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A cohort study on use of the spot urine calcium-creatinine ratio for prediction of antepartum preeclampsia among high-risk pregnant women in Delta State, Nigeria

Lloyd Jagu [1], V. Peter Orugbo [2], Patrick Okonta [1]

[1] Department of Obstetrics and Gynecology, Delta State University, Abraka, Delta State, Nigeria
[2] Department of Chemical Pathology, Delta State University, Abraka, Delta State, Nigeria

ORCID ID of the author(s)
LJ: 0000-0002-2290-5316
VPO: 0000-0001-6391-5732
PO: 0000-0001-6209-7336

Corresponding Author
V. Peter Orugbo
Department of Chemical Pathology, Delta State University, Abraka, Delta State, Nigeria
E-mail: drorugbopeter@gmail.com

Ethics Committee Approval
Ethical approval (Reference number, HREC/PAN/2019/006/0304; dated March 18, 2019) was sought and obtained from the Health Research Ethics Committee of CHW and DELSUTH and formal consent from the other centers.
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

Conflict of Interest
No conflict of interest was declared by the authors.

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Abstract

Background/Aim: Preeclampsia is a multisystemic disorder, which significantly contributes to maternal and fetal morbidity and mortality, especially in developing countries where it accounts for about one-third of maternal mortality cases. Predicting its occurrence will reveal a sizeable population of pregnant women who will undoubtedly benefit from prevention. The ideal screening marker for the disease is still being investigated. The urine calcium-creatinine ratio (CCR) is an inexpensive, simple, and easily assayed biomarker. This study determined the accuracy of the spot urinary calcium-creatinine ratio in predicting the occurrence of preeclampsia.

Methods: This was a prospective cohort study conducted in Delta State, which involved four healthcare facilities in Nigeria. A total of 138 pregnant women between 8 and 18 weeks gestation were recruited. Urine samples were obtained at 18 weeks to assay their CCR, and patients were followed up weekly for blood pressure measurement and dipstick urinalysis until delivery.

Results: The mean spot urine CCR in this study was 0.225 (0.101). It was significantly lower in women who developed preeclampsia compared to normotensive women ($P < 0.001$). Multiple logistics regression analysis showed that the association between urine CCR and occurrence of preeclampsia was statistically significant. At a receiver operating characteristic cutoff of ≤ 0.1065, CCR had a sensitivity of 75%, specificity of 91.3%, positive predictive value (PPV) of 35.3%, and negative predictive value (NPV) of 98.3%. The low PPV of 35.3% can be explained by the low prevalence of preeclampsia (5.78%) in the study population.

Conclusion: In conclusion, the poor PPV of the urine CCR was due to the low prevalence of preeclampsia in the study. However, in considering all women at risk, urine CCR may be a good prognostic marker when the illness prevalence is substantial.

Keywords: Preeclampsia, Urine calcium-creatinine ratio, Preeclampsia, Screening High-risk women, Significant proteinuria

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Introduction

Preeclampsia is a significant cause of maternal morbidity and mortality. It is defined as blood pressure ≥ 140/90 mmHg on two occasions at least 4 h apart, in the presence of significant proteinuria (≥ 300 mg in 24 h) or after 20 weeks of gestation [1–3]. According to the World Health Organization, preeclampsia is the second leading cause of maternal mortality globally with 76,000 maternal deaths estimated annually [4]. Its incidence is seven times higher in developing countries (2.8% of live births) than in developed countries (0.4%) [5, 6]. In Nigeria, the prevalence ranges between 2% to 16.7% [7–9]. While 10–15% of maternal deaths in developing nations are attributed to preeclampsia and its complications, the prevalence is 0–1.3% in industrialized nations [10–12]. The fetuses are not spared the numerous complications. In fact, about 500,000 neonatal deaths reported annually are a result of this disease [4]. It is estimated that 20 other women suffer from severe morbidity or disability from preeclampsia for every maternal death recorded. Near-miss cases are eight times more frequent in women with preeclampsia and 60 times more frequent if eclampsia occurs compared to women without these conditions [13]. These severe complications are multisystemic involving the central nervous, respiratory, and cardiovascular systems [5, 14, 15]. The fetus suffers from morbidities including intra-uterine growth restriction, oligohydramnios, placental abruption with evidence of fetal compromise, stillbirth, and prematurity [14, 16]. Long-term complications occur in both the mother and neonate and can be cardiovascular- or endocrine-related such as chronic hypertension, thromboembolism, and diabetes mellitus [5, 12]. Although its etiology is unknown, several theories have been proposed [16, 17]. One postulate is that distortion occurs during formation of the utero-placental unit in early pregnancy (8–18 weeks), resulting from a failure of trophoblastic invasion [1] and causing impaired placental perfusion with the generation and build-up of oxidative stress. Subsequent events are the development of systemic endothelial dysfunction leading to multisystemic disease [17-19], and possibly an imbalance between pro-angiogenic and anti-angiogenic factors [17]. There has been less reported about the prediction and prevention of preeclampsia than about the diagnosis and management of the disease. Nonetheless, prevention remains a core component in addressing this issue of public health relevance [1].

