



ORIGINAL ARTICLE

VARIATIONS IN THE BONE PROFILE AND VITAMIN D LEVELS DURING NORMAL PREGNANCY IN RURAL WOMEN

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Background: Pregnancy triggers significant physiological adaptations that alter maternal bone turnover and mineral metabolism to support foetal skeletal development. Investigating these biochemical shifts in specific demographics is essential for distinguishing normal gestational variations from true pathological deficiencies. The study was conducted to evaluate alterations in bone profile and vitamin D3 levels during pregnancy in normal women residing in rural areas of District Nowshera.

Methods: This cross-sectional study was conducted from April 2024 to March 2025 by the orthopaedic unit at Lady Reading Hospital in Peshawar, in collaboration with the health department of district Nowshera. A total of 90 pregnant women, aged between 18 and 35 years, from the three basic health units in the Nowshera district, were investigated. Twenty-five non-pregnant, non-lactating women who met the same inclusion and exclusion criteria were also examined. **Results:** Total serum calcium ($p=0.2944$), inorganic phosphorus ($p<0.0001$), and serum albumin ($p<0.0001$) concentrations were found to be lower in pregnant women in comparison to the control group, whereas alkaline phosphatase ($p<0.0001$) activity was elevated in pregnant women relative to non-pregnant women. **Conclusion:** The decrease in level of Calcium, Phosphorus, Albumin and Vitamin D 3, and increase in level of Alkaline Phosphatase in pregnant women underscore the necessity of creating pregnancy-specific reference ranges as needed for the professionals engaged in antenatal care.

Keywords: Bone profile, Nowshera, Pakistan, Pregnancy, Rural, Vitamin D levels, Women

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INTRODUCTION

Pregnancy constitutes a unique state in the lives of women, influencing numerous physiological, metabolic, and skeletal systems within the human body. All of these changes are essential for sustaining pregnancy, promoting foetal development, and facilitating postpartum lactation. Certain blood components generated during pregnancy are found in significantly higher concentrations, whereas others are present in lower amounts compared to women who are not pregnant.¹

Numerous changes begin shortly after conception and persist until the end of gestation. A previous report indicated that the physiological adaptations associated with pregnancy, result in numerous significant alterations in laboratory test values.¹ An increased plasma volume aids in the delivery of blood and nutrients to the foetus via the placenta.² The decrease in hematocrit and haemoglobin levels during pregnancy is referred to as physiological anaemia or dilutional anaemia.³ While the physiological rise in blood sugar during pregnancy is referred to as gestational diabetes.⁴

In females, skeletal development persists until approximately 20 years of age; however, bone mineralization may continue until the age of thirty-five.⁵ The bone profile encompasses four key blood parameters: serum calcium, serum phosphorus, serum

albumin, and serum alkaline phosphatase (ALP).⁵ A foetus at full term contains 30 grams of calcium and 20 grams of phosphorus, with around 80% of these minerals being accumulated during the third trimester of pregnancy.⁶

Vitamin D, often referred to as the ‘solar vitamin’, is crucial for the absorption of calcium and phosphate metabolism, as well as for the mineralization of bones. It is produced primarily through the direct exposure of sunlight to the skin (90%) or through dietary sources (10%) such as dairy products, eggs, and fish.⁷ An early study indicated that vitamin D deficiency is prevalent among females during pregnancy.⁸ Other researchers have pointed out that over 50% of mothers and their newborns experience some degree of vitamin D deficiency⁹, particularly among those of Asian descent.¹⁰ Understanding the variations in the stated blood parameters is crucial for the well-being of both mothers and their infants throughout the gestational period.¹¹

The present research was conducted to examine bone blood parameters, including vitamin D deficiency, during the three trimesters of pregnancy among women residing in rural areas of district Nowshera.

MATERIAL AND METHODS

This cross-sectional study was carried out by the Department of Orthopaedics across three Basic health units in the Nowshera district. These units were equipped with antenatal care facilities and were chosen randomly

for the study. The duration of the project spanned from April, 2024 to March, 2025.

