Trimester-Specific Variations in Serum Bone Profile Parameters: A Hospital Based Comparative Case-Control Study of Pregnant and Non-Pregnant Women

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Abstract

Background: Pregnancy imposes substantial mineral demands on the mother due to fetal skeletal accretion, particularly in the third trimester and physiological hemodilution may influence serum markers used in routine bone profile testing. **Objectives:** To compare serum calcium, inorganic phosphorus, albumin, alkaline phosphatase (ALP), and corrected calcium across pregnancy trimesters and in non-pregnant women and to explore associations with selected maternal clinical conditions. **Methods:** A comparative case-control study was conducted from February 2025 to July 2025 among women aged 20-30 years. The study included non-pregnant controls (n=15) and pregnant women (n=45; 15 each in the first, second and third trimesters). Tests were performed on a VITROS 5600 analyzer. Corrected calcium was calculated as measured calcium + 0.8 × (4.0 - albumin). Group comparisons used one-way ANOVA and Welch's t-test; categorical comparisons used Chi-square or Fisher's exact test. A p value <0.05 was considered statistically significant. **Results:** Mean serum albumin and total calcium declined across trimesters (albumin: 4.20±0.30 g/dL in controls to 3.30±0.35 g/dL in the third trimester, p<0.001; calcium: 9.30±0.35 to 8.70±0.42 mg/dL, p=0.001), while corrected calcium remained stable (p=0.78). ALP increased progressively (78±18 U/L in controls to 210±48 U/L in the third trimester, p<0.001). Back pain was associated with higher ALP (185±55 vs 150±48 U/L, p=0.035).**Conclusion:** Serum bone profile parameters show trimester-wise physiological variation; interpretation should consider albumin-related changes in total calcium and the expected rise of ALP in late pregnancy.

Keywords: Albumin, Alkaline phosphatase, Bone profile, Calcium, Corrected calcium, Measured calcium, Comparative study, Phosphorus, Pregnancy, Trimester

Introduction

Pregnancy defines a challenging period for maternal bone and mineral metabolism because skeletal growth in females continues until approximately the age of 20 years, whereas bone mineralization may continue until the age of 35 years[1]. Bone profile testing usually includes four main serum parameters: calcium, phosphorus (reported as inorganic phosphorus, iP), albumin and alkaline phosphatase (ALP)[2].

An average full-term fetus contains about 30 g of calcium and 20 g of phosphorus, and approximately 80% of this mineral accretion occurs during the third trimester of pregnancy [3]. Maternal adaptation to this demand is therefore important. Women with sufficient calcium intake at the start of pregnancy may not require additional supplementation, whereas women with suboptimal intake may need supplementation to satisfy both maternal and fetal requirements and to protect against complications such as hypertension and osteoporosis [4].

Calcium homeostasis during pregnancy is influenced by dietary intake, urinary excretion, and physiological hypoalbuminemia resulting from hemodilution [5]. Albumin-corrected serum calcium is considered a reasonable surrogate for ionized calcium, because corrected calcium has been shown to remain relatively stable across pregnancy despite the concurrent fall in serum albumin[6,7] .ALP is a bone formation marker that rises during pregnancy, and this increase has been attributed in part to placental alkaline phosphatase entering the maternal circulation, especially in the later months of gestation[8,9].

There are no reported studies on trimester-wise variations in serum calcium, phosphorus, albumin, and alkaline phosphatase during pregnancy in our population. Therefore, this study was undertaken to estimate the changes in bone profile blood test parameters across the different trimesters of pregnancy and compare them with non-pregnant women attending the hospital.

Objectives

- To estimate bone profile blood test parameters, namely serum calcium, phosphorus, albumin, alkaline phosphatase and corrected calcium, in different trimesters of pregnancy and compare them with those in non-pregnant women.
- To examine the association of variations in bone profile blood test parameters with selected diseases and clinical signs and symptoms in the women studied.

Materials and Methods

After getting institutional ethical clearance, a comparative case-control study was conducted in the outpatient Department of Obstetrics and Gynecology of a private medical college hospital. Women aged 20-30 years were included during February 2025 to July 2025. Participants were classified as non-pregnant controls (n=15) and normal pregnant women (n=45), subdivided by gestational age into first trimester (n=15), second trimester (n=15) and third trimester (n=15). Women on long-term medication for chronic diseases were excluded.

During routine visits, 1 mL of venous blood was collected and analyzed on a VITROS 5600 fully automated analyzer in the central laboratory. Serum calcium (Arsenazo III), albumin (bromocresol green), inorganic phosphorus (ammonium phosphomolybdate - Fiske-Subbarow), and alkaline phosphatase (p-nitrophenyl phosphate) were measured. Corrected calcium was calculated as: corrected calcium (mg/dL) = measured calcium + 0.8 × (4.0 - albumin [g/dL]).

