DEOXYPYRIDINOLINE (DPD), A MARKER OF BONE RESORPTION WHICH PREDICTS OSTEOPOROSIS

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ABSTRACT

With the improvements in medical facilities, the life expectancy of ladies has crossed fifty years in Pakistan. Because of this, problems related to menopause and senility are increasing. A cross-sectional study was conducted to determine the incidence of osteoporosis in Pakistani postmenopausal women residing at Rawalpindi. This was done by estimation of deoxypyridinoline (DPD), a marker of bone resorption, for its potential for early diagnosis; so that the patients are benefited by early treatment before the disease is severe and crippling. Seventy postmenopausal women having intact ovaries were selected randomly from the general public in the locality. Control group consisted of thirty healthy premenopausal women. The mean urinary DPD level in premenopausal ladies i.e Controls was (5.8 nmol/mmol of creatinine), while in postmenopausal women the mean DPD level was significantly higher (27.4 nmol / mmol of creatinine). The very high loser ladies (Urinary DPD level > 30 nmol / mmol of creatinine) were regarded to have osteoporosis. X-ray of the wrist remained a poor diagnostic marker as it was positive in only 15% of postmenopausal subjects. The frequency of osteoporosis was 13.5% in ladies between 50-59 years, while in ladies between 60 – 69 years the frequency was 78 % and 100% in ladies over 70 years. These figures are quite alarming.

Keywords: Postmenopause, osteoporosis, deoxypyridinoline (DPD)

INTRODUCTION

Postmenopausal osteoporosis is a preventable condition, responsible for considerable morbidity and mortality in older women. It usually becomes clinically apparent when a fracture occurs by which time the disease is well established and possibly irreversible [1]. As many as 25% ladies may require long term nursing home care [2]. Decline in physical functions and changes in appearance (kyphosis and height loss) due to osteoporosis, contribute to physical disability, social isolation, loss of self esteem and impaired quality of life [3,4]. This burden of osteoporosis will increase dramatically in the coming decades because of the growing population of the elderly ladies all over the world [5]. In postmenopausal ladies, estrogen deficiency is the key pathogenic mechanism responsible for osteoporosis [6]. The major predisposing factors for osteoporosis include; genetic predisposition, malnutrition with low dietary calcium intake, sedentary life style and multiple pregnancies with extended lactation, all leading to low bone mineral density at the time of menopause [7,8].

The rate of postmenopausal bone loss may be determined by biochemical bone resorption markers. A single biochemical bone resorption assessment done shortly after the menopause in conjunction with bone mineral density measurement may identify

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women with high bone turnover and a sustained high rate of bone loss. [9]. Type I collagen molecules in bone matrix are linked by pyridinoline cross links (pyridinoline or deoxypyridinoline) During osteoclastic bone resorption, pyridionoline crosslinks are released into circulation which are degraded and excreted through kidneys in the urine. Therefore, urinary deoxpyridinoline (DPD) levels are derived almost exclusively from bone osteoclastic degradation. The increased urinary DPD levels thus reflect the increased bone resorption caused by estrogen deficiency in potmenopausal women [10]. Therefore estimation of urinary deoxypyridinoline has proved to be a reliable marker for the early assessment of bone resorption as well as for monitoring the response to therapy in postmenopausal women [11]. As the life expectancy has crossed the age of fifty years in Pakistan, therefore, problems related to menopause and senility are increasing in our country [12] Moreover there is an increasing awareness of the problems of postmenopausal state in our elderly population.

The aim of the study was to determine the incidence of osteoprosis in Pakistani pastmenopausal women residing in Rawalpindi.

PATIENTS AND METHODS

A cross sectional study, spread over a period of one year from June 1999 to July 2000 was carried out in the Department of Chemical Pathology and Endocrinology, Armed Forces Institute of pathology Rawalpindi,to determine the incidence of osteoporosis in Pakistani postmenopausal women residing at Rawalpindi. This was done by estimation of urinary deoxypyridinoline, a biochemical marker for bone resorption which predicts risk for osteoporosis and its comparison was done with routine screening tests.

