Higher C-Reactive Protein Levels as Biochemical Determinant of Osteoporosis in Punjab

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Abstract

The present cross-sectional study involved 300 postmenopausal women to examine the role and relevance of C-reactive protein (CRP) and nitric oxide (NO) as the biochemical determinants of osteoporosis in the population of Punjab, India. Subjects were verified having osteoporosis on the basis of dual energy x-ray absorptiometry (DEXA) testing at hip (femoral neck) and lumbar spine (L1-L4 vertebrae). Enzyme linked immunosorbent assays (ELISA) were conducted for the quantitative analysis of CRP and NO. It was observed that women having higher levels of CRP (>3mg/L) were at substantially higher risk of osteoporosis (OR 2.29 95%CI: 1.44 – 3.87, P<0.001) than women having lower levels of CRP (≤3mg/L). Present study could not find any association of NO levels with the risk of osteoporosis. Linear regression analysis confirmed that higher the CRP levels, higher was the risk of low BMD at both lumbar spine (P<0.001) and femoral neck (P<0.001). In conclusion, present study has revealed that higher serum CRP levels are associated with the risk of osteoporosis, whereas, NO does not participate in it.

Keywords: *C-reactive protein; nitric oxide; bone mineral density; osteoporosis; postmenopausal women.*

1. Introduction

Osteoporosis is an age associated skeletal disorder, whereby low bone mass and deterioration of microarchitecture of bone leads to risk of fractures. It is multifactorial, complex and severe, especially in postmenopausal women [1]. Its higher prevalence impinges upon global health especially India, where every eighth man and every third woman is suffering from osteoporosis [2]. Although several risk factors such as advanced age, low body mass index (BMI), hypertension, abnormal lipid levels, unrestrained alcohol consumption and smoking influence its risk but lately, molecular studies have collected

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evidence that inflammation contributes significantly to bone remodelling, causing osteoporosis [3-5]. Several proinflammatory cytokines participate in the mediation of osteoblast and osteoclast differentiation thereby, activating the immune response in osteoporosis [6]. Many inflammatory conditions such as systemic lupus erythematosus (SLE), rheumatoid arthritis, human immunodeficiency virus (HIV) and inflammatory bowel disease increase the risk of osteoporosis, whereby increased cytokine profile and higher levels of C-reactive protein (CRP) regulate bone resorption [7].

CRP is synthesised in the liver and is upregulated by proinflammatory cytokines such as interleukin-1 (IL-1), IL-6 and Tumor necrosis factor-alpha (TNFα) [8]. It is a systemic inflammatory marker which has been observed to be associated with bone mineral density (BMD) in elderly women [9]. A clinical link between osteoporosis and inflammation exacerbates in postmenopausal women [5]. Another inflammatory regulator i.e. nitric oxide (NO) has also been associated with the risk of osteoporosis. It has been observed that cytokines (IL-1 and TNFα) activate NO production through inducible NO synthesis (iNOS) pathway, whereby NO inhibits osteoblastic differentiation and augments osteoblastic apoptosis [10].

Fewer studies have investigated the role of these proinflammatory markers vis-a-vis risk of osteoporosis, but largely this issue has remained overlooked and unforeseen in clinical arena, especially in India. Therefore, the present study has examined the role and relevance of CRP and NO as biochemical determinants for the risk of osteoporosis in postmenopausal women of Punjab, India.

2. Methods

Study participants

The present study involved 300 postmenopausal women of Punjab, out of which 150 women were categorised as 'osteoporotic' on the basis of dual energy x-ray absorptiometry (DEXA) testing and 150 women who were having normal bone mass were categorised as 'controls'. By cross-sectional design, the present study followed strict inclusion/exclusion criteria. Inclusion criteria were: postmenopausal women falling between 45-65 years of age and original inhabitants of Punjab. Exclusion criteria were: unwilling and non-consenting women, unverified menopause status, taking medicines like statins, psychotropics or multivitamins, having any pathology that may affect the result of the present study. All the study participants gave their written consent and the study was approved by ethical review committee of Punjabi University, Patiala.

Blood samples were collected from the recruited subjects with the help of paramedic staff in the anticoagulant free vacutainers. The serum was collected after incubation of blood sample at room temperature for 20 minutes and then centrifuged at 3000 rpm for 10 minutes at 4°C. After centrifugation, supernatant (serum) was transferred into falcon tubes and stored at -20°C for further analysis. Serum levels of CRP and NO were tested with commercially available kits (ThermoFisher Scientific, Waltham, MA, USA) using Enzyme Linked Immunosorbent Assay (ELISA) on a microplate reader (Biotek Instruments Inc., Winooski, VT, USA). Diagnostic sensitivities of the kits were 9.38 pg/mL and 1.75pg/mL for CRP and NO respectively. The inter and intra assay coefficients of variation for CRP and NO were 0.35 and 0.42 respectively.

