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The Effects of Alendronate and Calcitonin on Cytokines in Postmenopausal Osteoporosis: A 6-Month Randomized and Controlled Study

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The present study was designed to determine if levels of serum cytokines, such as interleukin (IL)-1β, IL-2, IL-2r, IL-6, IL-6r, IL-8, IL-10, and TNF-α are different in osteoporotic and non-osteoporotic postmenopausal women, and to evaluate the effects of calcitonin and alendronate therapies over a six month period on serum cytokine levels in postmenopausal osteoporotic women.

Serum levels of IL-2, TNF-α and IL-8 were found to be significantly higher ($p<0.05$), and serum IL-10, and IL-6r significantly lower in the calcitonin (N=60) and the alendronate (N=60) treatment groups than in the control group (N=50) ($p<0.05$). But, no significant difference was apparent between the calcitonin and alendronate treated groups before treatment. Statistically significant changes occurred in patients, with respect to the levels of serum IL-6r, and IL-8 after one month ($p<0.05$), in IL-2r, IL-6r, IL-8, IL-10 after three months, and in IL-1β, IL-6r, IL-8, IL-10 and TNF-α after six months of calcitonin therapy ($p<0.05$). No significant difference was observed in IL-6r after one month, in IL-8 and IL-10 after three months, and in TNF-α after six months in the calcitonin treated group and in the control group, whereas these parameters were significantly different at baseline. In the alendronate treated group, statistically significant changes occurred in the levels of serum IL-1α and IL-6 after three months, and in IL-1β, IL-6, IL-6r and TNF-α after six months ($p<0.05$). No significant difference was observed in IL-6r after one month, in IL-10 after three months or in TNF-α after six months between the alendronate treatment group and the control group, whereas these parameters were significantly different at baseline.

In conclusion, we suggest that; 1) not only IL-1, IL-6, TNF-α and IL-11 but also IL-2, IL-8 and IL-10 may have roles in the etiopathogenesis of osteoporosis, 2) calcitonin therapy have a more distinct influence on serum levels of some cytokines and have an earlier effect than alendronate therapy (especially upon IL-2r, IL-8, and IL-10). Nevertheless, further longitudinal studies are needed to identify the cytokines involved in the pathogenesis of postmenopausal osteoporosis and to evaluate the influence of different treatments on these cytokines.

Key Words: Alendronate, calcitonin, calcium, cytokines, and postmenopausal osteoporosis

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INTRODUCTION

Osteoporosis is a recognized major cause of morbidity in older people, and it is known that a large number of risk factors are associated with the development of osteoporosis. Moreover, these risk factors eventually must mediate their effects through modulation of bone remodelling. A variety of compounds, including hormones and nutrients, are known to modulate bone remodelling. In addition, to these well-characterized substances, the immune system plays a role in this process through the involvement of pro- inflammatory cytokines.[1] Much interest has been focused on the role of the immune system in bone remodelling, and in particular, on the potential influence of cytokines upon the autocrine and paracrine regulation of bone cell activity.[2-5] Cytokines produced in the microenvironment of the bone, such as interleukins 1 and 6 (IL-1 and 6), tumour necrosis factor (TNF), interferon-γ (IFN-γ), and granulocyte-macrophage colony-stimulating factor (GM-CSF) affect the bone-remodelling process by regulating cellular differentiation as well as by

regulating the activity of osteoblasts and osteoclasts.[2,5-10] Cytokines possess an important role in the regulation of bone resorption and formation during pathologic bone remodelling, and they also play a role during normal bone remodelling.[11]

The main consequence of increased cytokine production in the bone microenvironment is the expansion of the osteoclastic pool due to increased osteoclast formation and the elongation of their life-span.[12] In addition, enhanced cytokine production results in the increased activity of mature osteoclasts and in increased osteoblastic activity. The latter compensates, in part, for the consequences of increased bone formation upon bone mass. Cytokines exert their regulatory effects on bone turnover by stimulating both the secretory and proliferative activities of mature cells. However, they also condition the differentiation of immature cells, which leads to the emergence of new phenotypes that favour osteoclastogenesis.

Although, the number of cytokines increases day to day, the functions of the majority of cytokines are not fully understood. The studies of cytokines in pathogenesis of osteoporosis have been limited to IL-1, IL-6, TNF-α and IL-11.[13,14] In the present study, we studied cytokines more in detail and we tried to determine whether other cytokines, such as IL-2, IL-8 and IL-10, have any function in postmenopausal osteoporosis.[15]

Bisphosphonates and calcitonin are potent inhibitors of osteoclastic bone resorption, and are being successfully used in the treatment of postmenopausal osteoporosis. However, the mechanisms by which these medications exert their protective effects on bone are not clear, though it is generally accepted that bisphosphonates and calcitonin exert a direct inhibitory action on mature osteoclasts. A few longitudinal studies have reported upon the effects of calcitonin and alendronate therapies on cytokines in postmenopausal osteoporosis.

