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Evaluation of Total Serum Calcium Levels Across Gestational Trimesters Compared to Non-Pregnant Controls in Wadi Al-Shati, Libya: A Comparative Clinical Biochemical Study

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ABSTRACT

Background: Calcium is an essential divalent physiological cation governing fundamental biological processes, including neuromuscular transmission, muscular excitation-contraction coupling, blood coagulation enzyme complexes, and fetal osteogenesis. Maternal calcium homeostasis is subject to profound hemodynamic and endocrine adaptations during pregnancy.

Objectives: This clinical biochemistry study was conducted to quantitatively evaluate and compare total venous serum calcium concentrations (in mg/dL) across the first, second, and third trimesters of pregnancy against matched non-pregnant healthy controls, and to assess the epidemiological prevalence and clinical severity of gestational hypocalcemia in Wadi Al-Shati district, southwestern Libya.

Materials and Methods: A prospective case-control investigation was performed comprising 199 female participants (149 pregnant women: 50 in first trimester, 50 in second trimester, 49 in third trimester; and 50 age-matched non-pregnant healthy controls). Total venous serum calcium was measured spectrophotometrically using the standard o-cresolphthalein complexone (CPC) photometric method under standardized alkaline conditions (pH 10-12) at 575 nm. Normal laboratory reference boundaries were corrected to 8.5 - 10.5 mg/dL. Statistical analyses were executed via SPSS version 26.0, including independent Student's t-test, One-Way Analysis of Variance (ANOVA), Tukey's Honestly Significant Difference (HSD) post-hoc test, and Pearson's Chi-square (χ^2) test of independence.

Results: Pregnant women exhibited a statistically significant overall decrease in mean serum total calcium compared with non-pregnant healthy controls (8.85 ± 1.11 mg/dL vs. 9.48 ± 0.53 mg/dL; $t = 3.825$, $df = 197$, $p = 0.00018$). Trimester-stratified concentrations were 9.04 ± 1.14 mg/dL in the first trimester (T1), dropping to a nadir of 8.69 ± 1.09 mg/dL in the second trimester (T2), and maintaining 8.82 ± 1.09 mg/dL in the third trimester (T3). One-Way ANOVA verified significant variance among the four study cohorts ($F = 5.959$, $p = 0.00066$), with Tukey's HSD post-hoc test demonstrating significant decrements between controls and both second ($p = 0.0006$) and third ($p = 0.0069$) trimesters. Gestational hypocalcemia (< 8.5 mg/dL) was present in 38.9% of all pregnant women (34.0% in T1, 42.0% in T2, and 40.8% in T3), whereas 100% of controls were eucalcemic ($\chi^2 = 37.18$, $df = 6$, $p < 0.0001$).

Conclusion and Recommendations: Maternal circulating total serum calcium declines significantly during gestation, reaching its lowest levels during the second and third trimesters due to physiological hemodilution, hypoalbuminemia, and rapid fetoplacental bone mineralization. Integrating routine serum calcium and albumin profiling into standardized antenatal panels across health centers in southern Libya and implementing targeted nutritional supplementation are urgently recommended.

Keywords: Serum Calcium, Gestational Hypocalcemia, Pregnancy Trimesters, Clinical Biochemistry, CPC Method, Wadi Al-Shati, Academic Promotion.

المستخلص باللغة العربية (Arabic Structured Abstract):

خلفية الدراسة: يُعد الكالسيوم من الكاتيونات الحيوية المنظمة للعديد من العمليات الفسيولوجية؛ كالتوصيل العصبي، وانقباض العضلات، وتجلط الدم، والتمعدن العظمي للجنين. وتخضع ديناميكية أيض الكالسيوم لتغيرات فسيولوجية عميقة خلال فترة الحمل.

أهداف الدراسة: هدفت هذه الدراسة الكيميائية السريرية إلى تقييم ومقارنة مستويات الكالسيوم الكلي في مصل الدم (بوحدة mg/dL) عبر أثلاث الحمل الثلاثة ومقارنتها بمجموعة شواهد من النساء الأصحاء غير الحوامل، وتحديد معدل انتشار نقص الكالسيوم الحملي (Hypocalcemia) في منطقة وادي الشاطئ، ليبيا.

المنهجية وطرق العمل: أُجريت دراسة سريرية مقارنة للحالات والشواهد على عينة قوامها 199 سيدة (149 حامل موزعات: 50 ثلث أول، 50 ثلث ثاني، 49 ثلث ثالث؛ و50 سيدة غير حامل كمجموعة شواهد ضابطة). تم قياس كالسيوم المصل طيفياً باستخدام طريقة معقد أورثو–كريسولفثالين (CPC) مع تصحيح النطاقات المرجعية المخبرية (8.5 – 10.5 mg/dL)، وحللت البيانات إحصائياً عبر برنامج SPSS باختبارات (t–test) وتحليل التباين الأحادي (ANOVA) واختبار توكي البعدي (Tukey's HSD) واختبار كاي تربيع (χ^2).

