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STUDY PROTOCOL

Maternal Serum Vitamin D Levels and Pre-eclampsia among Kenyan Pregnant Women: protocol for a matched case-control pilot study

[version 1; peer review: awaiting peer review]

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Abstract

Background

Preeclampsia is considered among the top causes of avoidable maternal death in low- and middle-income countries (LMICs), making it a significant social, economic, and healthcare system burden. Maternal vitamin D deficiency could be a key factor in the pathophysiology of pre-eclampsia. Worldwide, the link between vitamin D deficiency and pre-eclampsia still has mixed results, with minimal data on this topic from Africa. This pilot study aims to determine the association between maternal serum vitamin D levels in pregnancy and the risk of preeclampsia in a Kenyan population.

Methods

This case-control study will be single-centered and conducted at Moi Teaching and Referral Hospital (MTRH), Kenya. A total of 120 women with confirmed viable intrauterine pregnancies at ≥28 weeks' gestation will be included. Sixty with pre-eclampsia (PE) as cases and 60 normotensive controls matched by parity, age, and gestation.

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Any reports and responses or comments on the article can be found at the end of the article.

Serum 25-hydroxyvitamin D [25(OH)D] will be quantified. The proportion of vitamin D deficiency (25(OH) D ˂30 nmol/L) among the study sample will be assessed. The vitamin D status among pregnant women with and without pre-eclampsia will be compared.

Results

We anticipate an enrollment of 120 pregnant women, 60 cases with PE and 60 matched controls. We hypothesize that pregnant women with PE will exhibit a significantly higher proportion of vitamin D deficiency compared to normotensive controls. Multivariable logistic regression is expected to demonstrate a significant association between vitamin D deficiency and odds of pre-eclampsia.

Conclusion

Maternal serum Vitamin D levels and any relationship with Pre-eclampsia among this cohort will be clarified.

Keywords
Vitamin D, Hypertension, Pregnancy-induced, Case-control , Pregnancy complications, Kenya

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Introduction

Hypertensive disorders of pregnancy (HDP) refer to a group of medical disorders characterised by high blood pressure occurring during pregnancy. HDP impacts up to 10% of pregnancies worldwide.[1] According to the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy, HDP is classified into 4 categories; Chronic hypertension, preeclampsia (and related disorders: eclampsia and haemolysis, elevated liver enzymes, low platelets (HELLP syndrome)), preeclampsia superimposed on chronic hypertension and gestational hypertension.[2]

In Kenya, approximately 1 in 5 direct maternal deaths result from hypertensive disorders, including pre-eclampsia.[3]

Pre-eclampsia (PE) aetiology can be divided into two distinct phases. The first phase is characterised by defective placentation. The progression into the second phase is marked by decreased vascularisation occurring at the placental site, which initiates a maternal inflammatory response. PE modifies angiogenic as well as antiangiogenic factors, e.g., serum placental growth factor (PIGF), soluble fms-like tyrosine 1 (sFlt-1), and soluble endoglin (sENG).[1]

Vitamin D is a family of chemically related secosteroid hormones. A primary way in which vitamin D is synthesised is through the skin's exposure to UVB radiation from sunlight (wavelength, 290 to 315 nm). Solar ultraviolet B radiation infiltrates the skin and converts 7-dehydrocholesterol into provitamin D3, t that is quickly transformed into vitamin D3. Both vitamin D from the skin and ingested through diet is metabolised in the liver into 25-hydroxyvitamin D (25(OH)D)—a vitamin D status biomarker & into 1,25-dihydroxyvitamin D ($1,25(OH)_2D$).

Although no consensus has been reached on vitamin D status definitions, The Endocrine Society considers deficiency as serum 25(OH) D < 20 ng/ml (50 nmol/L); in contrast The Institute of Medicine (IOM) and the National Osteoporosis Society adopt a more conservative 25(OH) D level threshold for bone health, defining deficiency as concentrations below 12 ng/ml (30 nmol/L).[4]

Pregnant women belong to the vulnerable population with vitamin D deficiency risk. Factors such as inadequate intake of vitamin D, high BMI, and a poor-quality diet further heighten this risk.