Biomarker analyses (clinical, biophysical, and biochemical) have been the focus of several research projects. Despite abundant research investigating the predictive accuracy of biomarkers, less has been done to identify the ideal biomarker. For most studies, outcomes appear unrealistic or have low replicability; other studies have poor validity indices or results that are too ambiguous to interpret [5, 6, 20]. Therefore, there is an urgent need for a marker that can predict preeclampsia in asymptomatic women in early pregnancy. In this study, the urine calcium-creatinine ratio (CCR) of pregnant women between 8 and 18 weeks gestation was analyzed in four healthcare centers in Delta State Nigeria.

Austdal et al. [21] showed that urine metabolomic profiles are better predictive markers than serum equivalents. Urine is an excretory product from which several biochemical analytes have been researched, many of which have been linked to disease processes. Normal urinary calcium concentration is 100 to 300 mg/24 h, which increases to 350–620 mg/dL in pregnancy., and normal urinary creatinine concentration is 1500 to 3000 mg/24 h [22]. The urine calcium concentration in women with preeclampsia is lower than that in their normotensive counterpart even when serum calcium levels are not significantly different [23–25]. Urine calcium is measured either from 24 h urinalysis or random spot samples. Urine calcium has a diurnal pattern that peaks at about midday [26]. The 24 h estimation is a more reliable estimate of total calcium; however, it has a number of drawbacks. Early morning spot urine samples correlate well with 24 h collection [27]. Estimation of early morning spot urine sample is time saving, convenient, and less cumbersome than 24 h estimation, which is error prone from contaminants arising mostly at the collection point. Random urine calcium is expressed as the ratio of calcium to creatinine called the CCR or fractional excretion of calcium [28-30]. Creatinine serves as a reference standard due to its relatively constant excretion rate throughout a 24 h period [29, 31-33].

The predictive value of urine CCR in preeclampsia has been shown in some studies; however the biomarker was mostly employed at ≥ 20 weeks gestation where it represented an early diagnostic tool rather than a predictive marker for the pathology [28-36]. Preeclampsia tends to develop between 8 and 18 weeks gestation, triggered by failure of trophoblastic invasion with resultant clinical manifestations occurring mostly after 20 weeks of gestation. Accordingly, certain preventive measures such as low-dose aspirin are recommended in the first trimester for women at high risk of preeclampsia. Therefore, the need to identify early in pregnancy, those likely to develop the disease cannot be overemphasized. Studies evaluating urine CCR as a predictive marker for preeclampsia in the African population were not found during our literature search. Thus, studies are needed to determine if the urine metabolome will be effective as a predictive biomarker for preeclampsia among asymptomatic Negroid women in early pregnancy (between 8 and 18 weeks). The results of this study will not only provide knowledge on the CCR of high-risk women in Nigeria but will also ascertain its relevance as a predictive marker of preeclampsia. Accurate prediction of preeclampsia will allow better counseling and closer monitoring of 'at risk pregnant' women and will facilitate prevention, early detection, and timely intervention, which will minimize the complications associated with the disease.