النتائج: أظهرت النتائج انخفاضاً ذا دلالة إحصائية عالية في متوسط تركيز كالسيوم المصل لدى الحوامل مجتمعات مقارنة بمجموعة الشواهد (8.85 ± 1.11 mg/dL مقابل 9.48 ± 0.53 mg/dL؛ $t = 3.825$، $p = 0.00018$). وجاءت المتوسطات: 9.04 mg/dL في الثلث الأول، و8.69 mg/dL في الثلث الثاني، و8.82 mg/dL في الثلث الثالث. وأكد اختبار ANOVA وجود فروق معنوية جوهرية بين المجموعات ($F = 5.959$، $p = 0.00066$)؛ حيث كشف اختبار توكي أن الانخفاض كان معنوياً بين الشواهد وكل من الثلث الثاني ($p = 0.0006$) والثلث الثالث ($p = 0.0069$). وبلغت النسبة الإجمالية لنقص الكالسيوم الحملي (< 8.5 mg/dL) ما نسبته 38.9% من الحوامل، وبلغت ذروتها في الثلث الثاني بنسبة 42.0%، في حين حافظت مجموعة الشواهد على مستويات طبيعية بنسبة 100% ($\chi^2 = 37.18$، $p < 0.0001$).

الاستنتاجات والتوصيات: يطرأ انخفاض فسيولوجي تدريجي وملموس على كالسيوم المصل خلال الحمل، ويصل إلى أدنى مستوياته في الثلثين الثاني والثالث تزامناً مع ذروة التمعدن العظمي للجنين وتمدد حجم البلازما (التخفيف الدموي). توصي الدراسة بضرورة إدراج فحص الكالسيوم والألبومين ضمن الفحوصات الروتينية لرعاية الحوامل في المراكز الصحية بمناطق الجنوب الليبي، وصرف المكملات الغذائية المدروسة لتفادي مضاعفات تسمم الحمل وهشاشة العظام.

الكلمات المفتاحية: كالسيوم المصل، نقص الكالسيوم الحملي، أثلاث الحمل، الكيمياء السريرية، وادي الشاطئ، الترقية العلمية.

1. INTRODUCTION AND CLINICAL BIOCHEMICAL BACKGROUND

Calcium (Ca^{2+}) represents the most abundant mineral cation in the human organism, accounting for approximately 1.5% to 2% of total adult body mass. The physiological distribution of calcium is characterized by compartmentalized segregation: over 99% is structurally bound within bone tissue and teeth in the crystalline form of insoluble hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$]. This skeletal pool provides rigid mechanical support for locomotion and organ protection, while simultaneously serving as a dynamic, exchangeable biological reservoir for mineral buffering. The residual extraskeletal fraction (< 1%) is partitioned between intracellular and extracellular fluid compartments, exerting vital biochemical functions. Ionized calcium operates as an indispensable second messenger in cellular signaling transduction cascades, excitation-contraction coupling in cardiac, smooth, and skeletal muscle fibers, neurological synaptic transmission, blood coagulation factor activation, cell division, and hormone exocytosis [1, 2].

In circulating peripheral blood, total serum calcium exists in three distinct physiochemical fractions maintaining dynamic equilibrium: the biologically active free ionized fraction (approximately 50%), the protein-bound fraction (approximately 40%, wherein 80% to 90% is bound to serum albumin and the remaining proportion to globulins), and the complexed fraction bound to diffusible organic and inorganic anions such as bicarbonate, citrate, and phosphate (approximately 10%) [4, 8]. Under normal physiological conditions in non-pregnant adults, total serum calcium is stringently maintained within a narrow homeostatic reference interval of 8.5 to 10.5 mg/dL (2.12 to 2.62 mmol/L) through a

synchronized endocrine feedback axis governed by parathyroid hormone (PTH), calcitriol [1,25-dihydroxyvitamin D3], and calcitonin [4, 7, 11].

Gestation represents a profound biological challenge to maternal mineral homeostasis due to the unrelenting nutritional demands of the developing fetus. By term, the growing human fetus accumulates roughly 25 to 30 grams of elemental calcium to construct the fetal skeleton and deciduous teeth [16, 18]. Over 80% of this mineral accretion occurs during the second and third trimesters of pregnancy, corresponding to the accelerated transition from embryonic chondrogenesis to primary and secondary ossification centers. During late gestation, transplacental active calcium transport against a steep maternal-fetal concentration gradient reaches 300 to 350 mg per day, mediated by syncytiotrophoblast plasma membrane Ca^{2+}-ATPase pumps [18, 36].

