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A Prospective Randomized Study on Serum Osteocalcin as a Diagnostic Biomarker for Primary Osteoporosis in Women

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ABSTRACT

Background: Osteoporosis is a worldwide disease with reduction of bone mass and decrease of bone strength to result in bone fragility and fracture. Based on the report of World Health Organization (WHO), the disease of osteoporosis has been diagnosed by bone mineral density (BMD) at the hip and/or the spine at least 2.5 standard deviations below in comparison with the bone mass of young healthy adults as determined by dual-energy X-ray absorptiometry (DXA). Osteocalcin (OC), also known as the bone Gla protein (BGP), is a 5.8 kDa, hydroxyapatite-binding protein that could be synthesized by osteoblasts, odontoblasts and hypertrophic chondrocytes and is the most abundant non-collagenous protein found in bone matrix. Aim of the study: To study serum osteocalcin as a diagnostic biomarker for primary osteoporosis in women. **Methods:** The present study was conducted in the Department of Orthopedics of the medical institution. For the study, we selected a total of 50 postmenopausal females between 40-70 years of age reporting to the outpatient department. They were subjected to BMD assessment by DEXA scan of upper end femur and lumbar spine. Those with t-score above -1 SD were taken as controls and with t-scores less than -1 SD were taken as cases. Both, control and case group comprised of 25 women. The sample was tested for serum level of osteocalcin by ELISA, serum calcium and serum alkaline phosphatase. The blood was centrifuged and serum was separated and stored in small capped vials for long term use at -200C until tested. The results of serum calcium, serum alkaline phosphatase and serum osteocalcin levels were compared with the BMD and their relationship was assessed. **Results:** In the present study, a total of 50 post-menopausal women were studied. Both, control and case group comprised of 25 women. We observed that the comparison of osteocalcin level and T-score at lumbar spine between cases and controls was statistically significant. We observed that osteocalcin level was highest in osteoporotic patients and lowest in controls. **Conclusion:** Within the limitations of the present study, it can be concluded that serum Osteocalcin level is fairly efficacious and economical method for screening of osteoporosis in women.

Keywords: Osteocalcin, bone mineral density, osteoporosis, DXA.

INTRODUCTION

Osteoporosis is a worldwide disease with reduction of bone mass and decrease of bone strength to result in bone fragility and fracture. Based on the report of World Health Organization (WHO), the disease of osteoporosis has been diagnosed by bone mineral density (BMD) at the hip and/or the spine at least 2.5 standard deviations below in comparison with the bone mass of young healthy adults as determined by dual-energy X-ray absorptiometry (DXA).[1] The people with osteoporosis are steadily increased because of aging society occurring worldwide. There are about 200 million people who had suffered from osteoporosis in the word and approximately 8.9 million fractures are caused by osteoporotic fracture.[2] In the osteoporotic fractures, hip fractures have led to mortality rates up to 20–24% within the first year and then the death rate has steadily increased for at least 5 years.[3] Postmenopausal osteoporosis (PMO) results from estrogen deficiency after menopause and is a major type of primary osteoporosis.[4] PMO may lead to fragility fracture and is one of the most disabling consequences of postmenopausal women. Early identification and effective therapeutic monitoring to PMO is necessary to reduce the public health burden of this pervasive disease.[5] Osteocalcin (OC), also known as the bone Gla protein (BGP), is a 5.8 kDa, hydroxyapatite-binding protein that could be synthesized by osteoblasts, odontoblasts and hypertrophic chondrocytes[6] and is the most abundant non-collagenous protein found in bone matrix. On the one hand, OC molecules can be released directly into blood after osteoblastic synthesis during bone formation; on the other hand it can also enter the circulation from osteoclastic

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bone matrix degradation during bone resorption. Therefore, circulatory OC may come from both bone formation and bone resorption[7,8] and serum osteocalcin (sOC) level may theoretically increase in PMO, which is characterized by high bone turnover status with both increased bone formation and bone resorption. Hence, the present study was conducted to study serum osteocalcin as a diagnostic biomarker for primary osteoporosis in women.