The present study was designed to determine if the levels of serum cytokines, such as IL-1β, IL-2, IL-2r, IL-6, IL-6r, IL-8, IL-10, and TNF-α are different in osteoporotic and non-osteoporotic postmenopausal women, and to evaluate the influences of calcitonin and alendronate treatments over a period of six months on serum cytokine levels in postmenopausal osteoporotic women. The present work is the first of its kind to study the serum levels of IL-2, IL-8, and IL-10 in postmenopausal osteoporosis and to investigate the effects of calcitonin and alendronate therapies on these cytokines.

MATERIALS AND METHODS

Serum IL-1β, IL-2, IL-2r, IL-6, IL-6r, IL-8, IL-10, TNF-α, BGP, alkaline phosphatase, creatine kinase, calcium and phosphorus, urine calcium and phosphorus levels were measured in 120 postmenopausal osteoporotic and 50 postmenopausal healthy women. All procedures were approved by the Human Studies Research Committee of the University of Dicle, Diyarbakır. All subjects were mobile and gave informed consent prior to entry.

All of the 120 patients in the study were postmenopausal osteoporotic women and were selected from the Department of Physical Therapy and Rehabilitation of Dicle University Hospital. The patients' ages ranged from 52 to 69 years (mean age: 60,15 ± 8, 64), and the controls' from 51 to 67 years (mean age: 58,76 ± 6, 12); the mean ages of these two groups were not significantly different.

Patients were required to have a bone mineral density (BMD) of 2 SD or more below the young adult mean at either the posteroanterior lumbar spine or at the femoral neck. Subjects were eligible for our study if they were age 50 years or older, with at least five years of menopause, and in good general health as determined by medical history and a routine clinical blood analysis (complete blood count and differential count). Subjects were excluded if they had:- 1) used any drug, or had any disease or condition known to affect bone or cytokine metabolism; 2) had taken corticosteroid medications during the previous 6 months; 3) had a history of chronic renal, hepatic, or gastrointestinal disease or traumatic lumbar compression fracture; or 4) evidence of collapsed or focal vertebral sclerosis. Exclusion criteria included other bone diseases, rheumatoid arthritis, disorders of calcium metabolism, malignancy, previous treatment with fluoride, recent treatment with specific therapy for osteoporosis other than calci-

um, active drug or alcohol abuse, uncontrolled hypertension or heart failure, significant rhinitis or sinusitis, recurrent renal calculi.

A total of 183 patients were evaluated for inclusion. BMD testing was performed on 153 patients, 120 met all of the eligibility criteria and were included in the study. The patients were then randomly assigned to either the calcitonin or the alendronate therapy groups.

We measured BMD, with postero-anterior projection, using standard dual energy X-ray absorptiometry (DEXA) (Hologic QDR model 1000, Hologic, Waltham, MA, USA). The variation coefficient of BMD for consecutive determinations on spine and femur images in our laboratory was 1.8% at lumbar spine and 1.5% at the femur. All spinal scans were reviewed for evidence of vertebrae with collapsed or focal sclerosis by an experienced radiologist.

Blood samples were obtained after an overnight fast; precautions were taken to avoid contamination. Freshly drawn blood (15 ml) samples were obtained and immediately centrifuged at 200xg (20 min at 24℃), and serum samples were stored at -75℃ until analysis at the end of the study. Serum levels of cytokines were determined using IMMULITE diagnostic kits (DPC-Diagnostic Products Corporation, USA). This diagnostic kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of human cytokines in serum. Serum and urinary chemical estimations were performed using Beckman-Synchron CX-5 technology.

All parameters were measured before therapy and again after 1, 3, and 6 months in all groups. 60 of the postmenopausal osteoporosis 120 patients were given 200 IU/day of intranasal salmon calcitonin and 60 patients were given 10-mg/day oral alendronate. All subjects received concurrent treatment with 1000 mg of oral elemental calcium daily, in the form of calcium lactate gluconate and calcium carbonate. Fifty postmenopausal healthy women were given only 1000 mg of oral elemental calcium daily.

Statistical analyses

Statistical analyses were carried out using SPSS 8.0. Results were expressed as means ± SD (standard deviation). Data were analysed for significance using One-way ANOVA and the post-hoc test for inter group comparisons. The effect of therapies on biochemical and urinary parameters over 6 months of treatment (at 1, 3, and 6 months) were evaluated using the paired-t test by comparing results at each time with the baseline (before treatment). Measurements at different times were considered to be independent comparisons between groups because there were sequential in nature. One-way analysis of variance (ANOVA) was used initially, and if any significance was found, the post hoc test was used. p values of < 0.05 were accepted as statistically significant.

RESULTS

Profiles of the demographic variables of the three groups are presented in Table 1. Baseline characteristics of the three study groups were found to be similar by ANOVA ($p > 0.05$). Serum levels of IL-2, TNF-α and IL-8 were significantly higher ($p < 0.05$), whereas levels of serum IL-10, IL-6r were significantly lower in the calcitonin and alendronate groups than in the control group ($p < 0.05$), and no significant difference was found between the calcitonin and the alendronate group (Table 2).

