Bone turnover markers and osteoprotegerin in uncomplicated pregnancy

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Abstract

Although calcium metabolism during pregnancy is well described the mechanisms involved in bone metabolism are not quite clear. Increase of osteoprotegerin (OPG) with elevated bone turnover is supposed to be a homeostatic mechanism limiting bone loss. The aim of the study was to assess bone turnover in pregnancy in relation to serum osteoprotegerin level. Osteocalcin (OC), beta-crosslaps (CTx), OPG, vitamin 25 OH D_3 , parathormone (PTH), and calcium (Ca) were determined in 30 healthy women at 1st and at 3rd trimester of pregnancy and 27 healthy age-matched non pregnant women.

In pregnant women average OPG, CTx and serum calcium concentrations were found to be highly elevated. During pregnancy OPG and bone resorption significantly rised whereas only slight increase in OC level was found with concomitant decrease in serum calcium. OPG correlated positively with OC and Ca only at 1st trimester. Serum CTx and OPG at 1st trimester seemed to be the only parameters to differentiate between elevated and normal bone turnover among pregnant women.

In pregnancy bone turnover increases mainly due to enhanced bone resorption. The determination of osteocalcin at the beginning of pregnancy seems to be of limited clinical use, whereas measuring bone resorption markers such as CTx and/or osteoprotegerin may have a good predictive value for later pregnancy-associated bone loss.

Key words: osteoprotegerin, bone turnover, pregnancy

Introduction

The attainment of peak bone mass in women typically takes place in the early 30s but pregnancy and lactation occur mostly during or before this period of life. It is considered that pregnancy could affect peak bone mass and increase the risk of developing osteoporosis later in life [1]. Bone loss during pregnancy may result in pregnancy-associated osteoporosis and vertebral fractures [2,3].

During pregnancy, about 30 g of calcium is transferred to a full term neonate [4]. Approximately 80% of calcium accumulates during the third trimester, when the fetal skeleton is rapidly mineralizing. Although maternal adaptations designed to meet the calcium needs of the fetus might begin early in pregnancy, they are most needed in the third trimester [5-6]. Calcium homeostasis appears to be attained by increased dietary intake with or without increased efficiency of absorption, decreased urinary excretion as a result of increased tubular calcium resorption and by elevated bone turnover with bone loss [7].

Several studies showed a decrease in BMD during pregnancy even up to 5%. Thus, there seems to be a good evidence that during pregnancy calcium is mobilized from the maternal skeleton to that of developing fetus. Development of biochemical markers enabled to asses bone turnover during normal pregnancy, when radiography or densitometry cannot be used [8-10].

The mechanisms regulating bone turnover during pregnancy are not well known [11]. RANK - a cellular receptor activator of NF-kappaB, RANK-ligand and osteoprotegerin (OPG) constitute a novel cytokine system that regulate activity of bone cells. Osteoprotegerin, is a soluble decoy receptor that inhibits bone resorption by binding to receptor activator of nuclear factor NF-kappaB ligand (RANKL) and in consequence inhibits osteoclast's maturation and activation [12]. RANKL produced by osteoblastic lineage cells and activated T lymphocytes is the essential factor for osteoclastogenesis, fusion, activation and survival of osteoclasts, thus effecting on bone resorption and bone loss. RANKL activates its specific receptor-RANK, located on osteoclasts and its signalling cascade involves stimulation of osteoclasts action. The effects of RANKL are counteracted by OPG which acts as a soluble neutralizing receptor. RANKL and OPG are regulated by various hormones (glucocorticoids, vitamin D, estrogens), cytokines (tumour necrosis factor alpha, interleukins 1,4,6,11 and 17) and various mesenchymal transcription factors. RANKL and OPG are also important regulators of vascular biology and calcification and of the development of a lactating mammary gland during pregnancy. OPG was also found in placenta [13]. All this indicates a crucial role for this system in extraskeletal calcium handling [14]. The discovery and

characterization of RANKL, RANK, OPG and subsequent studies have changed the concept of bone and calcium metabolism.