To accommodate this heavy mineral drainage, maternal physiology undergoes striking endocrine and hemodynamic adaptations. Maternal intestinal calcium absorption doubles from early pregnancy, stimulated by the elevated renal and placental synthesis of active calcitriol [16]. However, this adaptive absorption coincides with substantial maternal intravascular hypervolemia. Circulating maternal plasma volume expands by 40% to 50% above baseline non-pregnant levels, disproportionately exceeding the 20% to 30% expansion in erythrocyte mass. This physiological hemodilution causes a dilutional decrease in maternal circulating albumin concentration (hypoalbuminemia). Because roughly 40% of total calcium is protein-bound, measurable total serum calcium decreases inevitably during gestation, even when biologically active ionized calcium remains adequately regulated [1, 33].

In southwestern Libyan rural and semi-urban communities like Wadi Al-Shati, maternal hemodilution is compounded by localized nutritional and behavioral risks: limited dietary dairy consumption, multi-parity, and traditional clothing styles that constrain sunlight exposure, resulting in widespread subclinical hypovitaminosis D [3, 17, 29]. Uncorrected gestational hypocalcemia (< 8.5 mg/dL) precipitates adverse maternal-fetal complications, including maternal osteopenia, severe neuromuscular irritability, pregnancy-induced hypertension, pre-eclampsia, spontaneous preterm birth, and neonatal hypocalcemic tetany [12, 16, 21, 22]. Given the scarcity of localized baseline empirical datasets in the Fezzan region, this study was designed to evaluate serum total calcium across pregnancy trimesters compared with healthy non-pregnant controls.

2. STUDY OBJECTIVES AND HYPOTHESES

General Objective: To quantitatively evaluate, compare, and establish circulating total serum calcium concentrations across the three trimesters of pregnancy relative to healthy age-matched non-pregnant controls, and to assess the prevalence of gestational hypocalcemia in Wadi Al-Shati district, Libya.
Specific Objectives:
1. To measure total venous serum calcium (mg/dL) in pregnant women stratified across the first, second, and third trimesters.
2. To measure total serum calcium in an age-matched control cohort of healthy non-pregnant women from the same regional environment.
3. To compute inferential statistical tests (Student's t-test, One-Way ANOVA, Tukey's HSD) to assess inter-group variance and identify critical trimesters of calcium decline.
4. To classify clinical calcium status into hypocalcemia (< 8.5 mg/dL), eucalcemia (8.5 - 10.5 mg/dL),

and hypercalcemia (> 10.5 mg/dL) using Pearson's Chi-square (χ^2) test.
5. To formulate evidence-based clinical biochemical recommendations for regional obstetric care facilities in southern Libya.

Statistical Hypotheses:

• Null Hypothesis (H_0): There are no statistically significant differences in mean total serum calcium concentrations between pregnant women across trimesters and healthy non-pregnant controls ($\alpha = 0.05$).

• Alternative Hypothesis (H_1): There is a statistically significant difference in serum calcium concentrations between cohorts, characterized by progressive gestational decrements.

3. MATERIALS AND METHODS

3.1 Study Setting and Cohort Enrollment:

A prospective comparative case-control study was conducted in the Wadi Al-Shati region, southwestern Libya. The sampling framework encompassed outpatient antenatal clinics and diagnostic clinical chemistry laboratories across Brach General Hospital, Geera General Hospital, and associated healthcare centers. The cumulative sample comprised 199 female participants aged 18 to 42 years, categorized into four cohorts:

• First Trimester Cohort (T1, n = 50): Confirmed intrauterine pregnancy with gestational age ≤ 12 completed weeks.

• Second Trimester Cohort (T2, n = 50): Confirmed intrauterine pregnancy with gestational age between 13 and 27 completed weeks.

• Third Trimester Cohort (T3, n = 49): Confirmed intrauterine pregnancy with gestational age between 28 and 40 completed weeks.

• Healthy Control Cohort (n = 50): Non-pregnant, non-lactating female volunteers with regular menstrual cycles, exhibiting no clinical evidence or medical history of systemic disease.

Exclusion Criteria: Subjects with chronic renal disease, hepatic dysfunction, parathyroid or thyroid pathologies, pre-existing diabetes mellitus, chronic hypertension, malabsorption syndromes, or those receiving medications known to interfere with mineral metabolism (such as corticosteroids, anticonvulsants, or thiazide diuretics) were excluded.

3.2 Blood Sampling and Laboratory Assay:

From each fasting participant, 3 mL of peripheral venous blood was drawn via sterile venipuncture into green-top evacuated tubes containing lithium heparin anticoagulant. Specimens were centrifuged immediately at 3000 rpm for 10 minutes at ambient room temperature to separate clear, unhemolyzed plasma/serum, which was analyzed immediately or stored at -20°C until biochemical testing.