A statistically significant improvement occurred, in the levels of serum IL-6r, and IL-8 after one month ($p < 0.05$), in IL-2r, IL-6r, IL-8, and IL-10 after three months, and in IL-1β, IL-6r, IL-8, IL-10, and TNF-α after six months in patients receiving calcitonin therapy ($p < 0.05$). No significant difference was found in IL-6r after one month, in IL-8 or IL-10 after three months, or in TNF-α after six months between the calcitonin and control groups, whereas these parameters were significantly different from the baseline values (Table 3).

In the alendronate group, a statistically significant improvement was observed in the levels of serum IL-1β and IL-6 after three months, and in IL-1β, IL-6, IL-6r, and TNF-α after six months ($p < 0.05$), but no significant difference in these parameters was observed after one month. In addition, no significant difference was observed

Table 1. Baseline Characteristics of All Groups

Variable	Control (n=50)	Alendronate (n=60)	Calcitonin (n=60)
Age (years)	58.76 ± 6.31	60.55 ± 6.09	59.88 ± 7.12
BMI (kg/m^2)	25.64 ± 3.96	26.50 ± 4.11	25.26 ± 4.28
Age at menarche (years)	13.49 ± 1.16	13.25 ± 0.83	13.48 ± 1.07
Age at menopause (years)	45.08 ± 3.30	44.55 ± 4.98	44.31 ± 3.98
No. of fertile years	30.86 ± 3.29	31.33 ± 5.15	30.92 ± 4.06
Years since menopause	12.28 ± 6.89	13.56 ± 6.21	13.32 ± 6.49
Smoking (pack-years)*	22.33 ± 16.27 (15)	24.65 ± 17.83 (18)	24.36 ± 16.67 (17)
Alcohol intake (g/day)*	8.25 ± 6.21 (7)	9.13 ± 5.46 (10)	8.63 ± 6.37 (8)

*Second value in parentheses is number of women who consumed alcohol or smoked cigarette at any previous time or currently.
All values represent means ± standard deviation.
No significant differences were found between the groups by ANOVA.

IL-6r: interleukin-6 receptor; IL-8: interleukin- 8; IL-10: interleukin-10; TNF-α: Tumour necrosis factor-alpha)

Table 2. Comparisons of the Urinary and Biochemical Parameters at Baseline of the Three Groups

Variable	Reference Ranges	Control	Alendronate	Calcitonin
ALP (IU/L)	36 - 92	99.42 ± 25.29	94.86 ± 40.53	86.78 ± 18.46
sCa (mg/dl)	8.6 - 10.4	9.37 ± 0.59	9.56 ± 0.80	9.03 ± 1.50
sP (mg/dl)	2.4 - 4.7	3.27 ± 0.52	3.48 ± 0.63	3.31 ± 0.57
uCa (mg/24h)	100 - 300	150.06 ± 76.45	117.69 ± 79.55	135.67 ± 73.51
uP (g/24h)	0.4 - 1.3	1.11 ± 0.69	1.08 ± 0.76	1.09 ± 0.67
BGP (pg/ml)	13.0 - 19.5	15.05 ± 7.31	22.33 ± 13.47[a]	19.56 ± 9.15[b]
IL-1β (pg/ml)	0 - 5	5.03 ± 0.12	5.10 ± 0.47	5.07 ± 0.27
IL-2 (pg/ml)	0 - 14.5	14.48 ± 2.05	16.11 ± 2.63[a]	16.28 ± 4.56[b]
IL-2r (ng/ml)	223 - 710	498.20 ± 172.90	517.10 ± 178.20	551.90 ± 144.40
IL-6 (pg/ml)	0 - 5.4	6.23 ± 3.55	5.71 ± 3.41	5.61 ± 2.80
IL-6r (ng/ml)	6.59 - 30.7	26.07 ± 9.51	22.21 ± 8.95[a]	21.29 ± 7.71[b]
IL-8 (pg/ml)	0 - 62	16.90 ± 16.69	38.36 ± 57.10[a]	50.65 ± 71.41[b]
IL-10 (pg/ml)	0 - 14.1	5.27 ± 1.70	4.21 ± 1.07[a]	4.05 ± 1.75[b]
TNF-α (pg/ml)	4 - 8.1	12.04 ± 8.99	15.84 ± 12.72[a]	15.55 ± 10.08[b]

Values are shown as means ± standard deviation (SD) for all variables.
No significant differences were found between the values of the alendronate and the calcitonin groups.
[a]Alendronate and bcalcitonin groups are significantly different from the control group ($p < 0.05$).
(ALP: alkaline phosphatase; CPK: creatine phosphokinase; sCa: serum calcium; sP: serum phosphorus; uCa: urinary calcium; uP: urinary phosphorus; BGP: osteocalcin; IL-1β: interleukin-1 beta; IL-2: interleukin-2; IL-2r: interleukin-2 receptor; IL-6: interleukin-6; IL-6r: interleukin-6 receptor; IL-8: interleukin- 8; IL-10: interleukin-10; TNF-α: Tumour necrosis factor-alpha)

in; IL-6r after one month, in IL-10 after three months or in TNF-α after six months between the alendronate group and the control group, whereas these parameters were significantly different at baseline (Table 4). In addition, no significant difference was observed in any of these parameters in the control group, which received calcium alone (Table 3 and 4).