3. Statistical analysis

Odds ratio was generated by analysing an unadjusted model by taking normal levels of CRP and NO as referent. Linear regression was applied by taking BMD as target variable and C-reactive protein as predictive variable using SPSS software. P values <0.05 were considered significant with Bonferroni correction.

4. Results

Osteoporotic and control subjects were categorised according to the CRP and NO levels, values of which are shown in table 1. The categorisation of CRP levels were ≤3mg/L and >3mg/L whereas, NO levels were categorised into NO ≤10µmol/L and >10µmol/L. It was observed that women having CRP >3mg/L were at 2.29 times (OR 2.29 95%CI: 1.44 – 3.87, P<0.001) higher risk of osteoporosis than women having CRP levels ≤3mg/L. This relation was not evident for NO, as larger number of osteoporotic subjects had NO levels ≤10µmol/L as compared to controls. . Odds ratio analysis also suggested that NO did not participate for the risk of osteoporosis (OR 1.50 95%CI: 0.93 – 2.40, P=0.12).

Table 1. Association of CRP and NO with the risk of osteoporosis.

Biochemical parameters	Osteoporotic subjects (n=150)	Control subjects (n=150)	OR (95%CI)	P value
CRP ≤3 mg/L	71 (47.33)	101 (67.33)		
CRP >3 mg/L	79 (52.67)	49 (32.67)	2.29 (1.44 – 3.87)	**<0.001**
NO ≤10 µmol/L	102 (68)	88 (58.87)		
NO >10 µmol/L	48 (32)	62 (41.33)	1.50 (0.93 – 2.40)	0.12

CRP: C-reactive protein, NO: Nitric oxide

To understand the relationship between serum CRP levels and BMD, linear regression analysis was performed (figures 1 and 2) which revealed that serum CRP levels were negatively correlated with the BMD at both lumbar spine and femoral neck. As evident from figure 1 the gradual increase in BMD of lumbar spine (P<0.001) and femoral neck (P<0.001) was negatively associated with gradual increase in CRP levels. It suggested that higher CRP levels were inversely associated with BMD at lumbar spine and femoral neck. Figure 2 demonstrated that NO levels were not correlated with both BMD at lumbar spine (P=0.63) and femoral neck (P=0.74).

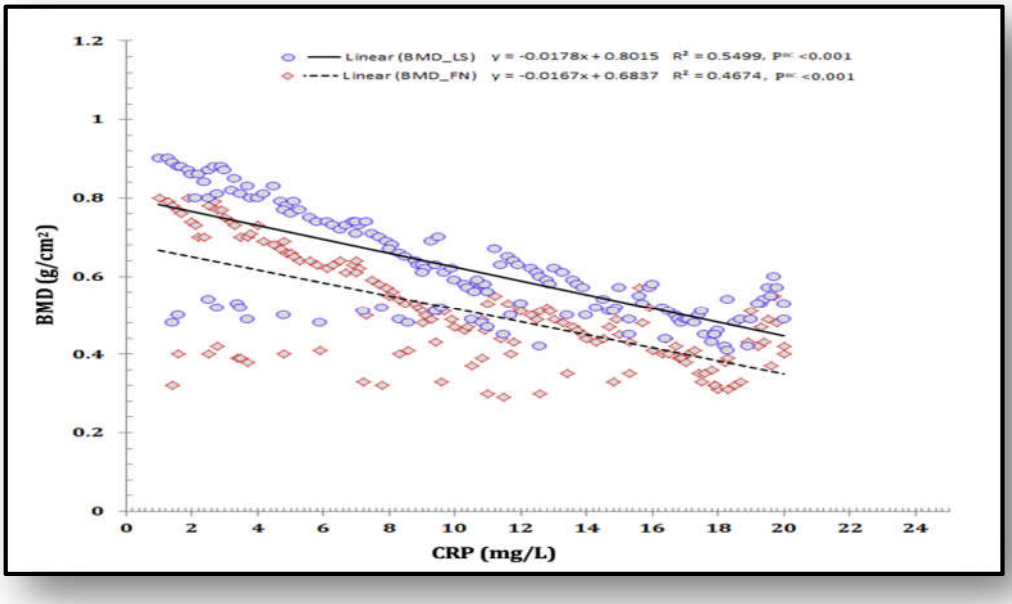

Figure 1. Linear regression analysis between bone mineral density (BMD_LS and BMD_FN) and C-reactive protein (CRP). P values are corrected with Bonferroni (P^{BC}) for correlation coefficients (r^2). BMD_LS: bone mineral density at lumbar spine, BMD_FN: bone mineral density at femoral neck, CRP: C-reactive protein.