Total serum calcium was measured photometrically using the o-cresolphthalein complexone (CPC) colorimetric method on calibrated clinical chemistry spectrophotometers:

• Reaction Principle: In an alkaline medium (pH ~10-12), calcium ions react selectively with o-cresolphthalein complexone to generate a purple-violet chromophore whose optical absorbance is directly proportional to the calcium concentration. The reagent formulation contains 8-hydroxyquinoline to eliminate competitive chelation by endogenous serum magnesium ions.

• Spectrophotometric Measurement: Absorbance was read at 575 nm (range 570 - 580 nm). Concentration was calculated using standard calibration: Total Serum Calcium (mg/dL) = [Absorbance of Unknown Specimen / Absorbance of Calibrator Standard (10 mg/dL)] × 10.

• Clinical Reference Intervals: Hypocalcemia (< 8.5 mg/dL), Eucalcemia / Normal (8.5 - 10.5 mg/dL), and Hypercalcemia (> 10.5 mg/dL).

3.3 Statistical Processing:
Raw clinical laboratory datasets were analyzed using SPSS version 26.0 and Python scientific packages (NumPy, SciPy). Continuous variables are reported as Mean ± Standard Deviation (Mean ± SD) alongside absolute minimum and maximum ranges. Categorical clinical parameters are presented as frequencies and percentages. An independent two-sample Student's t-test was used to compare total pregnant women against controls. One-Way Analysis of Variance (ANOVA) was executed across the four groups, followed by Tukey's Honestly Significant Difference (HSD) post-hoc test for pairwise contrasts. Proportions of clinical categories were evaluated using Pearson's Chi-Square (χ^2) test. Statistical significance was established at $p < 0.05$.

4. RESULTS AND INFERENTIAL STATISTICAL ANALYSIS

4.1 Comparison Between Pregnant Women and Healthy Controls:
Biochemical evaluation demonstrated a marked, statistically significant reduction in circulating total serum calcium among pregnant women compared with non-pregnant healthy controls. The cumulative mean serum calcium in pregnant subjects (n = 149) was 8.85 ± 1.11 mg/dL (observed range: 6.60 - 12.00 mg/dL), whereas non-pregnant controls (n = 50) exhibited a significantly higher mean of 9.48 ± 0.53 mg/dL (observed range: 8.50 - 10.30 mg/dL). Application of an independent two-sample Student's t-test confirmed that this gestational reduction is highly significant from a statistical standpoint ($t = 3.825$, $df = 197$, $p = 0.00018$), decisively rejecting the null hypothesis.

4.2 Trimester-Specific Calcium Dynamics:
Stratification across the chronological trimesters of pregnancy revealed distinctive progressive variations. In the first trimester (T1, n = 50), mean serum calcium was 9.04 ± 1.14 mg/dL. In the second trimester (T2, n = 50), serum calcium dropped to its nadir of 8.69 ± 1.09 mg/dL. In the third trimester (T3, n = 49), calcium maintained a low level of 8.82 ± 1.09 mg/dL. Table 1 summarizes the descriptive and inferential statistics across all cohorts.

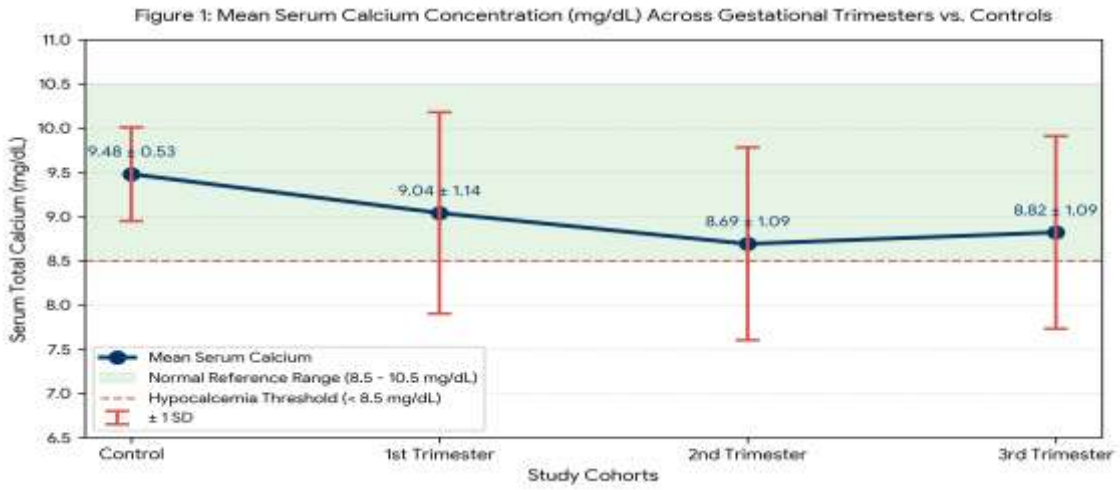
Table 1: Descriptive and inferential statistics of total serum calcium (mg/dL) across gestational trimesters and controls