Therefore, this study investigated the predictive value of urine CCR among high-risk women in early pregnancy in hospitals in Delta State Nigeria.

Materials and methods

Study design

This was a prospective cohort study that determined the indices of validity of the spot urine CCR ratio for the prediction of preeclampsia in high-risk pregnant women. Participants included consecutively recruited women with risk factors for preeclampsia at the antenatal clinic or in the ward. All recruits were followed up for four times weekly with blood pressure measurement and dipstick urinalysis conducted at each antenatal visit until delivery to monitor the development of preeclampsia.

A spot urine sample, which is the first morning urine, was obtained from each recruit at 18 weeks and analyzed for urine CCR.

Study location

This study took place between November 2019 and September 2020 and involved the following four healthcare facilities in close proximity to one another in Delta State: Delta State University Teaching Hospital (DELSUTH), Oghara; Central Hospital, Warri; General Hospital, Oghara; and Central Hospital, Sapele. DELSUTH and Central Hospital, Warri have accreditation for residency training in Obstetrics and Gynecology by the National Postgraduate Medical College of Nigeria. There is a memorandum of understanding between hospitals that are part of the Hospital Management Board and Delta State University Teaching Hospital. They all have similar protocols for the diagnosis and management of preeclampsia. As at the time of this study, the Department of Obstetrics and Gynecology at DELSUTH had 9 consultants and 23 resident doctors at different stages of postgraduate training. The Department of Obstetrics and Gynecology in Central Hospital, Warri has 4 consultants and 10 resident doctors also at different stages of training. Central Hospital, Sapele has two consultants. Together, the institutions have a combined annual delivery rate of 7,500, and residents rotate among the hospitals. They collaborate in the training of medical students of DELSUTH and the training of resident doctors at all levels. DELSUTH and Central Hospital, Warri are located about 40 km apart, along the federal east-west highway and provide specialist Obstetrics and Gynecological care to patients. They are the major referral centers to Delta State as well as neighboring towns/villages in Edo and Bayelsa States. Patients are usually referred from private medical centers, government-owned healthcare centers, and general hospitals as well as from other departments in these hospitals.

Recruitment of study participants

The study population consisted of pregnant women attending antenatal clinics in the study centers who had risk factors for preeclampsia. The sample size was calculated using the formula for the cohort Study [37], using a previous study by Rashmi and Indu [35], which share similar characteristics as the study references. With an attrition rate of 20%, an additional 12 women were recruited per group, yielding a total of 72 women per study group. Thus, a total of 144 participants were recruited for the study. Research assistants assisted resident doctors and nurses in recruiting women from antenatal clinic and wards and following up with them until delivery in all four centers. Study personnel also included a chemical pathologist at DELSUTH and Central Hospital, Warri. Training sessions were held to demonstrate how participant recruitment, follow-up, sample collection, handling, and processing were done.

Selection of cases

Consecutive sampling technique was employed for the selection of participants who met the inclusion criteria. Following counseling, those who provided written informed consent were enrolled.

Inclusion criteria

The inclusion criteria were women between 8 and 18 weeks gestation who had at least one preeclampsia risk factor including maternal age ≥ 40 years, obesity (pre-pregnancy or first trimester body mass index [BMI] > 35 kg/m²), family history of preeclampsia (mother or sister), interpregnancy interval of more than 10 years, multiple gestation, and admission systolic blood pressure (SBP) > 130 mmHg but < 140 mmHg or diastolic blood pressure (DBP) > 80 mmHg but < 90 mmHg; or those who had two or more of risk factors such as primigravidity, family history of early-onset cardiovascular disease, interpregnancy interval of less than 2 years, use of assisted reproductive technologies, and a new partner.

Exclusion criteria

Women were excluded from the study if they were of gestational age (GA) > 18 weeks; had a previous history of early onset preeclampsia (< 34 weeks); were hypertensive; had diabetes mellitus; had a history of renal disease, vitamin D deficiency, autoimmune disease, or coagulation disorders; were on calcium supplementation or medications other than iron and folic acid supplementation that altered or interfered with serum calcium or its metabolism or excretion (e.g., thiazide diuretics, lithium, anti-epileptic drugs); were on drugs that affect the bioavailability of creatinine such as antibiotics (e.g., cephalosporins and aminoglycosides, cisplatin, phenytoin, deriphyllin, levofloxacin) that affect alkaline picrate methods; or refused to provide informed consent.