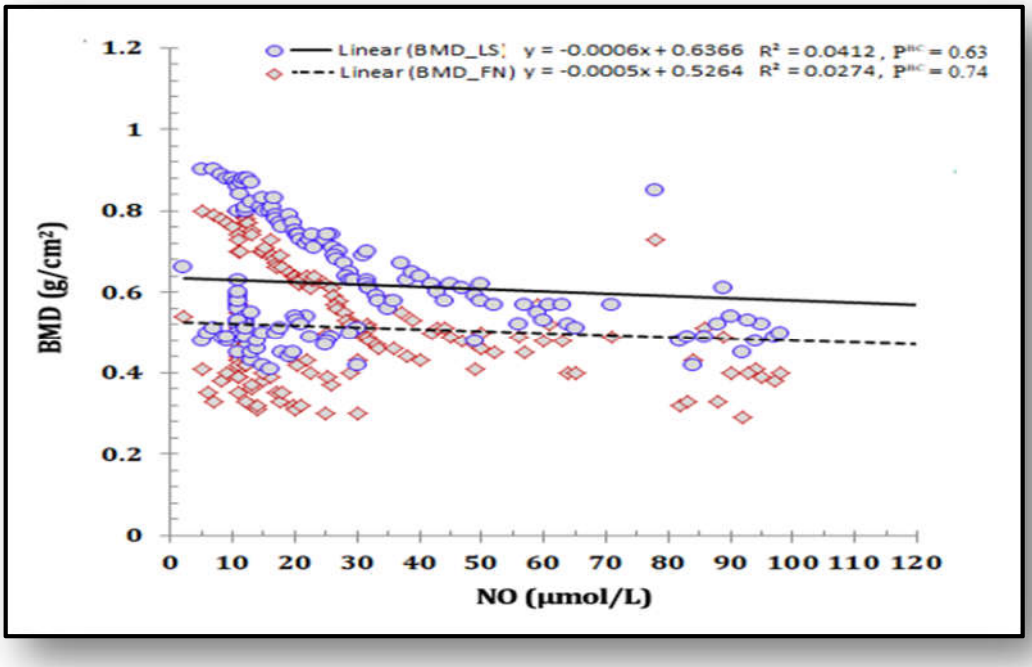

Figure 2. Linear regression analysis between bone mineral density (BMD_LS and BMD_FN) and nitric oxide (NO). P values are corrected with Bonferroni (P^{BC}) for correlation coefficients (r^2). BMD_LS: bone mineral density at lumbar spine, BMD_FN: bone mineral density at femoral neck, NO: Nitric oxide

5. Discussion

Present study has exposed the role and relevance of two important biochemical parameters i.e. CRP and NO for the risk of osteoporosis in postmenopausal women of Punjab. This is the first study from India, which has revealed that higher CRP levels are significantly associated with low bone mass, whereas no association of NO levels and osteoporosis is evident. Several studies have already highlighted that process of bone resorption is mediated by higher levels of CRP induced inflammation [11-14]. It has also been observed that those women who have higher levels of CRP (>3mg/L) experience higher bone loss than those women who are having lesser CRP levels (≤3mg/L) [12]. Moreover, higher CRP levels are associated with increased risk of fractures and higher mortality risk [12, 14]. Furthermore, several studies have confirmed that higher CRP levels in osteoporotic subjects make them vulnerable for other affiliated diseases such as type II diabetes mellitus, ankylosing spondylitis, atherosclerosis, chronic obstructive pulmonary disease, chronic pancreatitis and depression [15-20].

Present study has not found any association of nitric oxide levels with the risk of osteoporosis. Molecular studies have suggested that CRP interacts with NO to regulate the systemic inflammation in several diseases [21, 22]. Interestingly, higher CRP levels inhibit NO production by attenuating eNOS gene expression within endothelium [23]. Higher levels of NO are associated with down regulation of osteoblastic activity and increased osteoblast apoptosis [24]. Therefore, it is reasonable to say that these two peers of inflammation have opposite consequences.

Apropos to these discussed studies, present study has revealed that higher CRP levels are significantly associated with the risk of osteoporosis whereas, NO does not participate in it.

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7. Disclosure Statement

No potential conflict of interest was reported by the authors.

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