The objective of the study was to assess bone turnover in pregnancy by measuring biochemical bone markers in the serum in relation to osteoprotegerin level.

Participants and sample collection

Thirty healthy, pregnant women during their first visit for prenatal care participated in our study. Exclusion criteria included assisted conception or any diseases or use of medication known to affect bone metabolism. All pregnant women were primiparas of mean age 24.5±3.8 yrs (20-36 yrs) and body mass index (BMI) before pregnancy 20.3±2.8 kg/m2 (16.7-30.9). Five women were smokers, 23 were physically active. Only one woman fulfilled the daily requirement of calcium intake. Most of women fulfilled 50-75% of recommended daily calcium intake.

27 healthy, non pregnant women, before first pregnancy, (mean age, 25±3.4 yrs; range 21-33 yrs, mean BMI 20.9±2.9 ; range 17.6-29.8) served as controls. In this group 5 women were smokers, 15 were physically active. The average calcium intake was on the level of 50-75% of daily requirement.

The study protocol was approved by the local Bioethical Committee of Collegium Medicum, N.C. University in Bydgoszcz. All participants gave their informed written consent.

Materials and methods

Fasting blood samples from pregnant women were collected, between 8-9 am, at 1st trimester (6-14 wks) and at 3rd trimester (31-37 wks) of pregnancy. In control group fasting blood samples were taken once in autumn/winter season. Serum was immediately separated after blood clotting and kept deep frozen until assayed. Osteoprotegerin and vitamin 25 OH D_3 were assayed by ELISA (Biomedica, Austria). Reference value for OPG at age 20-36 yrs was 44.5 ± 21.2 pg/ml, reference range vitamin 25 OH D_3 in winter and summer were 14-42 ng/ml and 15-80 ng/ml, respectively. N-mid osteocalcin (OC), a bone formation marker and beta-Crosslaps (βCTx), a bone resorption marker were determined by ECLIA (Roche Diagnostics). Reference values for OC in premenopausal women were 4-35 ng/ml and for βCTx 0.299 ± 0.137 ng/ml. Intact PTH was assayed by ECLIA (Roche Diagnostics), expected values were 15-65 pg/ml. Serum calcium was measured by colorimetric method (Roche Diagnostics) and accepted reference values were 2.15-2.55 mmol/L.

Statistical analysis

Data were expressed as means (SD). Pearson correlation tests and cluster analysis (K-means) were performed. The data collected at 1st trimester and during 3rd trimester were compared by Wilcoxon test. P values equal to or less than 0.05 were considered statistically significant.

Results

The average concentrations of CTx, OPG and calcium were elevated in pregnant women comparing to expected reference values (Table 1). At 3rd trimester serum CTx and calcium levels were significantly higher than in age-adjusted non pregnant women ($p < 0,004$; $p < 0,001$ respectively). Mean OC values were only slightly increased during pregnancy and comparable with these in non-pregnant women. Serum vitamin D3 (1st trimester 71,0± 28,0; 3rd trimester 87,0±38,0 ng/ml) in pregnant women were found to be in the upper reference range whereas PTH (16,0± 8,0 and 19,0± 8,8 pg/ml; respectively) was in the lower. A strong relationship between both markers of bone turnover OC and CTx ($r=0,76$; $p < 0,00001$) and positive but weak correlations between OPG and OC ($r=0,54$; $p < 0,04$), OPG and Ca ($r=0,55$; $p < 0,03$) were found at 1st trimester.

Serum CTx and OPG significantly increased during pregnancy ($p < 0,002$; $p < 0,004$) whereas calcium slightly decreased. The average concentration of measured parameters, except calcium, were higher in 8 women in which blood was collected at the very end of pregnancy (36-37 weeks) (Table 1). At 3rd trimester no correlation between OPG and OC or Ca was found, but there was still a strong positive relationship between OC and CTx ($r=0,69$; $p < 0,00002$).