Follow-up study procedure

A detailed medical and obstetric history was obtained from each participant. Their weight (in kilograms) and height (in meters) were measured with the adult analogue weight-measuring scale fitted with height-measuring stadiometers (ZT-160®; Techmel, Los Angeles, CA, USA). The BMI was calculated. Blood pressure was measured with mercury sphygmomanometer using the appropriate sized cuff. General and systemic examinations were conducted. Participants were followed up four times weekly until delivery. Blood pressure measurements and urinalysis were done at each visit.

At 18 weeks gestation, participants were given universal bottles to take home. They were instructed to void the first morning urine into the bottles after normal washing of the genitals; the midstream urine specimen was collected at least 2–3 s from the start of urination. The bottle cover was tightly sealed and returned to the hospital the same day. Research assistants received samples at the clinics and wards and sent them to the laboratory for analysis. Samples retrieved at the Central Hospital, Warri were analyzed there, whereas those retrieved from other centers were analyzed at the DELSUTH laboratory.

Quality assurance measures were taken at all times through the pre-analytic, analytic, and post-analytic phases [38]. In the pre-analytic phase, participants were given all necessary instructions from recruitment to sample collection. They were instructed to avoid medications that could alter serum calcium levels, its metabolism and excretion such as calcium supplements and diuretics. The first morning urine was collected. The samples were returned to the laboratory same day as collection. Participants and research assistants were instructed to avoid exposing the sample to extreme weather conditions at all times. Then one milliliter of 6 M hydrogen chloride was added and the mixture was thoroughly stirred to dissolve any sediment and keep the calcium in solution. Samples were analyzed upon return. Those that could not be analyzed on that day were stored

at 4°C. They were analyzed the following day. Urine calcium is stable in solution for up to 3 days at temperatures ≤ 4°C and for at least 3 or more weeks at ≤ -20°C. Samples were thoroughly stirred before they were analyzed. During analysis, laboratory standard operating procedures and/or quality assurance plans were adhered to. Reagent kits were stored in their ideal environment at all times. Universal safety measures and laboratory guidelines were followed.

Preeclampsia was diagnosed when blood pressure was ≥140/90 mm Hg on two occasions at least 4 h apart in the presence of a dipstick test showing at least 1+ protein in a random clean catch urine sample [20, 28, 32]. Participants who had only one of either finding of hypertension or proteinuria were excluded from the analysis, whereas those who developed preeclampsia were managed according to departmental protocol. Those diagnosed with mild preeclampsia were conservatively managed on oral antihypertensives for blood pressure control and seen more frequently at antenatal visits for fetomaternal monitoring. This was terminated when the disease progressed despite conservative management. Those with severe preeclampsia were admitted for stabilization (blood pressure control, seizure prophylaxis, judicious intravenous fluid, management of clinical and laboratory findings) and then delivery through the most expeditious route.

The midstream urine dipstick for protein assessment was done using the Multistix 10SG urinalysis strip. The following were the grades of proteinuria and the corresponding protein concentration provided by the manufacturers: 0, trace (10–20 mg/dL), 1+ (30 mg/dL), 2+ (100 mg/dL), 3+ (300 mg/dL), and 4+ (1000 mg/dL). A 1+ of midstream clean catch urine protein by dipstick was considered significant proteinuria [28].

Laboratory analysis

Samples collected from participants were sent to the laboratory where analyses were carried out under the supervision of the chemical pathologists.

Spot urine calcium assay

Urine calcium was analyzed using the colorimetric method in which under alkaline conditions, the metal-complexing dye, orthocresolphthalein, formed a purple-red chromophore with calcium. The color intensity was directly proportional to the total calcium concentration. Total urinary calcium was quantified using spectrophotometry to measure the color intensity of the reaction at 340 nm [39].

