficiency, therefore results in a longer life span of osteoclasts. Intestinal calcium absorption decreases in postmenopausal women.[(9)] Calcium, well known as a bone resorption marker, is recommended as biochemical marker for osteoporosis screening.

The calcium measured in the test comes mainly from bone and reflects net bone resorption. A raised fasting urinary ca/cr indicates an increase in net bone resorption which is frequently found in normal postmenopausal women.

Estrogen can suppress collagen synthesis in glomerular messengial cells by attenuating angiotensin II induced mitogen –activated protein kinase activity. (MAPK). The expression of the transcription factor AP-1 indirectly inhibit proliferation of glomerular messengial cells , which might limit the progression of glomerulosclerosis, because increased generation and deposition of extracellular matrix proteins is considered as an initial step in glomerular injury and loss of renal function.[(10,11,12)]

Microalbuminuria (MA) is defined an excretion of 30-300mg of albumin per 24 hr or 20-200 mcg/min or 30-300 mcg/mg creatinine on 2or 3 urine collection.[(13)] When the permeability of albumin is high in the renal glomerulus, the chances of microalbuminuria increase to a greater extent. MA is usually considered as a common risk factor for renal diseases, but recent studies have shown that MA could also occur due to general arterial diseases. In this context the purpose of this study was to determine serum calcium, microalbuminuria and calcium creatinine ratio in postmenopausal women for early detection of osteoporosis as well as renal parenchymal diseases and to prevent further complication.

Aim and Objectives

To study microalbuminuria, serum calcium, calcium creatinine ratio in postmenopausal women and To compare and correlate the different parameters of cases and controls.

Material and Methods

The present study was undertaken in the department of Biochemistry, VIMSAR Burla in collaboration with O&G department of VIMSAR Burla, between Sept 2013 to Sept 2015. The study included 50 healthy individuals of postmen-pausal women without diabetes and hypertension and 50

healthy indivusals of premenopausal women as control. Approval from the institutional ethical committee was obtained before the study and informed consent was obtained from individuals cases and controls.

Blood pressure was measured by standard sphygmomanometer with right arm in supine position. A structured questionnaire was used to obtain data on family h/o DM, h/o hypertension, past and present illness, dietary pattern, addiction and medication.

Inclusion Criteria

- Postmenopausal women of (50- 65)yrs age group without diabetes and hypertension are taken as cases And Premenopausal women of (35-50)yrs age group of healthy individuals are taken as control.

Exclusion Criteria

- Patients with diabetes mellitus, Patients with hypertension, obesity, nephropathy, Patients taking medications of calcium, Vit D3 & hormone replacement therapy.
- Physical examination:
- Height ,Weight, Bloodpressure, Pulse, Body mass index(BMI)

Sample collection and preparation:

After an overnight fast, 5ml fasting venous blood was collected aseptically in metal – free plastic tubes from each individual for following test.

Biochemical investigations: Following biochemical investigations were carried out in both the study groups by standard methods.

Routine blood test:

Fasting blood sugar: Glucose oxidase and peroxidase method.

Serum creatinine: Initial rate method using Alkaline Picrate

Serum Na+ and K+ -ISE Method.

Urine –routine and microscopic examination

Special tests

Urine creatinine: Alkaline picrate method (Bonsnes and Taussky, 1945)

Urine calcium: ISE Method

Serum calcium: ISE Method

Urine Microalbumin (turbidimetric immunoassay)

Statistical Analysis

The significance of difference of parameters in cases and controls was tested using students t-test analysis, Association between variables was determined using the pearsons correlation analysis on Microsoft Excel .Values of different parameters presented as mean± SD, A p value <0.05 was considered statistically significant.

Results and Discussion

The present study was undertaken in the department of Biochemistry, VIMSAR Burla in collaboration with O&G department of VIMSAR, Burla. The study included 50 healthy individuals of postmenopausal women without diabetes and hypertension and 50 healthy indivusals of premenopausal women as control. Mean age in healthy controls was 39.4±3.04 yrs with a range of 35-50yr and for cases 51.2±4.64yrs wit.a range of 50-65 yrs. In cases mean age of menopause was 52.9± 4.64.

Table -1 Demographic and anthropometric characteristics of the Cases and Controls:

Parameters	Healthy Controls (N=50)		Cases (N=50)		'P' Value
	Mean±SD	Range	Mean±SD	Range	
Age(Years)	39.4±3.04	35-50	51.2±4.64	50-65	-
Sbp(Mmhg)	107±7.54	100-118	108±6.89	102-120	-
Dbp(Mmhg)	69.4±7.9	70-82	71.2±7.62	70-84	-
Fasting Blood Sugar (Mg/Dl)	89±10.9	68-106	96.3±7.0	78-106	<0.005
Bmi (Kg/M^2)	21.16±1.6	20.56-22.76	22.05±3.45	18.7-25.0	