The study population consisted of a group of seventy postmenopausal women having intact ovaries randomly selected from the general urban population of Rawalpindi. The age ranged between 50 – 78 years with no history of bone, endocrine or any chronic disorder. They were not taking any medication known to influence bone metabolism. Details regarding the parity status and breast feeding to their children were recorded for each postmenopausal subject.

Thirty pre-menopausal healthy ladies between 15-40 years and having regular menstrual cycle were taken as control. They were neither pregnant nor lactating and were not taking any medication. Detailed clinical history and complete physical examination were carried out in all subjects. X-ray of the wrist was done to identify decreased bone density. Informed consent was obtained from all the subjects.

Specimen Collection

Venous blood was collected without stasis from each subject for the estimation of serum calcium, inorganic phosphate, alkaline phosphatase and creatinine. The serum was separated from the clotted blood after centrifugation. The serum specimens were analyzed on the same day for calcium, inorganic phosphate, albumin, alkaline phosphatase and creatinine. Early morning first voided urine sample was collected for the estimation of deoxypyridinoline (DPD), creatinine and spot urinary calcium level from each subject. The samples were stored at – 20^{o}C until analyzed.

Urinary DPD Estimation

The urinary DPD level was measured by a quantitative assay on "Immulite" Automated Immunoassay Analyzer". using Immulite kit Catalog number: LK PD1 (Diagnostic Product Corporation, USA). The assay was based on a solid phase, two site chemiluminescence enzyme immunometric

assays. Before analysis, the samples were thawed to room temperature (15-28o C). Turbid urine sample were cleared by centrifugation before analysis. The assay had a measurable range of urinary DPD from 3.3-300 nmol/l, with analytical sensitivity of 4.4 nmol/1.

DPD values were normalized to the urinary creatinine concentration and expressed as nmol DPD / mmol creatinine. Those with DPD level between 3.4-7.4 nmol /mmol creatinine were considered to have normal levels. The grading of urinary DPD levels was made according to criteria of Riis et al into one of the following three groups where very high losers were presumed to have osteoporosis.

- Low losers - DPD level between 7.4-15 nmol/mmol creatinine
- High losers - DPD level between 16-30 nmol/mmol creatinine
- Very high losers - DPD level >30 nmol/ mmol creatinine

STATISTICAL ANALYSIS

Data was analyzed using SPSS version 10.0. Mean and standard deulation were calculated to describe different variables. T-test was used to compare the groups.

RESULTS

The study group comprised seventy postmenopausal ladies. Most of them were having more than five children and all gave history of breast feeding to their children. X-Ray of the wrist showed osteoporotic changes in only 15 % of the ladies in study group and most of them were over 60 years of age. There is a highly significant difference in serum calcium levels, serum inorganic phosphate and alkaline phosphatase have been observed between the two groups (P<0.001) (table-1).

The ratio of urinary calcium to creatinine shows a highly significant excretion of urinary calcium in postmenopausal ladies as compared to controls (P < 0.001), while for urinary phosphate/creatinine ratio, the difference is only highly significant among the two groups. Urinary DPD / creatinine ratio denotes an increased DPD excretion by the study group with mean + 1SD values of (27.4 + 9.5nmol/ mmol creatinine) (table-2).

Table-1: Results (mean ± 1 SD) of serum calcium, inorganic phosphate and alkaline phosphatase (ALP)

Group	Calcium (mmol/1)	Inorganic phosphate (mmol/1)	ALP (U/L)
Premeno pausal (n=30)	2.33 ± 0.05	1.06 ± 0.05	190 ± 9
Postmen opausal (n=70)	2.57 ± 0.04	1.20 ± 0.06	204 ± 10.2
'P' Value	<0.001*	<0.05	<0.05

**P<0.001 = Highly Significant P<0.05 = Significant P>0.05 = Not Significant*

Table-2: Results of ratio (mean ± 1 SD) for urinary calcium, phosphates and DPD with urinary creatinine

Group	U.Calcium/ Creatinine ratio (nmol/mmol)	U. Phosphate/ Creatinine ratio (nmol/mmol)	U.DPD / Creatinine ratio (nmol/mmol)
Premeno pausal (n=30)	0.104±0.0049	0.37 ± 0.0143	5.48 ± 1.05
Postmeno pausal (n=70)	0.133±0.0072	0.39 ± 0.0134	27.4 ± 9.5
P-Value	P<0.001*	P< 0.001*	P<0.001*

*P<0.05 = Significant *P<0.001= Highly Significant P>0.05 = Not Significant*

Table-3: Urinary DPD level (mean ± 1 SD) in controls and sub grouped postmenopausal women.

