Research Article

Systemic Inflammation: A Risk Factor for Osteoporosis in Pre- and Post-menopausal Women

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Citation: Yasovanthi Jeedigunta, Shehnaz Sultana, Rajender Rao Kalashikam, Raghunath Manchala. Systemic Inflammation: A Risk Factor for Osteoporosis in Pre- and Post-menopausal Women. Journal of Women's Health and Development 3 (2020): 423-431.

Abstract

Background: Inflammatory markers like Interleukin 6 (IL-6), tumor necrosis factor α (TNF α) and high sensitivity C-reactive protein (hs CRP), play an important role in the pathogenesis of osteoporosis. The present study aims to investigate the relationship between markers of inflammation, BMD and to correlate these markers with estradiol levels in pre- and post-menopausal women.

Methods: A total of 169 subjects with low BMD along with 170 age matched controls were studied. Serum concentrations of hs CRP, IL-6, TNF α and estradiol levels were measured by ELISA method.

Results: Bone specific ALP, acid phosphatase, hsCRP, IL-6 and TNF α, levels were significantly high in osteoporotic subjects ($p < 0.05$) when compared with controls. hsCRP levels were positively correlated with IL-6 (r = 0.247, $p < 0.001$) and TNF α levels (r = 0.397, $p < 0.001$) in osteoporotic women.

Conclusion: Inflammation is associated with low bone mass and therefore an independent risk factor for osteoporosis.

Keywords: Osteoporosis; CRP; Interleukin-6; Tumor necrosis factor α; Estradiol

1. Introduction

Osteoporosis is characterized by the loss of bone mass and deterioration of bone microarchitecture with a resulting increase in bone fragility and, therefore, susceptibility to fractures [1]. Currently Osteoporosis is attributed to various endocrine, metabolic and environmental factors. Emerging clinical and molecular evidence suggests that inflammation also exerts significant influence on bone turnover, inducing osteoporosis. Numerous pro-inflammatory cytokines have been implicated in the regulation of osteoblast and osteoclasts and an activated immune profile has been hypothesized as an important risk factor in the occurrence of the disorder [2].

During the past decade, considerable evidence has accumulated that estrogen deficiency dramatically alters the dependency of bone cells on several cytokines, including interleukin 6 (IL-6), interleukin -1(IL-1), and tumor necrosis factor α (TNFα) for the role of cytokines in bone loss due to estrogen deficiency [3-5]. Although these data provide strong evidence for the involvement of cytokines due to estrogen deficiency most of the results have been derived from in vitro and animal studies. However, evidence that these inflammatory cytokines play a similar role in human studies is limited. Preliminary studies on cytokines and bone mineral density [6-9] or bone loss [10, 11] in post-menopausal women have shown inconsistent and contradictory results.

Further the associations between circulating inflammatory markers, estradiol levels and BMD in pre-menopausal women have not been reported. Therefore, the aim of this study was to estimate the serum bone specific alkaline phosphate (ALP, bone formation marker), bone specific tartrate resistant acid phosphatase (TRAP, bone resorption marker) and to determine the association between serum hsCRP ,IL-6 ,TNF α (markers of inflammation) and bone mineral density as well as estradiol levels in pre and post-menopausal women.

2. Materials and Methods

2.1 Study subjects

A total of 169 osteoporotic Indian women from a high socio- economic group attending the bone health program of Vijaya Diagnostic Centre, Himayat Nagar, Hyderabad were included in the study. Subjects affected by disorders of mineral metabolism or by conditions known to cause secondary osteoporosis, such as hyperthyroidism, cortisol excess, malabsorption syndromes and malignancies were excluded. Likewise, individuals on drugs known to affect calcium metabolism, including corticosteroids, Thyroid hormones, anticonvulsants, diuretics as well as bisphosphonates, Fluorides were excluded from the study.