Table -2 Comparision between Different Clinical Parameters

Parameters	Controls (N=50)		Cases (N=50)		'P' Value
	Mean±Sd	Range	Mean±Sd	Range	
Serum Calcium(Mg/Dl)	9.24±0.512	8.4-10.1	8.07±0.88	6.6-9.5	<0.005
Urinary Calcium (Gm/24hr) Mean±Sd	0.130±0.518	1.00-0.3	1.63±0.726	0.300-3.2	<0.0001
Urinary Creatinine (Gm/24 Hr) Mean±Sd	0.729±0.265	0.11-1.31	1.07±0.509	0.150-2.00	<0.0001
Urinary Ca/Cr Ratio Mean±Sd	0.212±0.112	0.100-0.334	1.40±0.413	0.60-2.15	<0.0005
Microalbuminuria (Mg/24 Hr) Mean±Sd	22.5±8.89	8-45	74±30.2	10-122	<0.0001

Table – 3 Correlation of Serum Calcium with Age

Parameters	'R' Value	'P' Value
Serum Calcium	-0.197	<0.05
Age		

Table – 4 Correlations between Serum Creatinine with Urine Creatinine

Parameters	'R' Value	'P' Value
Serum Creatinine	-0.105	<0.05
Urine Creatinine		

Fig-1 Correlation of Serum Calcium with Age

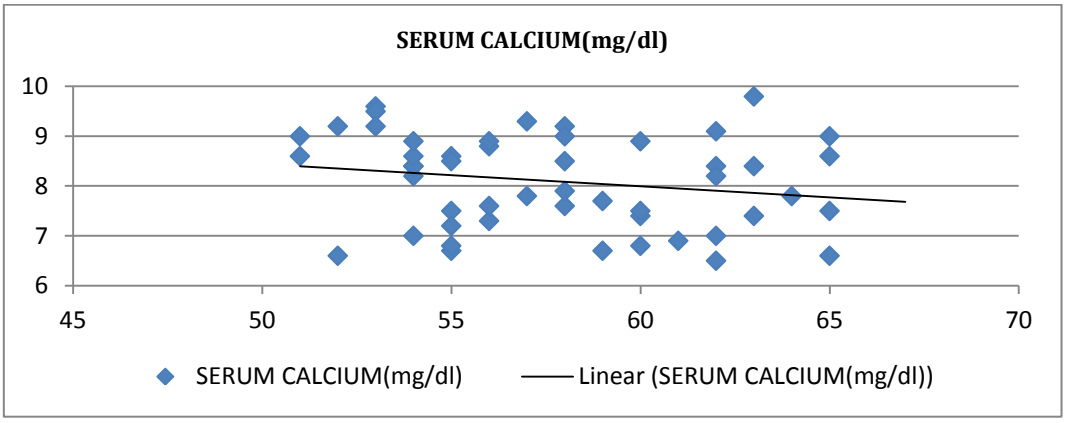
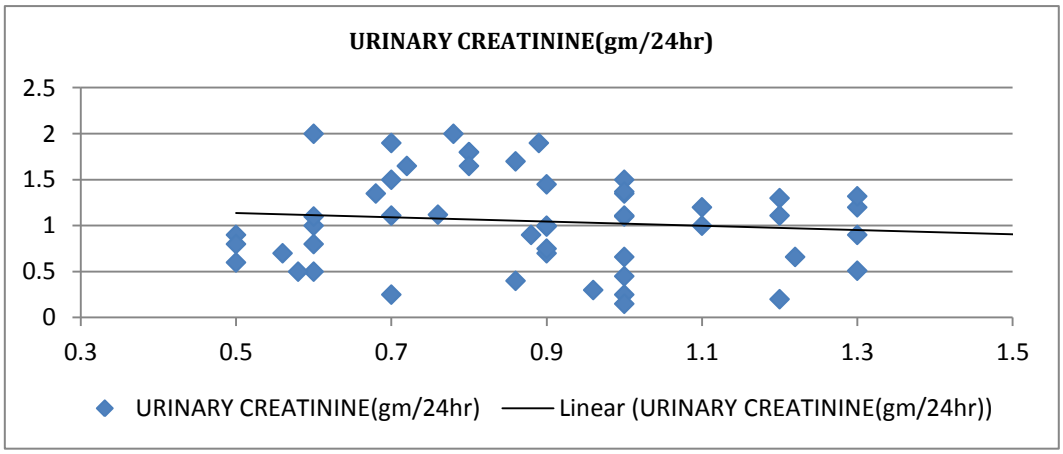


Fig-2 Correlation of Urinary Creatinine with Serum Creatinine



Study by Bharadwaj JK and Vaidya PR shows the mean age of menopause of Indian women is 45.03yr.[14] and a study conducted by institute for Social and Economic changes Bangalore shows the mean age of menopause in Indian women is 44.3years[15]. A study by Hidayat NM et al., it revealed that the semi urban women reached menopause significantly earlier than urban ones

(41.22± 5.64 year compared with 47.25± 5.12 year) (t=7.66, p< 0.001.[16]

Mean SBP in healthy controls was 107±7.54 mmHg with a range of 100-118mmHg and for cases 108±6.89mmHg with a range of 102-120mmHg.Mean DBP in healthy controls was 69.4±7.9mmHg with a range of 70-82mmHg and in cases 71.2±7.62 mmHg with a range of 70-84mmHg.

