HIGH SERUM CONCENTRATION OF INTERLEUKINE-6 AND RANK-LIGAND AS RISK FACTORS FOR OSTEOPOROSIS IN ESTROGEN DEFICIENCY POST-MENOPAUSAL WOMEN

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ABSTRACT

Osteoporosis in post-menopausal women is not merely due to deficient estrogen hormone production. The development of osteoporosis is due to increased bone resorption by osteoclasts. The osteoclast's number and activity is controlled by activating factors such as IL-6 and RANK-L. The objective of this study was to determine that high IL-6 and RANK-L serum concentrations are risks for osteoporosis in estrogen deficient post-menopausal women. The serum concentration of ß-CrossLaps (CTx) was measured to determine bone resorption rate. This is an observational analytical study using case and control design conducted at Sanglah General Hospital of Denpasar. The sample size was calculated using the paired case-control study formula. There were 41 osteoporotic and 41 non-osteoporotic (control) estrogen deficient post-menopausal women involved in the study.

Data were analyzed by using the t-paired and McNemar tests. Mean serum concentration of IL-6 among the osteoporotic women was significantly higher as compared to that of the controls (3.47±1.75 pg/ml vs 2.51±1.13 pg/ml, p = 0.001). Mean serum concentration of RANK-L among the osteoporotic women was also significantly higher as compared to that of the controls (320.66±122.44ng/ml vs 249.94±82.41 ng/ml, p = 0.002). To qualify as risk factors for osteoporosis, the cut-off point for IL-6 was 2.17 pg/ml (OR = 4, CI 95%: 1.23-14.24; p = 0.032); the cut-off point for RANK-L was 275.165 ng/ml (OR = 8, CI 95%: 1.84-34.79; p = 0.001). Analysis of both high serum concentration of IL-6 and RANK-L was associated with an odd ratio of 9 (CI 95%: 4,27-18,96, p=0,000). CTx concentration in the osteoporotic women was significantly higher than in the controls (0.60±0.22ng/ml vs 0.46±0.16ng/ml, p = 0.004).

We found that the high IL-6 and RANK-L serum concentrations were risk factors in estrogen deficient post-menopausal women. CTx being a marker for osteoclastic bone resorption activity, increased in concentration higher in osteoporotic than in non-osteoporotic women. The high serum concentrations of IL-6 and RANK-L could be used as predictors for osteoporosis in estrogen deficient post-menopausal women.

Keywords: Post-menopausal osteoporosis, estrogen, osteoclast activity, ß-Cross, (CTx), Interleukine-6, RANK-Ligand

INTRODUCTION

Osteoporosis is a major health problem second only to cardiovascular disease (WHO, 1994). The prevalence of osteoporosis based on the study of Djoko Roeshadi (1997) by measuring bone mineral density is 26%. Osteoporosis in post-menopausal women is thought to be due to estrogen deficiency. However many estrogen deficient post-meno pausal women are not afflicted with osteoporosis. Wardiana (2004) found that 78,9% post-menopausal women had low estradiol concentration (< 40 pg/ml) in whom only 25% were associated with increased bone resorption rate leading up to osteoporosis.

Osteoporosis is due to the osteoclasts being more active than osteoblasts (Manolagas, 2000). IL-6 dan RANK-L are mediators which stimulate the differentiation process from osteoclast precursor cell to become active osteoclast which resorps bone as shown by the increase of CTx serum concentration and then ends up in osteoporosis. (Baylink, 1999; Jilka, 2001; Aubin & Bonnelye 2002; Jones *et al*, 2002; Manolagas *et al*, 2002).

Until recently, it is not yet known whether IL-6, RANK-L and CTx serum concentrations are higher in estrogen deficient post-menopausal women asso ciated with osteoporosis.

Study Design

This study is an observational analytical study using case and control design with estrogen deficient post-menopausal women with osteoporosis as the case and without osteoporosis as the control group.

RESULTS

IL-6, RANK-Ligand, and CTx concentrations

The difference in IL-6, RANK-Ligand and CTx concentrations between the case and control was analyzed using the paired–test with significant level $\alpha = 0.05$. Data analysis showed that IL-6, RANK-Ligand and CTx concentrations were higher in the case as compared to those in the control. They were statistically significant as the p value from mean difference from those three variables were less than the α value ($p < 0.05$, Tabel 1).

Table 1 Mean difference between IL-6, RANK-Ligand, and CTx concentrations between case and control

Independent variable	Mean and SD		T statistic	P value
	Case	Control		
IL-6 (pg/ml)	3.47±1.75	2.51±1.13	3.558	.001
RANK-Ligand (ng/ml)	320.66±122.44	249.94±82.41	3.364	.002
CTx (ng/ml)	0.60±0.22	0.46±0.16	3.098	.004

Osteoporosis Risk Factors
Bivariate Analysis (McNemar)

IL-6 and Osteoporosis

Table 2 Case-control cross table according to IL-6 concentration

Case	Control		Total
	> 2.17	< 2.17	
> 2.17	18	12	30
< 2.17	3	8	11
Total	21	20	41

OR = 4 (CI 95%: 1.23-14.24) and p = 0.032

Using 2.17 as cut-off point for IL-6 concentration, we found the OR between estrogen deficient post-menopausal women with IL-6 concentration same as or above 2.17 and below 2.17 was 4 (CI 95%: 1.23-14.24). Statistically the OR was significant with $p < 0.05$ ($p = 0.032$) using McNemar test (Table 2).