MATERIALS AND METHODS

The present study was conducted in the Department of Orthopedics of the medical institution. The ethical clearance for the study was approved from the ethical committee of the hospital. For the study, we selected a total of 50 postmenopausal females between 40-70 years of age reporting to the outpatient department. An informed written consent was obtained from the participants after explaining them the protocol of the study. They were subjected to BMD assessment by DEXA scan of upper end femur and lumbar spine. Those with t-score above -1 SD were taken as controls and with t-scores less than -1 SD were taken as cases. Both, control and case group comprised of 25 women. The Case group subjects were graded as osteopenic, mild osteoporosis and severe osteoporosis on the basis of t-scores. Osteopenia was defined as t-score between -1 SD and up to -2.5 SD of normal adult bone mineral density, and osteoporosis was defined as t-score below -2.5 SD. T scoreless than -2.5 SD along with fragility fracture was labeled as severe osteoporosis. Five ml whole venous blood sample of all the recruited subjects was drawn in syringe taking all aseptic precautions between 1400 hours-1600 hours. The sample taken was kept in plain vial at room temperature before sending in to laboratory. The sample was tested for serum level of osteocalcin by ELISA, serum calcium and serum alkaline phosphatase. The blood was centrifuged and serum was separated and stored in small capped vials for long term use at -200C until tested. The results of serum calcium, serum alkaline phosphatase and serum osteocalcin levels were compared with the BMD and their relationship was assessed.

The statistical analysis of the data was done using SPSS version 11.0 for windows. Chi-square and Student's t-test were used for checking the significance of the data. A p-value of 0.05 and lesser was defined to be statistically significant.

RESULTS

In the present study, a total of 50 post-menopausal women were studied. Both, control and case group comprised of 25 women. [Table 1] shows the demographics, bone density and blood tests. We observed that the comparison of osteocalcin level and T-score at lumbar spine between cases and controls was statistically significant ($p<0.05$). [Table 2] shows the comparison of osteocalcin level in controls, osteoporosis and osteopenia cases. We observed that osteocalcin level was highest in osteoporotic patients and lowest in controls. The results on comparison were statistically significant. ($p<0.002$)

Table 1: Demographics, bone density and blood tests

	Controls (n=25)	Cases (n=25)	p-value
Mean age (years)	53.68	52.82	0.52
Mean weight (kg)	69.68	65.29	0.21
Mean BMI (kg/m^2)	26.92	27.51	0.62
Total calcium (mg/dL)	22.92	18.51	0.26
Osteocalcin level (ng/ml)	15.29	21.98	0.002
T score at lumbar spine	1.49	-2.98	0.001

Table 2: Osteocalcin level in controls, osteoporosis and osteopenia cases

Group	Number of patients	Mean osteocalcin level (ng/ml)
Controls	25	15.26
Osteopenia	12	18.92
Osteoporosis	13	24.39

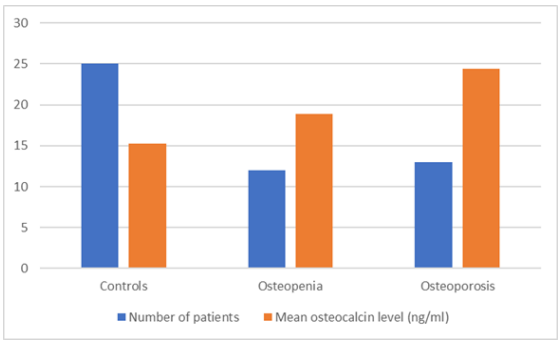

Figure 1: Osteocalcin levels

DISCUSSION

In the present study, we studied 50 post-menopausal women to check the efficacy of osteocalcin as a biomarker for osteoporosis. The mean age of controls was 53.68 years and controls were 52.82 years. The mean weight was 69.68 kg in controls and 65.29 kg in cases. The osteocalcin level was 15.29 ng/ml in controls and 21.98 ng/ml in cases. Furthermore, the T score at lumbar spine was 1.49 in controls and -2.98 in cases. We observed that the comparison of osteocalcin level and T-score at lumbar spine between cases and controls was statistically significant. Furthermore, we observed that osteocalcin level was highest in