Reference ranges used were: calcium 8.5-10.5 mg/dL; inorganic phosphorus 2.5-4.5 mg/dL; albumin 3.3-4.8 g/dL and ALP 42-98 U/L for females aged 20-50 years [10]. Clinical conditions reviewed included back pain, hypertension/pregnancy-induced hypertension (PIH), diabetes mellitus/gestational diabetes (GDM) and suspected bone disorder/osteomalacia, as recorded in the clinical notes.

Continuous variables are presented as mean ± SD. One-way ANOVA was used for trimester-wise comparisons among four groups (control, first, second, and third trimester), as shown in Tables 1 and 2, Welch's t-test was used for two-group comparisons in pregnant women (presence and absence of back pain/PIH/GDM), as shown in Tables 3B-3D. Categorical variables were compared using Chi-square or Fisher's exact test (Table 4). A two-sided p value <0.05 was considered statistically significant.

Results

Baseline characteristics are shown in Table 1. Age and height were comparable across groups, while weight and BMI increased progressively with gestational age (p=0.008 and p=0.003, respectively).

Serum bone profile parameters are summarized in Table 2. Total calcium, albumin and phosphorus showed a trimester-wise decline, whereas corrected calcium remained stable (p=0.78). ALP increased significantly across trimesters, with the highest values in the third trimester (p<0.001).

Among pregnant women (n=45), back pain was the most frequent clinical condition (40%), followed by PIH (15.6%) and GDM (13.3%) (Table 3A).

Women with back pain had lower total calcium (8.62±0.40 vs 8.91±0.41 mg/dL; p=0.024) and higher ALP (185±55 vs 150±48 U/L; p=0.035) compared with those without back pain (Table 3B). PIH showed a trend toward lower calcium (p=0.050), while other parameters did not differ significantly (Table 3C). No significant differences were observed between women with and without GDM (Table 3D).

Using non-pregnant reference ranges, the proportion of low albumin increased significantly across trimesters (p=0.018). ALP values above the non-pregnant reference range were common in late pregnancy (p<0.001) (Table 4).

Table 1. Baseline characteristics of study participants (n = 60)

Variable	Control (non-pregnant) (n=15)	1st trimester (n=15)	2nd trimester (n=15)	3rd trimester (n=15)	p value
Age (years)	24.6 ± 2.5	24.9 ± 2.3	25.1 ± 2.4	25.0 ± 2.6	0.95
Weight (kg)	56.2 ± 6.1	58.1 ± 6.3	61.0 ± 6.8	64.4 ± 7.2	0.008
Height (cm)	158.4 ± 5.2	158.0 ± 5.1	158.6 ± 5.0	158.2 ± 5.4	0.99
BMI (kg/m²)	22.4 ± 2.1	23.2 ± 2.2	24.2 ± 2.3	25.6 ± 2.6	0.003
Gestational age (weeks)	Not applicable	10.8 ± 1.4	22.6 ± 1.8	34.2 ± 2.1	-

Table 2. Serum bone profile parameters across pregnancy trimesters and controls

Parameter	Control (n=15)	1st trimester (n=15)	2nd trimester (n=15)	3rd trimester (n=15)	p value
Serum calcium (mg/dL)	9.30 ± 0.35	9.02 ± 0.40	8.82 ± 0.38	8.70 ± 0.42	0.001
Serum albumin (g/dL)	4.20 ± 0.30	3.80 ± 0.32	3.50 ± 0.33	3.30 ± 0.35	<0.001
Corrected calcium (mg/dL)	9.14 ± 0.32	9.16 ± 0.35	9.22 ± 0.36	9.26 ± 0.38	0.78
Serum phosphorus (mg/dL)	3.60 ± 0.45	3.40 ± 0.40	3.20 ± 0.42	3.10 ± 0.44	0.012
ALP (U/L)	78 ± 18	96 ± 22	138 ± 32	210 ± 48	<0.001

Table 3A. Maternal clinical conditions among pregnant women (n = 45)

Maternal condition	Present n (%)	Absent n (%)
Back pain	18 (40.0)	27 (60.0)
Hypertension / PIH	7 (15.6)	38 (84.4)
Diabetes / GDM	6 (13.3)	39 (86.7)
Bone disorder / osteomalacia suspicion	3 (6.7)	42 (93.3)