Several mechanisms can elucidate the protective effects of vitamin D in PE. Firstly, vitamin D modulates pro-inflammatory responses, a key factor in the pathogenesis of PE. This occurs by activation of receptor elements in vascular endothelial growth factor (VEGF), promoting angiogenesis and supporting placental perfusion.[5] Additionally, vitamin D plays a role in cholesterol uptake by vascular smooth muscle cells of the arterial walls and macrophages, a pathology which is observed in utero in placental vessels of patients with preeclampsia.[5] Vitamin D also contributes to blood pressure reduction through the renin-angiotensin system (RAAS) by regulating endothelial and vascular smooth muscle cell proliferation.[6]

A systematic review in 2020 that included pooled data from twenty-seven randomised controlled trials (RCTs) involving 4,777 participants reported that vitamin D supplementation resulted in a 63% risk reduction in the occurrence of PE.[7] Even so, the studies included in the review did not strictly select participants on the basis of vitamin D status, nor did they evaluate the achievement of optimum vitamin D levels post-supplementation. The authors also noted that the results could not be generalised to an African population, given the absence of studies that included this cohort.

Genetic and phenotypic diversity in vitamin D metabolism has been observed among different races, with individuals of European ancestry exhibiting higher Vitamin D concentrations.[8] Skin pigmentation negatively influences vitamin D synthesis as melanin acts as an absorbent filter (scattering UVR), reducing the efficiency of vitamin D production by UV-induction & subsequent circulating levels of 25(OH) D.[9,10] Mogire et al. observed that many African countries lacked studies measuring vitamin D levels.[11]

This study aims to ascertain the rate of vitamin D deficiency among parturients with viable pregnancies and to compare the vitamin D levels between women with & without preeclampsia.

The research will allow us to identify the link between vitamin D & PE within a Kenyan context. These findings will provide evidence for the potential value of antenatal screening for maternal vitamin D status, which does not exist currently. Moreover, these results will provide a basis for further research (in Kenya and comparable settings) into the effectiveness of vitamin D as a preventive and/or therapeutic intervention for PE.

Broadly, the findings will potentially inform broader maternal health measures, enhancing maternal and pregnancy outcomes.

Aims and objectives
The study aims to describe vitamin D status during pregnancy and to examine its association with PE.

Objectives
1. To describe the proportion of vitamin D deficiency among the study sample of women with viable pregnancy at Moi Teaching and Referral Hospital (MTRH).

2. To compare vitamin D status among pregnant women with and without pre-eclampsia.

Methods
Study design and setting
This will be a single-center matched case-control study, conducted at The Moi Teaching and Referral Hospital (MTRH) in Eldoret, Kenya. MTRH is a public multi-speciality public tertiary healthcare facility with about 12,000 deliveries annually. The referral population includes western Kenya region, parts of eastern Uganda, southern Sudan, northern Tanzania, and the Democratic Republic of Congo. The study is intended to span October 1st 2025 to March 31st 2026.

Cases will include pregnant women with viable pregnancies (≥28 weeks gestation) diagnosed with PE. Controls will comprise normotensive pregnant women matched to cases by maternal age (±3 years), gestational age (±1 week), and parity (0, 1, ≥2).

Eligibility criteria
Inclusion criteria will be: (a) maternal age of ≥ 18 years at the time of consent, (b) confirmed singleton pregnancy of 28 weeks gestation or greater (confirmed by ultrasound), (c) delivery at MTRH (d) ability to provide written informed consent.

Exclusion Criteria: (a) pre-existing chronic kidney disease, (b) pre-existing parathyroid conditions, (c) those on cardiac medication therapy that includes calcium channel blockers, (d) pre-existing thrombophilia, (e) participating in a similar interventional study.

Recruitment and sampling strategy
Recruitment of cases will occur at the MTRH-Antenatal Ward (ANW). Cases will be identified prospectively through a daily review of the ANW admission register. The ANW admission register includes details such as patient name, age, inpatient number, room of admission and diagnosis impression.

Controls will be recruited concurrently from the ANW, matched 1:1 to the enrolled cases via the criteria previously described.