70 premenopausal women (mean age 40.7 ± 5.1 years; range 30 -45 years) having regular cycles and T score ≤ -2.5 at spine were considered as osteoporotic. The women were non pregnant, non-lactating at the time of sample collection. Postmenopausal women with cessation of menses for at least one year (mean age 56.6 ± 7.8; range 50-70

years) and T score ≤ -2.5 at spine were taken as osteoporotic individuals for the study. Individuals were excluded if they had undergone hysterectomy prior to natural menopause. None of the women were on estrogen replacement therapy. None of the subjects were alcoholic nor smokers. All the subjects were interviewed in accordance with a structured questionnaire in order to collect clinical data, including a history of fracture which was verified by medical records. Osteoporotic fractures included clinically recognized fractures of the hip, spine, or distal fore arm which resulted from minimal or moderate trauma. Among the 169 osteoporotic individuals none of them had fractures at the time of sample collection. Written informed consent was obtained from all the individuals and the study was approved by the ethical committee of the Institute. Standing height and weight were recorded. Physical built was expressed as body mass index, calculated as the weight (in kilograms) divided by the square of the height (in meters).

2.2 BMD measurements

Bone mineral density was obtained from anterior –posterior lumbar spine (L2-L4) scanning with Dual energy X-ray absorptiometry (Lunar GE medical systems, Prodigy 6.80, DF+10243). Subjects were categorized according to the WHO definition, osteopenia was diagnosed at $-2.5 <$ T -score < -1.0 SD, and osteoporosis was diagnosed at T-score ≤ -2.5 SD at any site. T-score ≤ 1 SD is considered normal. All the individuals were analyzed by the same DEXA machine to avoid minor variations if any.

2.3 Biochemical measurements

Serum total alkaline phosphatase and bone specific alkaline phosphatase, total tartrate resistant acid phosphatase and bone acid phosphate, were measured by spectroscopy. [12-14] Serum calcium and phosphorus levels were determined by atomic absorption spectrophotometry (AAS). Enzyme linked immunosorbent assay (ELISA) test was carried out to determine the serum levels of IL-6, TNF α, hs CRP and estradiol using kits obtained from Pharmingen opt EIA[TM] BD Biosciences, Diagnostics Biochem, Canada Inc.and Equipar Diagnostici Italy respectively.
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2.4 Statistical analysis

The results are expressed as mean ± SD. Differences in the parameters between the Pre- and post-menopausal women were compared using Students t-test. Pearson's correlation co-efficient was used to find the strength of the association between serum IL-6, TNF α and hs CRP. Relationship between estradiol levels and IL-6, TNF α and hs CRP were performed by Pearson correlation. All statistical analysis was performed with SPSS statistical software (version 15), with p value less than 0.05 considered statistically significant.

3. Results

The clinical characteristics of the study subjects are shown in Table 1. The mean age of the pre-menopausal women was 40.7 ± 5.1years in osteoporotic group and 39.2 ± 7.4 years in the control group, in post-menopausal women it was 58.6 ± 7.8 years in osteoporotic group and 56.7 ± 6.8 years in the controls. Weight and BMI were significantly higher (p<0.05) in pre-and post-menopausal osteoporotic subjects when compared to the controls.

Variables	Premenopausal women (n=140)			Post-menopausal women (n=199)		
	Controls	Osteoporotics	P- value	Controls	Osteoporotics	P-value
	N=70	N=70		N=99	N=100	
Age (years)	39.2 ± 7.4	40.7 ± 5.1	0.16	56.7 ± 6.8	58.6 ± 7.8	0.06
BMI(kg/m2)	23.1 ± 5.0	24.1 ± 4.8	0.04	25.4 ± 5.1	26.0 ± 3.6	0.004
Duration of menopause (years)	NA	NA	NA	8.4 ± 6.2	11.0 ± 6.1	0.003
Age at menopause (years)	NA	NA	NA	49.5 ± 5.3	45.8 ± 5.3	0.001
T-score	-1.0 ± 0.7	-2.6 ± 0.3	<0.001	-1.2 ± 0.4	-2.98 ± 0.6	0.007
Z-score	-1.5 ± 0.9	-2.1 ± 0.6	0.009	-1.5 ± 0.5	-3.6 ± 1.1	<0.001

Table 1: Clinical characteristics of the study subjects.

We could not find any statistical significance ($p > 0.05$) in serum calcium and phosphorus levels between osteoporotics and controls (Table 2). Total ALP and bone ALP were significantly high in osteoporotics when compared to controls ($p<0.05$). Tartrate resistance acid phosphatase was also significantly high in patients when compared to controls (Table 2). There was no significant difference ($p>0.05$) in serum estradiol levels of premenopausal osteoporotic women in comparison with their controls whereas statistically significant values were obtained in postmenopausal osteoporotic women when compared to controls. Markers of inflammation, IL-6, TNF α and hs CRP levels were significantly high ($p<0.001$) in all osteoporotic subjects in comparison with controls. (Table 2).

