Section: Miscellaneous

Original Research Article

A COMPARATIVE STUDY OF CALCIUM LEVEL IN NORMOTENSIVE PREGNANT WOMEN AND PREECLAMPTIC WOMEN

Pragati Divedi[1], Adil Rahman[2], Sushil kumar shukla[3], Akshay jouhary[4]

[1]*Associate Professor, Department of Obstetrics and Gynaecology, Uttar Pradesh University of Medical Sciences, Saifai Etawah, Uttar Pradesh, India*
[2]*Professor & Head, Department of Biochemistry, Uttar Pradesh University of Medical Sciences, Saifai, Etawah, Uttar Pradesh, India*
[3]*Professor (Statistics), Department of Community Medicine, Uttar Pradesh University of Medical Sciences, Saifai, Etawah Uttar Pradesh, India*
[4]*PGJR-3, Department of Biochemistry, Uttar Pradesh University of Medical Sciences, Saifai, Etawah, Uttar Pradesh, India*

Received *: 05/04/2025*
Received in revised form *: 18/05/2025*
Accepted *: 09/06/2025*

Corresponding Author:
Dr. Akshay Jouhary,
PGJR-3, Department of Biochemistry, Uttar Pradesh University of Medical Sciences, Saifai, Etawah, Uttar Pradesh, India
Email: akshayjouhary135690@gmail.com

DOI: 10.70034/ijmedph.2025.2.405

Source of Support: Nil,
Conflict of Interest: None declared

Int J Med Pub Health
2025; 15 (2); 2240-2244

ABSTRACT

Background: Preeclampsia is a hypertensive disorder of pregnancy associated with significant maternal and fetal morbidity. Emerging evidence suggests a possible link between altered calcium metabolism and the development of preeclampsia. The objective is to compare total serum calcium levels in normotensive pregnant women and those diagnosed with preeclamptic women.
Materials and Methods: This analytical cross-sectional study included 116 pregnant women aged 18–40 years, with gestational age from 30 weeks to term. The participants were divided into 58 normotensive pregnant women and 58 preeclamptic women, based on standard clinical criteria (blood pressure ≥140/90 mm Hg). Women with chronic medical conditions that could influence calcium levels were excluded. Blood samples were analyzed for total serum calcium using standard biochemical methods.
Results: A significant difference in total serum calcium levels was observed between the two groups. Preeclamptic women had notably lower calcium levels compared to normotensive pregnant women.
Conclusion: The study demonstrates a clear association between reduced total calcium levels and preeclampsia, supporting the hypothesis that calcium deficiency may play a role in its pathogenesis. These findings highlight the potential importance of calcium monitoring and supplementation in pregnancy to reduce the risk of preeclampsia and improve maternal outcomes.
Keywords: Preeclampsia, Normotensive pregnancy, Total serum calcium, Hypertensive disorders, Calcium deficiency, Maternal health, Pregnancy outcomes, Calcium supplementation.

INTRODUCTION

Preeclampsia is a complex pregnancy disorder causing global maternal and fetal complications.[1] Preeclampsia is known as "the disease of theories".[2] Preeclampsia causes maternal-fetal risks, prematurity, and long-term maternal heart disease.[3] Preeclampsia's cause is unclear despite research; it harms the mother and fetus. Pregnancy hypertension: systolic ≥140 mmHg and/or diastolic ≥90 mmHg.[4] Severe hypertension: systolic ≥160 or diastolic ≥110 mmHg. ACC/AHA defines hypertension in nonpregnant adults as ≥130/80 mmHg; may apply to pregnancy.[4] Preeclampsia is more commonly observed in developing countries than in developed countries.[5] Preeclampsia affects 2–8% of pregnancies worldwide, varying by race and region.[6] Studies show higher preeclampsia rates in Latin America, Africa and lower in Asians.[7] Unknown cause of pregnancy hypertension; immune changes and poor placental perfusion are suggested. The exact cause of hypertensive disease in pregnancy is unknown, but immunologic changes and placental dysfunction leading to poor utero-placental perfusion are proposed factors. In normal pregnancy, cytotrophoblasts invade the myometrium and spiral arteries to form vascular anastomoses that perfuse the

