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ASSOCIATION OF BONE MINERAL DENSITY WITH URINARY HYDROXYPROLINE AND SERUM ESTROGEN IN POSTMENOPAUSAL WOMEN.

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ABSTRACT

OBJECTIVE: *To assess the association of bone mineral density with urinary hydroxyproline creatinine ratio and serum estrogen in pre and postmenopausal women.*
DESIGN: *Cross sectional study.*
SETTING: *The study was carried out in Department of Biochemistry, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC) Karachi.*
METHOD: *Females (n = 90) were assessed with measurements of Body Mass Index (BMI), Menstrual History, 24 hour urinary excretion of hydroxyproline Creatinine ratio, serum estrogen. Bone Mineral Density of Women was evaluated by Dual Energy X - ray Absorptiometrey (DEXA) and its association was analyzed with urinary hydroxyproline creatinine ratio and serum estrogen.*
RESULTS: *A significant number (44%) of postmenopausal women were found to be osteoporotic while 48% were osteopenic. Bone Mineral Density was normal in premenopausal women. The mean urinary hydroxyproline creatinine ratio increased in postmenopausal women (23.59 mg/gm creatinine) compared to premenopausal women (13.62 mg/gm creatinine). Serum estrogen was significantly decreased in postmenopausal women (45.082 ± 3.44 pg/mi) compared with (106.41± 6.69 pg/mi) in premenopausal women.*
CONCLUSION: *This study showed that there is growing incidence of osteoporosis with increasing postmenopausal years. Twenty four hours urinary hydroxyproline creatinine ratio is directly proportional whereas serum estrogen is inversely proportional to bone mineral density.*

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KEY WORDS: Postmenopausal women, Osteoporosis, Urinary hydroxyproline, Serum estrogen.

INTRODUCTION:

Osteoporosis is a condition in which bone mass decreases, causing abnormality in structure and reducing bone strength resulting in an increased risk of fracture [1].

Osteoporosis is classified as primary if it occurs as part of the normal human aging phenomenon and menopause. Secondary causes includes a large number of medical illnesses, endocrinopathies medications and life style factors significantly contribute to bone loss and fracture risk [2].

The bone mineral density decreases markedly immediately after menopause [3]. With the cessation of ovarian function estrogen levels decreases after menopause, beside other effects on body one major effect of estrogen deficiency is osteoporosis.

The characteristic low trauma osteoporotic fractures often occur only when a considerable amount of bone has been lost [4]. It is estimated that approximately 40% women will experience one or more fractures after the age of 50 years [5]. The rate of clinically serious fractures of hip and spine increases as much as 20 fold after the first fragility fracture [6]. Studies conducted on ovariectomized rats have found that discontinuation of 17 â estradiol administration to ovariectomized rats was associated with a striking reduction in trabecular bone formation. The rate of formation or degradation of bone matrix can be assessed either by measuring prominent enzymatic activity of the bone forming or resorbing cells or by measuring bone matrix components released into circulation during formation or resorption [8].

Urinary hydroxyproline levels have been considered marker of bone status [9]. Hydroxyproline is found mainly in collagens. Most of the endogenous hydroxyproline present in biologic fluids is derived from degradation of various forms of collagen. During

Table 1:
BIOPHYSICAL PARAMETERS IN PRE AND POSTMENOPAUSAL WOMEN.

S.No.	Parameters	Premeno-pausal	Postmeno-pausal
1	Age (years)	28.45 ± 0.806	56.06 ± 1.1.171*
2	Postmenopausal years	—-	9.12 ± 1.019*
3	Weight (kg)	60.45 ± 1.455	55.18 ± 1.454
4	Height (m)	1.576 ± 0.011	1.536 ± 0.014
5	Body Mass Index (BMI)	24.06 ± 0.437	24.25 ± 0.685

*** P< 0.001**

Table II:
BIOCHEMICAL PARAMETERS IN PRE AND POSTMENOPAUSAL WOMEN.

S.No.	Biochemical Parameters	Premenopausal women n = 40	Postmenopausal women n = 50
1	Urinaryhydroxyproline (mg/gm creatinine)	13.62 ± 0.544	23.59 ± 0.899*
2	Serum estrogen (pg/ml)	106.41 ± 6.69	45.082 ± 3.44*

*** P < 0.001**

Table III:
CORRELATION COEFFICIENT OF BIOCHEMICAL PARAMETERS WITH BMD IN POSTMENOPAUSAL WOMEN.

S.No.	Biochemical Parameters	Value at hip	R value at lumber spine
1	Urinary hydroxyproline mg/gm creatinine	– 0.18	– 0.40
2	Serum estrogen pg/ml	0.04	0.04

resorption of bone, the collagen structure is degraded [10]. As half of the human collagen resides in bone excretion of hydroxyproline in urine is regarded as a marker of bone resorption [11].

The radiologic bone mineral measurement at various sites and using different methods are useful in predicting fracture risk in postmenopausal women [12]. While multiple technologies exist to measure bone mineral density whereas, Dual Energy X-ray Absorptiometry (DEXA) has been reported by many investigators as gold standard for measurement of bone mass density [13].

