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A Comparative Study of Calcium, Phosphorus and Alkaline Phosphatase in Pre- and Post-Menopausal Women with and without Diabetes

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ABSTRACT

Background: Globally, osteoporosis has become a major health issue, particularly among postmenopausal women, and leading to a high morbidity and mortality. Recent meta-analyses and cohort studies confirm that bone turnover and skeletal integrity were affected negatively by diabetes, and that diabetes was associated with a higher risk of fracture.

Objectives: This study was aimed to evaluate the risk of accelerated bone mass loss by assessing bone markers, such as alkaline phosphatase (ALP), serum calcium and phosphorus in pre and post-menopausal women with and without diabetes.

Materials and Methods: Total of 92 subjects including 37 non-diabetic, 15 diabetic premenopausal women and 25 diabetic and 15 non-diabetic postmenopausal women without any major medical illness, who gave consent were included for study. Fasting blood samples were collected and analyzed for glucose, calcium, phosphorus and alkaline phosphatase by semi-auto analyzer using commercial kits. Values were reported as mean ± standard deviation (SD). The data were analysed by one-way ANOVA with Tukey-Kramer Post Hoc test using SPSS version 20 and p value of < 0.05 was taken as statistically significant at 95% confidence interval.

Results: Results of the study showed that calcium levels were significantly lower whereas phosphorus and alkaline phosphatase were increased in postmenopausal women with and without diabetes compared to premenopausal women without diabetes as well as premenopausal women with diabetes.

Conclusions: The study was concluded that the observed low levels of calcium and increased phosphorus and alkaline phosphatase in postmenopausal women indicating a supplementation of calcium may improve quality of life in postmenopausal women.

Keywords: Menopause, Diabetes, Calcium, Phosphorus, Alkaline Phosphatase.

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INTRODUCTION

Osteoporosis is a disorder of increased bone fragility and low bone mass with a consequent increase in fracture risk.[1] Age is an important factor affecting bone metabolism. Globally, osteoporosis is occurring at an alarming rate and is becoming a major health issue, particularly among postmenopausal women, and this leads to high morbidity and mortality.[2] It is mainly due to the prime deficiency of calcium, as low dietary intake of calcium leads to risk of fractures in later stages[3], and vitamin D, which plays an important role in calcium absorption and osteoclastic activity.[4] In earlier study vitamin D has been shown to be low in postmenopausal women.[5] Phosphorus is the main mineral in the bone, where it is deposited together with calcium.[6] In a population-based cohorts, serum P levels were positively and significantly associated with fracture risk in both sexes.[7]

Serum alkaline phosphatase (ALP) is the most commonly used biomarker of bone formation. ALP is a ubiquitous enzyme that plays an important role in osteoid formation and bone mineralization. The serum ALP pool consists of several dimeric isoforms that originate from various tissues, such as the liver, bone, intestine, spleen, kidney, and placenta.[8]

Menopause is defined as the permanent cessation of menses resulting from reduced ovarian hormone secretion.[9] The post-menopausal stage in women is essentially an oestrogen-deficient state.[10] Estrogen plays a fundamental role in skeletal growth, and bone homeostasis and its deficiency makes prominence of osteoporosis in postmenopausal women.[11] Estrogen deficiency is correlated with a rapid reduction in bone mineral density.[12] Thus, osteoporosis is more common in post-menopausal women. Both

menopause and aging are associated with an accelerated loss of bone mass. Menopause occurs when the balance between bone formation and resorption is upset and resorption become excessive, resulting in a negative remodeling balance.[13]

Osteoporosis and Osteopenia have been ascribed to diabetes without residual insulin secretion and high insulin requirement. However, it is not known if this is partially due to disturbance in the Insulin - like growth factor system, which is a key regulator of bone cell function.[14] Bone metabolism in diabetes is influenced by many factors, including depressed osteoblast activity and decreased numbers of osteoclasts as a result of abnormal insulin secretion and/or insulin action.[15] Researchers who evaluated bone mineral density (BMD) in diabetic and nondiabetic patients did not observe differences in BMD between the 2 groups but did find higher osteoporosis incidence in those with diabetes.[16] Recent meta-analyses and cohort studies confirm that bone turnover and skeletal integrity are affected negatively by diabetes, and that diabetes is associated with a higher risk of fracture.[15,17,18] Our earlier study shown the lower vitamin D in postmenopausal women compared to premenopausal women with or without diabetes.[5]

Thus, the aim of the present study is to evaluate the risk of accelerated bone mass loss by assessing bone markers, such as alkaline phosphatase (ALP), serum calcium and phosphorus in pre and post-menopausal women with and without diabetes.