Changes in the serum levels of IL-1β, IL-6, IL-6r, IL-8, IL-10, and TNF-α of all groups during the treatment period are presented in Fig. 1-6.

No side-effects that were serious enough to discontinue medication were seen in any of the patients during 6 months of follow-up period.

DISCUSSION

Postmenopausal osteoporosis is a progressive disorder characterized by a decreased bone mass and an increased susceptibility to fractures. The estrogen deficiency that occurs after a natural or surgically induced menopause leads to a uncoupling of the activities of bone resorbing cells and the bone forming cells, and this is responsible for accelerated bone loss. Although it is well established that estrogen deficiency plays a causal role in this condition, our understanding of the mechanism by which oestrogen prevents bone loss is incomplete. Recent evidence suggest that estrogen may modulate the secretion of factors that are produced in the bone microenvironment, which in turn influence bone remodelling.[16,17]

The involvement of both osteoclast and osteoblast precursors in the response to estradiol points to a complex cell communication model.[16] These findings may have implications at the physiopathological and pharmacological levels. Indeed, it is not completely understood why some women lose bone rapidly after menopause,

Table 3. Changes in Urinary and Biochemical Parameters from Baseline and at the First, Third and Sixth Months of Treatment for the Control and the Calcitonin Groups

Variables	Control group				Calcitonin Treatment Group			
	Before Treatment	1st Month	3rd Month	6th Month	Before Treatment	1st Month	3rd Month	6th Month
ALP (IU/L)	99.42±25.29	95.36±23.32	96.48±56.25	98.37±36.41	86.78±18.46	87.81±19.15	89.96±22.43	95.83±29.49
sCa (mg/dl)	9.37±0.59	9.38±0.57	9.45±0.55	9.32±8.63	9.03±1.5024	8.71±1.11	8.96±33.89	9.19±17.61
sP (mg/dl)	3.27±0.52	3.32±0.44	3.30±0.49	3.33±0.51	3.31±0.57	3.31±0.62	3.45±33.87	3.16±67.54
uCa (mg/24h)	150.06±76.45	155.09±72.15	152.48±69.62	154.55±73.32	135.67±73.51	129.48±65.48	148.21±69.82	142.54±67.31
uP (g/24h)	1.11±0.69	1.13±0.56	1.10±0.74	1.09±0.65	1.09±0.67	1.0±0.73	1.11±0.69	1.09±0.58
BGP (pg/ml)	15.05±7.31	16.25±6.31	15.85±7.83	15.25±6.92	19.56±9.15	18.59±8.67*	14.74±9.50[b]	14.74±9.50[c]
IL-1β (pg/ml)	5.03±0.12	5.02±0.29	5.06±0.14	5.04±0.46	5.07±0.27	5.01±0.14	5.14±0.51	5.43±10.58[c]
IL-2 (pg/ml)	14.48±2.05	14.70±1.64	14.64±1.46	14.77±1.70	16.28±4.56	16.29±3.31*	16.62±2.97*	15.57±3.27
IL-2r (ng/ml)	498.20±172.90	499.6±162.9	493.2±159.3	498.3±155.8	551.9±144.4	512.5±164.5	404.7±184.3*[b]	497.5±190.9
IL-6 (pg/ml)	6.23±3.55	6.18±3.23	6.34±3.36	6.21±3.19	5.61±2.80	5.72±2.35	5.65±5.51	5.56±1.47
IL-6r (ng/ml)	26.07±9.51	25.47±9.02	26.18±9.03	25.89±8.62	21.29±7.71	25.31±9.55[a]	28.15±10.07[b]	27.05±8.71[c]
IL-8 (pg/ml)	16.90±16.69	17.09±15.12	17.29±14.35	16.88±15.32	50.65±21.41	30.44±14.99*[a]	22.53±17.31[b]	17.26±14.81[c]
IL-10 (pg/ml)	5.27±1.70	5.36±1.62	5.25±1.61	5.39±1.51	4.05±1.75	4.55±1.43*	5.16±1.38[b]	5.22±1.33[c]
TNF-α (pg/ml)	12.04±8.99	11.81±8.41	11.75±8.39	11.65±8.24	15.55±10.08	16.58±6.15*	16.99±12.29*	12.14±10.05[c]

*Follow-up values of calcitonin group are significantly different from the values of the control group in the corresponding month ($p<0.05$). [a]First month, [b]third month, [c]sixth month values of the calcitonin group are significantly different from the baseline values of the same group ($p<0.05$).