osteoporotic patients and lowest in controls. The results on comparison was statistically significant. The results were compared with previous studies from the literature and were found to be consistent. Vs K et al,[9] assessed the association between the biochemical markers of bone remodeling and osteocalcin with the bone mineral density in non-osteoporotic and osteoporotic women among post-menopausal subjects. Sixty postmenopausal women whose ages ranged from 55-65 years included in this study, were further divided into group 1 (thirty non osteoporotic subjects) and group 2 (thirty osteoporotic subjects). For all the subjects, serum osteocalcin was measured by ELISA. BMD was measured by the Dual Energy X- Ray Absorptiometry (DXA) scan. A negative correlation was found between the osteocalcin level and the bone mineral density in post-menopausal women. The mean values of both serum osteocalcin and BMD between the osteoporotic and the non-osteoporotic subjects were statistically significant. Liu SZ et al,[10] investigated the significance of sex hormones and some biochemical indicators related to bone metabolism in the genesis and development of osteoporosis. The plasma samples were collected from 244 post-menopausal women of Xi'an urban area, and their plasma contents of testosterone, estradiol, calcitonin, osteocalcin and N-terminal propeptide of type I procollagen were detected by ELISA. The concentrations of the biochemical indicators were compared among the three groups (normal bone mass group, osteopenia group and osteoporosis group). The comparison results of blood biochemical indicators of BMD-based groups showed that the plasma contents of estradiol, testosterone and calcitonin decreased more significantly in the osteoporosis group, but the content of osteocalcin increased significantly in osteoporosis group than those in the other groups. The correlation analysis between BMD of different parts and the blood biochemical indicators showed that there was a significant positive correlation between estradiol and the BMD of lumber vertebra, femoral neck and great trochanter. Significant positive correlations between calcitonin and BMD of lumber vertebra and femoral great trochanter, and between testosterone and BMD of femoral great trochanter were also observed. In addition, there existed significant negative correlations between osteocalcin and BMD of lumber vertebra, femoral neck, and great trochanter, and between the activity of tartrate-resistant acid phosphatase and BMD of femoral great trochanter. The partial correlation analysis also showed that there were significant correlations between estradiol, calcitonin, osteocalcin and BMD when the influence of age was excluded. The Pearson correlation analysis of biochemical indicators showed there were positive correlations between the contents of testosterone and calcitonin, testosterone and osteocalcin, calcitonin and osteocalcin, calcitonin and PINP, calcitonin and NO, osteocalcin and NO, and PINP and NO, but negative correlations between the contents of testosterone and PINP, estradiol and calcitonin, estradiol and osteocalcin, and estradiol and NO. The blood contents of sex hormones and calcitonin significantly influence BMD and osteoporosis development, and the increase of osteocalcin contents could be used as a biomarker to indicate the degree of osteoporosis in post-menopausal women.

Lateef M et al,[11] studied the significance of serum osteocalcin, a marker of bone formation, and C-terminal telopeptide of type I collagen, a marker of bone resorption, in evaluating osteoporotic patients and to find out their relationship with bone mass density. One hundred and fifty (150) females; 50 premenopausal (age=31.13 +/- 1.29), 50 postmenopausal (age = 54.36 +/- 0.81) and 50 postmenopausal osteoporotic patients (age = 58.6 +/- 0.701) were included in this study. A lower BMD in postmenopausal subjects, with and without osteoporosis, indicating increased bone loss with aging and menopause, was observed. A negative correlation was found between age and BMD. No correlation was found between osteocalcin and BMD among these groups, suggesting heterogeneity of osteocalcin fragments in serum that limits its significance in the evaluation of osteoporosis. A positive correlation was found between osteocalcin and telopeptide-C. A positive correlation of telopeptide-C with age and a negative correlation with BMD was observed indicating increased bone resorption in postmenopausal control and postmenopausal osteoporotic patients. They concluded that C-terminal telopeptide of type I collagen appears to be a significant determinant of bone loss and may be used as a valuable tool in the assessment of postmenopausal osteoporotic patients. Mohamed AS et al,[12] compared between periostin and osteocalcin as biomarkers in Egyptian postmenopausal women with osteoporosis and to explore their possible relationship with fracture risk. This study included 90 postmenopausal females recruited from Al-Hussein University Hospital, Cairo, Egypt; divided into three groups; 35 postmenopausal osteoporotic females with low fracture risk (group I), 35 postmenopausal osteoporotic females with high fracture risk (group II), and 20 apparently healthy controls. The diagnostic performance of periostin for discriminating high fracture risk from low fracture risk groups showed the specificity of (68.6 %) and sensitivity of (100 %), while for osteocalcin the specificity was (51.4 %) and the sensitivity was (68.6 %) respectively. Moreover, the multi Receiver Operating Characteristics (multi-ROC) curve for periostin and osteocalcin together

revealed improved specificity and sensitivity of (100 %) each. They concluded that Periostin was superior to osteocalcin in discriminating high fracture risk from low fracture risk postmenopausal osteoporotic groups. Moreover, dual use of both markers gave the highest discriminative power between low and high fracture risk groups with 100 % specificity and sensitivity.

CONCLUSION

Within the limitations of the present study, it can be concluded that serum Osteocalcin level is fairly efficacious and economical method for screening of osteoporosis in women.

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