All participants will be identified by a trained research assistant who will then approach and introduce the study to them. This will be done in a private and comfortable manner at the ANW to ensure confidentiality. The purpose of the study and potential risks and benefits will be explained in a detailed manner in the patients' preferred language. Opportunity and time will be given for any clarifications or questions. Informed consent will be obtained prior to enrolment. The patients will be assured of their right to withdraw their study participation at any time, without impact on their medical management.

Purposive sampling will be applied, targeting women who will satisfy eligibility during the study window.

Sample size calculation
Sample size was calculated to detect a 25-or-more percentage point difference in Vitamin D deficiency (serum 25(OH) D <30 nmol/L) between cases and controls.

Baseline rate of vitamin D deficiency was set at 58·54% based on a systematic review by Mogire et al.[11] Confidence interval was set at 95%, power at 0.80, alpha = 0.05, and an allocation ratio of 1:1 was used. Using Stata software command: . *sampsi 0.5854 0.8354, power(0.8) alpha(0.05) ratio(1)*, a total sample size of 118 participants was determined as appropriate. To cater for potential attrition, sample size was increased to 120 participants (60 cases and 60 controls). The study participants will be recruited using purposive sampling technique.

Research outcomes, covariates and measures

Primary exposure: Maternal serum 25(OH) D levels measured by ELISA (Lancet Laboratory, ISO15189-certified). Vitamin D status will be defined as normal (≥50 nmol/L), insufficient (30-49 nmol/L) or deficient (<30 nmol/L), as per the 2011 classification guidelines of the Institute of Medicine.[12]

Primary outcome: Pre-eclampsia status (binary: yes/no) confirmed through medical file review for diagnosis.

Pre-eclampsia will be defined prospectively according to the guidelines of the International Society for the Study of Hypertension in Pregnancy[11]; i.e., new-onset hypertension, defined as systolic blood pressure of 140 mmHg or greater and/or diastolic blood pressure of 90 mmHg or greater (based on an average of two measurements) in a patient who was previously normotensive, accompanied by at least one other symptom and sign related to pre-eclampsia. These symptoms and signs include proteinuria (≥300 mg/mmol or more than 0.3 g/day), acute kidney injury (creatinine levels at or greater than 90 µmol/l), liver involvement (elevated transaminases, for example ALT or AST exceeding 40 IU/l), neurological symptoms (such as altered mental status, blindness, stroke, severe headaches, persistent visual scotomata), haematological abnormalities (including thrombocytopenia i.e. platelet count below 150,000/µl, disseminated intravascular coagulation, haemolysis), cardiorespiratory complications (pulmonary oedema, myocardial ischaemia or infarction, oxygen saturation below 90%, use of 50% or more inspired oxygen for over an hour, intubation not related to caesarean delivery), or uteroplacental dysfunction (foetal growth restriction, angiogenic imbalance, placental abruption) occurring after 20 weeks gestation.

Key covariates: Demographic factors, including maternal age, parity, gestational age, and pre-pregnancy Body Mass Index (BMI) data, will be collected. Gestational age will be calculated from the first day of the last menstrual period. Parity will be defined as the number of times the participant has given birth to a foetus of ≥ 24 weeks gestational age, regardless of pregnancy outcome. BMI will be calculated as the ratio of weight in kilograms (kg) to height squared in metres squared (m^2). The use of Vitamin D supplements, including antenatal multivitamins and cod liver oil, will be defined as consumption of relevant pills for at least one month during pregnancy.

Participant and medical files reporting relevant medical diagnoses (chronic hypertension, diabetes) data will also be collected.

Data collection procedures and tools

Structured interviewer-administered questionnaires and review of medical files will be used to collect data.

The questionnaire will include socio-demographic, medical and reproductive history information. Dietary intake over the past year will be evaluated using the core questions from the WHO STEPS Instrument.[13] Information on sunlight exposure will be sought using questions adopted from the validated Sunlight Exposure Measurement Questionnaires (SEMQ).[14] Hospital files will be reviewed to provide additional details on medical and reproductive history. Interviews and medical file review will be carried out concurrently.

Interviewers and phlebotomists will receive training prior to commencement of the study to ensure precision in the data and sample collection process.