RANK-L and Osteoporosis

From association analysis between RANK-L concentration and osteoporosis in the estrogen deficient post-menopausal women using cut off point 275.165, we found the OR = 8 (CI 95%: 1.84–34.79) and $p < 0.05$ ($p = 0.001$, Table 3).

Table 3 Case-control cross table according to RANK-L concentration

RANK-L concentration (Case)	RANK-L concentration (Control)		Total
	> 275,165	< 275,165	
> 275,165	12	16	28
< 275,165	2	11	13
Total	14	27	41

OR = 8 (CI 95%: 1.84–34.79) and p= 0.001

Both high IL-6 and RANK-L toward Osteoporosis

From association analysis, both high IL-6 and RANK-L concentrations gave OR as high as 9 (CI 95%: 4.27-18.96, p=0,000) for osteoporosis in estrogen deficient post-menopausal women (Table 4).

Table 4 Case and control cross table for both high IL-6 and RANK-L concentrations

IL-6 and RANK-L concentrations in Case		IL-6 and RANK-L concentrations in Control		Total
		IL-6 and RANK-L increase	Both not increase	
Case	IL-6 and RANK-L increase	4	18	22
	Both not increase	2	17	19
Total		6	35	41

OR = 9 (CI 95%: 4.27–18.96) and p= 0,000

DISCUSSION

High IL-6 serum concentration as risk factor for osteoporosis

From this study we found the mean concentrations and standard deviation for case and control were respectively 3.47±1.75pg/ml and 2.51±1.13pg/ml. Assessed by using the t-paired test with significant level $\alpha = 0.05$, we found that those differences were statistically significant with p value of less than $\alpha = 0.05$ ($p = 0.001$). For risk factor, analysis using McNemar test was conducted and using 2.17 as cut-off point for IL-6 concentration, we found the OR between IL-6 concentration equal to or above 2.17 and below 2.17 was 4 (CI 95%: 1.23-14.24). Statistically the OR was significant with $p < 0.05$ ($p = 0.03$, Table 2).

Interleukine-6 in spite of its roles in inflammatory and immunologic process, also plays a part in bone meta bolism through osteoclastogenesis induction and stimulate osteoclast activity (Keller, 1996). Interleukine-6 increases the formation of osteoclast especially when there is decreased estrogen hormone concentration (Roitt *et al,* 1998; Pfeilschifter *et.al,* 2002). From these studies it was thought that IL-6 was one among many cytokines which had important role in bone resorption through the increased osteoclast formation and activity.

Maggio *et al,* (2006) conducted study in elderly males on the association

between testosterone hormone concen tration with IL-6 concentration. They found significant association: the older the person and the lower the testosterone level the higher the IL-6 concentration and lesser bone mass. It showed that systemic inflammatory sign especially CRP (C-reactive protein) and IL-6 could predict bone mass loss (Ding *et al*, 2008). HIV infection was also related to bone mass loss in the spine and hip and an increase of bone turnover due to increased pro-inflammatory factor (such as IL-6) (Dolan, *et al,* 2006).

So from this study we inferred that in estrogen deficient post-menopausal women, high IL-6 was considered as risk factors for osteo porosis. We found that estrogen deficient post-menopausal women with IL-6 concentration ≥ 2.17 pg/ml (cut off point) had OR as high as 4 (CI 95%: 1.23–14.24, p = 0.03), meaning that their risk to develop osteoporosis was 4 times higher than the those with < 2.17 pg/ml.

High RANK-L serum concentration as risk factor for osteoporosis

We found that RANK-L mean concentration in the osteoporotic women was higher than in the controls: 320.66±122.44 ng/ml vs 249±82.41 ng/ml. Analysis using the t-paired test with level of significance α = 0.05, revealed that the mean difference was significant (p = 0,002, Table 1). To determine that RANK-L is a risk factor for osteoporosis in estrogen deficient post-menopausal women, the association between RANK-L concentration and osteoporosis data were analyzed using the McNemar test.and RANK-L concentration as high as 275.165ng/ml used as the cut off point. We found OR as high as 8 (CI 95%: 1.84-34.79) with p = 0.001 (p< 0.05) which was statistically significant (Table 3).

Osteoporosis develops due to increased osteoclastic bone resorption. Osteoclast is a member of monocyte or macrophage family and its different tiation and activation depend on essential cytokines such as: RANK-L and M-CSF (Teitelbaum, 2007). Buxton *et al.* (2004) found increase of RANK-L and IL-6 concentrations and decrease of Osteoprotegerin (OPG) level on 51 post-menopausal women who had received PTH (parathyroid hormone) for 12 months. RANK-L was more stable and consistent as osteoclast differentiation and activation factors and produces more risk for osteoporosis. Even Nakchbandi, *et al.* (2008) found that certain level of RANK-L serum concentration can be used as parameter to determine bone loss in primary hyperparathyroid patients.