The age of participants was between 18 and 35 years. Eligible pregnant women attending the selected antenatal clinic and were residents of the rural areas of Nowshera, and willing to participate in the study were included in the study. A non-probability consecutive sampling technique was used. All of them had regular ovulatory cycles, normal obstetric histories, and never used any contraceptives during their reproductive lives.

Women with poor general health, a history of smoking, any chronic disease, any hormonal disorder, or who did not give birth to healthy singleton infants at term were excluded. The sample size was calculated using the formula: $n = Z^2 P (1-P) / d^2$. The expected prevalence was 50%, with a confidence interval of 90% and an alpha error of 10%. The determined sample size 69 was subsequently raised to 90, to account for potential non-responses.

A total of 90 pregnant women, comprising 60 primigravidas and 30 multigravidas, from the middle socioeconomic group with a nearly identical lifestyle, were included in the study. A control group consisting of 25 non-pregnant, non-lactating women with same criteria was recruited. Blood sample in the control group was performed during the early follicular phase of the menstrual cycle (days 4–10). A smaller control group was recruited because the primary objective was to evaluate the laboratory changes across pregnancy trimesters while non-pregnant women served only as a reference group.

The research was approved by the Institutional Research and Ethics Board, Postgraduate Medical Institute (PGMI), Hayatabad Medical Complex, Peshawar before start of study (Reference No. 6683 dated 3 Jun 2024). The goals and procedure of the project were communicated to the participants, and were assured about their privacy and confidentiality. All participants volunteered and provided a written informed consent for participation in the study.

Then, all participants completed a validated and pre-tested questionnaire that gathered information on age, family composition, occupation, onset of puberty, age at marriage, gestation duration, age at first childbirth, lactation history, and parity.

A total of six millilitres of blood samples were obtained in the morning, between 8:00 and 9:00 AM, during the first trimester (1–13 weeks), the second trimester (14–26 weeks), and the third trimester (27+ weeks) of pregnancy. The serum was separated from all specimens following the clotting process. Calcium, phosphorus, albumin, and alkaline phosphatase levels were measured using the automatic cobas® c 311 analyser from Roche, while Vitamin D was estimated with ARCHITECT-Plus I1000SR® analyser from Abbott Diagnostics.

The following formula was used to calculate the corrected calcium concentration¹²:

$$\text{Calcium corrected (mg/dL)} = \text{Total calcium measured (mg/dL)} + 0.8(4 - \text{albumin (g/dL)})$$

Data were entered and analysed on SPSS-17. Continuous variables were presented as Mean±SD. Normality was assessed with Shapiro-Wilk test. Comparison among the four groups was performed with one-way ANOVA for normally distributed data; $p \leq 0.05$ was considered significant. Frequency distribution tables were generated using the Mean±SD.

RESULTS

The results of the current research are displayed in Table-1. The mean levels of calcium, phosphorus, and albumin were observed to decrease as gestational age progressed through the three trimesters of pregnancy. The level of alkaline phosphatase activity rose with the progress of gestational age. The vitamin D levels in pregnant women were found to be lower when compared to the control group of non-pregnant women.

A comparison of the bone profile and vitamin D levels between pregnant and non-pregnant women is presented in Table-2. The conventional SI units/reporting formats is instrument generated and appropriate for this study as the comparison is not between the given parameters. All parameters were notably modified during pregnancy, with the exception of calcium and corrected calcium. Moreover, the patterns observed in our study group's primigravidas and multigravidas did not differ for any of the parameters examined. Furthermore, age at marriage and parity had no effect on the above estimated parameters in both the control and study groups.

Table-1: Blood parameters among controls and three trimesters of pregnancy (Mean±SD)

Analyte	Non-pregnant	1 st trimester	2 nd trimester	3 rd trimester
Total Calcium (mg/dL)	8.7±1.4	8.6±2.0	8.2±1.9	8.0±1.8
Albumin (g/dL)	4.4±0.2	4.3±0.1	3.8±0.1	3.7±0.1
Phosphorus (mg/dL)	3.16±0.09	2.82±0.15	2.91±0.09	2.57±0.08
ALP (U/l)	62±2.60	61±1.24	73±2.60	130±5.03
Corrected Ca ⁺⁺ (mg/dL)	8.4±0.21	8.4±0.17	8.4±0.21	8.2±0.30
Vitamin-D3 (ng/mL)	47.8±12.2	31.5±10.4	16.5±6.8	14.2±8.8