placenta and fetus.[8] In pre-eclampsia, cytotrophoblasts lack an invasive phenotype, leading to poor spiral artery invasion and inadequate anastomosis formation.[9] Preeclampsia involves elevated NK cells, inflammation markers, Th1-driven cytokines, autoantibodies, and oxidative stress, worsening ischemia, and tissue damage.[10] Abnormal ischemia and placentation may trigger preeclampsia by releasing anti-angiogenic and pro-inflammatory proteins into maternal circulation.[11] This leads to endothelial dysfunction, causing preeclampsia. Key biomarkers linked to its onset are PlGF and sFlt-1.11. Calcium, the body's most abundant mineral, is essential for bone formation, muscle contraction, and hormone and enzyme activity. In extracellular fluid, it exists in three forms: ionic, protein-bound, and complexed.[12] Plasma, with a mean calcium level of 9.5 mg/dL (2.38 mmol/L), holds nearly all blood calcium in three forms: ionized (50%, biologically active), protein-bound (40%—80% to albumin, 20% to globulins), and complexed.[13] Calcium is intra- or extracellular. Extracellular Ca supports intracellular Ca, bone mineralization, coagulation, and membrane potential. It stabilizes membranes, influencing permeability and excitability. Low plasma-free Ca increases neuromuscular excitability, risking tetany.13 Calcium deficiency is a key factor in pre-eclampsia, a pregnancy complication. Growing research links low Ca to increased risk, highlighting the role of supplements in preventing pre-eclampsia and improving maternal and fetal outcomes.[14]

Diagnostic Criteria[15]

Condition	Criteria Required
Gestational Hypertension	BP > 140/90 mm Hg after 20 weeks in previously normotensive women
Preeclampsia: Hypertension plus	
• Proteinuria	≥ 300 mg/24h, or Urine protein: creatinine ratio ≥ 0.3, or Dipstick 1+ persistent
• Thrombocytopenia	Platelets < 100,000/μL
• Renal insufficiency	Creatinine > 1.1 mg/dL or doubling of baseline
• Liver involvement	Serum transaminase levels are twice normal
• Cerebral symptoms	Headache, visual disturbances, convulsions
• Pulmonary edema	May be ++

Biochemistry of Calcium: With a mean calcium concentration of —9.5 mg/dL (2.38 mmol/L), plasma contains almost all of the calcium in blood. Three physiological states of calcium are present in plasma: i)The free (ionized) fraction (50%) is the biologically active form.ii) protein-bound calcium (40%)- 80% of which is associated with albumin and the remaining 20% with globulins. iii) complexed.[13]

Physiological functions of Calcium: Calcium is classified as intracellular or extracellular. Both intracellular and extracellular calcium are important for hormone secretion, muscle contraction, cell division, and glycogen metabolism. The intracellular Ca concentration in the cytosol of unstimulated cells is about 0.1 μmol/L, which is lower than 1/20,000 of the extracellular fluid concentration. The extracellular Ca supplies calcium ions for the maintenance of intracellular Ca, bone mineralization, blood coagulation, and plasma membrane potential. Ca stabilizes plasma membranes & affects permeability & excitability. There is a decrease in plasma-free Ca concentration raises neuro-muscular excitability and can result in tetany.[13]

Calcium Homeostasis: There is a unified hormonal system that regulates calcium transport in the stomach, bone, and kidney, which plays a major part in maintaining calcium homeostasis. It includes serum ionized Ca & calcium-sensing receptor (CaR), as well as the two main calcium-regulating hormones with their receptors, Parathyroid hormone (PTH) with PTH receptor (PTHR) & 1,25(OH)2D with vitamin D receptor (VDR).