Variables	Premenopausal women (n=140)			Post-menopausal women (n=199)		
	Controls	Osteoporotics	P- value	Controls	Osteoporotics	P-value
	N=70	N=70		N=99	N=100	
Calcium(mg/dl)	8.74 ± 0.43	9.09 ± 0.40	NS	9.26 ± 0.31	9.38 ± 0.35	NS
Phosphorus(mg/dl)	3.48 ± 0.43	4.0 ± 0.52	NS	4. 19 ± 0.62	4.52 ± 0.75	NS
Total ALP(IU/L)	69.40 ± 4.60	80.0 ± 7.54	<0.001	101.0 ± 6.70	103.0 ± 5.05	0.005
Bone ALP(IU/L)	24.50 ± 5.48	38. 0 ± 4.20	0.02	40.20 ± 4.10	47.0 ± 6.53	0.01
Total TRAP (IU/L)	7. 0 ± 3.60	10.5 ± 2.63	0.002	8.1 ± 1.26	11.5 ± 1.78	0.007
Bone TRAP (IU/L)	3.40 ± 0.78	4.92 ± 1.36	0.001	3.65 ± 1.20	5.3 ± 1.85	0.002
hsCRP (mg/l)	0.7 ± 0.21	1.90 ± 0.20	0.001	1.3 ± 0.20	2.2 ± 0.30	0.001
IL-6 (pg/ml)	2.40 ± 0.41	3.0 ± 0.40	0.001	2.8 ± 0.41	4. 0 ± 0.92	0.001
TNF α (pg/ml)	2.73 ± 0.40	3.5 ± 0.71	0.002	2.8 ± 0.40	5.2 ± 1.10	0.001
17ß Estradiol (pg/m)	89.65 ± 14.50	75.87 ± 11.82	NS	36.0 ± 3.0	21.0 ± 4.5	0.007

**Table 2:** Biochemical and inflammatory markers of the study subjects.

When we compared pre-menopausal osteoporotic women with post-menopausal osteoporotic women all the above said inflammatory markers were significantly high in post-menopausal osteoporotic women with p value <0.05 (Table 3).

Variables	Premenopausal Osteoporotic women	Postmenopausal Osteoporotic women	P value
Estradiol pg/ml	89.65 ± 14.50	21.0 ± 3.4	<0.0001
hs CRP mg/l	1.9 ± 0.2	2.2 ± 0.3	<0.004
IL-6 Pg/ml	3.0 ± 0.4	4.0 ± 0.9	<0.001
TNF α (pg/ml)	3.5 ± 0.7	5.2 ± 1.1	<0.001

The results are given as mean ± SD, p value <0.05 is considered significant

Table 3: Comparison of inflammatory markers according to menopausal status.