MATERIALS AND METHODS

Samples

The postmenopausal women selected were those with a history of natural menopause, who had cessation of menstruation for a minimum of one year, and premenopausal women who were studied were those who had regular menstruation. In the present study the total number of participants were 92. The age group of pre-menopausal group of women was between 25 – 50 and for the post-menopausal women it was between 45 – 75. The first group consisted of 37 premenopausal women without diabetes, and second group consisted of 15 premenopausal women with diabetes, third group had 15 postmenopausal women without diabetes and fourth group consisted of 25 postmenopausal women with diabetes.

Inclusion and Exclusion Criterion

Diabetic and non-diabetic women in the study were selected depending on the exclusion criterion. Women with any type of hormonal abnormality, cardiac problems, pregnancy, hormonal therapy, heavy exercise, and familial hypertriglyceridemia were excluded.

Sample Collection

After an overnight fasting for 12 -14 hours, sample was collected from the subjects. About 5 ml of venous blood was drawn under aseptic precaution in a sterile plain vacutainer from selected subjects. Sample for glucose estimation was separately taken in fluoride, oxalate vial and remaining sample was collected into a plane vial. Glucose was estimated in plasma and calcium, phosphorus and alkaline phosphatase in serum. As soon as the sample was collected, serum was separated, and estimations were done on the same day. Glucose, calcium, phosphorus and alkaline phosphatase estimations were done using Erba-chem-5 plus 2 semi-automated analyser. The quality control was checked using control sera of two levels. Glucose was estimated by GOD/PAP method[19], calcium by OCPC method[20], phosphorus by molybdate UV method[21], alkaline phosphatase by pNPP- AMP (IFCC), kinetic assay.[22]

Results were reported as mean ± standard deviation (SD). The data were analyzed by one-way ANOVA with Tukey-Kramer Post Hoc test using SPSS version 20 and p value of < 0.05 was taken as statistically significant at 95% confidence interval.

Ethical Considerations

Sample was collected after taking written/oral consent from the subjects. This project has been approved by the ethical committee of Shadan Institute of Medical Sciences.

Table 1: Number and age of subjects in study groups

Study groups	No of Subjects	Age range (Mean ± SD)
Premenopausal women without diabetes	37	29 – 54 (39.2 ± 6.6)
Premenopausal women with diabetes	15	32 - 49 (39.5 ± 5.7)
Postmenopausal women without diabetes	15	45 - 62 (52.4 ± 5.8)
Postmenopausal women with diabetes	25	50 - 75 (58.8 ± 7.9)
Total number of subjects	92	29-75*

*Minimum and Maximum years of age

Table 2: Fasting Blood Glucose, Calcium, Phosphorus and Alkaline Phosphatase in the study groups

Study groups	Fasting Blood Glucose (mg/dl)	Calcium (mg/dl)	Phosphorus (mg/dl)	Alkaline Phosphatase (U/L)
Premenopausal women without diabetes	95.2 ± 14.1	9.41 ± 0.80	3.63 ± 0.58	82.4 ± 16.6
Premenopausal women with diabetes	151.7 ± 32.9*	9.31 ± 0.54	3.81 ± 0.42	85.6 ± 15.6
Postmenopausal women without diabetes	92.3 ± 12.4@	8.61 ± 0.60*@	4.43 ± 0.58*@	108.6 ± 15.0*@
Postmenopausal women with diabetes	154.9 ± 61.4*#	8.41 ± 0.62*@	4.50 ± 0.54*@	112.7 ± 15.5*@

