Hormonal Role in Osteoporosis among Post – Menopausal Uremic Women

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Abstract: Osteoporosis is the most prevalent bone disorder in the general population, particularly in the middle and older age groups. Although more than half of the prevalent dialysis population is within these age groups, little concern has been given to the possible role of estrogen deficiency in the pathogenesis of bone disease in end stage renal disease (ESRD). It is often referred to as the "silent epidemic," it is a global problem that is increasing in significance as the population of the world both increases and ages. The purpose of this study is to evaluate both estradiol (E2) and parathormone (iPTH) as evidence that supports a potential role of the postmenopausal state in the pathogenesis of bone disease in ESRD to search for treatment. Subjects and Methods : A total of 20 women below 50 years and had persistent amenorrhea with ESRD (under prolonged hemodialysis) were selected from Faysal Kidney Dialysis Unit Giza, Egypt (ESRD group) and a control group of 20 women matched with same age were selected from the healthy volunteers of the medical staffs. For all subjects, bone mass density (BMD) analysis by dual x-ray absorptiometry (DEXA) was done. Routine chemistry as serum creatinine, calcium, inorganic phosphorus and alkaline phosphatase were estimated using chemistry autoanalyzer. Serum E2 and iPTH levels were also measured by enhanced chemiluminescence technique. Results: It was shown that although the risk factors for fracture in ESRD were similar to the general population, the incidence was four folds higher in the cases. In ESRD cases the lower E2 level, the higher iPTH level ($r = -0.861$; $p < 0.001$), and consequently the higher incidence of osteoporosis and fractures. Recent evidence on the risk of HRT therapy should caution about its use in ESRD patients. In conclusion, osteoporosis should be recognized as an important entity that may modify the current conception of renal osteodystrophy in postmenopausal patients with ESRD. Low serum E2 and high iPTH levels are risk factors in decreased BMD in postmenopausal women on dialysis. Recommendations: Early detection of osteoporosis leads to good prevention of the disease. The use of selective estrogen receptor modulators (SERM) which may increase bone mass without significant secondary effects needs further clinical studies in order to propose strategies that may reduce postmenopausal osteoporosis in the dialysis population and may be an essential part of post-renal-transplant care. [Journal of American Science. 2010;6(10):284-291]. (ISSN: 1545-1003).

Keywords: Bone mineral density, hormone replacement therapy hemodialysis, iPTH, E2, osteoporosis.

1. Introduction

Little concern has been given to the possible role of estrogen deficiency in the pathogenesis of uremic bone disease or the existence of post-menopausal osteoporosis in end-stage renal disease (ESRD) patients. Osteoporosis is most common in women after menopause, where it is called postmenopausal osteoporosis, but may also develop in men, and may occur in anyone in the presence of particular hormonal disorders and other chronic diseases. The term renal osteodystrophy, widely accepted to define the bone lesions associated with chronic renal failure includes diseases that affect the control of bone remodeling, like high turnover bone disease caused basically by secondary hyperparathyroidism, or low turnover in the form of adynamic bone disease and osteomalacia, usually associated to vitamin D deficiency (*Elder, 2002).*

In osteoporosis the bone mineral density (BMD) is reduced, bone microarchitecture is disrupted, and the amount and variety of non-collagenous proteins in bone is altered. Bone resorption is the process by which osteoclasts break down bone and release the minerals, resulting in a transfer of calcium from bone fluid to the blood. Osteoporosis is a disease of bone that leads to an increased risk of fracture , it is defined by the World Health Organization (WHO) in women as a bone mineral density 2.5 standard deviations below peak bone mass (20-years-old healthy female average) as measured by Dual energy X-ray absorptiometry

(DEXA). It is responsible for millions of fractures annually, mostly involving the lumbar vertebrae, hip, and wrist (*WHO 1994 & WHO 2003*). The term "established osteoporosis" includes the presence of a fragility fracture. It is estimated that 1 in 3 women and 1 in 12 men over the age of 50 worldwide have osteoporosis (*Siris et al., 1998*). In the U.S. the costs associated with osteoporosis-related fractures are equivalent to those of cardiovascular disease and asthma (*Burge et al., 2007*).