Table-2: Comparison of Serum parameters among non-pregnant and pregnant women (Average of three trimesters, Mean±SD)

Analyte	Non-Pregnant	Pregnant	p
Total Calcium (mg/dL)	8.7±1.4	8.27±1.9	0.2944
Albumin (g/dL)	4.4±0.2	3.93±0.1	<0.0001
Phosphorus (mg/dL)	3.16±0.09	2.7±0.11	<0.0001
ALP (U/L)	62±2.60	88±2.96	<0.0001
Corrected Ca ⁺⁺ (mg/dL)	8.4±0.21	8.3±0.23	0.0527
Vitamin-D3 (ng/mL)	47.8±12.2	20.7±8.67	<0.0001

DISCUSSION

Metabolic processes are closely associated with skeletal health. Particularly the mineral metabolism may alter due to foetal requirements during pregnancy. In the present study we investigated the alterations in minerals and vitamin D levels.

Lower serum vitamin D3 levels were observed among pregnant women in the present study. Previous research has produced similar results.¹³ The low levels of vitamin D3 may be associated with limited dietary intake of vitamin D rich food and reduced sunlight exposure. A woman whose vitamin D levels are below 15 ng/mL has a fivefold increased risk of developing pre-eclampsia.¹³ Certain researchers demonstrated that women who consumed 4,000 IU of vitamin D each day had the advantages in reducing the risk of preterm birth, bone fragility, and infections.¹⁴

The level of serum calcium decreased during the three trimesters of pregnancy. Similar findings were observed in an earlier study¹⁵, likely attributed to nutrient deficiency and the heightened needs of both the mother and the infant. Hypocalcaemia during late pregnancy is prevalent, occurring in 59% of women of reproductive age.¹⁶ Another study has indicated that pregnant women exhibit elevated maternal serum calcium levels compared to non-pregnant women; however, this finding originates from a developed country.¹⁷

The serum Albumin levels were lower in pregnant than in non-pregnant women. Similar results were observed in another study carried out on Sudanese pregnant females.¹⁸ This phenomenon may be ascribed to the dilutional effect resulting from the rise in maternal plasma volume.¹⁹ A decrease in serum albumin levels in the pregnant women may also be attributed to hepatic dysfunction affecting albumin synthesis.¹⁹ Elevated levels of steroid hormones also influence the metabolic, synthetic, and excretory functions of the liver during pregnancy.²⁰

The serum phosphorus also showed a declining trend across pregnancy with the lowest values observed in the third trimester. A prior study revealed comparable findings.²⁰ The lower phosphorus levels observed in our study may be associated with reduced dietary intake, and increased foetal requirements.¹⁸ Furthermore, our results are supported by an earlier study which indicated that maternal urinary phosphorus excretion rises during the third trimester of pregnancy.¹⁸

The activity of serum ALP exhibits a markedly significant rise during pregnancy when compared to the control group of non-pregnant women. The rise observed during pregnancy is mainly attributed to the production of placental ALP iso-enzyme activity, rather than solely with hepatic iso-enzymes.²¹ There is a report that the blood samples from women who experienced preterm delivery exhibited higher levels of ALP placental iso-

enzyme when compared to those who delivered at term.²² Therefore elevated ALP levels in late pregnancy should be interpreted with caution, as they may reflect physiological changes related to the placental production in addition to other potential sources of ALP.²²

The constraint of this research was noted in the budgeting aspect. The limited budget restricted the research to a minimal number of samples. The authors carried out the study prioritizing the community's welfare, and they have no conflicts of interest to report.

CONCLUSION

The decrease in level of Calcium, Phosphorus, Albumin and Vitamin D 3, and increase in level of Alkaline Phosphatase in pregnant women underscore the necessity of creating pregnancy-specific reference ranges as needed for the professionals engaged in antenatal care.

RECOMMENDATIONS

Comprehensive studies are required across various regions of the country to validate the aforementioned variation concerning this subject.

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Authors approved the draft and are accountable in ensuring that questions related to accuracy or integrity of the work are duly investigated and resolved.

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