Serum Ca homeostasis has developed to permit calcium to move to and from essential stores while also keeping extracellular ionized calcium levels within the physiological range. Reduced serum calcium causes the parathyroid glands' CaR to become inactive, increasing PTH secretion. This, in turn, increases tubular Ca reabsorption inside the kidney and net bone resorption in the bone via acting on the PTHR. The increased PTH further encourages the kidney to increase 1,25(OH)2D secretion, which activates VDR in the gut to enhance the calcium absorption, in bone to increase resorption, and in parathyroid glands to decrease PTH secretion. To enhance calcium reabsorption and intensify the effects of PTH, the drop in serum calcium most likely also deactivates the kidney's CaR. The negative feedback loop is closed and serum calcium is restored by this coordinated hormonal response. These effects are reversed when serum Ca levels rise, and the unified hormonal response lowers serum calcium levels. Collectively, these negative feedback mechanisms upkeep total serum Ca levels in healthy individuals somewhat within a slight physiologic range of ~10%.16Increased bone turnover, raised Ca excretion in urine, and increased intestinal calcium absorption are all components of the Ca homeostatic response in pregnancy. The skeletal system of a newborn baby contains around 20–30 g of Ca. The major part of fetal skeletal growth takes place by midpregnancy onward, and maximal Ca accretion occurs during the third trimester.

It was initially reported in 1980 that a negative correlation occurred between calcium consumption and pregnancy-related hypertension problems. The finding that Mayan Indians in Guatemala customarily soak the corn in lime before cooking it. So, they consume a lot of Ca & a low rate of pre-eclampsia and eclampsia observed in them. A minimal

prevalence of pre-eclampsia is reported from Ethiopia, where people consume high levels of Ca in food.[15-17]

MATERIALS AND METHODS

The study was approved by the Institutional Ethical Committee of Uttar Pradesh University of Medical Sciences, Saifai, with written informed consent obtained from all participants after full explanation.

Study Design: Comparative cross-sectional.

Population: Pregnant women aged 18–40 years, gestation from 30 weeks to delivery.

Location: Gynaecology and Obstetrics OPD, IPD, and Biochemistry Department at Uttar Pradesh University of Medical Sciences, Saifai, Etawah.

Duration: 1and half year

Inclusion Criteria:

- Written informed consent obtained.
- Pregnant women aged 18–40 years.
- Blood pressure ≥140/90 mm Hg.

Exclusion Criteria:

- History of chronic hypertension, diabetes, renal, liver, thyroid, or heart disease.
- Previous preeclampsia or any condition affecting calcium or magnesium levels.
- Sample Size Estimation: —The minimum sample size was calculated considering the prevalence (p) of 3.6% using the formula $4pq/l^2$, where:
- p = prevalence of preeclampsia (3.6%)
- q = 100 - p =(100 - 3.6)
- Allowable precision error (l) = 5

The minimum sample size was found to be 4 × 3.6 × (100 - 3.6) / 25 = 55.5264.

Therefore, the study was carried out with 58 normotensive pregnant women and 58 preeclamptic women.

Total sample size -116

Sample Collection and Processing

Blood collection: After taking consent of participants, with all aseptic precautions, we withdrew 3ml of venous blood from antecubital vein by sterile disposable plastic syringe and transferred the sample immediately into a plain test tube from the inpatients and outpatients' department of Obstetrics and Gynaecology to the central lab of biochemistry immediately. There, we kept the sample for 30 minutes to clot. After 30 minutes, Centrifugation of sample at 3000 rpm for 10 minutes was done to prepare serum, then the serum was separated and stored and analysed immediately. If by any reason there was delay, it was stored at 2-8° C. It was analysed after that to evaluate the Total Calcium level.

Processing And Method of Estimation of Total Calcium.[18,19]

Principle of the Method: Calcium in the sample reacts with arsenazo III, forming a coloured complex that can be measured by spectrophotometry.