Spot urine creatinine assay

The principle of analysis of creatinine was based on Jaffe's alkaline picrate reaction in which picric acid reacts with creatinine in alkaline medium to produce a red complex, the absorbance of which is proportional to the creatinine concentration. Urine creatinine was quantified using spectrophotometry to measure the color intensity from the reaction at 520 nm [41].

Spot urine CCR calculation

CCR was calculated as: urine calcium (mg/dL)/urinary creatinine (mg/dL).

Statistical analysis

Participants' sociodemographic data, blood pressure, dipstick urine protein, and urine CCR results were collated using specially designed data collection proforma. At the end of the study, data were entered into the computer and analyzed using SPSS statistical software (version 22; IBM Inc., Chicago, IL, USA). Descriptive statistics were used to calculate mean, standard deviation, and minimum and maximum values. A multiple linear regression model was used to analyze effects of age, BMI, and parity on spot urine CCR. The receiver operating characteristic curve for urine CCR was generated from the data entered into the SPSS statistical software (version 22; IBM Inc., Chicago, IL, USA), and the optimal cutoff was determined. This was used to estimate the indices of validity of urine CCR in this study. This was possible by comparing with a cutoff ≤ 0.04 [20, 27, 33], which is used in many studies.

Ethical approval

Ethical approval (Reference No. HREC/PAN/2019/006/0304; dated March 18, 2019) was obtained from the Health Research Ethics Committee of Community Health Workers and DELSUTH, and formal consent was obtained from the other centers. Informed consent was obtained from study participants after counseling. Patient participation in the study was voluntary and those who declined to participate in the study were not penalized and received the care that was due to them. There was no added cost to the participants for their participation in the study.

Results

A total of 183 women were recruited from four healthcare centers. Their data were collected through questionnaires, and they were given clean plastic bottles to take home and return at 18 weeks with their first morning urine. They were all followed up through phone calls. Seventeen of the women did not return with their samples and were reminded on the phone to do so. Some said they were traveling; others could not be reached by phone. An additional three declined to continue with the study due to their spouse's refusal. The remaining participants were followed up until delivery. Forty-seven women delivered outside their primary facility; seventeen of whom could not be contacted due to their phone being unreachable, turned off, or the wrong phone number. Another eight women did not provide the needed data concerning their delivery such as their blood pressure or protein in urine. Complete data were obtained from 138 women; the others were excluded. Among the 138 women followed up with complete data, 127 (92%) remained normotensive, 3 (2.17%) had gestational hypertension, and 8 (5.78%) developed preeclampsia. The three women with gestational hypertension were excluded from the analysis. Table 1 summarizes the anthropometric and clinical characteristics of the women. It showed a statistically significant difference in maternal age, GA at delivery, and SBP and DBP at delivery. The GA at the time of recruitment (P = 0.393), parity (P = 0.198), BMI (P = 0.558), SBP (P = 0.119), and DBP (P = 0.173) at the time of entry into the study did not significantly differ. The significant majority of the women (97%) were between 18 and 39 years old, with only 3% aged ≥ 40 years old. The majority of women were primigravida (44.2%) followed by primipara (37.7%) and multipara (25%).

Table 1: Anthropometric and clinical characteristics (means)

	Normotensive (n = 127)	Preeclamptic (n = 8)	*P*-value
Age	26.23 (4.79)	33.25 (7.29)	0.030*
Parity	1.00 (1.00)	1.00 (2.00)	0.198
GA (at recruitment)	14.19 (2.33)	14.75 (1.67)	0.393
BMI (at recruitment)	27.60 (4.26)	28.99 (6.23)	0.558
SBP (mm Hg upon joining the study)	114.20 (10.61)	119.88 (8.84)	0.119
DBP (mm Hg upon joining the study)	66.06 (7.65)	70.25 (7.67)	0.173
SBP (mm Hg at delivery)	121.22 (9.89)	163.13 (8.89)	0.000*
DBP (mm Hg at delivery)	71.63 (7.76)	100.38 (7.21)	0.000*
GA (at delivery)	37.65 (2.20)	34.50 (2.07)	0.003*

*statistically significant

The mean urinary calcium, urinary creatinine, and urinary CCR of the cohort (n = 135) were 21.74 (14.36) mg/dL, 103.3 3(48.89) mg/dL, and 0.225 (0.101) mg/dL, respectively. Table 2 illustrates the urinary levels of the analytes in both arms of the study. The urinary levels of calcium and CCR were significantly lower in the preeclamptic group than the normotensive group. On the other hand, the normotensive group had higher urine creatinine levels, although not statistically significant.