Table 4. Changes in Urinary and Biochemical Parameters from Baseline and at the First, Third and Sixth Months of Treatment for the Control and the Alendronate Groups

Variable	Control group				Alendronate Treatment Group			
	Before Treatment	1st Month	3rd Month	6th Month	Before Treatment	1st Month	3rd Month	6th Month
ALP (IU/L)	99.42±25.29	95.36±23.32	96.48±56.25	98.37±36.41	94.86±40.53	94.41±30.81	93.13±34.33	95.90±33.82
sCa (mg/dl)	9.37±0.59	9.38±0.57	9.45±0.55	9.32±8.63	9.56±0.80	9.57±0.94	9.15±1.45	9.35±0.65
sP (mg/dl)	3.27±0.52	3.32±0.44	3.30±0.49	3.33±0.51	3.48±0.63	3.43±1.14	3.34±0.44	3.15±0.41
uCa (mg/24h)	150.06±76.45	155.09±72.15	152.48±69.62	154.55±73.32	117.69±79.55	147.64±76.47	157.43±72.35	151.76±65.23
uP (g/24h)	1.11±0.69	1.13±0.56	1.10±0.74	1.09±0.65	1.08±0.76	1.10±0.83	1.09±0.76	1.12±0.72
BGP (pg/ml)	15.05±7.31	16.25±6.31	15.85±7.83	15.25±6.92	22.33±13.47	19.32±10.15*	13.12±8.02[b]	12.12±7.07[c]
IL-1β (pg/ml)	5.03±0.12	5.02±0.29	5.06±0.14	5.04±0.46	5.10±0.47	5.09±0.35	5.35±0.81[b]	5.90±1.42*[c]
IL-2 (pg/ml)	14.48±2.05	14.70±1.64	14.64±1.46	14.77±1.70	16.11±2.63	16.53±3.39*	16.27±4.96*	16.28±4.33*
IL-2r (ng/ml)	498.20±172.90	499.6±162.9	493.2±159.3	498.3±155.8	517.1±178.2	506.0±171.1	463.1±167.7	464.2±223.4
IL-6 (pg/ml)	6.23±3.55	6.18±3.23	6.34±3.36	6.21±3.19	5.71±3.41	5.59±1.44	5.31±0.82*[b]	5.22±4.84[c]
IL-6r (ng/ml)	26.07±9.51	25.47±9.02	26.18±9.03	25.89±8.62	22.21±8.95	23.92±8.13	24.81±10.16	28.70±9.76[c]
IL-8 (pg/ml)	16.90±16.69	17.09±15.12	17.29±14.35	16.88±15.32	38.36±57.10	41.21±16.68*	44.80±15.10*	48.15±15.84*
IL-10 (pg/ml)	5.27±1.70	5.36±1.62	5.25±1.61	5.39±1.51	4.21±1.07	4.37±1.33*	4.76±1.28	4.81±1.74
TNF-α (pg/ml)	12.04±8.99	11.81±8.41	11.75±8.39	11.65±8.24	15.84±12.72	16.29±12.56*	17.37±13.20*	12.39±9.94[c]

*Follow-up values of the alendronate group are significantly different from values of the control group in the corresponding month ($p<0.05$).
[a]First month, [b]third month, [c]sixth month values of the alendronate group are significantly different from the baseline values of the same group ($p<0.05$).

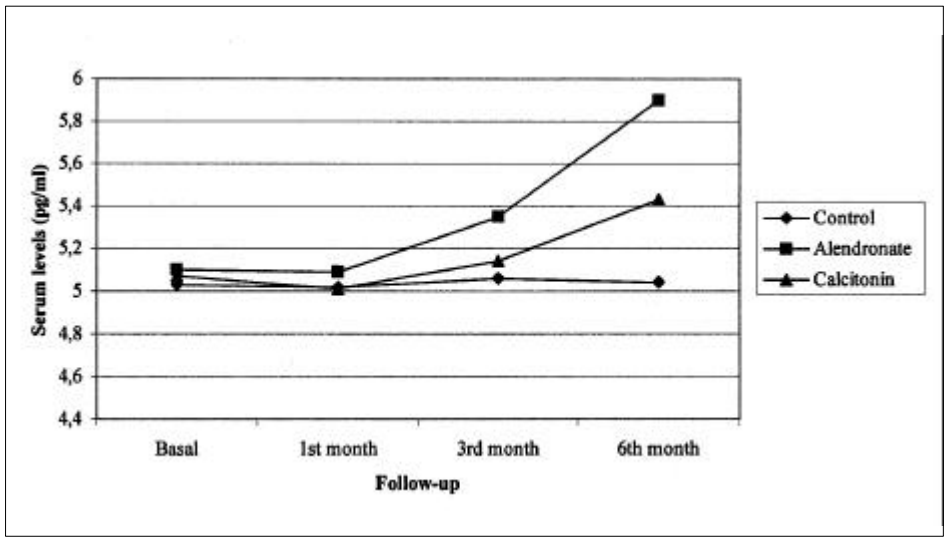

Fig. 1. Changes in the serum levels of IL-1β in all groups according to follow-up.