Composition: Reagent-Arsenazo III 0.2mmol//L, imidazole 75 mmol/L

Storage and stability: —Store at 2-8c.

On board stability: Reagents open and kept in the refrigerated compartment of the analyzer are stable for 2 months.

Reagent preparation: Reagents are provided ready to use. (Manufactured by BioSystems)

Calibration: Manufactured by BioSystem.

Quality Control: Control -Human I, II, Two time run/day

Metrological characteristics: Obtained using a BA400 analyzer and following the guidelines of the Clinical and Laboratory Institute

Detection limit: 0.42mg/dl=0.105mmol/L

Linearity limit: 18mg/dl=4.5mmol/L

Precision.

Serum Mean Concentration	Repeatability (CV)	Within-Laboratory (CV)
10.6 mg/dL=2.65 mmol/L	0.7%	1.0%
14.3mg/dL=3.57mmol/L	0.7%	0.9%

RESULTS

Our study included a total of 116 participants (pregnant women of 18-40 years of age), which were divided in the following groups: 1. Normal pregnant women (n=58), 2. Preeclamptic women (n=58) illustrate the comparison of serum mineral levels in both groups. There was a highly significant depression in total calcium levels ($p<0.01$) observed in preeclamptic as compared to normal pregnant women.

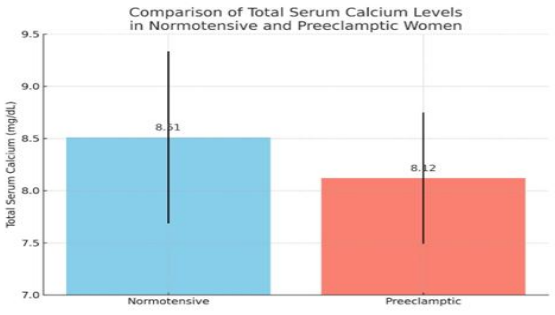


Table 1: Comparative analysis of serum Calcium levels in Normal pregnant and preeclamptic women.

Parameter	Normal pregnant women (Mean ± SD)	Preeclamptic women (Mean ± SD)	p-value
Total Calcium (mg/dL)	8.51 ± 0.825	8.12 ± 0.628	0.002**

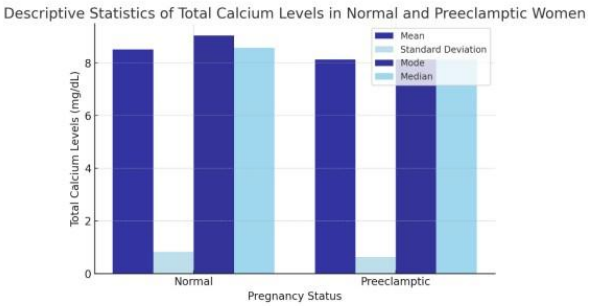
STATISTICAL ANALYSIS

Data was Analyzed using Statistical package for social science software (SPSS) version [24.0]. Descriptive statistics were used to summarize the data. Comparison was done by Student's unpaired t-test‖ taking both groups. The chi- square test was used to find out association among the variable. The results were expressed as Mean ± SD , percentage

(%) and the p- value of p≤0.05 was considered to be significant.

Table 2: Descriptive statistics of Total Calcium Level in Normal pregnant and preeclamptic women

Pregnancy Status	Total Calcium (mg/dL)			
	Mean	Standard Deviation	Mode	median
Normal	8.51	0.825	9.04	8.57
Preeclamptic	8.12	0.628	8.12	8.12



