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ORIGINAL RESEARCH

Impaired renal function and increased urinary isoprostane excretion in Ghanaian women with pre-eclampsia

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Background: The cause of pre-eclampsia remains largely unknown, but oxidative stress (an imbalance favoring oxidant over antioxidant forces) has been implicated in contributing to the clinical symptoms of hypertension and proteinuria. Assessment of oxidative stress in pre-eclampsia using urinary isoprostane has produced conflicting results, and it is likely that renal function may affect isoprostane excretion. The aim of this study was to determine the role of oxidative stress in the pathophysiology of pre-eclampsia and to assess the effect of renal function on isoprostane excretion in pre-eclampsia in the Ghanaian population.

Methods: This was a case-controlled study, comprising 103 pre-eclamptic women and 107 normal pregnant controls and conducted at the Korle-Bu Teaching Hospital between December 2006 and May 2007. The study participants were enrolled in the study after meeting the inclusion criteria and signing their written informed consent. Oxidative stress was determined by measuring urinary excretion of isoprostane and total antioxidant capacity using an enzyme-linked immunosorbent assay technique. Renal function was assessed by calculating the estimated glomerular filtration rate using the Modification of Diet in Renal Disease formula.

Results: The pre-eclampsia group had significantly ($P = 0.0006$) higher urinary isoprostane excretion (2.81 ± 0.14 ng/mg creatinine) than the control group (2.01 ± 0.18 ng/mg creatinine) and a significantly ($P = 0.0008$) lower total antioxidant power (1.68 ± 0.05 mM) than the control group (1.89 ± 0.04 mM). Urinary isoprostane excretion showed a positive correlation with both mean arterial pressure ($r = 0.261$) and microalbuminuria ($r = 0.510$) in the pre-eclampsia cases. The pre-eclampsia group had a significantly lower estimated glomerular filtration rate than the control group ($P < 0.001$), indicating more renal impairment.

Conclusion: The increased urinary excretion of isoprostanes and decreased total antioxidant power in the in pre-eclampsia group suggest increased production of oxidants and depletion and/or reduction of maternal antioxidants. Increased oxidative stress may be important in the pathophysiology of pre-eclampsia by contributing to endothelial dysfunction, proteinuria, and hypertension.

Keywords: pregnancy, Ghana, pre-eclampsia, oxidative stress

Introduction

Pre-eclampsia is the most ubiquitous hypertensive disorder of human pregnancy, in which the normal hemodynamic response to pregnancy is compromised. It remains a leading cause of maternal morbidity (5%–7% of all pregnancies) and perinatal mortality.[1–3] In Ghana, pre-eclampsia is responsible for 15%–25% of maternal deaths.[4] Pre-eclampsia is diagnosed primarily by the onset of hypertension and proteinuria in the latter half of gestation. Currently, there are no conclusive preventive treatments available because both the etiology and pathophysiology of pre-eclampsia are poorly understood.

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An emerging theory explaining this portentous disease involves oxidative stress, which is an imbalance between pro-oxidant production and antioxidant defenses.[5]

The classical symptoms of pre-eclampsia are hypertension and proteinuria. It is thought that free radicals produced in the placenta gain access to the maternal circulation and induce pathologic changes in endothelial cells of the vasculature, leading to hypertension, and in the renal glomerular capillaries, increasing their permeability to plasma proteins and leading to proteinuria.[6] Alterations in the biosynthesis, secretion, and excretion of isoprostane in physiologic and pathophysiologic states are attributable to several endogenous and exogenous regulatory mechanisms that control availability of the precursors required for isoprostane synthesis. Some of these regulatory mechanisms include dietary and tissue arachidonic acid content, oxygen concentration, and generation of various free radical species.[7] While products of lipid peroxidation such as isoprostanes are utilized as biomarkers of oxidative stress in pre-eclampsia studies,[8,9] their detection in urine samples may be affected by renal mechanisms, but few studies have demonstrated the effect of renal impairment, which is a common phenomenon in pre-eclampsia, on isoprostane excretion. Whilst the role of oxidative stress in pre-eclampsia has been investigated in some African populations, none of these studies has considered isoprostane excretion and total antioxidant power (TAP) as indices of oxidative stress in pre-eclampsia in a homogeneous black African population. A study by Bowen et al evaluated markers in Africans of mixed race and another conducted in Egypt recruited women of predominantly Arab origin.[10,11]