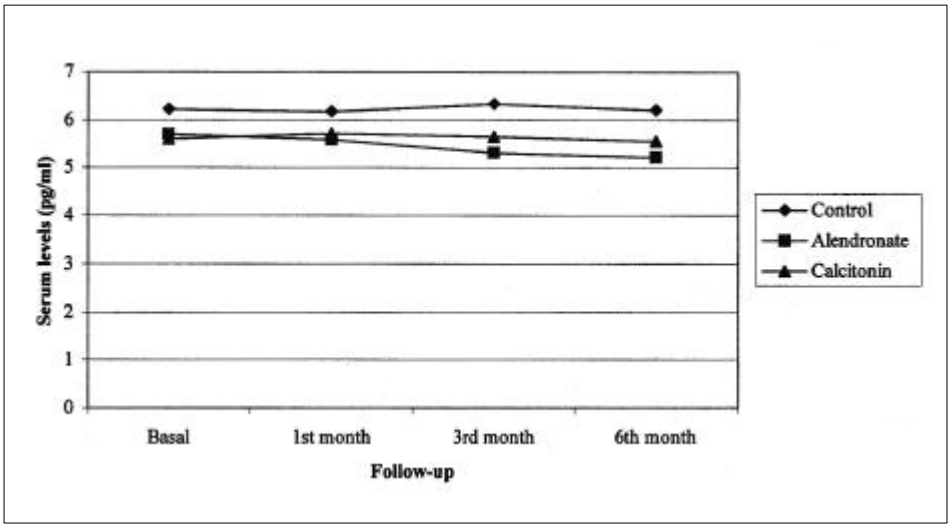

Fig. 2. Changes in the serum levels of IL-6 in all groups according to follow-up.

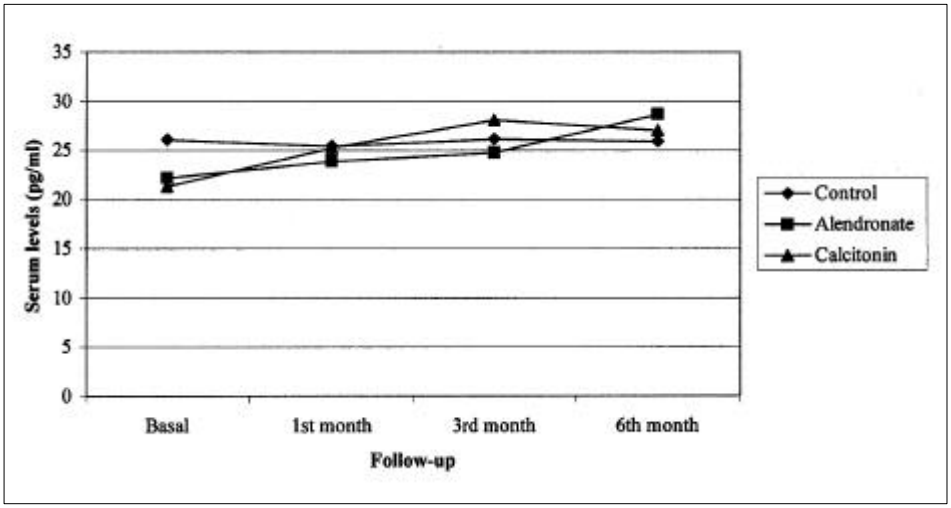

Fig. 3. Changes in the serum levels of IL-6r in all groups according to follow-up.

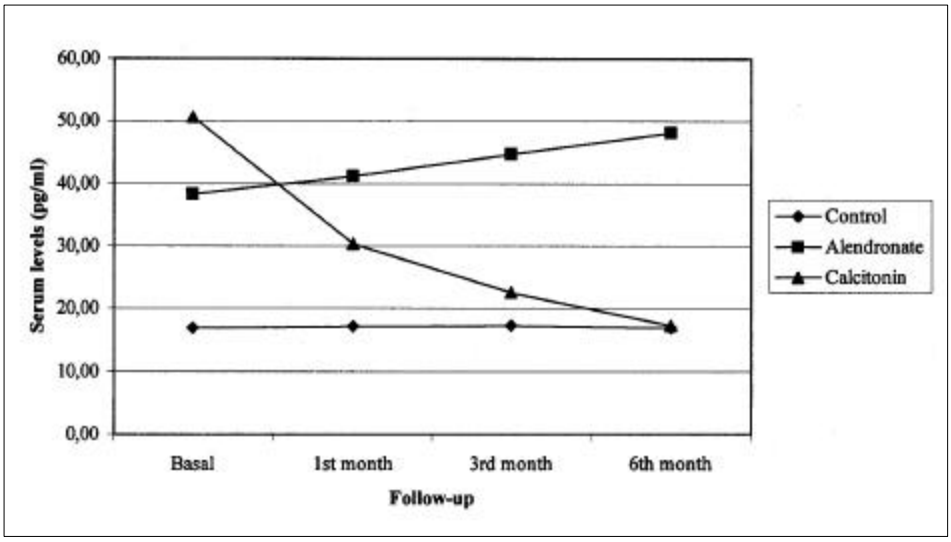

Fig. 4. Changes in the serum levels of IL-8 in all groups according to follow-up.