DISCUSSION

Pre-eclampsia is a hypertensive disorder in pregnancy, specified by high blood pressure & proteinuria, which can lead to maternal & fetal morbidity and fatality. It is believed to be a multifactorial disorder involving vascular dysfunction, oxidative stress, and inflammation. Calcium (Ca) is an important mineral involved in various physiological functions and has been implicated in the pathogenesis of preeclampsia. Previous studies had reported alterations in total Ca level in normo-tensive pregnant women in contrast to preeclamptic women. The aim of our study was to compare the Total Calcium level in Normotensive Pregnant Women and Preeclamptic Women.[18,19]

Above Table describes that the mean total Ca level in normal pregnant women was 8.51 ± 0.825 mg/dL, whereas in preeclamptic women, it was 8.12 ± 0.628 mg/dL, which is a statistically significant difference ($p = 0.002$). Similarly, in their research, Jayaraman Nambiar et al [20] (2023) had reported that the mean total Ca level in normotensive women is 8.87 ± 0.37 mg/dL. For preeclamptic women, the value is slightly lower at 8.74 ± 0.74 mg/dL; value differences are not statistically significant, though. The finding of significantly lower calcium levels in preeclamptic women was also observed in study by Uddin et al,[21] (2023) and the meta-analysis by Eslamzadeh et al,[22] (2023) indicating a consistent finding that calcium levels were lower in preeclamptic women and, therefore, further confirming the hypothesis of calcium deficiency as a potential risk factor for preeclampsia.[23]

Future research should emphasize early detection biomarkers, larger populations, and the effectiveness of mineral supplementation in preventing adverse pregnancy outcomes. The burden of preeclampsia-related complications can be reduced by implementing public health strategies to promote adequate maternal nutrition, which can improve maternal and neonatal health outcomes. There is necessity of research to refine prevention and management approaches for this complex pregnancy disorder.

Limitations of the Study

This study has some limitations: The sample size was small, so the results may not apply to all pregnant women. A study on large population would provide stronger conclusions.

We only measured calcium level at one time in pregnancy. Repeated follow up can give good insight about association of mineral levels with severity of disease.

We did not consider dietary intake or supplements, which could affect the results.

Future research should include larger groups, dietary factors, and additional biochemical markers to better understand preeclampsia and its prevention.

CONCLUSION

This study provides compelling evidence of a significant association between reduced serum calcium levels and preeclampsia in pregnant women. By comparing normotensive and preeclamptic women, we observed a consistent pattern of lower calcium concentrations in the preeclamptic women group, underscoring the possible role of calcium deficiency in the development or exacerbation of hypertensive disorders during pregnancy. The findings align with previous research suggesting that calcium plays a critical role in vascular smooth muscle function, endothelial stability, and blood pressure regulation. Hypocalcemia may contribute to increased vascular resistance, altered placental perfusion, and systemic endothelial dysfunction — all hallmark features of preeclampsia.

The clinical implications of these results are significant. Monitoring calcium levels during routine antenatal check-ups could help in the early identification of women at higher risk for preeclampsia. Furthermore, dietary counseling and calcium supplementation in deficient individuals may serve as a simple and cost-effective preventive strategy, particularly in resource-limited settings where preeclampsia remains a major cause of maternal and fetal morbidity and mortality.

However, while this study strengthens the observed correlation between calcium levels and preeclampsia, it does not establish causality. Further randomized controlled trials and mechanistic studies are warranted to explore the efficacy of calcium supplementation in preventing or mitigating preeclampsia. In conclusion, maintaining optimal calcium levels during pregnancy should be considered a priority in prenatal care, as it may play a crucial role in improving pregnancy outcomes and maternal health.