Subjects	Controls Premeno-pausal	Study group Postmenopausal (Age groups in years)		
Age	20-40	50-59	60-69	70-79
DPD level nmol/mmol creatinine	5.48±1.05	20±3.7	50±5.1	63± 4.7
P-Value	-	< 0.001*	< .001*	< .001*

**Highly Significant*

The mean ± 1SD urinary DPD level in control group is 5.48±1.05 nmol/mmol of creatinine. There is a steep rise in urinary excretion of DPD with increasing age (table-3).

Fig.1 shows that among postmenopausal women, 30% are low losers, i.e DPD excretion between 7.4-15 nmol/mmol creatinine and 31.5% are very high losers, thus presumed to have postmenopausal osteoporosis.

Fig.2 shows that among postmenopauasal ladies between 50 – 59 years, only 13.5 % (n=7) are very high losers, while the ladies between 60 – 69 years, 78.6% are very high losers, thus presumed to have postmenopausal osteoporosis.

All the ladies above 70 years of age fall in the group of very high losers.

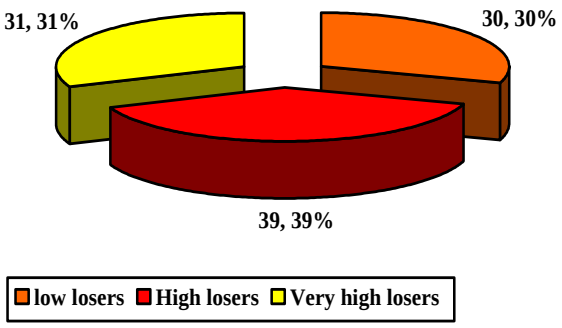

Fig. 1: Percentage distribution of postmenopausal women into groups depending upon urinary DPD.

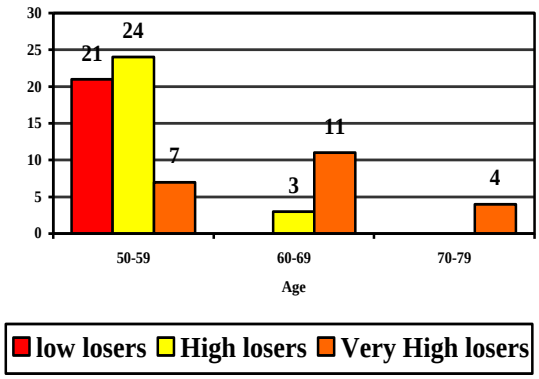

Fig. 2: Decade wise distribution of the three groups i.e low losers, high losers and very high losers.

DISCUSSION

Postmenopausal osteoporosis is a common health problem that may lead to serious disability, increased mortality and significant health care costs. Biochemical markers of bone remodeling are adjunctive tests that predict rate of bone loss and monitor effectiveness of antiresorptive therapy.

In our study, X-Ray of the wrist showed osteoporotic changes in 15 % of postmenopausal subjects. It remained a poor diagnostic marker because osteoporosis becomes radiologically apparent when more than 30 % bone mass is lost. Therefore, specialized radiological techniques like photon absorptiometry and X-ray absorptiometry i.e single emission X-ray absorptiometry (SEXA) and dual emission X-ray absorptiometry (DEXA) have been recommended for evaluation of bone mineral density and osteoporosis. In our study, a relative rise in serum calcium level (table-1) as well as increased urinary calcium excretion (table–2) was observed in postmenopausal women as compared to controls. These findings are consistent with various other studies which also reported increased serum calcium levels and high urinary calcium excretion after menopause [16,17,18]. However, in our study no significant change in serum calcium level and urinary calcium excretion was observed either with increasing age or decade wise distribution of postmenopausal ladies. This reflects that serum calcium level and its urinary excretion are poor predictors of osteoporosis.