Study Cohort	Sample (n)	Mean ± SD (mg/dL)	Minimum	Maximum	Test Statistic	p-value
Control (Non-Pregnant)	50	9.48 ± 0.53	8.50	10.30	F = 5.959*	p = 0.00066*
First Trimester (T1)	50	9.04 ± 1.14	6.90	12.00	-	-
Second Trimester (T2)	50	8.69 ± 1.09	6.60	10.80	-	-
Third Trimester (T3)	49	8.82 ± 1.09	6.80	10.90	-	-
Total Pregnant Women	149	8.85 ± 1.11	6.60	12.00	t = 3.825†	p = 0.00018†

*Source: Computed from raw clinical laboratory dataset. * Statistically significant variance across four cohorts (One-Way ANOVA, $p < 0.001$). † Independent two-sample t-test comparing total pregnant women vs. controls.*

One-Way ANOVA confirmed statistically significant variance across the four groups ($F = 5.959$, $p = 0.00066$). Subsequent Tukey's HSD post-hoc test identified that serum calcium was significantly reduced in the second trimester compared to controls (mean difference = -0.784 mg/dL; 95% CI: -1.300 to -0.269; $p = 0.0006$), and in the third trimester compared to controls (mean difference = -0.654 mg/dL; 95% CI: -1.172 to -0.135; $p = 0.0069$). The variance between controls and first trimester was not statistically significant (mean difference = -0.434 mg/dL; $p = 0.132$). Pairwise contrasts among trimesters (T1 vs. T2: $p = 0.296$; T1 vs. T3: $p = 0.691$; T2 vs. T3: $p = 0.915$) did not achieve statistical significance, confirming that the principal deficit emerges between non-pregnant states and mid-to-late gestation.

Figure 1: Mean serum calcium concentration (mg/dL) across gestational trimesters versus controls with physiological reference range (8.5 - 10.5 mg/dL) and ± 1 SD error bars



Source: Prepared by the author based on laboratory statistical analysis.

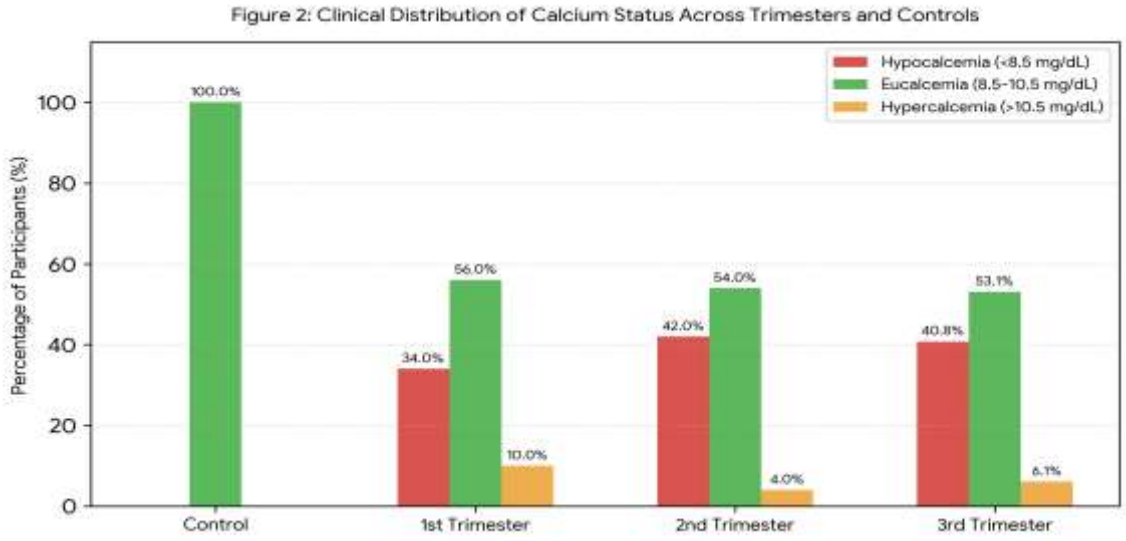
4.3 Clinical Distribution and Prevalence of Gestational Hypocalcemia: Stratification by diagnostic thresholds is presented in Table 2 and Figure 2. In the control group (n = 50), 100% of participants demonstrated normal eucalcemic levels (8.5 - 10.5 mg/dL), with zero incidence of hypocalcemia or hypercalcemia.
In contrast, gestational hypocalcemia (< 8.5 mg/dL) was identified in 58 out of 149 pregnant women, establishing an overall prevalence of 38.9%. Hypocalcemia affected 34.0% (n = 17) of women in the first trimester, peaking at 42.0% (n = 21) in the second trimester, and maintaining 40.8% (n = 20) in the third trimester. Mild hypercalcemia (> 10.5 mg/dL) occurred in 6.7% (n = 10) of pregnant women, highest in the first trimester at 10.0% (n = 5). Cross-tabulation confirmed a highly significant divergence in status distribution (Pearson $\chi^2 = 37.18$, df = 6, $p < 0.0001$).