According to WHO classification when the subjects were categorized into normal, osteopenia and osteoporotic, we found that the serum hs CRP ($F=29.90$, $p=0.000$) IL-6 ($F=16.30$, $p=0.000$) and, TNF α ($F=3.754$, $p=0.05$) levels were significantly high in osteoporotic subjects compared to controls. The significant decrease in bone mineral density and increase in the levels of inflammatory markers in post- menopausal osteoporotic women indicate a rapid bone loss than pre- menopausal osteoporotic women (Table 2). Osteopenic subjects had also higher concentrations of inflammatory markers hs CRP ($F=5.073$, $p=0.026$) and IL-6 ($F=6.604$, $p=0.016$) when compared to controls but TNF α levels did not reach statistical significance ($F=0.390$, $p=0.533$) in these subjects. Serum hs CRP (a), IL-6 (b) and TNF α (c) concentrations among controls, osteopenics and osteoporotic subjects are shown in figure 1. Data reported as estimated mean ± S.E values in all the subjects.
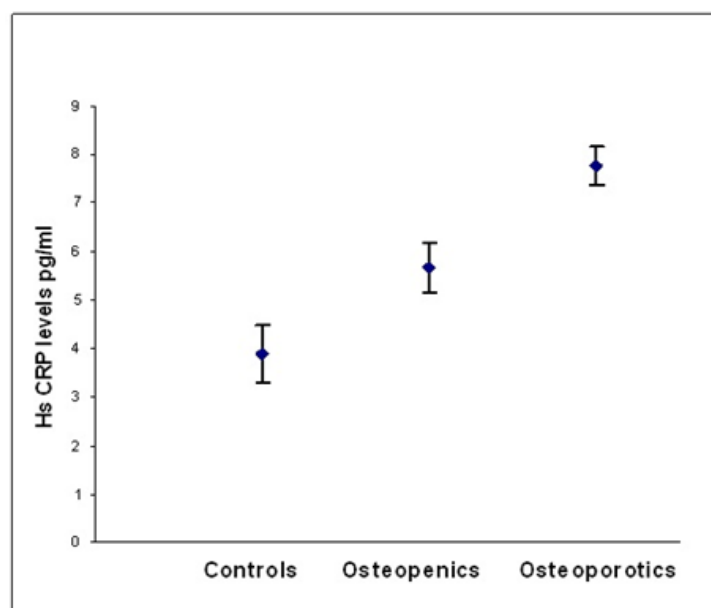
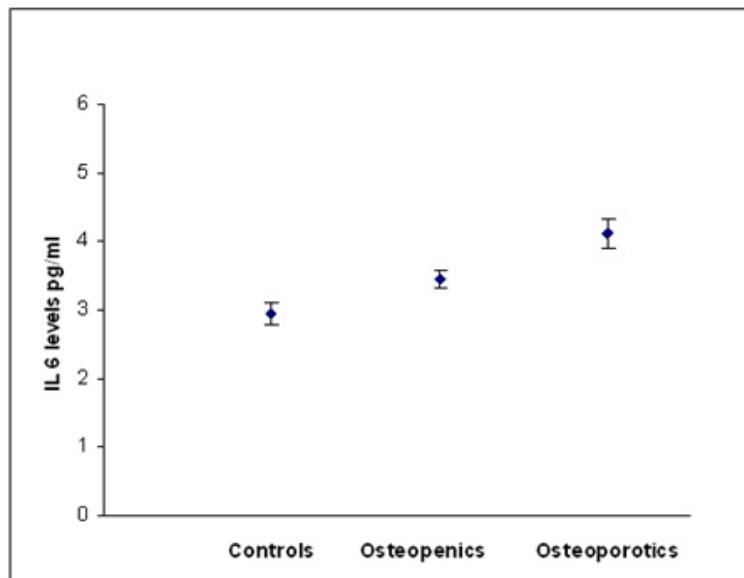**Figure 1a:** Relationship between hs CRP levels and BMD status.

Figure 1b: Relationship between IL-6 and BMD status.
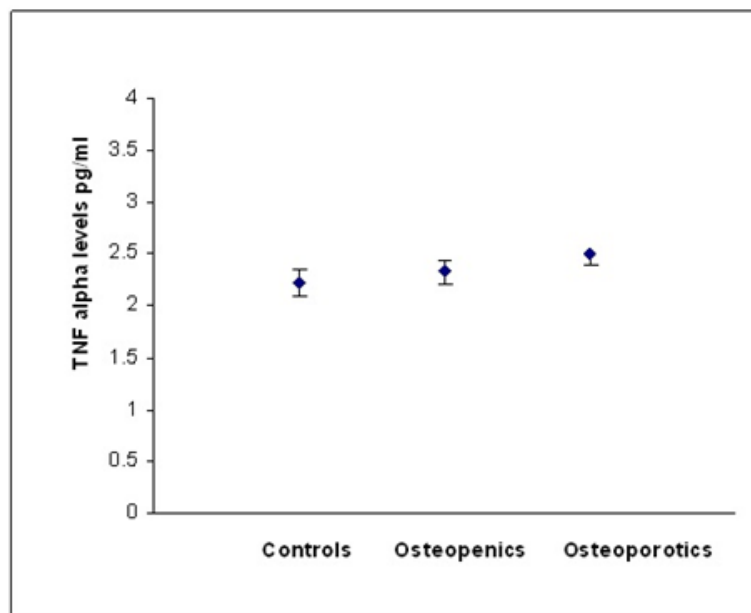
Figure 1c: Relationship between TNF alpha and BMD status.