Parathyroid hormone (PTH), produced by the parathyroid gland, is the major circulating factor regulating extracellular calcium concentration. Abnormally low ionized calcium concentrations trigger the secretion of PTH. The PTH molecules bind to type 1 parathyroid hormone receptors in target tissues and initiate a sequence of reactions that results in an increase in extracellular calcium concentrations. PTH stimulates osteoclastic bone resorption resulting in the release of calcium from bone. PTH stimulates transcellular calcium reabsorption from the renal tubules and stimulates the kidney to produce 1, 25-dihydroxyvitamin D which acts on the intestines to increase calcium reabsorption (*Friedman, 2000).*

Although the prevalence of menopause in women on dialysis is very high and premature amenorrhea occurs frequently in young women with ESRD, post-menopausal osteoporosis has not been traditionally included under the term of renal osteodystrophy (*Lindberg and Moe, 1999).* But, it seems possible that post-menopausal osteoporosis increases the burden of bone alterations in patients already affected by the multiple factors that determine renal osteodystrophy. Bone remodeling rates double at menopause, triple 13 years later, and remain elevated in osteoporosis (*Recker, 2006).* Several studies have suggested that patients with ESRD are at increased risk for bone fractures. It has been shown, that like in the general population, the risk for fracture increases with age, female gender, Caucasian race, lower body mass index, and decreased bone mass (*Mussolino et al., 1998 & Atsumi et al., 1999).* It has been proposed that factors such as renal osteodystrophy and metabolic acidosis may determine the increased risk for fractures observed in these patients, it is possible that the relatively high incidence and prevalence of ESRD in the middle age and older population may also play an important role. *Alem et al., 2000* described that the overall incidence of hip fracture among patients, who had undergone dialysis for seven years, was about fourfold higher than expected in the general population *(Ball et al., 2002).* Nevertheless, renal transplant patients exceed the dialysis risk of hip fracture during the first 1-3 years after transplantation (*Hodsman, 2001***)**. Although the majority favors that a low BMD can predict the incidence of hip or vertebral fractures (*Alem et al., 2000* & *Jamal et al., 2002),* an evidence shows that BMD analysis by DEXA does not identify patients with dialysis-dependent renal failure who have fractures (*Hsu et al., 2002***)**. Similarly, a large epidemiological study in patients with mild to moderate chronic renal insufficiency demonstrated that subjects with worse renal function had significantly lower femoral BMD, but this association was explained by confounding factors, principally gender, age and weight (*Kaji et al., 2002***).**

Gynecological issues in the uremic women: Early amenorrhea and hypoestrogenism dialysis patients

Most of the gynecological interest in women with ESRD treated or not with dialysis has been related to the problem of reduced fertility and libido. It was postulated that a defect in the hypothalamic regulation of gonadothrophin secretion would result in lower estradiol peaks, lower follicle stimulating hormone /luteinizing hormone ratios and higher prolactin concentration leading to anovulatory cycles and persistent amenorrhea (*Cochrane & Regan, 1997***)**. Thus, women with chronic renal disease are frequently hypoestrogenic as a result of the disease or its treatment. An increased prevalence of low bone mineral density has been reported in patients with major depressive disorder (MDD), mostly women. The possible contribution of immune or inflammatory imbalance to low BMD in premenopausal women with MDD remains to be clarified (Eskandari, et al., 2007). Osteoblasts have estrogen receptors and respond directly or indirectly, inducing the production of growth factors such as IGF and TGF-β. Estrogen deficiency increases the production of IL-1, IL6, IL-11 and TNF- α, which in turn increase osteoclastic activity (*Pereira et al., 1993***)**. Several studies have demonstrated that estrogen prevent osteoporosis through a blockade of IL-1 and other cytokines production in the bone micro ambient. Menopause increases IL-1 production, whereas estrogen replacement decreases it (*Kanatani et al., 1998***)**. This cytokine promotes osteoclastogenesis by favoring the maturation of Osteoblasts precursors, whereas the antagonist of the IL-1 receptor prevents the activation of mature osteoclasts and the differentiation and proliferation of Osteoblasts precursors. Another study indicates that low radial BMD but not vertebral BMD, increased serum levels of alkaline phosphatase, and lower oral calcium carbonate intake, were important predictors of hip fractures among Japanese patients on dialysis (*Coco & Rush, 2002***)**.