We conducted a study comparing urinary isoprostane excretion and TAP in women with pre-eclampsia and normotensive pregnant controls and examining the influence of renal function on isoprostane excretion in a Ghanaian population. Most studies using isoprostanes as a marker of oxidative stress in pre-eclampsia have measured levels in plasma, exhaled breath condensates, saliva, and urine. In this study, isoprostane excretion was measured in urine because there is no reported evidence of in vitro generation of lipid peroxidation products, which is characteristic of plasma samples because of their high lipid content.[12]

Materials and methods

Subjects

Participants in this study, conducted between December 2006 and May 2007, included pregnant women aged 18–45 years who were enrolled for antenatal care in the obstetrics clinic at the Korle-Bu Teaching Hospital in Accra, Ghana. Pregnant women with serious nonobstetric problems including lupus, chronic hypertension, type 1 or 2 diabetes mellitus, seizure disorders, malignancies, or drug or alcohol abuse were excluded. Two groups of women were recruited for the study, comprising 103 pre-eclamptic patients and 107 normotensive pregnant controls. Pre-eclampsia was defined as systolic blood pressure (BP) 140 mmHg and/or diastolic BP 90 mmHg for the first time after 20 weeks of gestation. In this study, we determined BP as the average of the last five recordings obtained over a period of one hour after the first measurement in the semi-Fowler's position after hospital admission for delivery but before medications or clinical interventions that could alter BP. Mean arterial pressure (MAP) was then calculated as: MAP = diastolic BP + 1/3 (systolic BP − diastolic BP).[9] Proteinuria was confirmed by a urine dipstick value of ≥+1 in women with no known history of hypertension or renal disease, and whose BP returned to normal levels by 3 months postpartum. The study was approved by the ethical and protocol review board of the University of Ghana Medical School, and written informed consent was obtained from all participants. The potential risks of the study were explained to the participants and they were informed that failure to take part in the study would not affect their management in any way.

Biochemical and hematologic variables

Venous blood samples were taken at the antecubital fossa from all the pre-eclamptic women and controls after application of a tourniquet. Blood urea nitrogen, uric acid levels, and liver function tests were measured using a Microlab 300 chemistry semiautomated analyzer (ELITechGroup, Puteaux, France). A full blood screen and white blood cell differential were measured using a hematology analyzer (Sysmex, Mundelein, IL) in the department of biochemistry at the Ghana Medical School.

Measurement of urinary albumin

Urinary albumin concentrations were determined using an albumin blue fluorescent assay kit (Active Motif, Carlsbad, CA). Lyophilized fluorescent dye was resuspended in isopropanol to create the concentrated stock solution. Standards and samples were then added, and fluorescence was read in a fluorescence spectrophotometer (Krontron, Everett, MA) with excitation at 560 nm and emission at 620 nm. Fluorescence intensity directly reflects

albumin concentration. The standards gave an almost linear standard curve from which microalbuminuria in the urine samples was determined.

Measurement of urinary isoprostane

A urine specimen was collected over 24 hours in a sterile container from study participants who met the inclusion criteria. Samples were aliquoted and stored at −70°C until the assays were done. Collection of a 24-hour urine specimen was started as soon as the diagnosis of pre-eclampsia was made. A 15-isoprostane-F2t enzyme-linked immunosorbent assay kit (Oxford Biomedical Research, Oxford, MI) was used to measure urinary isoprostane excretion according to the manufacturer's instructions. Briefly, 100 µL of diluted 15-isoprostane F2t conjugate was added to each well containing 100 µL of samples and standards and plates were incubated for two hours at room temperature. After washing, 200 µL of TMB substrate was added to each well and the plates were incubated for 20–40 minutes. Next, 50 µL of 3 M sulfuric acid was added to each well to stop the reaction and the plate was read at 450 nm.

Measurement of urinary and serum creatinine

Urinary creatinine was measured using a commercial kit (CR01, Oxford Biomedical Research) according to the manufacturer's instructions. Serum levels of creatinine were determined using the alkaline picrate method. Reaction of creatinine in samples with picrate in alkaline medium formed a colored complex that was measured by spectrophotometry (520 nm) using the Microlab 300 semiautomated chemistry analyzer.