In our study we found that RANK-L serum concentration was higher in the estrogen deficient post-menopausal women with osteoporosis as compared to than in the controls, thus RANK-L was a risk factor for osteoporosis. Subjects with RANK-L concentration ≥ 275.165 ng/ml (cut off point) had OR as high as 8 (CI 95%: 1.84-34.79, p=0.001), meaning that the subjects had risk for osteoporosis 8 times higher than those with less than 275.165 ng/ml.

IL-6 and RANK-L combined as risk factors for osteoporosis

To determine that both IL-6 and RANK-L high concentrations are risk factors for osteoporosis in estrogen deficient post-menopausal women, the association between both IL-6 and RANK-L high

concentration and osteoporosis was analyzed using McNemar test using the IL-6 and RANK-L cut off points. We found the OR as high as 9 (CI 95%: 4.27-18.96, p=0.000) which was statistically significant (Table 4).

The results imply that estrogen deficient post-menopausal women with IL-6 and RANK-L concentrations above the cut off point had increased risk for osteoporosis up to 9 times as compared to those with level below the cut off point. This is due to the synergic nature of cytokines (Karnen, 2000; Siti, 2001). So in this study we found that IL-6 and RANK-L had synergic effect to influence the activity of the osteoclasts.

CTx concentration is higher in estrogen deficient post-menopausal women with osteoporosis compared to those without osteoporosis

We measured the levels of CTx as indicator of bone resorption rate and compared the levels in the estrogen deficient post-menopausal women with osteoporosis and in the controls; and we found that CTx concentration in the osteoporotic women was higher than in the control, 0.60±0.22 ng/ml vs 0.46±0.16 ng/ml. Analysis using the t-paired test with level of significance $\alpha = 0.05$, reveled that the mean difference for CTx between the case and the control was statistically significant ($p = 0.004$, Table 1). This implies that bone resorption rate due to osteoclast activity was higher in estrogen deficient post-menopausal women with osteoporosis as compared to those without osteoporosis.

In fact Riggs & Khosla (1995) has already suggested that in measuring women with high risk for osteoporosis, the most important thing is to determine the increase of bone resorption rate. CTx, a byproduct of bone collagen type I, is a good indicator to determine bone resorption rate. (Kaniawati & Moeliandari, 2003; Roche Diagnostics, 2003b). CTx as bone resorption marker was shown by decreasing osteoclast activity. Giving anti-resorption drug such as Zoledronic acid to osteoporotic post-menopausal women for 1 month, they found a decrease in CTx serum concentration as compared to those with placebo (Reid *et al,* 2002). It implies that CTx as byproduct of bone resorption was not produced in that study.

From all studies cited above, we may infer that CTx serum concentration is a good indicator for bone resorption rate. Our study revealed that CTx in estrogen deficient post-menopausal women with osteoporosis was significantly higher in the case than in the control. This implies that osteoclast induced resorption of was more active in the osteoporotic women as compared to the control.

Novelty of the Study

The developmental mechanism of osteoporosis in estrogen deficient post-menopausal women was shown to be closely associated with increase of IL-6 and RANK-L serum concentrations. This implies that high serum concentrations of IL-6 and RANK-L will stimulate osteoclast differentiation and activation. The osteoclast's number and activity lead to increased bone resorption thus increasing the risk for osteoporosis. CTx as bone collagen type I derivation which serves as bone resorption indicator can be detected in the serum.

Therefore we infer that high serum concentrations of IL-6 and

RANK-L are risk factors for osteoporosis in estrogen deficient post-menopausal women

CONCLUSIONS

IL-6 and RANK-L concentra tions were significantly higher in osteoporotic estrogen-deficient post-menopausal women as compared to those without. osteoporosis.

Being a marker for osteoclastic bone resorption activity, CTx concen tration was shown to be higher in osteoporosis.

The developmental mechanism of osteoporosis in estrogen deficient post-menopausal women appears to be closely related to increased IL-6 and RANK-L serum concentrations.

IL-6 and RANK-L could be used as predictors to increase our awa reness for the possible occurrence of osteoporosis in post-menopausal women.

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SUGGESTIONS

It is suggested that high IL-6 and RANK-L serum concentrations are risk factors for osteoporosis which might have immediate clinical implications; this implies that changing the factors might have some impact on the develop pment of osteoporosis..

To qualify increased IL-6 and RANK-L as risks for osteoporosis in estrogen deficient post-menopausal women, a study with a larger sample is required.

Morrow and de Lennox (2007) have set out three criteria a biomarker should fulfill to be useful clinically: (1) accurate, repeated measurements must be available to the clinician at a reasonable cost and with short turnaround times, (2) biomarker must provide information that is not already available from a careful clinical assessment, and (3) knowing the measured level should aid in medical decision making.

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