Estimation of urinary deoxypyridinoline has emerged as a specific marker for bone turnover [19]. At present, most analysis for pyridinium crosslinks of bone matrix collagen are done using urine, primarily due to the fact that concentrations in serum are low. In this study the urinary DPD levels of

premenopausal healthy controls are between 3.7–7.9 nmol/mmol of urinary creatinine. Almost similar reference range for urinary DPD has been quoted by different researchers in Western healthy premenopausal ladies [13]. This shows that DPD reference range does not differ in our population from Western ladies though belonging to different ethnic groups [20].

The comparison of mean ± ISD values of urinary DPD of postmenopausal women (27.4+9.5) with the mean DPD levels of premenopusal women (5.48+1.05) revealed that there is significantly higher urinary excretion of DPD in postmenopausal women ($P < 0.001$). This is in agreement with results of various other studies [13]. To assess the severity of the problem, the postmenopausal subjects were divided into three groups of, low losers, high losers and very high losers depending upon urinary DPD/urinary creatinine ranges of 7.4-15, 16-30 and more than 30 nmol of DPD/mmol of creatinine respectively. This is in accordance with the criteria laid down by Riis et at [14]. It was found that among the postmenopausal ladies, about 1/3rd of the subjects were low losers, while 2/3rd of the subjects belonged to high and very high DPD loser groups.

While further dividing the study group decade wise, it was observed that between 50-59 years, 135% (n=7) subjects were very high losers. Between 60-69 years, very high losers were 78.6% (n=11) while over 70 years of age, almost all were found to be very high losers. These findings are consistent with Scottish study in which females between 40-80 years of age revealed prevalence of osteoporosis between 5-85% in different age groups [21]. A recent study by Habiba et al' done on Pakistani population has also revealed an alarming predisposition to osteoporosis ranging from 55 % to over 90 % in the age range of 45 years to over 70 years in postmenopausal ladies [22].

In our study, it was observed that after 60 years of age, there was a marked increase in DPD excretion and the mean value of DPD excretion became more than double the normal reference rage, while in the decade between, 70-79 years, the mean value of DPD excretion was very high i.e 10 to 20 times the normal reference range. These ladies were presumed to have osteoporosis. This is in accordance with the EPIDOS study [23] where postmenopausal subjects showed increasing urinary DPD excretion with the advancing age. Grading the subjects as low, high or very high losers is of clinical importance. It has been shown in the 'EPIDOS' study that women; having a hip fracture, show a high urinary DPD excretion than non fractured controls [23]. Thus in a clinical setting, identification of high risk patients is very important so that effective preventive and therapeutic measures could be instituted to prevent further progress of the disease and subsequent fractures. This can be done by measurement of bone mass by special radiological techniques and bone resorption by biochemical markers like DPD, independently or by both the measures in combination.

CONCLUSION

It is concluded that Post menopausal osteoporosis is also becoming a major health concern in our elderly population because of the fact that life expectancy in Pakistan has crossed the age of fifty years. Multiparity, lactation and poor diet during reproductive life are the major risk factors leading to later on development of osteoporosis. The very high DPD excretion (78%) has been documented in postmenopausal ladies between 50 – 59 years of age suggesting a high frequency of postmenopausal osteoporosis in our country. In the absence of specific radiological techniques like 'SEXA' and DEXA, urinary measurement of DPD

appears as the most sensitive marker for predicting osteoporosis in the clinically suspected patients.

REFERENCES

1. Jone G, Nguyen T, Sambrook PN, Kelly PJ, Gilbert C, Eisman JA, et al. Symptomatic fracture incidence in elderly men and women: The Dubbo osteoporosis Epidemiology study (DOES). **Osteoporosis Int 1994; 4: 277 – 282**.

2. Schurch MA, Rizzoli R, Mermillod B, Vasey H, Michel JP, Bonjour JP, et al. A prospective study on socioeconomic aspects of fracture of the proximal femur. **J Bone Miner Res 1996; 11: 1935 – 1942**.

3. Ross PD Clinical consequences of vertebral fractures. **Am J Med 1997; 103 (Supply 2A): 305– 435.**

4. Ross PD, Ettinger B, Davis JW, Melton LJ 3rd, Wasnich RD, et al. Evaluation of adverse health outcomes associated with vertebral fractures. **Osteoporosis Int 1991; 1:134-140**.