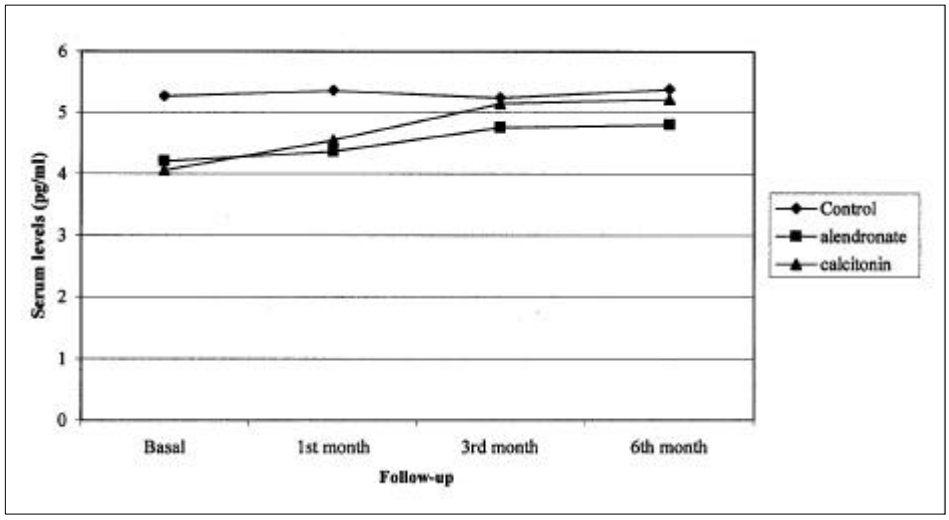

Fig. 5. Changes in the serum levels of IL-10 in all groups according to follow-up.

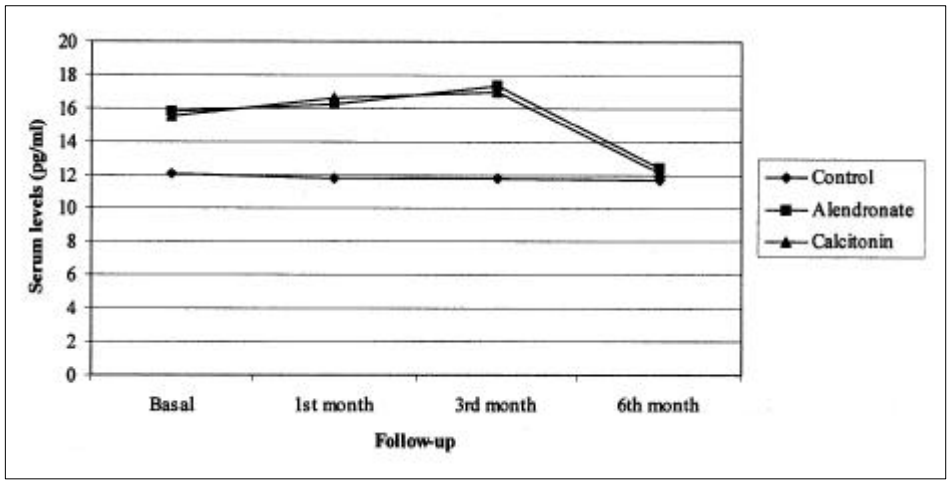

Fig. 6. Chunges in the serum levels of TNF-α in all groups according to follow-up.

whereas others show only slow bone loss. This difference could be dependent on the level of cytokine secretion.

It is currently believed that during the premenopausal years, estrogen in the bone marrow microenvironment regulate the expressions of certain cytokines, most notably IL-6. In the presence of estrogen, IL-6 expression is suppressed, but in the absence of estrogen, its level increases. Significantly, IL-6 is a potent activator of ostoclasts and bone resorption. Similarly, other cytokines, such as IL-1, IL-11 and TNF influence ostoclast function and the age associated dysregulation of these cytokines may also contribute to the development of osteoporotic bone disease.[1]

The roles of IL-1 and IL-6 as mediators of the deleterious effect of estrogen withdrawal on the skeleton have been frequently debated. Pacifici et al.[18] reported that circulating mononuclear cells, from subjects with high turnover osteoporosis and from early postmenopausal women, in whom bone remodelling is higher than normal, produce increased amounts of IL-1 and that oestrogen replacement therapy blocks this increased production of IL-1.[19] This article by Pacifici, suggests that changes in the bioactivities of cytokines may be significant even if interleukin levels do not show any significant differences versus controls. Recently, Girasole et al.[20] showed that IL-6 is produced by a murine clonal bone-derived stromal cell line, and that it can be considered as an osteoblast precursor. Chen et al.[21] investigated

151 healthy women whose menstruation status differed and found that levels of soluble IL-6 receptor were substantially higher in postmenopausal women than in premenopausal and perimenopausal women. Papudopoulos et al.[22] investigated IL-6 and the biochemical parameters of bone metabolism in postmenopausal women, but were unable to find any significant relationship between the biochemical parameters. Kimble[23] suggested that cytokine levels increase because of the lack of estrogen, which fulfils an important role in the development of postmenopausal osteoporosis. Bellido et al.[24] showed that IL-6 disregulation has an important role in the development of osteoporosis, and found that IL-6 disregulation increases osteoclastogenesis in ovariectomized mice, but can be inhibited by androgens or IL-6 neutralising antibody.

