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Serum tumour necrosis factor alpha in osteopenic and osteoporotic postmenopausal females: A cross-sectional study in Pakistan

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RESEARCH ARTICLE

Serum tumour necrosis factor alpha in osteopenic and osteoporotic postmenopausal females: A cross-sectional study in Pakistan

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Abstract

Objective: To compare biochemical parameters serum tumour necrosis factor alpha, calcium, magnesium, bone-specific alkaline phosphatase and vitamin D in postmenopausal women.

Methods: This cross-sectional study was carried out from June 2015 to July 2016 at Jinnah Medical and Dental College, Karachi, and comprised postmenopausal women. Bone mineral density done by dual energy X-ray absorptiometryscan categorised subjects by World Health Organisation classification into normal (T score $\geq$ -1) osteopenic (T score between -1 and -2.5) and osteoporotic (T score $\leq$ -2.5). Biochemical parameters like tumour necrosis alpha, calcium, magnesium, bone-specific alkaline phosphatase and vitamin D were measured by solid phase enzyme amplified sensitivity immunoassay method. SPSS 16 was used to analyse the data.

Results: Of the 146 women, 34(23%) were normal, 93(67%) were osteopenic and 19(13%) were osteoporotic. There was significant difference in mean body mass index, serum tumour necrosis factor alpha and calcium in all the three groups ($p<0.01$). Significant mean difference was observed in serum calcium levels between normal and osteopenic, and between normal and osteoporotic group ($p<0.05$ each) without any significant mean difference between osteopenic and osteoporotic groups ($p>0.05$). A significant difference was observed for mean tumour necrosis factor alpha values between normal and osteoporotic groups ($p<0.05$). Tumour necrosis factor alpha showed negative correlation with bone mineral density in osteopenic and osteoporotic groups ($p>0.05$).

Conclusion: Increased bone turnover in postmenopausal osteopenic women can be predicted by increased serum cytokine.

Keywords: TNF alpha, Osteoporosis, Postmenopausal, Osteopenia, BMD, Vitamin D. (JPMA 68: 428; 2018)

Introduction

Osteoporosis is a disorder of the skeleton involving a decrease in bone strength culminating in increased bone fractures and is prevalent among postmenopausal women.[1] Globally there is increased proportion of elderly and postmenopausal women. In future there will be increased number of women suffering from osteoporosis and osteoporosis related fractures.[2] A survey conducted in 2009 in Pakistan revealed the overall prevalence of osteoporosis which was found to be 16% and that of osteopenia was 34%.[3]

In postmenopausal state there is seizure of ovarian function leading to oestrogen deficiency. oestrogen is known to act on osteocytes, osteoblasts and osteoclasts, via these cells it inhibits bone remodelling, stimulates bone formation and inhibits bone resorption.[4] Low oestrogen levels after menopause are associated with increased osteoblasts apoptosis and production of pro-inflammatory cytokines.[5] These pro-inflammatory cytokines like interleukins (IL-1β, IL-6, IL-8) and tumour necrosis factor alpha (TNF-α) are mediators of bone resorption and play an important role in oestrogen deficiency-related bone loss in postmenopausal women. These pro-inflammatory cytokines act both directly and indirectly to increase bone resorption, prevent or inhibit bone formation.[6] The molecular mechanism underlying the phenomenon of bone resorption, induced by these proinflammatory cytokines specially TNF-α is related to its effect on mesenchymal progenitor cells, responsible for the formation of osteoblasts and osteoclasts. TNF-α affects bone metabolism by stimulating the differentiation of progenitor cells into osteoclasts thus causing bone resorption.[7]

Bone remodelling being a crucial physiological mechanism helps in bone repair, increases bone strength and calcium and phosphate homeostasis.[8] Vitamin D has an important role in bone mineralisation through its endocrine effects on bone, intestine and parathyroid gland, as its insufficiency leads to deregulation of calcium and phosphate homeostasis.[9]

Increased bone turnover seen in postmenopausal women results in decreased bone strength.[10] This is a combination of both bone mineral density (BMD) and bone quality and is frequently measured by dual energy

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X-ray Absorptiometry (DEXA) scan.[11] According to World Health Organisation (WHO) recommendation, diagnosis of osteoporosis is made when T score on BMD measurement by DEXA is -2.5 or lower. They also suggested that the term 'osteopenia' or 'low bone mass' be applied when T scores are from -1.0 to -2.5.[12] As a result of the increased bone turnover, as seen in postmenopausal women, the biochemical bone marker concentration is also altered in blood and urine.[13]

TNF-α and other cytokines are important risk factors for osteoporosis. Little information is known regarding risk factors for osteoporosis especially in Pakistani and Indian women. Previous results highlighting mainly Western and Caucasian women could not be compared with women of Indian subcontinent. Also, due to lack of diagnostic facilities in Pakistan, evaluation of various risk factors becomes important to avoid future risk of osteoporotic fragility fractures.