Table 2: Biochemical characteristics (mean)

	Normotensive (n = 127)	Preeclamptic (n = 8)	*P*-value
Urinary calcium (mg/dL)	22.37 (14.56)	11.73 (3.60)	< 0.001*
Urinary creatinine (mg/dL)	99.63 (40.42)	166.34 (107.36)	0.122
Urinary CCR	0.233 (0.099)	0.100 (0.065)	< 0.001*

*statistically significant

The ROC curve plotted for the CCR produced a cutoff value of 0.1065 (Youden's index) with an area under the curve of 0.885 (P < 0.001). This produced a sensitivity of 0.75 and specificity of 0.08 (Figure 1).

Figure 1: ROC curve of urine CCR at 18 weeks

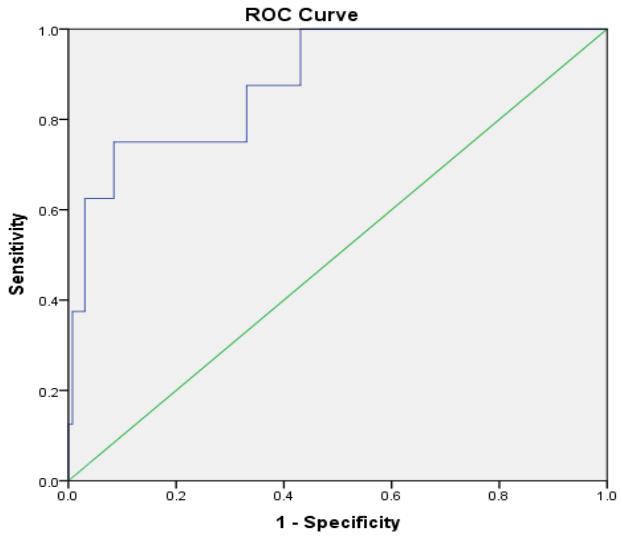

Table 3 depicts the logistic regression of the association between preeclampsia (categorical dependent variable) and a set of independent or explanatory variables (maternal age, parity, BMI, and urine CCR). For the outcome (preeclampsia), there was a statistically significant difference between CCR < 0.1065 and CCR > 0.1065 (P = 0.001), adjusted for parity, BMI, and maternal age. It can be inferred that a unit change in the value of CCR caused an inverse change in preeclampsia by log (0.018), and all other factors and covariates were constant. It also showed that there was a statistically significant difference between maternal age ≥ 40 years and less than, in predicting preeclampsia, all other factors, that is, parity, BMI and urine CCR, being adjusted for.

Table 3: Multiple logistics regression analysis

Outcome; Preeclampsia	Regression Coefficient (B)	Chi-Square	*P*-value	Odds Ratio (95% Confidence Interval)
Intercept	6.184	0	0.042	0.8 (0.098–6.739)
Nulliparity	-0.21	0.038	0.846	0.05 (0.000–0.195)
Age > 40 years	-1.941	1.394	0.005*	0.144 (0.06–3.20)
CCR < 0.1065	-3.933	15.491	0.001*	0.018 (0.002–0.207)
BMI	0.073	0.385	0.537	1.076 (0.853–1.357)

*statistically significant

Table 4 shows the distribution of the outcome with CCR ≤ 0.1065 being a positive test and CCR > 0.1065, a negative test. Of the 135 women administered the test, 17 tested positive (CCR ≤ 0.1065), 6 (4.34%) of whom had preeclampsia (true positive), and 11 (8.14%) who did not (false positive). Of the remaining 118 (85.5%) with CCR > 0.107 (test negative), 2 (1.45%) had the disease (false negative) and 116 (84.1%) did not (true negative).