The purpose of this study is to evaluate both estradiol and parathormone as evidence that supports a potential role of the postmenopausal state in the pathogenesis of bone disease in ESRD to search for treatment.

2. Material and Methods

One hundred women with chronic renal failure were questioned (36 of them on chronic dialysis) about different gynecological aspects. Menstrual disorders were present in 85 % of the patients. Menopause was found in one third of the patients, but only one of these women had been offered hormonal replacement therapy. A total of 20 women below 50 years and had persistent amenorrhea with ESRD (under prolonged hemodialysis, more than 5 years) were selected from Faysal Kidney Dialysis Unit Giza, Egypt, cases with hysterectomy or ovariotomy were excluded. A control group of 20 women matched with same age were selected from the healthy volunteers of the medical staffs. The World Health Organization has established that by DEXA (T-score -1.0 or greater is "normal", T-score between -1.0 and -2.5 is "low bone mass" or "osteopenia" T-score -2.5 or below is osteoporosis [WHO 1994 & WHO 2003].

The study measured the following for all subjects:
- BMD analysis by DEXA.
-Routine chemistry as serum creatinine, calcium, inorganic phosphorus and alkaline phosphatase were estimated using chemistry autoanalyzer Olympus 400.
- Serum estradiol-6III (*Johnson et al., 2001*) (E2) levels were measured by fully automated enhanced chemiluminescnce technique, ADVIA Centaur using ready pack® primary reagent packs containing ADVIA Centaur® E2-6 Lite Reagent and Solid Phase with master curve calibration.
- Serum intact parathormone (iPTH) (*National Committee, 2001*) levels were also measured by enhanced chemiluminescnce technique, ADVIA Centaur. The ADVIA Centaur Intact PTH assay is a two-site sandwich immunoassay using direct chemiluminometric technology, which uses constant amounts of two anti-human PTHantibodies in the Lite Reagent. The first antibody is a polyclonal goat anti-human PTH (N-terminal 1–34) antibody labeled with acridinium ester. The second antibody is a biotinylated polyclonal goat anti-human PTH (39–84 regions) antibody. Streptavidin in the Solid Phase is covalently coupled to paramagnetic latex particles.

All blood samples were collected observing universal precautions for venipuncture and allowed to clot adequately before centrifugation. Tubes were stoppered and upright at all times. Specimens were tightly capped and refrigerated at 2 to 8°C if the assay was not completed within 8 hours. Samples were freezed at or below -20°C if the sample was not assayed within 48 hours. Samples were freezed only once and mixed thoroughly after thawing and before placing samples on the system we ensured that: samples were free of fibrin or other particulate matter and were free of bubbles. We tried to avoid hemolyzed, lipemic and icteric specimens because they show demonstrate of <10% change in results (*Johnson et al., 2001*). Interpretation of iPTH values were taking into account serum calcium results. Measurement of iPTH is useful in differentiating between hypercalcemia due to hyperparathyroidism and hypercalcemia of malignancy (*Johnson et al., 2001*).

Statistical analysis:

Data of the results were collected coded and analyzed using SPSS software Version 15 under windows Xp, The following statistical tests were used: Student t- test, Mann Whitney test for not normally distributed data. Correlation was investigated between two parameters using Pearson correlation. Significant level was considered at 0.05.

3. Results

As shown in table 1, the mean age of control group was 39.10 +3.61 yrs vs. 40.45 +5.09 yrs in the ESRD group. Menstrual cycles were regular in the control group vs. persistent amenorrhea in ESRD cases. Incidence of fractures in ESRD group was fourfold higher than in the control group (4/20 vs. 1/20). DEXA-T-score was -1 in the control group vs. -2.5 in the ESRD group.

Table (1): Clinical data of all studied female subjects.