Both, serum CTx and OPG seemed to be the parameters that allowed to differentiate between elevated and normal bone turnover among pregnant women (Table 2). When cluster analysis was applied, with CTx and OPG as dimensions, two subgroups were obtained. At 1st trimester 14 out of 30 women could be included into the subgroup with increased bone resorption according to CTx values. The average OPG concentration in this subgroup was highly elevated, calcium was slightly increased while osteocalcin was still within the reference range. In the second subgroup the values of biochemical parameters were found to be within the accepted reference range.

Table 1. Mean (± SD) of biochemical parameters measured in pregnant and non-pregnant women

	OC (ng/ml)	CTx (ng/ml)	OPG (pg/ml)	Ca (mmol/L)
1st trimester (6-14 wks) n= 30	20,6 ± 9,1	0,391 ± 0,185	86 ± 50	2,52 ± 0,22
3rd trimester (31-37 wks) n= 30	23,2 ±9,6	0,543 * ±0,216	113 *** ± 58	2,46 ± 0,15
Pregnant women (36-37) wks n= 8	32,0 ± 11,9	0,635 ± 0,173	131 ± 68	2,42 ±0,08
Non-pregnant women n= 27	17,9 ±6,5	0,348 ** ±0,144	69 **** ±19	2,24***** ± 0,25

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*1st vs 3rd trimester $p < 0.002$; ** 3rd vs non-pregnant $p < 0,004$; *** 1st vs 3rd $p < 0,004$; **** 3rd vs non-pregnant $p < 0,03$; *****1st, 3rd vs non-pregnant $p < 0,001$

At 3rd trimester bone resorption was highly elevated in 10 (7 at 31-35 wks and 3 at 36-37 wks) out of 30 pregnant women (Table 2). In these women the average OPG concentration was also elevated, whereas osteocalcin was found to be in the upper reference range and calcium was normal. In the second subgroup with CTx in the upper reference range only OPG concentration was strongly elevated.

The bone markers were also analyzed in relation to serum calcium level (Table 3). In pregnant women at 1st and 3rd trimester the mean values of bone markers, except OPG, were lower in the lowest quartile of calcium concentrations and higher in Q3. The relationship of OPG and calcium has changed between 1st and 3rd trimester. At the end of pregnancy higher OPG concentration was related to lower calcium.

Table 2. Osteoprotegerin, osteocalcin and calcium concentration in two CTx subgroups at 1st and 3rd trimester of pregnancy (cluster analysis with CTx and OPG as dimensions; K-means)

1st trimester		CTx ng/ ml	OPG pg/ml	OC ng/ ml	Ca mmol/l
Subgroup with increased CTx n=14	Mean	0,538	109	27,5	2,58
	SD	0,180	49	7,0	0,11
Subgroup with normal CTx n=16	Mean	0,263	57	14,8	2,47
	SD	0,066	12	2,9	0,08

3rd trimester		CTx ng/ ml	OPG pg/ ml	OC ng/ml	Ca mmol/l
Subgroup with increased CTx n=10	Mean	0,783	101	32,1	2,47
	SD	0,159	40	8,7	0,093
Subgroup with normal CTx n=20	Mean	0,430	120	19,3	2,44
	SD	0,167	58	5,3	0,166

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Table 3. Serum osteoprotegerin and bone marker mean values in relation to calcium level (quartiles) in pregnant women at 1st and 3rd trimester

Ca (mmol/L)	OPG (pg/ml)	OC (ng/ml)	CTx (ng/ml)
1st trimester			
Q1 1,56-2,47	45,7	17,2	0,326
Q3 2,56-2,65	75,4	18,1	0,367
3rd trimester			
Q1 2,11-2,38	138,3	21,7	0,549
Q3 2,47-2,55	118,6	26,4	0,591

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Discussion

During pregnancy dynamic changes occur in maternal bone and calcium metabolism, but the effect of pregnancy upon the bone mass is not fully understood [15]. Two mechanisms: intestinal calcium absorption and urinary calcium excretion help to satisfy the increased demand for calcium during pregnancy. But they are not sufficient enough, because there is evidence that pregnancy affects also bone mass. Many authors infer that pregnancy is followed by loss in bone mass up to 5% [8,9,11,16]. Some pregnant women become prone to excessive bone loss and even fractures [17].