According to a study in Chinese women increasing age in women were associated with increased body mass index, waist to hip ratio, systolic blood pressure, diastolic blood pressure, fasting blood sugar and lipid profile. BMI, FBS and BP was slightly higher in cases than compared with that of controls but within the normal range. There was a progressive increase with age in the prevalence of glucose intolerance, hypertension, dyslipidemia and obesity. These changes suggest that age has an important and independent effect on cardiovascular risk in chinese women as in Caucasians, the onset of menopause might further increase this risk. [17] BMI in healthy controls was 21.16±1.6 kg/m^2 with a range of 20.56- 22.76 kg/m^2 and for cases 22.05± 3.45 kg/m^2 with a range of 18.7 – 25.0 kg/m^2. BMI was higher in cases as compared to the healthy controls which was statistically significant (p< 0.005). Mean fasting blood sugar in healthy controls was 89±10.9 mg/dl with a range of 68- 106mg/dl and for cases 96.3±7.0 mg/dl with a range of 78- 106 mg/dl. Fasting blood sugar was higher in cases as compared to the healthy controls which was statistically significant (p <0.005).

Mean serum calcium in cases was 8.07±0.88mg/dl with a range of 6.6-9.5mg/dl and in controls it was 9.24±0.512mg/dl with a range of 8.4-10.1mg/dl .serum calcium level was lower in cases as compared with that of controls which was statistically significant (p<0.005).In 1941, Albright et al first detected that estrogen deficiency is a major pathogenic factor of osteophorosis in post menopausal women. Estrogen deficiency during menopause causes rapid bone loss. Especially in the early years after menopause. Ribot et al (France), Hediund and Gallagher (Omaha) worked on their country women and reported that rapid bone loss was observed especially in the first 4-7yrs after menopause. [18]

Mean microalbuminuria in healthy controls was 22.5 ± 8.89 mg/24hr with range of 8 – 45 mg/24hr and for cases 74±30.2 mg/24hr with a range of 10-122 mg/24hr. microalbumin level was higher in cases as compared to the healthy controls which was statistically significant.(p<0.0001)Smaller quantities of proteinuria and albuminuria are still very powerful predictors for CVD both in the general population as well as the specific diseased populations.[8] Mark Roset et al have found that excessive urinary Albumin levels were associated with future cardiovascular mortality in postmenopausal women. [19].

Urinary calcium levels were, however, strongly correlated with age, and also serum and urine creatinine, older patients had lower urine calcium compared to the younger population; characterizes the significant positive correlation between urine calcium and urine creatinine; and shows that there is a negative correlation between serum creatinine and urine calcium. The 24-hour urine calcium correlates with serum creatinine and urine creatinine because complexed (calcium bound to phosphate, citrate, carbonate, and other anions) and ionized calcium is freely filterable in the glomerulus as urinary ultrafiltrate, and 98% is reabsorbed along the renal tubule. Increased urinary creatinine (reflected by increased creatinine clearance and GFR) explains the increase in calcium excretion. Also, renal insufficiency, reflected by decreased creatinine clearance, decreases renal calcium filtration. [20]

Mean urinary calcium of the controls was 0.130± 0.515 gm/24hr with a range of 0.3 -1.00 gm/24hr and in cases was 1.63± 0.726 gm/24hr with a range of 0.300 -3.2 gm/24hr. urinary calcium was higher in cases as compared with that of controls which was statistically significant .(p< 0.0001).

Mean urinary creatinine in cases was 0.729± 0.265 gm/24 hr with a range of 0.110 -1.31 gm/24 hr and in controls was 1.07± 0.509 gm/24 hr with a range of 0.150 -2.00 gm/24 hr. urinary creatinine was higher in controls as compared with that of

cases which was statistically significant. (p<0.0001)Several studies demonstrate that lower urinary creatinine excreation rates on timed urine specimens are associated with greater mortality risk, CVD events, and progression of CKD, independent of albuminuria and eGFR. Mean urinary ca/cr ratio in normal healthy controls was 0.212± 0.112 with a range of 0.100 -0.334, and in cases was 1.40± 0.413 with a range of 0.60 – 2.15. Mean urinary ca/cr ratio was higher in cases as compared with that of controls which was significant.(p<0.0005)

Conclusion

The study was conducted to evaluate the correlation between serum calcium level as well as urine calcium and urine creatinine (Ca/Cr) ratio and Bone Mineral Density (BMD) in postmenopausal women. So an increased urinary albumin excretion rate must be regarded as a useful and rather inexpensive clinical tool for the identification of patients with higher cardiovascular risk and organ damage in postmenopausal women. Therefore a greater effort can be made to prevent cardiovascular complications and osteoporosis in post menopausal women by giving estrogen replacement, calcium and Vit.D3 to avoid higher risk of bone resorption. Other drugs such as HRT, estrogen agonists, calcitonin, PTH are decided as per the affordability and availability of treatment option. Major gaps still remain as the diagnosis and management of osteoporosis and highlights the need for more structured research in the area.

Present study was performed in a small no of cases in a short period of time and further extensive studies are required to have any conclusive statement.

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