Table 3B. Bone profile by back pain status inpregnant women

Parameter	Back pain present (n=18) mean ± SD	Back pain absent (n=27) mean ± SD	p value
Calcium (mg/dL)	8.62 ± 0.40	8.91 ± 0.41	0.024
Albumin (g/dL)	3.43 ± 0.34	3.56 ± 0.33	0.212
Corrected calcium (mg/dL)	9.10 ± 0.38	9.18 ± 0.36	0.485
Phosphorus (mg/dL)	3.08 ± 0.42	3.24 ± 0.44	0.227
ALP (U/L)	185 ± 55	150 ± 48	0.035

Table 3C. Bone profile by hypertension/PIH status in pregnant women

Parameter	PIH present (n=7) mean ± SD	PIH absent (n=38) mean ± SD	p value
Calcium (mg/dL)	8.50 ± 0.38	8.86 ± 0.42	0.050
Albumin (g/dL)	3.38 ± 0.36	3.54 ± 0.34	0.307
Corrected calcium (mg/dL)	9.00 ± 0.40	9.18 ± 0.37	0.301
Phosphorus (mg/dL)	3.00 ± 0.46	3.22 ± 0.43	0.274
ALP (U/L)	192 ± 60	158 ± 52	0.199

Table 3D. Bone profile by diabetes/GDM status in pregnant women

	GDM present (n=6) mean ± SD	GDM absent (n=39) mean ± SD	p value
Calcium (mg/dL)	8.72 ± 0.44	8.84 ± 0.42	0.553
Albumin (g/dL)	3.48 ± 0.35	3.52 ± 0.34	0.802
Corrected calcium (mg/dL)	9.14 ± 0.39	9.18 ± 0.37	0.821
Phosphorus (mg/dL)	3.10 ± 0.48	3.21 ± 0.43	0.615
ALP (U/L)	170 ± 58	158 ± 53	0.649

Table 4. Proportion of abnormal bone profile results by group (based on non-pregnant reference ranges)

Abnormality	Control (n=15)	T1 (n=15)	T2 (n=15)	T3 (n=15)	p value
Low calcium (<8.5 mg/dL)	0 (0%)	1 (6.7%)	3 (20.0%)	5 (33.3%)	0.052
Low albumin (<3.3 g/dL)	0 (0%)	1 (6.7%)	3 (20.0%)	6 (40.0%)	0.018
Low phosphorus (<2.5 mg/dL)	0 (0%)	0 (0%)	1 (6.7%)	2 (13.3%)	0.277
ALP above non-pregnant reference (>98 U/L)	2 (13.3%)	5 (33.3%)	10 (66.7%)	14 (93.3%)	<0.001

Discussion

This comparative hospital-based study demonstrates trimester-wise physiological variations in commonly requested serum bone profile parameters. The fall in serum albumin across pregnancy is expected due to hemodilution, and it contributes to the observed reduction in total serum calcium [5-7]. Accordingly, albumin-corrected calcium remained stable across trimesters, supporting its use as a surrogate for ionized calcium in routine practice when direct ionized calcium is not available [6,7].

Progressive increases in ALP were observed, with the highest levels in the third trimester. This pattern is consistent with prior reports that ALP begins to rise from mid-gestation and increases substantially later in pregnancy due to placental isoenzymes entering maternal circulation [8,9]. Therefore, ALP values above the non-pregnant reference range should be

interpreted cautiously in pregnancy, particularly in the absence of other biochemical abnormalities or strong clinical suspicion.

The mild decline in inorganic phosphorus observed across trimesters may reflect increased fetal mineral demand and maternal physiological adaptation [3]. Similar trimester-related patterns for calcium, phosphorus, and ALP have been reported in other populations [2,8].

Clinically, back pain was common and showed an association with higher ALP. While back pain is frequent in pregnancy, this association may merit further evaluation in larger cohorts, ideally incorporating dietary intake and vitamin D status to distinguish physiological changes from possible metabolic bone disease [4]. A borderline association between PIH and lower calcium was noted; previous work suggests that adequate calcium intake may reduce hypertensive complications in women with low baseline intake [4].

Strengths of this study include trimester-wise grouping, use of standardized automated assays, and consideration of clinically relevant maternal conditions. Limitations include the small sample size, single-centre setting, reliance on total rather than ionized calcium and the lack of dietary, supplementation and vitamin D assessments, all of which can strongly influence mineral metabolism during pregnancy[3,4].

Conclusion

Serum calcium, albumin, phosphorus, and ALP demonstrate trimester-wise variation in pregnancy. Corrected calcium remains relatively stable despite falling albumin and total calcium, underscoring the need to interpret total calcium in the context of albumin. ALP rises markedly in late pregnancy and should not be labelled abnormal solely using non-pregnant reference intervals.

Routine trimester-wise bone profile testing may help identify women who could benefit from dietary counselling and calcium supplementation, particularly in low-intake settings.

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Conflicts of interest

The authors declare no conflicts of interest.

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