Review of medical records will be done to verify gestational age, comorbidities, and pre-eclampsia diagnosis.

Biological sampling and measurement of serum vitamin D: Maternal blood will be collected in the non-fasting condition by trained phlebotomists after the completed questionnaire. The phlebotomist will collaborate with clinical staff at MTRH to identify participants who are already scheduled for other blood tests. The aim being to have the study related blood sample collection performed during the same venipuncture session as their other required blood tests, whenever feasible.

Approximately 5 ml of a sample of whole blood will be obtained via venipuncture into serum separator tubes (Becton Dickinson, Franklin Lakes, NJ, USA). Once samples are obtained, they will be allowed to clot at room temperature for 30 minutes, then placed in an insulated cool box for transportation to Lancet laboratory (ISO15189 certified) for processing. The transportation temperature of the cool box shall be maintained at 2-8°C, ensured by the use of a temperature monitor. The research team phlebotomists will have comprehensive training to ensure adherence to Standard Operating Procedure (SOP) for sample handling and transportation. The transportation will take place within 2 hours. The circulating form of vitamin D - 25(OH)D3- is stable for up to two months independent of the storage temperature.[15] 25(OH) D is stable for up to 4 hours at room temperature and up to 24 hours at 2–8 °C[16] (Figure 1).

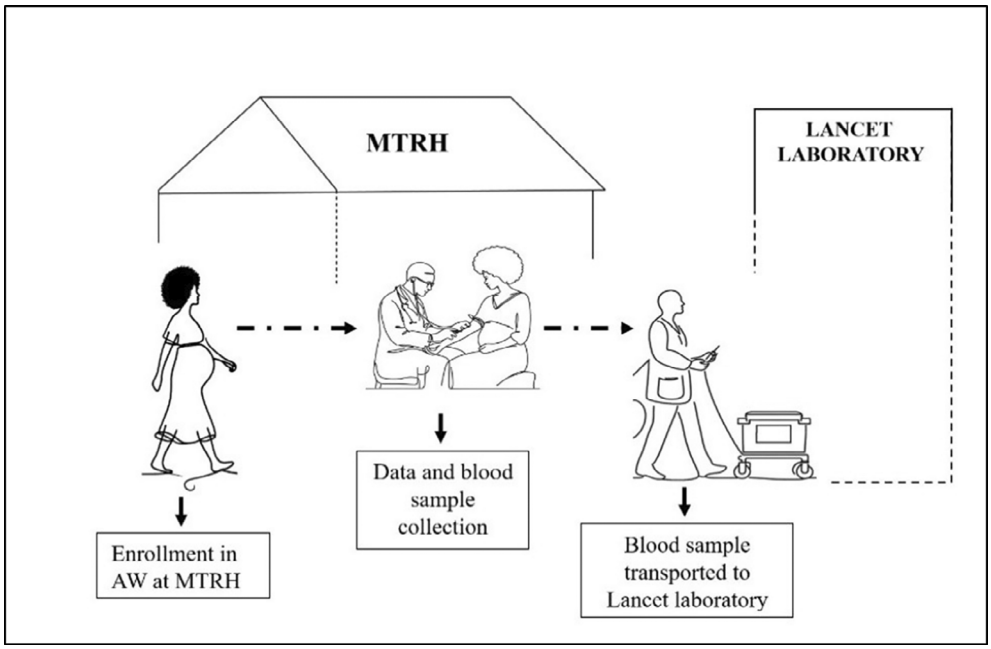

Figure 1. Participant flow diagram.

Measurement of serum 25(OH) D, [25(OH)D2 and 25(OH)D3 combined] will be conducted using a commercial ELISA kit.

Data will be recorded into a password-protected Microsoft Access 2021 database (Microsoft Corporation, Redmond, WA). Unique alphanumeric identifiers will be allocated to each participant; all personal identifiers will be removed from analytical datasets. Scheduled weekly audits, as well as regular systems backups, will be conducted to ensure data integrity.

Statistical analysis plan

All statistical analyses will be conducted using STATA software version 15.0 (Texas, USA).