Table 4: Distribution of patients according to urinary CCR = 0.1065

	Preeclamptic	Normotensive	Total
CCR ≤ 0.1065	6	11	17
CCR > 0.1065	2	116	118
Total	8	127	135

R = 0.1065; X^2 = 31.09; P < 0.001

Table 5 analyzed the distribution of test outcome, taking the common CCR cutoff ≤ 0.04 as positive test. As shown above, two women had a positive test, one of whom had preeclampsia and the other was normotensive. A CCR > 0.04 indicated a negative test. In all, 133 women had a negative test result, 7 of whom had the disease and 126 of whom were normotensive.

Table 5: Distribution of patients according to urinary CCR = 0.04

	Preeclamptic	Normotensive	Total
CCR ≤ 0.04	1	1	2
CCR > 0.04	2	126	133
Total	8	128	135

X^2=7.274, P = 0.026

Measures of validity of CCR for predicting preeclampsia

Table 6 compares the measures of validity between the tests CCR ≤ 0.1065 and CCR ≤ 0.04. At CCR ≤ 0.1065, urine CCR is a more sensitive test. It is by far a better diagnostic test among those who have the disease. On the other hand, CCR > 0.1065 and CCR > 0.04 are equally as good in screening out those not likely to have preeclampsia.

Table 6: Measures of validity of CCR for predicting preeclampsia

	Sensitivity	Specificity	PPV	NPV
CCR ≤ 0.1065	75.0	91.3	35.3	98.3
CCR > 0.04	12.5	99.2	50.0	90.0

Discussion

This was a prospective cohort study that investigated spot urine CCR as a tool for predicting the occurrence of preeclampsia among gravid women in south southern Nigeria. The sensitivity, specificity positive predictive value, and negative predictive values were the measures assessed to determine the validity of CCR as a predictive modality.

In this study, preeclampsia had a prevalence rate of 5.78% among women at risk who were recruited. These women had significantly lower urine calcium and CCR assayed at 18 weeks gestation compared to their normotensive counterparts. Few studies have explored this biomarker in early gestation among those at risk. Additionally, they are rare in the Negroid population. Mandira et al. [26] investigated the predictive validity of urine CCR of serial assays from 16 weeks using 24 h urinary samples. In their findings, urine calcium and CCR were

lower significantly among women who developed preeclampsia compared to their normotensive counterpart at the various times of urine calcium and creatinine assay from 16 weeks. Urine CCR progressively declined with advancing GA to 0.26, 0.21, 0.14, and 0.12 at 28, 32, 36, and 40 weeks, respectively. Most studies on the predictive validity of this biomarker were conducted in women at or beyond 20 weeks gestation, when failure in utero-placental trophoblastic invasion had already occurred and the pathology was already activated in those who will develop the disease. The findings of reduced urine CCR among women, who developed preeclampsia compared to their normotensive counterparts, have nonetheless been consistent [22, 27, 33-35]. The changes are thought to be due to alterations in calcium homeostasis in its microenvironment among those who have developed preeclampsia [40]. Mechanisms such as decreased distal tubular reabsorption, decreased glomerular filtration rates, and decreased intestinal calcium absorption have been postulated to explain this phenomena [41, 42].

In this study, the mean GA of women who developed preeclampsia was 14.19 (2.33) weeks at the time of recruitment and 14.75 (1.67) weeks for those who remained normotensive. However, as was the methodology of the study, urine CCR was assayed at 18 weeks gestation in all participants. This was to avoid confounding the value of urine CCR attributed to differences in GAs in women at recruitment. We achieved the desired sample size; however, difficulty was envisaged due to the late booking nature of women in the study area. The drawback was the limited benefit in starting aspirin prophylaxis at 18 weeks. In most studies investigating CCR as a predictive marker for preeclampsia, the spot urine biomarker assay was studied over a wide range GAs. While a ready cohort of women would be recruited with ease, the drawback is that the relevance of the predictive biomarker at a given GA is blurred.