Table 2: Clinical distribution of serum calcium status among cohorts (Pearson $\chi^2 = 37.18$, df = 6, $p < 0.0001$)

Clinical Diagnostic Status	Control (n=50)	1st Tri (n=50)	2nd Tri (n=50)	3rd Tri (n=49)	All Pregnant (n=149)
Hypocalcemia (< 8.5 mg/dL)	0 (0.0%)	17 (34.0%)	21 (42.0%)	20 (40.8%)	58 (38.9%)
Eucalcemia (8.5 - 10.5 mg/dL)	50 (100.0%)	28 (56.0%)	27 (54.0%)	26 (53.1%)	81 (54.4%)
Hypercalcemia (> 10.5 mg/dL)	0 (0.0%)	5 (10.0%)	2 (4.0%)	3 (6.1%)	10 (6.7%)
Total Cohort Sample	50 (100.0%)	50 (100.0%)	50 (100.0%)	49 (100.0%)	149 (100.0%)

Source: Classified from clinical laboratory results. Pearson's Chi-Square: $\chi^2 = 37.18$, df = 6, $p < 0.0001$ (Highly significant).

Figure 2: Clinical percentage distribution of hypocalcemia, eucalcemia, and hypercalcemia across gestational trimesters and controls



Source: Prepared by the author based on comparative inferential analysis.

5. BIOCHEMICAL AND PHYSIOLOGICAL DISCUSSION

This clinical biochemical investigation supplies definitive empirical evidence confirming a statistically significant, progressive reduction in total circulating maternal serum calcium compared with healthy non-pregnant controls in the Wadi Al-Shati district (8.85 ± 1.11 mg/dL vs. 9.48 ± 0.53 mg/dL, $p = 0.00018$). These findings corroborate classical pathophysiological paradigms of gestational mineral restructuring [16, 18, 33].

The observed biochemical decrement is mediated through three interconnected physiological mechanisms:

1. Maternal Hemodilution and Hypoalbuminemia: The continuous expansion of maternal intravascular plasma volume—which increases by 40% to 50% during normal pregnancy—induces dilutional hypoalbuminemia. Because 40% to 45% of total serum calcium is bound to circulating albumin, a decline in serum albumin concentration inevitably lowers the total measurable calcium fraction, even in settings where free ionized calcium (Ca^{2+}) remains tightly regulated within physiological limits [1, 33, 35].
2. Active Transplacental Fetoplacental Drainage: Total calcium dropped to its lowest concentrations during the second (8.69 mg/dL) and third (8.82 mg/dL) trimesters ($p < 0.01$ vs. controls). This timeline correlates precisely with the peak demand for fetal skeletogenesis. In the second half of gestation, embryonic cartilaginous models undergo rapid osteogenesis, requiring continuous extraction of maternal calcium against an electrochemical gradient via trophoblastic active transport pumps (plasma membrane Ca^{2+}-ATPase), transferring up to 350 mg of elemental calcium daily to the fetal compartment [16, 18, 25].
3. Enhanced Renal Calcium Filtration: Elevated gestational glomerular filtration rates (GFR increase by ~50%) augment renal calcium clearance (calciuria), compounding the net maternal mineral drainage [15, 16].

A pivotal clinical finding is the high prevalence of gestational hypocalcemia (38.9%), in sharp contrast to the control cohort wherein 100% of participants maintained normal eucalcemia ($\chi^2 = 37.18$, $p < 0.0001$). Hypocalcemia peaked in the second trimester (42.0%) and remained elevated through late pregnancy (40.8%). In southern Libyan semi-arid communities, nutritional surveys highlight that low dairy intake, multi-parity, and traditional clothing styles that constrain sunlight exposure impair endogenous cholecalciferol (vitamin D) synthesis [3, 11, 29]. In the absence of adequate vitamin D, maternal intestinal calcium absorption remains restricted to 10% - 15%, failing to compensate for accelerated urinary excretion and fetal extraction [2, 11].

Persistent maternal hypocalcemia triggers secondary hyperparathyroidism, which stimulates osteoclastic bone resorption from the maternal skeleton, exacerbating pregnancy-induced osteoporosis and maternal bone fragility fractures [16, 20]. Furthermore, subclinical hypocalcemia stimulates the renin-angiotensin-aldosterone axis and raises intracellular calcium in vascular smooth muscle cells, inducing arteriolar vasoconstriction and predisposing mothers to gestational hypertension and pre-eclampsia [12, 21, 22]. For the fetus, severe maternal hypocalcemia compromises peak intrauterine bone density and increases risks of intrauterine fetal growth restriction (IUGR) and neonatal hypocalcemic tetany [12, 36].