Data normality will be assessed by the Kolmogorov-Smirnov test; skewed variables will undergo log transformation, with both raw and transformed results reported.

Baseline characteristics will be summarised and compared by pre-eclampsia status.

Continuous variables will be expressed as mean values with standard deviation or median with interquartile range (IQR) (maternal age, pre-pregnancy BMI, gestational age), and categorical variables as numbers and percentages. The means of the continuous variables will be compared using t-test, and if the normality assumption is violated, Mann-Whitney U test instead. The distribution of categorical variables will be examined using chi-square (χ^2) statistics or Fisher's exact test, as appropriate.

Proportion of vitamin D deficiency (25(OH) D <30 nmol/L) will be calculated as a percentage of the total sample (n = 120), along with a 95% confidence interval. Unadjusted χ^2 statistics will be used to compare proportions of women with vitamin D deficiency between cases/controls. Adjusted multivariable logistic regression will be applied to assess the odds of pre-eclampsia associated with vitamin D deficiency, adjusting for a priori confounders: maternal age, parity, gestational age and vitamin D supplementation. A forward stepwise model will retain covariates with $p < 0.2$ in univariate analysis and biological plausibility.

Multivariable linear regression will estimate the mean difference in vitamin D-25(OH) D (nmol/L) based on pre-eclampsia status with the same covariates.

Missing data will be handled by complete case analysis. The impact of missing data on results will be assessed through sensitivity analyses. All models will report effect sizes along with their 95% confidence intervals.

Discussion

PE remains a significant health concern in Kenya. The research aims to assess any link between PE and vitamin D. These findings will support any potential value of antenatal screening for maternal vitamin D status.

Given the chosen case-control study design and the relatively small sample size, the findings cannot be generalised to the broader pregnant population at MTRH. Interpretation of the results should remain preliminary and exploratory.

Ethical considerations

Written informed consent will be taken from each participant before enrolment, explaining the rationale, benefits and risks of the study, in accordance with Good Clinical Practice (GCP) principles and the Declaration of Helsinki.

Autonomy will be upheld by providing participants all the necessary information and freedom to withdraw from the study at any point without need for justification.

Confidentiality and privacy will be guaranteed. All data will be maintained as confidential, and participants will not be identified in any reports of the findings. Alphanumeric codes will be used in the structured proforma to protect the privacy of participants. Computers used for data entry and analysis will be password protected, accessible only to the principal investigator. Printed research data will be secured in a locked room with restricted access.

Risk assessment and mitigation

There is a risk of failed vitamin D tests and participant dropout: this will be mitigated by enrolling more participants than the minimum sample size required.

Risk of loss of data due to technical failures or human error will be addressed through a hard disc backup, weekly data integrity checks, and training of the research staff on data management protocols.

Ethical/Regulatory approvals

The study was prospectively registered with the Pan African Clinical Trial Registry (PACTR); registration number PACTR202505516303679. Protocol review and ethical approval were obtained from the Institutional Review & Ethics Committee of Moi University/MTRH (IREC approval number 0005019). Written authorisation was obtained from the management of MRTH to conduct the study (reference ELD/MTRH/R&P/10/2/V.2/2010). A research licence was also granted from the National Commission for Science, Technology, and Innovation (NASCOTI) Kenya, before commencement of the study (Licence number NACOSTI/P/25/417821).

Sex and gender considerations

As pre-eclampsia is biologically specific to gestational carriers, all participants will comprise pregnant individuals assigned female at birth. Sex will be confirmed via clinical obstetric history and medical records. Outcomes will be exclusively for women, with acknowledgement that findings are not generalisable to male or non-pregnant populations. Sex-stratified analysis will also not be applicable.

Plans for dissemination

Results will be disseminated through:

We intend to publish this work in a peer-reviewed open-access publication as well as community feedback sessions at MTRH for the healthcare providers and participating women.

Disclosure

Curie AI-powered language editing tool was used to assist with English language editing.

Data availability

No underlying data is associated with this protocol. Upon completion of the study, de-identified data and study instruments will be deposited in Zenodo (https://zenodo.org).

Reporting guidelines

This protocol was developed in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines.

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