Parameter	Control group	ESRD group
Age (yrs)	39.10 ±3.61	40.45 ±5.09
Menstrual cycles	Regular	Persistent amenorrhea
Incidence of fractures	1/20	4/20
DEXA-T-score	-1	-2.5

As shown in table 2-a and 2-b; figures 1 and 2:

The mean serum creatinine in control group was 0.78±0.17 mg/dl vs. 11.33 ±2.03 mg/dl in the ESRD group with high significant difference ($p<0.001$). The mean serum calcium in control group was 9.83±0.60 mg/dl vs. 8.52 ±1.30 mg/dl in the ESRD group with high significant difference

(p<0.001). The mean serum inorganic phosphorus in control group was 3.52±0.30 mg/dl vs. 6.16 ±1.50 mg/dl in the ESRD group with high significant difference (p<0.001). The mean serum alkaline phosphatase in control group was 71.65±15.57 U/L vs. 628.50 ±3831.90 U/L in the ESRD group with high significant difference (p<0.001). The mean serum E2 in control group was 17.45 ±3.34 pg/ml vs. 9.90 ±2.25 pg/ml in the ESRD group with high significant difference (p<0.001). The mean serum iPTH in control group was 52.50 ±15.53 pg/ml vs. 935.90 ±583.26 pg/ml in the ESRD group with high significant difference (p<0.001).

Table (2): Comparison between controls and ESRD cases (a and b):

a) T-Test

Group Statistics

	Group	N	Mean	Std. Deviation
Creatinine mg/dl	Cases	20	11.3350	2.03270
	Controls	20	.7800	.17351
S. Calcium mg/dl	Cases	20	8.5200	1.30125
	Controls	20	9.8300	.60271
S. Inorganic Phosphorus	Cases	20	6.1650	1.50097
	Controls	20	3.5200	.30366
S. Alkaline Phosphatase U/L	Cases	20	628.5000	383.90069
	Controls	20	71.6500	15.57419
S. E2 pg/ml	Cases	20	9.9000	2.25318
	Controls	20	17.4500	3.34782
S. Parathormone pg/ ml	Cases	20	935.9000	583.26034
	Controls	20	52.5000	15.53095

Independent Samples Test

		t-test for Equality of Means	
		t	Sig. (2-tailed)
Creatinine mg/dl	Equal variances assumed	23.138	.000
S. Calcium mg/dl	Equal variances assumed	-4.085	.000
S. Inorganic Phosphorus	Equal variances assumed	7.724	.000
S. E2 pg/ml	Equal variances assumed	-8.367	.000

B- Mann-Whitney Test

Ranks

	Group	N	Mean Rank	Sum of Ranks
S. Alkaline Phosphatase U/L	Cases	20	30.50	610.00
	Controls	20	10.50	210.00
	Total	40		