It is generally agreed that the bone loss leading to osteoporosis and its associated fractures can be delayed or prevented. For preventive measures to be successful, however, they must be initiated before on soon after menopauses. This requires identification of those individuals at greatest risk for fractures [14].

MATERIAL AND METHOD:

The study was conducted from December 2006 to July 2007 in the Department of Biochemistry, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre Karachi, in collaboration with Karachi Institute of Radiotherapy and Nuclear Medicine (KIRAN). A group of 90 healthy females (40 premenopausal and 50 postmenopausal) were selected from general population belonging to different socioeconomic status and ethnicity. Age group of premanopausal women was between 20 – 35 years whereas postmenopausal women were in 46 – 75 years of age. All women had normal liver and renal function and were not taking any drug known to affect bone metabolism.

Twenty four hour urinary hydroxyproline was estimated by the spectrophotometric method of Bergman and Loxley. Participants were given clean plastic containers and told regarding the time specification of the sample. Subjects were also advised to avoid meat, fish, poultry, meat extracts and all gelatin containing food 24 hours before collection of urine. Hydroxyproline was analyzed and calculated per gram of creatinine to check the renal function.

Serum estrogen level was estimated by DRG Estradiol Enzyme Immunoessay Kit (Cat. Nr / Kat Nr: EIA 2693). Six ml. of blood was collected from each subject by vein puncture using plastic disposable syringes under aseptic measures and was transferred to clean test tube allowing to clot at 37 ^{0}C. Serum was separated after centrifugation for 10 minutes within an hour of blood collection. Serum was transferred to clean plastic serum cups and stored at – 20 ^{0}C. Body Mass Index (BMI) of subjects were calculated by measurements of height in meters and weight in kg.

$$BMI = \frac{\text{Wt. (Kg.)}}{\text{Ht. (m}^2\text{)}}$$

Bone Mineral Density of subjects was measured by Dual Energy X-ray Absorptiometry (DEXA) at two sites, lumber spines ($L_1 - L_4$) and hip bone. Hip bone measurements included at total hip, femoral neck, trochanter, intertrochanter and Ward's triangle. Data obtained was analysed on SPSS.

RESULTS:

Statistically no significant difference was observed in height, weight and BMI of premenopausal and postmenopausal women, whereas a significant higher mean age (p value < 0.001) was observed in postmenopausal women. The biophysical parameters of pre and postmenopausal women are shown in table 1.

Highly significant increase in urinary hydroxyproline levels mean ± s.e.m. = 23.59 ± 0.899 mg / gm creatinine observed in postmenopausal women compared to premenopausal women 13.62 ± 0.544 mg / gm creatinine. P value < 0.001. As shown in table II there is significant decrease in serum estrogen level in postmenopausal women 45.082 ± 3.44 pg/ml.

Data of Dual Energy X-ray Absorptiometry (DEXA) conducted on pre and postmenopausal women showed normal Bone Mass Density (BMD) in all the 40 premenopausal women at lumber spines mean ± s.e.m. 0.939 ± 0.016 gm/cm^2 and at hip mean ± s.e.m. 1.088 ± 0.026 gm/cm^2. a significant reduction in BDM is noted in postmenopausal women both at spines (mean ± s.e.m. 0.794 ± 0.017 gm/cm^2) and at hip (mean ± s.e.m. 0.774 ± 0.017 gm/cm^2). Table III shows strong inverse correlation between BMD and urinary hydroxyproline excretion in postmenopausal women r = – 0.40 at lumber spine and r = – .18 at hip. A positive correlation was found between serum estrogen and bone mineral density r = 0.04 at lumber spine and r = 0.04 at hip.

DISCUSSION:

Many workers [1235] have reported osteoporosis as a major public health concern due to increased fracture risk particularly among postmemenopausal women.

Our study confirms the findings of Akesson K et al[10] who showed that increased urinary excretion of hydroxyproline in postmenopausal women is associated with decrease bone mineral density. This reduction in bone mineral density and increased hydroxylproline excretion in urine occurs as a result of collagen degradation in bone. Similar findings were reported by George BO[9] and Siris ES[13]. Study of serum estrogen levels showed weak correlation with bone mineral density in Pakistani postmenopausal women. These findings differ with results of Jane Cauley 2001[16] and Quigley 1986[17] who reported a direct correlation between estrogen and BMD.

The decrease in BMD among Pakistani postmenopausal women in our study is also in line with findings of other workers[369 10], who reported dramatic increase in the bone turnover with an imbalance between bone formation and bone resorption after menopause with accelerated rate of bone loss.

CONCLUSION:

The human life expectancy has increased all over the world. This has also increased the segment of postmenopausal women population. Now a woman has to live more postmenopausal years than before and thus exposed to bone loss and increased fracture risk. There is also massive increase in annual expenditure all over the world for the management of complications occurring due to osteoporotic fractures. Our study showed a direct association of increased urinary excretion of hydroxyproline and decreased bone mineral density in postmenopausal women. Increased fragility of bones due to loss of mineral mass can be predicted if women nearing menopausal age screened for BMD and/or hydroxyproline levels in urine. High risk women can be appropriately treated, preventing or delaying the process of demineralization from bones. A number of women can be prevented from fractures and crippling old age miseries.

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