4. Discussion

Emerging clinical and molecular evidence suggests that factors involved in inflammation are linked with those critical for bone physiology and remodeling, supporting the theory that inflammation significantly contributes to the etiopathogenesis of osteoporosis. [15, 16]. Decline in ovarian function with menopause is associated with spontaneous increase in pro inflammatory cytokines. Experimental and clinical studies strongly support a link between the increased state of pro inflammatory cytokine activity and postmenopausal bone loss. Cytokines in the

microenvironment of the bone, such as interleukins 1 and 6 (IL-1 and IL-6), tumor necrosis factor (TNF), interferon (IFN), affect the bone-remodeling process by regulating the differentiation as well as the activity of osteoblasts and osteoclasts [17-20]. Over the past decades, numerous reports have been published demonstrating that natural or surgical menopause increases blood, bone marrow, and monocytic levels of IL-1, IL-6, TNF, and the related factors IL-1R [4, 21] In spite of these observations, controversy persists concerning the specific contribution of each of these factors to bone loss. Recent data from a number of laboratories have indicated a potential critical role for certain pro inflammatory cytokines, both in the normal bone remodeling process and in the pathogenesis of peri menopausal and late-life osteoporosis [21, 22].

However, there is no consensus about serum IL-6 levels in menopause. Previous studies have failed to demonstrate a regulatory effect of ovarian steroids on IL-6 levels in human bone cells and animals [23-25]. Furthermore, McKane et al also suggested that there was no difference in serum IL-6 levels between pre- and post-menopausal women [26]. However, Rachon et al reported that estrogen deprivation results in IL-6 production by the peripheral blood mononuclear cells in postmenopausal women [27]. They reported that the mean serum levels of IL-6 in postmenopausal women were significantly higher than in young women. Abrahamsen et al reported that serum IL-6 was associated with an increase in BMD at lumbar spine in pre-menopausal women [10]. In contrast Salamone et al reported that the production of IL-1, IL-6 and/or TNF-α by peripheral blood monocytes positively correlated with bone resorption or spinal bone loss in healthy pre- [28] and postmenopausal women [29].

In the present study all the three markers of inflammation (hs CRP, IL-6 and TNF α) were found to be significantly high in osteoporotic subjects when compared to controls. When we compared the above said markers in pre-menopausal osteoporotic and post-menopausal osteoporotic women we found significantly higher levels of hs crp , IL-6 and TNF α in the latter group. Our study corroborates earlier findings that estrogen deficiency dramatically alters the dependency of bone cells on several cytokines, including interleukins 6 (IL-6) IL-1 and tumor necrosis factor (TNF) [3-5]. Tartre resistant bone acid phosphatase a marker of bone resorption is significantly high (p<0.001) in osteoporotic patients when compared to controls and also the elevated levels of IL-6, hs CRP and TNF α in pre- and post-menopausal osteoporotic women suggest that inflammation is associated with low bone mass. Further we also found that the serum hs CRP levels correlated positively with both IL-6 and TNF α concentrations in pre and postmenopausal osteoporotic women.

Results of the present study reveal that inflammation is involved in bone metabolism. However, the mechanisms linking bone metabolism are not clear. Accelerated bone loss due to menopause –induced estrogen deficiency is the major cause of postmenopausal osteoporosis [30] where inflammatory processes are known to up-regulate many cytokines, such as IL-1, IL-6 and TNF-α, which strongly stimulate CRP production from the liver as well as induce bone resorption [31, 32]. Increased bone resorption may result in increased bone turnover and decreased BMD [33-36]. But the mechanism involved in pre-menopausal women is not clear. The strong family history of osteoporosis, especially in the premenopausal women, may provide further support for current theories of a genetic predisposition to osteoporosis.

5. Conclusion

Our observations clearly indicate that elevated levels of serum hs CRP, IL-6 and TNF α concentrations in both pre and postmenopausal women are associated with low BMD. Thus, inflammation alone could be an important risk factor in the occurrence of the disease and targeted anti-inflammatory therapy may play a potential role in the prevention of rapid bone loss.

Conflict of Interest

All the authors declare no conflict of interest.

Acknowledgements

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