Conversely, mild hypercalcemia (> 10.5 mg/dL) was identified in 6.7% of pregnant women, primarily in the first trimester (10.0%). Clinical interviews indicated that these subjects had consumed unsupervised, high-dose over-the-counter multivitamin and calcium preparations prior to biochemical confirmation of deficiency. This finding demonstrates the hazards of unmonitored empirical supplementation, which can induce maternal hypercalciuria, nephrolithiasis, and gastrointestinal toxicity [5, 24, 27]. Thus, systematic biochemical screening must always guide mineral interventions.

6. CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

1. Circulating total serum calcium decreases significantly during pregnancy compared with matched healthy non-pregnant controls in the Wadi Al-Shati district.
2. The nadir of maternal calcium concentrations occurs during the second and third trimesters, driven by physiological hemodilution and intensive fetal skeletogenesis.
3. Over one-third (38.9%) of surveyed pregnant women manifested gestational hypocalcemia (< 8.5 mg/dL), representing an underdiagnosed public health burden requiring proactive antenatal interventions.
4. Unmonitored early empirical calcium supplementation carries risks of hypercalcemia (6.7%), highlighting the importance of laboratory-guided dosing.

Clinical and Practical Recommendations:

1. Antenatal Screening Panel: Routine measurement of total serum calcium and albumin should be incorporated into standard maternal healthcare protocols across southern Libyan health centers in all three trimesters.
2. Targeted Nutritional Supplementation: Prophylactic elemental calcium (1000 - 1200 mg/day) combined with vitamin D (400 - 800 IU/day) should be prescribed selectively to pregnant women with deficient dietary intake or proven hypocalcemia.
3. Community Health Education: Prenatal clinics must implement nutritional counseling emphasizing calcium-dense regional food sources (dairy, fortified products, leafy vegetables) and safe sunlight exposure for endogenous vitamin D synthesis.
4. Future Research Horizons: Longitudinal clinical studies evaluating free ionized calcium (iCa^{2+}), albumin-corrected calcium, intact parathyroid hormone (iPTH), and 25-hydroxyvitamin D [25(OH)D] are recommended to establish localized gestational reference intervals.

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APPENDIX: COMPLETE INDIVIDUAL CLINICAL DATASET (n = 199)

الملحق الإحصائي الشامل: كافة البيانات المخبرية الفردية لعينات الدراسة (149 حوامل + 50 شواهد)

This appendix contains the complete individual patient raw laboratory records across all study cohorts, evaluated via the CPC spectrophotometric assay (mg/dL) in Wadi Al-Shati district clinical laboratories.

Control Group (n=50)	1st Trimester T1 (n=50)	2nd Trimester T2 (n=50)	3rd Trimester T3 (n=49)
#1: 9.74 mg/dL	#1: 9.41 mg/dL	#1: 7.15 mg/dL	#1: 9.09 mg/dL
#2: 9.41 mg/dL	#2: 8.60 mg/dL	#2: 8.23 mg/dL	#2: 9.20 mg/dL
#3: 9.82 mg/dL	#3: 8.27 mg/dL	#3: 8.32 mg/dL	#3: 8.08 mg/dL
#4: 10.29 mg/dL	#4: 9.74 mg/dL	#4: 7.82 mg/dL	#4: 9.07 mg/dL
#5: 9.36 mg/dL	#5: 10.22 mg/dL	#5: 8.51 mg/dL	#5: 9.14 mg/dL
#6: 9.36 mg/dL	#6: 10.10 mg/dL	#6: 9.13 mg/dL	#6: 8.04 mg/dL
#7: 10.30 mg/dL	#7: 8.08 mg/dL	#7: 10.75 mg/dL	#7: 10.85 mg/dL
#8: 9.89 mg/dL	#8: 8.69 mg/dL	#8: 8.88 mg/dL	#8: 9.34 mg/dL
#9: 9.23 mg/dL	#9: 9.42 mg/dL	#9: 8.97 mg/dL	#9: 7.52 mg/dL
#10: 9.77 mg/dL	#10: 10.15 mg/dL	#10: 8.61 mg/dL	#10: 9.54 mg/dL
#11: 9.23 mg/dL	#11: 8.49 mg/dL	#11: 6.60 mg/dL	#11: 7.76 mg/dL
#12: 9.23 mg/dL	#12: 8.83 mg/dL	#12: 8.66 mg/dL	#12: 9.68 mg/dL
#13: 9.61 mg/dL	#13: 7.78 mg/dL	#13: 8.76 mg/dL	#13: 10.08 mg/dL
#14: 8.50 mg/dL	#14: 7.68 mg/dL	#14: 10.80 mg/dL	#14: 7.93 mg/dL
#15: 8.57 mg/dL	#15: 9.97 mg/dL	#15: 8.48 mg/dL	#15: 9.87 mg/dL
#16: 9.18 mg/dL	#16: 10.59 mg/dL	#16: 9.02 mg/dL	#16: 9.27 mg/dL
#17: 8.94 mg/dL	#17: 8.96 mg/dL	#17: 8.65 mg/dL	#17: 9.72 mg/dL
#18: 9.65 mg/dL	#18: 10.18 mg/dL	#18: 7.42 mg/dL	#18: 10.89 mg/dL
#19: 9.00 mg/dL	#19: 9.45 mg/dL	#19: 9.94 mg/dL	#19: 8.55 mg/dL
#20: 8.73 mg/dL	#20: 8.30 mg/dL	#20: 9.51 mg/dL	#20: 8.00 mg/dL
#21: 10.26 mg/dL	#21: 9.45 mg/dL	#21: 9.55 mg/dL	#21: 7.85 mg/dL
#22: 9.36 mg/dL	#22: 10.79 mg/dL	#22: 7.70 mg/dL	#22: 7.93 mg/dL
#23: 9.52 mg/dL	#23: 9.00 mg/dL	#23: 10.22 mg/dL	#23: 8.74 mg/dL
#24: 8.72 mg/dL	#24: 10.82 mg/dL	#24: 7.16 mg/dL	#24: 9.19 mg/dL