Although IL-1β and IL-6 may be involved in the bone remodelling process, we did not find any significant difference when we compared their serum levels in normal postmenopausal and postmenopausal osteoporotic patients. It should be borne in mind that several disputes exist among researches concerning cytokines and pathologic bone remodelling, especially concerning the secretion of cytokines into the peripheral blood. Some differences may be attributed to the different methods used, or perhaps they are related to different ages, essentially they reflect our need to learn more about the connections between bone remodelling, the immune system, and the ageing process.[11]

IL-2, IL-8, and IL-10 have not been previously investigated , except in the present study. In previous studies, interestingly, these interleukins were found to be quite different in control and postmenopausal osteoporotic groups. We conclude that IL-2, IL-8 and IL-10 must be studied in more detail to identify the role of these cytokines in the pathogenesis of osteoporosis.

IL-2 is present in nearly all rheumatoid arthritis fluids but absent in fluids from patients with osteoarthritis or reactive arthritis.[25,26] Sera of patients with rheumatoid arthritis have elevated levels of IL-2 and soluble IL-2rα, but this is rarely observed in nonrheumatoid arthritis, including psoriatic arthritis.[27] The occurrence of juxtaarticular osteoporosis in rheumatoid arthritis,[28-30] and lack of juxtaarticular osteoporosis in psoriatic arthritis,[30] reactive arthritis[26,27] and osteoarthritis,[30] and the undetectable levels of IL-2 in these pathologies, suggest that IL-2 may play a role in the pathogenesis of osteoporosis.

IL-8 is a chemokine of importance in inflammatory processes, and causes an increase in the levels of parathyroid hormone (PTH) mRNA. This suggest that IL-8 and inflammatory events may play a role in bone homeostasis by acting upon the parathyroid gland.[31]

When discovered, IL-10 was referred to as the cytokine synthesis inhibitory factor. IL-10 has been shown to suppress the levels of the inflammatory cytokines, IL-1α, IL-1β, TNF-α, IL-6, IL-8, and IL-2 and to inhibit the synthesis of nitric oxide, gelatinase, and collagenase.[32] Moreover, the specific neutralization of IL-10 resulted in an increase in the production of IL-1 and TNF-α.[33]

In the present study, serum levels of IL-2, IL-8 and TNF-α in patients were found to be higher than in the control group, while serum levels of IL-10 were found to be lower than in the control group. This situation may be interpreted in patients with postmenopausal osteoporosis, because of the insufficiency of serum IL-10. Because the organic matrix of the bone has a collagenous structure and IL-10 inhibits the collagenase synthesis. The insufficient inhibition of collagenase synthesis because of the low IL-10 level may influence osteoporosis development. In addition, several studies[34,35] have addressed the role of nitric oxide as a mediator of cytokine effects on bone cell activity *in vitro*. Therefore, the fact that nitric oxide production cannot be inhibited because of the low IL-10 levels also may influence osteoporosis development. In fact, all of these hypotheses are possible and further studies are needed before this issue is fully understood.

The data presented in this report indicate that calcitonin inhibits the production of IL-8 and TNF-α, and stimulates the production of IL-1β, IL-6r and IL-10, while alendronate inhibits the production of IL-6 and TNF-α, and stimulates the production of IL-1β and IL-6r. In addition, we found that calcitonin therapy has a more significant influence on the serum levels of cytokines and an earlier effect than alendronate therapy. The fact that calcitonin increases the IL-10 level

and that alendronate does not significantly change the IL-10 levels, may mean that the underlying effect of calcitonin on cytokines concerns its induction of IL-10 levels.

It is likely that the during the current decade the development of orally active, tissue selective cytokine inhibitors will lead to new strategies for the prevention and treatment of postmenopausal osteoporosis. Studies on these factors will help further our understanding of the pathophysiology of bone diseases, although it should be kept in mind that no specific bone cytokine has yet been found. This point is important when considering the possibility of future pharmacological intervention by administering these effectors to patients.

In conclusion, we claim that:- 1) not only IL-1, IL-6, TNF-α and IL-11 but also IL-2, IL-8 and IL-10 may have a role in the etiopathogenesis of osteoporosis, 2) calcitonin therapy has a more significant influence on the serum levels of certain cytokines and has an earlier effect on cytokine levels than alendronate therapy (especially upon IL-2r, IL-8, and IL-10 levels), and 3) further longitudinal studies are required to elucidate the roles of these cytokines in the pathogenesis of postmenopausal osteoporosis, and to evaluate the effects of different treatments on these cytokines.

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