5. Cooper C, Campion G, Melton LJ. Hip fractures in the elderly: A world wide projection. **Osteoporosis Int 1995; 5: 389-409**.

6. Cumming SR, Nevitt MC, Browner WS, Stone K, Fox KM, Ensrud KE, et al. Risk factors for hip fracture in white women. Study of osteoporotic fractures research group. **N Eng J Med 1995; 332:762-73**.

7. Johnell O, Gullberg B, Kanis JA, Allander E, Elffors L, Dequeker J, et al. Risk factors for hipfracture in European women: The MEDOS study. **J Bone Miner Res 1995; 10:1802-15**.

8. Lydick E, Cook K, Turpin J, Melton M, Stine R, Byrnes C, et al. Development and validation of a simple questionnaire to facilitate identification of women likely to have low bone density. **Am J Maneged care 1998; 4:37-48.**

9. Eastell R, Col well A, Hampton L, Rew J. Biochemical markers of bone Resorption compared with estimates of bone Resorption from radiakinetic studies in osteoporosis **J Bone Miner Res 1997; 12:59-65**.

10. Robin SP, Woitge H, Hesley R, Ju J, Seibel MJ. Direct enzyme linked immunoassay for urinary deoxy-pyridinoline as a specific marker for measuring bone Resorption. **J Bone Miner Res 1994;1643-9**

11. Garnero P, Shih WJ, Delmas PD. Comparison of new biochemical markers of bone turnover in late postmenopausal osteoporotic women in response to alendronate treatment. **J Clin Endocrinol Metab 1994; 79: 1693-1700**.

12. Sultan A, Salam A, Zamir M. Effects of menopause on serum calcium, Phosphorous, Alkaline Phosphates in relation to urinary calcium phosphorous and hydroxyl Proline in **Pak J Med Res 1994; 33: 184 – 86**.

13. Delmass PD, Gineyts E, Bertholin A, Garnero P, Marchand F, et al. Immunoassay of pyridinoline crosslink in normal adults and in pagets disease. **J Bone Miner Res 1993; 8: 643-648**.

14. Riis Brazier M, Nevi V, et al. Measurement of biochemical markers. Methods and limitations **J Bone Miner Res 1995; 10: 385 – 392.**

15. Nordin BEC. Diagnostic Procedures in diagnosis of calcium metabolism. **Clinical Endocrinology 1988; 8: 55-67**.

16. Young MM, Nordin BEC. Effects of natural and artificial menopause on

plasma and urinary calcium and phosphorus. **Lancet 1976; 2: 118-20**.

17. Frumar AM, Meldrum DR, Geola F,Defts LJ, Judd HL. Relationship of fasting urinary calcium to circulating estrogen and body weight in postmenopausal women. **J Clin Endocrinol Metab 1980; 50-70**.
18. Ouyang PC, Huang C, Chen R, Chows. Serum total estrogen level and urinary excretion of calcium and hydroxyproline in pre and postmenopausal chines women. **Asia Oceana J Obstet Gynaecol 1984; 1: 467**.
19. Delmas PD. Biochemical markers for the assessment of bone turnover. In: Riggs BL, Melton LG, Editors. Osteoporosis: etiology, diagnosis and management. **Philadelphia: Lippin Cott-Raven, 1995: 319-33**.
20. Garnero P, Delmas PD. Clinical usefulness of markers of bone remodeling in osteoporosis In: Maunier PJ (ed). Osteoporosis: diagnosis and management. **London: Martin Dunitz Ltd. 1998; 79-101**.
21. Davei DA. The menopause and chimectric. In: Dewhurstis Text Book of Obstetrics and Gynaecology for postgraduates by charles R. Whit field 25 John Street **London WC N 2 BL 5th edi, 1994: 609 – 639.**
22. Habiba U, Ahmed S and Hassan L. Predisposition to osteoporosis in Postmenopausal women. **J Coll Phy Surg Pak 2002; 12(5): 297 – 301**.
23. Garnero P, Hausherr E, Chapuy MC, Marcelli C, Grandjean H, Muller C, et al. Markers of bone Resorption predict hip fracture in elderly women: The EPIDOS prospective study. **J Bone Miner Res 1996; 11: 1531-8**.