#25: 9.19 mg/dL	#25: 6.90 mg/dL	#25: 9.33 mg/dL	#25: 9.12 mg/dL
#26: 9.54 mg/dL	#26: 9.98 mg/dL	#26: 10.80 mg/dL	#26: 9.72 mg/dL
#27: 8.87 mg/dL	#27: 9.14 mg/dL	#27: 7.61 mg/dL	#27: 8.83 mg/dL
#28: 9.68 mg/dL	#28: 8.70 mg/dL	#28: 8.07 mg/dL	#28: 10.40 mg/dL
#29: 9.16 mg/dL	#29: 9.14 mg/dL	#29: 8.80 mg/dL	#29: 8.53 mg/dL
#30: 9.33 mg/dL	#30: 6.90 mg/dL	#30: 8.14 mg/dL	#30: 10.90 mg/dL
#31: 9.16 mg/dL	#31: 8.79 mg/dL	#31: 7.00 mg/dL	#31: 9.50 mg/dL
#32: 10.30 mg/dL	#32: 9.45 mg/dL	#32: 8.76 mg/dL	#32: 7.89 mg/dL
#33: 9.47 mg/dL	#33: 10.72 mg/dL	#33: 7.53 mg/dL	#33: 7.65 mg/dL
#34: 8.92 mg/dL	#34: 8.45 mg/dL	#34: 9.21 mg/dL	#34: 9.35 mg/dL
#35: 9.92 mg/dL	#35: 8.12 mg/dL	#35: 7.69 mg/dL	#35: 8.58 mg/dL
#36: 8.83 mg/dL	#36: 8.47 mg/dL	#36: 10.38 mg/dL	#36: 9.60 mg/dL
#37: 9.59 mg/dL	#37: 10.08 mg/dL	#37: 7.84 mg/dL	#37: 9.34 mg/dL
#38: 8.50 mg/dL	#38: 9.41 mg/dL	#38: 8.34 mg/dL	#38: 8.74 mg/dL
#39: 8.78 mg/dL	#39: 8.44 mg/dL	#39: 9.58 mg/dL	#39: 7.90 mg/dL
#40: 9.58 mg/dL	#40: 9.63 mg/dL	#40: 7.35 mg/dL	#40: 7.17 mg/dL
#41: 9.87 mg/dL	#41: 9.15 mg/dL	#41: 8.94 mg/dL	#41: 8.33 mg/dL
#42: 9.57 mg/dL	#42: 10.14 mg/dL	#42: 10.11 mg/dL	#42: 9.75 mg/dL
#43: 9.42 mg/dL	#43: 8.24 mg/dL	#43: 6.94 mg/dL	#43: 9.05 mg/dL
#44: 9.32 mg/dL	#44: 8.67 mg/dL	#44: 8.89 mg/dL	#44: 7.46 mg/dL
#45: 8.70 mg/dL	#45: 8.59 mg/dL	#45: 8.97 mg/dL	#45: 9.01 mg/dL
#46: 9.10 mg/dL	#46: 7.37 mg/dL	#46: 9.54 mg/dL	#46: 9.24 mg/dL
#47: 9.24 mg/dL	#47: 9.38 mg/dL	#47: 7.34 mg/dL	#47: 7.86 mg/dL
#48: 10.04 mg/dL	#48: 9.34 mg/dL	#48: 7.25 mg/dL	#48: 8.99 mg/dL
#49: 9.66 mg/dL	#49: 9.05 mg/dL	#49: 9.26 mg/dL	#49: 8.88 mg/dL
#50: 8.55 mg/dL	#50: 8.77 mg/dL	#50: 9.01 mg/dL	-