Test Statistics

	S. Alkaline Phosphatase U/L
Mann-Whitney U	.000
Asymp. Sig. (2-tailed)	.000

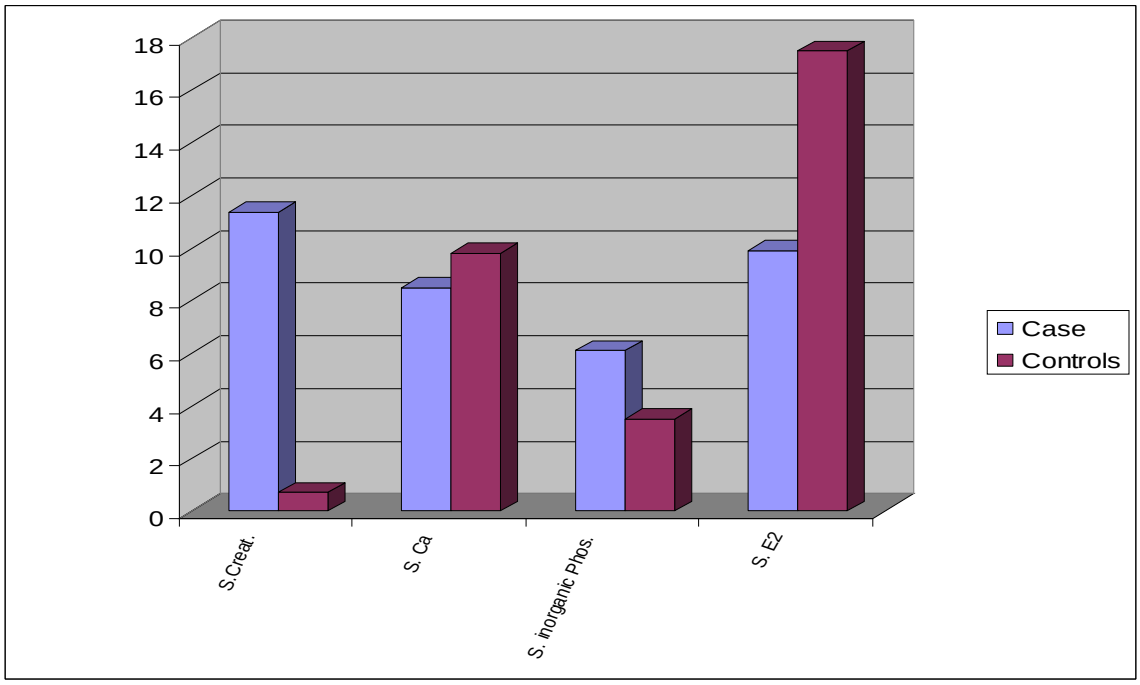

Figure (1): Serum creatinine, calcium, inorganic phosphorus and E2 between controls and ESRD cases.

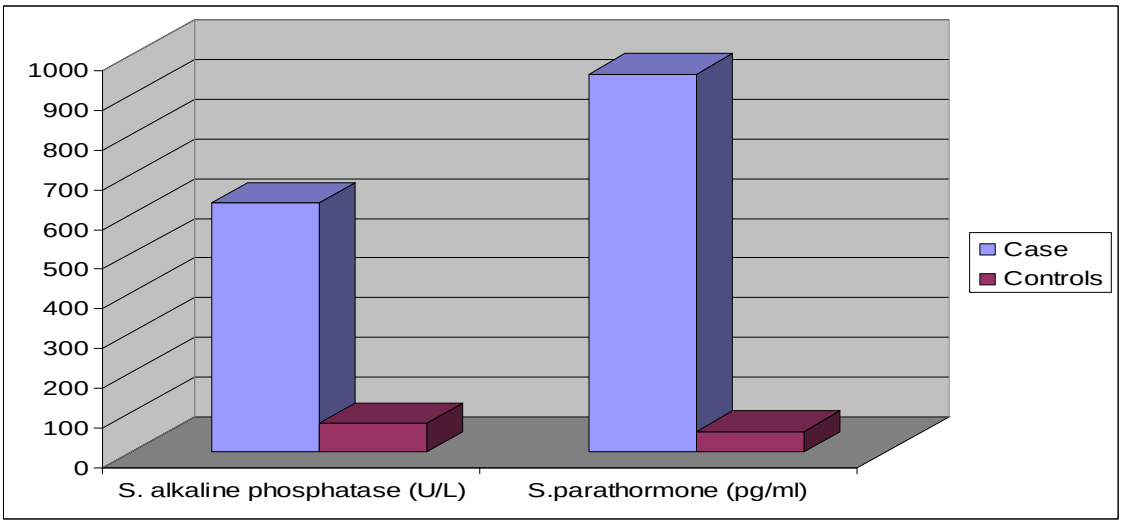

Figure (2): Serum alkaline phosphatase and parathormone between controls and ESRD cases.

There was a negative correlation between serum E2 and serum iPTH levels in ESRD cases which was highly significant (r= -0.861; p<0.001).

4. Discussions

Previous studies have shown that young women with anovulatory cycles have lower bone mass compared with regularly menstruating women (*Weisinger et al., 2000).* A large proportion of dialysis women below 50 years had persistent amenorrhea and estradiol levels significantly lower than age-matched women with normal menstruation (*Manolagas, 2000*). A large proportion of them had persistent amenorrhea with estradiol levels significantly lower than identical women with normal menstruations. Although the adverse effects of hypoestrogenism, and the beneficial effects of estrogens replacement on postmenopausal osteoporosis have been widely recognized, the mechanisms are not fully defined. Estrogen may affect bone metabolism through an effect on PTH synthesis. Indeed, parathyroid glands express estrogen receptors that respond to physiologically relevant doses of estradiol increasing PTH mRNA and PTH secretion (*Hsu et al., 2002*) and modulating PTH action on bone. Indeed, in-vitro studies have demonstrated that estrogen inhibits PTH-stimulated Osteoblasts formation by directly affecting hemopoietic blasts cell precursors by a cAMP-mediated mechanism *(Coco & Rush., 2002).* Intermittent administration of parathyroid hormone (PTH) stimulates bone formation, and continuous infusion of PTH stimulates bone resorption *(Ito, 2007*).