The aim of having a predictive marker is to detect early disease as evidenced by altered urine CCR levels in large numbers of apparently healthy women who had risk factors as a basis for commencing prophylaxis. Urine CCR assayed at 18 weeks had a sensitivity of 75%. This indicates that CCR ≤ 0.1065 is good at establishing the presence of preeclampsia among those who developed the disease excluding those who remained normotensive despite being positive. However, the positive predictive value (PPV) of the test was low (35.3%). This seemingly indicates that the probability of a woman with a positive test (CCR ≤ 0.1065) developing preeclampsia is low, after excluding those with preeclampsia who tested negative (CCR > 0.1065). On the other hand, the screening test (CCR > 0.1065) had a specificity of 99.2% and NPV of 90%. This indicated that CCR > 0.1065, which represented a negative test at 18 weeks, was a good marker in predicting that women will not develop preeclampsia at delivery excluding normotensive women whose CCR ≤ 0.1065 and represented a probability of 9 of 10. Prevalence however impacts the PPV and NPV of a test such that as the prevalence decreases, the PPV decreases while the NPV increases [43]. The poor PPV in this study can thus be attributed to the low preeclampsia prevalence rate of 5.78% in this study. Again, the low prevalence in this study is likely the result of the exclusion of women with high-risk factors such as a previous history of early onset preeclampsia, histories of hypertension, diabetes, renal and autoimmune diseases. Thus, it is likely that if these cohorts of women were factored in, the PPV value of the test would be high.

When a CCR ≤ 0.04 was applied as a predictive marker, its sensitivity, specificity, PPV, and NPV were 12.5%, 99.2%, 50%, and 90% respectively. This cutoff has been widely used in studies assessing the predictive accuracy of CCR. A CCR ≤ 0.04 was a poor predictor of the disease among those who had preeclampsia (screened population) but among those with positive results, it had a performance of 50% in identifying those with preeclampsia. Again, the relatively higher PPV at CCR ≤ 0.04 is attributed to the low prevalence rate of disease in this study. However, it performed well in identifying those without preeclampsia who had a negative test as did a CCR ≤ 0.1065. The poor sensitivity of CCR ≤ 0.04 compared to CCR ≤ 0.1065 emphasize the fact that urine CCR cutoff is an entity defined by the ethnic and sociodemographic characteristic of populations and is thus is not generally applicable to other populations with different characteristics. The cutoff of urine CCR = 0.04 ,which gave high diagnostic accuracies in studies by David [27], Sheela [33] and Rashmi [35], were due to similarities in the sociodemographic features of the study populations in those communities in India.

The accuracy measures of urine CCR at 16 weeks using Youden's index with cutoff point > 220 mg in the study by Mandira et al. [26] were sensitivity, specificity, PPV, and NPV of 73%, 97%, 96%, and 78%, respectively. In their analyses, orthocresolphthale and Jaffe's alkaline picrate reactions were used for calcium and creatinine estimation as was done in this study. Although assaying the calcium and creatinine from 24 h urinary samples is the gold standard, the more suitable reason for the high values in validity measures in their study, especially the PPV and NPV, was the high preeclampsia prevalence of 16.6%.

This study was carried out in four centers in Delta State with the analysis of the urine biomarkers done in two laboratories, unlike other studies that involved just one facility where the bioassays were likely done. Although standards and protocols were similar and quality control measures were followed as much as possible, the subtle deficiencies and errors attributable to laboratory and personnel in different laboratories is possibly a limitation as this may affect the measurements and thus predictive validity measures of the assayed biomarker. Again, in the centers where the participants had delivered outside the four antenatal care facilities, the outcome variables assessed such as blood pressure and dipstick proteinuria could have been error prone, as standard techniques that were taught to the research assistants in the four centers would not have been followed. These may be a source of bias in the results. Others factors that would have produced bias are faulty instruments and use of instruments without correct calibrations, among others.

Although the urine CCR had a seemingly low PPV in this study, is still likely to be a good predictive marker of preeclampsia when utilized in populations where all at risk women are screened.

Conclusion

At 18 weeks, the urine CCR was significantly lower in those who developed preeclampsia compared to those who remained normotensive. The low PPV of urine CCR is

attributable to the low prevalence of preeclampsia in the study. Urine CCR is still capable of being a good predictive marker when all women at risk are factored in, at which time the disease prevalence is high.

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