The current study was designed to compare the serum levels of proinflammatory cytokine like TNF-α along with biochemical and biophysical parameters in postmenopausal women stratified into normal, osteopenic and osteoporotic groups on the basis of BMD and also to examine the relationship between TNF-α and BMD in postmenopausal females.

Subjects and Methods

This cross-sectional study was carried out from June 2015 to July 2016 at Jinnah Medical and Dental College (JMDC), Karachi, in collaboration with the Department of Biochemistry, Bahria University Medical & Dental College, Karachi, and Department of Biological and Biomedical Sciences, Aga Khan University Hospital, Karachi, after approval from the JMDC ethical review committee. The sample size was calculated using Open Source Epidemiologic Statistics for Public Health with 16% prevalence of osteoporosis in postmenopausal women at a confidence interval of 90%.[3]

Subjects were recruited by organising health camps in different districts of Karachi through convenience sampling. Postmenopausal women age >50 years, with history of menopause for more than 5 years were included from the general population from different socioeconomic statuses and ethnicities. The subjects were not matched because the target was to know the impact of biophysical profile on biochemical markers in all BMD groups.

Females with premature menopause, history of vertebral fractures or suffering from any systemic illness like hyperthyroidism, diabetes mellitus, hyperparathyroidism, renal failure or taking medications known to affect bone metabolism like bisphosphonates, calcium and Vitamin D supplementation, calcitonin, hormone replacement therapy or anabolic steroids were excluded from the study.

BMD measurement was done by DEXA scan at the lumbar spine and proximal femur. Results were recorded as T score. Although it was not a case-control study but to see the outcome response in each group, the subjects were further categorised on the basis of BMD according to WHO classification into normal (T score $\geq$ -1), osteopenic (-2.5< T score, < -1) and osteoporotic (T score $\leq$ -2.5). Age, marital status, menstrual history, number of children, and years since menopause were recorded. Height, weight and body mass index (BMI) were calculated. Biochemical markers like serum TNF-α, calcium, magnesium, bone-specific alkaline phosphatase and Vitamin D were measured by solid phase enzyme amplified sensitivity immunoassay method (commercial kit DIA source immunoassay, Belgium).

SPSS 16 was used to analyse data. One-way analysis of variance (ANOVA) was used to compare the differences in mean values of BMD, biochemical and biophysical variables across three groups. P<0.05 was considered significant. Post hoc analysis was done using Tukey's honest significant difference (HSD) test for multiple comparisons between groups.

Results

Of the 146 women, 34(23%) were normal, 93(67%) were osteopenic and 19(13%) were osteoporotic. Higher weight and BMI were found in the normal group compared to the other two groups (p<0.01) (Table-1). Calcium and TNF-α mean values were significantly different across the groups (p<0.01). However, no significant difference was found in serum magnesium, alkaline phosphatase and vitamin D levels (Table-2). Calcium was significantly different between normal and osteopenic groups, and between normal and

Table-1: Biochemical parameters in normal, osteopenic and osteoporotic females.

	Normal (34) Mean±S.D	Osteopenic (93) Mean±S.D	Osteoprotic (19) Mean±S.D	p-value
Age	55.65±2.3	56.89±3.3	56.5±2.4	NS
Weight	78.88±14.40	72.52±11.53	63.42±16.47	<0.01*
Height	1.61±0.03	1.61±0.05	1.59±0.05	NS
BMI	31.15±5.46	28.18±4.12	24.90±5.42	<0.01*

*p<0.05 was considered significant using one way ANOVA.
ANOVA: Analysis of variance
BMI: Body Mass Index
SD: Standard deviation.

Table-2: Biophysical parameters in postmenopausal females.

	Normal Mean±S.D	Osteopenic Mean±S.D	Osteoprotic Mean±S.D	P-value
Calcium	9.29±0.64	8.93±0.59	8.75±0.38	<0.01*
Magnesium	1.68±0.30	1.82±0.40	1.75±0.22	0.174
Alkaline PO4	211.70±38.63	231.77±71.65	223.25±42.84	0.322
TN-alpha	116.23±24.99	141.48±52.37	167.70±64.18	0.01*
Vitamin D	18.54±9.85	18.83±9.96	11.69±2.19	0.052

*p<0.05 was considered significant using one way ANOVA.
PO4: Phosphate.
TN: Tumour necrosis.
SD: Standard deviation.

Table-3: Comparison of biochemical parameters in osteopenic and osteoporotic females.