The issue of HRT with opposed estrogen-progestin therapy in uremic women is still controversial, especially after the recent Women's Health Initiative (WHI) Study demonstrating increased risk of breast cancer, pulmonary embolism, coronary and cerebrovascular disease after a long-term combination of estrogen and progestin in normal postmenopausal women (*Cochrane & Regan, 1997).* New evidence suggests that selective estrogen receptor modulators may increase bone mass without significant secondary effects. Estrogen receptor (ER) polymorphism could predict the response of BMD to selective estrogen receptor modulators (*Weisinger, 2000).* Although bisphosphonates are widely used to reduce the high bone turnover of severe secondary hyperparathyroidism so reducing fracture risk in patients with osteoporosis, the use of bisphosphonates to treat dialysis patients with osteoporosis have never been tested prospectively as it may significantly suppress bone turnover, favoring the development or worsening of adynamic bone disease (*Weisinger et al., 2000*). These compounds could reduce the high bone turnover of severe secondary hyperparathyroidism, as has been shown in recent studies in which bone deposition was related to bone turnover. However, the indiscriminate use of the compounds may significantly suppress bone turnover, favoring the development or worsening of adynamic bone disease (*Jose and Ezequiel, 2003*).

The studies have shown that although the risk factors for fracture in end-stage renal disease are similar to the general population, the incidence is four fold higher. The high prevalence of older population, the frequently observed premature amenorrhea and early menopause in dialysis patients may play a role. *Ebeling, 2007* supported our results and added that the fracture risk for kidney transplant recipients is 4 times that of the general population and higher than for patients on dialysis. He noted that organ transplant candidates should be assessed and pre-transplantation bone disease should be treated. Preventive therapy initiated in the immediate post-transplantation period is indicated in patients with osteopenia or osteoporosis, as further bone loss will occur in the first several months following transplantation. Long-term organ transplant recipients should also have bone mass measurement and treatment of osteoporosis. Bisphosphonates are the most promising approach for the management of transplantation osteoporosis. Active vitamin D metabolites may have additional benefits in reducing hyperparathyroidism, particularly after kidney transplantation. The author stated that large, multi-center treatment trials with oral or parenteral bisphosphonates and calcitriol are recommended.

Palmer et al., 2007 assessed the use of interventions for treating bone disease following kidney transplantation. Few or no data were available for combined hormone replacement, testosterone, selective estrogen receptor modulators, fluoride or anabolic steroids. We observed that beside the lack of attention of some treating physicians, women with ESRD are usually stoical and rarely complain about gynecological problems that may seem trivial in comparison to their renal disease and the burden of dialysis. As a consequence, HRT is not a frequently discussed issue between the patients and their physicians. Because of increasing life expectancy after renal transplantation, the prevention of long-term complications, such as bone disease, has become an essential part of post-renal-transplant care. Bone disease is one of the possible long-term complications that can significantly influence quality of life. Compared to members of the normal population of the same age, the fracture rate in renal transplant patients is four times higher. In addition, there is a risk for avascular bone necrosis after transplantation, which mainly affects weight-bearing

bone structures, such as femoral heads, and as a rule, can only be treated by hip replacement with endoprosthesis. Histological evidence of abnormal bone structure, osteodystrophy and osteopaenia is already present shortly after transplantation in almost all transplant recipients (*Kunzendorf et al., 2008).*

5. Conclusion:

Postmenopausal osteoporosis has been recognized as an important entity associated to renal osteodystrophy and efforts have been started to treat the reduced BMD and increased fracture rate. Low serum E2 and high iPTH levels share in decreased BMD in postmenopausal women on dialysis. Although fracture risk reduction data are needed, preliminary observations on the effect of HRT as well as estrogen receptor modulators on BMD and bone resorption markers in uremic women are encouraging. Nevertheless, due to the pharmacokinetics of these drugs in ESRD and the recent observations of increased side effects of some of these compounds, clinical studies to guide the prescription of these drugs in women on dialysis, including short and long-term studies are needed. Because vertebral fractures occur in the same proportion in men and women on hemodialysis, it will be necessary to design studies to evaluate the role of sex steroids in the bone loss of male dialysis patients. Estrogen receptor (ER) polymorphism could predict the response of BMD to selective estrogen receptor modulators.

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