Statistical analysis done by one-way ANOVA with Tukey-Kramer Post Hoc test

Values are Mean ± SD; statistically significant = p< 0.05

*Statistically significant when compared to premenopausal women without diabetes; @Statistically significant when compared to premenopausal women with diabetes; #Statistically significant when compared to postmenopausal women without diabetes

RESULTS

Table 1 gives the number and age of subjects in study groups. Premenopausal women without diabetes group was with 37 number of subjects having 39.2 ± 6.6 mean ± standard deviation years of age and premenopausal women with diabetes group was with 15 subjects having 39.5 ± 5.7, Postmenopausal women without diabetes group was 15 number of subjects having 51.0 ± 7.7 mean ± standard deviation years of age and postmenopausal women with diabetes group was 58.8 ± 7.9. Overall, the subjects were from 29 to 75 years of age.

Fasting Blood Glucose, Calcium, Phosphorus and Alkaline phosphatase in the study groups were shown in Table 2. The analysis of results by ANOVA indicated the statistically significant mean values ($p<0.05$) for all the parameters. Fasting blood glucose levels were clearly showed an increased level in pre- and post-menopausal women with diabetes. Calcium levels were significantly lower in postmenopausal women with and without diabetes compared to premenopausal women without diabetes as well as premenopausal women with diabetes. Alkaline phosphatase and phosphorus levels were significantly higher in postmenopausal women with and without diabetes compared to premenopausal women without diabetes as well as premenopausal women with diabetes.

DISCUSSION

General Health and bone related problems are common among post-menopausal women, and more in women with diabetes. Osteoporosis is a serious health problem among postmenopausal women that leads to an increased risk of fracture, which increases with age.[23]

Estrogen is the best stimulator of bone growth and is responsible for maintaining the bone mass in the female. Estrogen deficiency that usually occurs among menopausal women may lead to calcium loss due to decreased intestinal calcium absorption and decreased renal calcium conservation.[24] Estrogen deficiency induces calciuria by increasing the filtered load of Ca^{2+}. Estrogen receptors have been demonstrated on renal tubules and it may directly act on kidney to promote renal calcium conservation.[25]

Insulin, together with insulin-like growth factor; stimulates bone matrix synthesis. The stimulatory effect of insulin on bone matrix results from its action on the differentiating function of osteoblasts. Insulin is also necessary for normal bone mineralization. Bone metabolism in diabetes is influenced by many factors, including depressed osteoblast activity and decreased numbers of osteoclasts as a result of abnormal insulin secretion and/or insulin action.[15] The present study did not observe significant difference in bone markers studied between diabetic and nondiabetic groups. A group of studies showed similar results regarding hyperglycemia and the bone markers.[26,27] Serum calcium, phosphorus and alkaline phosphate can be used as biochemical markers to assess the bone health and bone turnover. Hence these markers were estimated in present study to evaluate the bone health in pre and postmenopausal women with and without diabetes. The results showed a decrease in calcium in postmenopausal women with and without diabetes compared to premenopausal women with or without diabetes. This clearly indicates that, the risk factor that clearly associated with osteoporosis in postmenopausal women may be estrogen deficiency. Estrogen deficiency, which is common during menopause, induces synthesis of cytokines by osteoblasts, monocytes, and T cells and thereby stimulates bone resorption by increasing osteoclastic activity. Similar results were published in some articles where statistically significant association with the reduced serum calcium levels among postmenopausal women compared to premenopausal women were found.[28-30]

The age-related factors of bone loss in postmenopausal women may be due to inadequate level of vitamin D and progressive increase in parathyroid hormone with increasing age.[31,32] Inadequate level of vitamin D has been shown as the most common reason for women with osteoporosis. Earlier study demonstrated a low levels of vitamin D in postmenopausal women compared to premenopausal women.[5]

Osteoporosis in postmenopausal women can remain asymptomatic for a long time, with the only indication being changes in biochemical markers of bone turnover. Thus estimation of the bone markers like calcium, phosphorus and alkaline phosphatase should be done periodically in postmenopausal women and calcium supplementation may be given to improve quality of life in postmenopausal women.

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