Dependent Variable	Main Group	Comparison With	Mean Difference	P-value
Calcium	Normal	Osteopenic	0.36	0.011
		Osteoporotic	0.54	0.021
	Osteopenic	Osteoporotic	0.17	0.578
TN-ALPHA	Normal	Osteopenic	26.22	0.055
		Osteoporotic	51.46	0.016
	Osteopenic	Osteoporotic	25.24	0.277

*The mean difference is significant at the 0.05 level.
Multiple comparisons made by using Tukey's HSD test
TN: Tumour necrosis
HSD: Honest significant difference.

osteoporotic groups (p<0.05 each), but no significant mean difference was observed in osteopenic and osteoporotic groups (p>0.05).Similarly, as there was a significant difference in mean TNF-α values between normal and osteoporotic groups (p<0.05), and between normal and osteoporotic groups (p<0.05) (Table-3).

There was an inverse relationship between serum TNF-α and BMD in the osteopenic (r=-0.253; p<0.01) and osteoporotic(r=-0.557; p=0.039) groups.

Discussion

The study has highlighted the changes occurring in the serum concentration of a TNF-α and biochemical markers in postmenopausal women. The results have shown higher mean value of serum TNF-α in postmenopausal osteoporotic women compared to normal postmenopausal and osteopenic postmenopausal women. TNF-α is implicated in number of experimental studies in mediating joint damage and systemic bone loss by stimulating osteoclastogenesis.[14] Apart from transition to menopausal period, aging is also associated with increased serum levels of the pro-inflammatory cytokines seen in elderly individuals.[15] A study highlighted increased serum concentration of inflammatory cytokines in elderly postmenopausal women related to changes in the immune system.[16] Our results also showed negative correlation of TNF-α with BMD in osteopenic and osteoporotic postmenopausal women. Similar findings have been reported in another study.[17] It shows that higher levels of osteoclastogenic cytokines like TNF-α increases bone loss and decreases BMD.[18]

In the present study biochemical parameters like alkaline phosphatase, Vitamin D and magnesium showed no significant difference in all the three studied groups except serum calcium level which showed significant mean differences in all the groups and was found to be low in postmenopausal osteopenic and osteoporotic groups compared to the normal group. Aging and menopause is associated with reduced production of oestrogen and progesterone which is related to increased concentration of serum calcium levels in postmenopausal women.[19] In postmenopausal women oestrogen deficiency stimulates production of cytokines by osteoblasts, monocytes and T cells causing osteoclastogenesis and bone loss. This phenomenon leads to modification in bone mineralisation causing increased resorption and excretion of calcium, raising its serum levels.[20] A recent cross-sectional study found that serum calcium levels were significantly reduced in postmenopausal osteoporotic women but, contrary to our results, found increased serum alkaline phosphatase levels in postmenopausal women.[10]

The role of Vitamin D is well established for bone mineralisation. Previous studies showed that 25-hydroxyvitamin D level below 10ng/ml (25 nmol/L) compared to the normal cut-off value of vitamin D >32 ng/mL (80 nmol/L) may be associated with an increased risk of falling in the elderly.[21] In our study Vitamin D levels were on the lower side in osteoporotic women but no significant statistical difference was found in the three groups. This was perhaps due to low calcium intake and inadequate exposure to sunlight in elderly women, causing deficiency of Vitamin D and decreased calcium absorption. This may lead to secondary hyperparathyroidism causing increased bone turnover and bone loss.[22] Unlike calcium, serum levels of magnesium were not significantly different in the three groups in our study which is similar to some previous findings[22] and is in contrast to another study which showed decreased serum levels of magnesium in osteoporotic women compared to the normal group.[23]

The subjects were not matched in the current study on the basis of age and BMI since the target was to see the

impact of age and BMI in all groups. There was significant mean difference among the groups. A higher weight and BMI was found among postmenopausal women with normal BMD compared to the osteopenic and osteoporotic groups. Low BMI and low weight have been implicated as risk factors in previous studies for osteoporosis and osteoporosis related fractures.[24] Similar findings were given by another study[25] which highlighted association of biochemical variables and BMI with change in BMD measured at different skeletal sites.

Due to limited number of patients, the current study could not draw definitive conclusions from the findings. Further studies with a larger sample size are needed to evaluate the role of TNF-α as an important risk factor in the pathogenesis of postmenopausal bone loss.

Conclusion

Increased calcium level due to intensified bone turnover in postmenopausal women did not demarcate the differences in osteopenic and osteoporotic groups. Serum levels of TNF-α correlated with BMD in both osteopenic and osteoporotic groups. Hence, serum levels of TNF-α may be used as a better marker to evaluate the risk of osteoporosis and future fragility fractures.

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Conflict of Interest: None

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