REFERENCES

1. Dymara-Konopka W, Laskowska M. The role of nitric oxide, asymmetric dimethylarginine, and homocysteine in the etiopathogenesis of preeclampsia. Int J Mol Sci. 2019 Jun 5;20(11):2757.
2. Onyegbule OA, Meludu SC, Dioka CE, et al. Comparison of serum levels of calcium and magnesium among preeclamptic and normotensive pregnant women at Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria. Int J Res Med Sci. 2014 Apr;2(2):404–8.
3. Phipps EA, Thadhani R, Benzing T, Karumanchi SA. Pre-eclampsia: pathogenesis, novel diagnostics and therapies. Nat Rev Nephrol. 2019 May;15(5):275–89.
4. Sisti G, Colombi I. New blood pressure cut off for preeclampsia definition: 130/80 mmHg. Eur J Obstet Gynecol Reprod Biol. 2019;240:322.
5. Winarno GN, Pribadi A, Maruli HJ, et al. Ratio of serum calcium to magnesium levels on pregnancy with and without preeclampsia. Med Sci Monit. 2021;27:e932032-1.
6. Chu H, Ramola R, Jain S, Haas DM, Natarajan S, Radivojac P. Using association rules to understand the risk of adverse pregnancy outcomes in a diverse population. Pac Symp Biocomput. 2023;28:209–20.
7. Osungbade KO, Ige OK. Public health perspectives of preeclampsia in developing countries: implication for health system strengthening. J Pregnancy. 2011;2011:481095
8. Redman CWG, Staff AC, Roberts JM. Syncytiotrophoblast stress in preeclampsia: the convergence point for multiple pathways. Am J Obstet Gynecol. 2022;226(1 Suppl):S907–27.
9. Ridder A, Giorgione V, Khalil A, Thilaganathan B. Preeclampsia: the relationship between uterine artery blood flow and trophoblast function. Int J Mol Sci. 2019;20(1):1–14.
10. Amaral LM, Wallace K, Owens M, LaMarca B. Pathophysiology and current clinical management of preeclampsia. Curr Hypertens Rep. 2017;19(8):61.
11. Rana S, Lemoine E, Granger JP, Karumanchi SA. Preeclampsia: pathophysiology, challenges, and perspectives. Circ Res. 2019;124(7):1094–112.
12. Kumar A, Kaur S. Calcium: a nutrient in pregnancy. J Obstet Gynaecol India. 2017 Oct;67(5):313–8.
13. Nader R, Horvath AR, Wittwer CT. Tietz fundamentals of clinical chemistry and molecular diagnostics. 8th ed. St. Louis: Elsevier; 2023.
14. Hassan SM, Khashan AS. Nutritional deficiencies and pregnancy complications. Nutrients. 2015;7(5):4218–29.
15. Cunningham FG, Leveno KJ, Bloom SL, et al. Williams obstetrics. 26th ed. New York: McGraw-Hill; 2022.
16. Nader R, Horvath AR, Wittwer CT. Tietz fundamentals of clinical chemistry and molecular diagnostics. 8th ed. St. Louis: Elsevier; 2023.
17. Peacock M. Calcium metabolism in health and disease. Clin J Am Soc Nephrol. 2010 Jan;5(Suppl 1):S23–30.
18. Kumar A, Kaur S. Calcium: a nutrient in pregnancy. J Obstet Gynaecol India. 2017 Oct;67(5):313–8.
19. Burtis CA, Ashwood ER, Bruns DE. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 4th ed. Philadelphia: WB Saunders Co.; 2005.
20. Michaylova V, Ilkova P. Photometric determination of micro amounts of calcium with Arsenazo III. Anal Chim Acta. 1971;53:194–8.
21. Jayaraman Nambiar, Patil P. Serum calcium, magnesium and calcium–magnesium ratio in women with preeclampsia: a prospective observational study. Indian Obstet Gynaecol. 2023 Jan-Mar;13(1):1–5.
22. Uddin SN, Haque M, Barek MA, et al. Analysis of serum calcium, sodium, potassium, zinc, and iron in patients with pre-eclampsia in Bangladesh: a case–control study. Health Sci Rep. 2023 Feb;6(2):e1097.
23. Eslamzadeh A, Kianfar H, Hasanpour M, et al. Serum calcium and magnesium levels in preeclampsia: a systematic review and meta-analysis. J Hypertens. 2023;41(5):1026–34.