It is not known whether osteoprotegerin is involved in the regulation of bone turnover during pregnancy. In earlier study Uemura et al have found that   circulating OPG levels increased with gestational age and especially before the delivery, after 36weeks [18]. The tissue source of OPG in pregnancy is unknown, but the placental source was suggested [19]. The breast is also a potential source of maternal serum OPG and the RANK-RANKL signalling pathway appears to be involved in the development of lactating mammary tissue [20,21]. The presence of OPG in human breast milk was previously described [22]. However, the rapid postpartum decline in maternal OPG toward preconception values in both breast-feeding and non-breast-feeding women suggests that the breast is not the primary contributor to maternal serum OPG during pregnancy [23].

In our study serum OPG concentration in non pregnant women and those at 1st trimester of pregnancy was similar, what suggests that OPG levels gradually increased as gestational age progressed [18,23]. This may be related with the increasing level of estradiol found

during pregnancy. In postmenopausal women a significant, positive relationship between OPG and estradiol was found [24,25], but such a correlation was not confirmed in pregnant women [19]. Contrary to the others [18,19] we found a weak positive correlation between OPG and OC but only at the 1st trimester.

We noticed a significant increase in OPG during pregnancy. It is consistent with previous observations in women [18,19]. Similarly to earlier findings [18], we observed much higher rise in OPG at the end of 3rd trimester with concomitant decline of serum calcium.

The association of serum calcium and osteoprotegerin level changed during pregnancy. At the 3rd trimester, when the calcium demands of the fetus are the greatest, OPG was higher in the lowest quartile of calcium whereas in Q3 osteoprotegerin was lower. This was also observed earlier [18].

 Data on bone turnover markers during pregnancy are inconsistent. Among bone formation markers bone alkaline phosphatase was shown to rise with gestational age [10,18,19] whereas osteocalcin did not change or similarly to N-terminal propeptide of collagen type I showed a biphasic pattern with decrease from 1st to second trimester, followed by increase in the 3rd [10,16,18].   We have measured biochemical markers in fasting morning samples only twice, at 1st and 3rd trimester and observed the elevation in OC during pregnancy, especially at 36-37 wks.

 Bone resorption, reflected by serum CTx, increased significantly during pregnancy with peak levels at the end of 3rd trimester that confirms data by other authors [5,10,11,19]. This was accompanied by a decrease in serum calcium, especially before the delivery (36-37wks).

 Serum CTx and OPG seemed to be the only parameters to differentiate between elevated and normal   bone turnover. According to the nomogram proposed for the Polish premenopausal women serum CTx value over 0,490 ng/ml and OC > 34 ng/ml (>95th percentile) reflect the elevated bone turnover [26]. From our study we may conclude that, at least, abnormal CTx during 1st trimester may be a good predictor for faster bone loss during pregnancy.

Our results confirm that serum OPG and bone turnover markers levels increase during pregnancy and clearly show that bone resorption precedes bone formation. In pregnancy many   factors known to influence on the bone mass undergo changes: increased calcium demand, change in nutritional habits, changes in body weight and fat content, changed levels of physical activity and hormones with potential to affect bone metabolism [27]. This may be the main reason for difficulties in finding the exact role of OPG in relation to bone turnover during pregnancy. While the determination of osteocalcin at the beginning of pregnancy,

seems to be of limited clinical use, measuring OPG as a factor related to bone turnover or a bone resorption marker, such as CTx, may have a good predictive value for later pregnancy-associated bone loss or osteoporosis.

Conclusions

In pregnancy bone turnover increases mainly due to enhanced bone resorption. The determination of CTx and/or osteoprotegerin at the beginning of pregnancy may have a good predictive value for later pregnancy-associated bone loss.

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