Measurement of TAP

A colorimetric microplate assay kit (TA01, Oxford Biomedical Research) was used to assay TAP in urine samples from the pregnant women. The method measures colorimetrically the amount of Cu^{+} derived from Cu^{2+} by the action of all antioxidant moieties in the sample. The Cu^{+} produced by the reaction complexes with bathocuproine (the chromogenic reagent) form a stable complex proportional to the concentration of all antioxidants in the sample. The complex has an absorption maximum at 490 nm and was detected using a microplate reader. A known concentration of uric acid was used to create a reference curve to compare readings obtained by the samples. TAP in the urine samples was expressed as mM uric acid equivalents.

Assessment of renal function

Renal function was determined by calculating the estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease formula:[13]

$$\text{eGFR (mL/min/1.73 m}^2\text{)} = 186 \times (\text{serum}_{\text{creatinine}})^{-1.154} \times (\text{age})^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if race is black}).$$

Statistical analysis

Generation of standard curves for the enzyme-linked immunosorbent assay data was done using GraphPad Prism statistical software version 3.0 for Windows (GraphPad Software, San Diego, CA). Because these data were not normally distributed, the data was log transformed for analyses. Continuous variables were compared between the pre-eclamptic and normal pregnant women using the Student's *t*-test for unpaired data. $P < 0.05$ was interpreted as being statistically significant.

Results

A total of 210 pregnant women were enrolled, of whom 103 were diagnosed with pre-eclampsia and 107 were normotensive pregnant controls. The mean maternal age at sampling was similar (30.2 years for the pre-eclamptic women and 28.99 years for the normotensive pregnant controls, $P > 0.05$) but a significant difference in gestational age at the time of sampling was observed between the two study groups (33.82 weeks for pre-eclampsia and 29.83 weeks for normal pregnancy, $P < 0.001$).

The pre-eclamptic women had an average BP on admission of 165.49/109.13 mmHg and the control group had an average BP of 112.32/67.82 mmHg ($P < 0.001$). Women with pre-eclampsia had a significantly higher MAP than the normotensive pregnant controls (127.69 ± 1.44 mmHg versus 82.65 ± 0.88 mmHg, $P < 0.001$) and higher urinary protein (76.31 ± 0.99 g/L per 24 hours versus 57.93 ± 1.98 g/L per 24 hours, respectively). Table 1 shows the clinical and laboratory data for the normotensive and pre-eclamptic groups at admission. Serum protein, uric acid, and creatinine were elevated in the pre-eclamptic women whereas serum albumin was significantly reduced (Table 1). Total white blood cell counts and neutrophil levels were similar between the study groups. However differential lymphocytes were significantly elevated in the women with pre-eclampsia, whilst platelet counts were higher in the normotensive pregnant controls.

Table 1 Demographic, laboratory, and clinical data for pregnant women with pre-eclampsia and normotensive pregnant women

Investigations	Pre-eclampsia (n = 103)	Normal pregnant women (n = 107)	*P* value
Maternal age (years)	30.20 ± 0.55	28.99 ± 0.48	0.0503
Clinical characteristics			
Gestational age (weeks)	33.82 ± 0.45	29.83 ± 0.68	<0.001*
Systolic BP (mmHg)	165.49 ± 2.10	112.32 ± 1.16	<0.001*
Diastolic BP (mmHg)	109.13 ± 1.27	67.82 ± 0.89	<0.001*
MAP (mmHg)	127.69 ± 1.44	82.65 ± 0.88	<0.001*
Hematology			
Hemoglobin (g/dL)	11.25 ± 0.16	11.21 ± 0.14	0.4269
RBC (10^{12}/L)	4.33 ± 0.06	4.00 ± 0.05	<0.001*
Hematocrit (%)	35.17 ± 0.45	32.96 ± 0.35	0.0003*
Total WBC (10^9/L)	8.35 ± 0.31	7.94 ± 0.18	0.1269
Differential neutrophils (%)	63.33 ± 1.11	64.92 ± 0.77	0.1190
Differential lymphocytes (%)	28.32 ± 1.09	25.87 ± 0.63	0.0257*
Platelets (10^9/L)	191.76 ± 7.35	223.73 ± 7.79	0.0016*
Biochemistry			
ALP (μ/L)	317.97 ± 14.52	196.20 ± 11.71	<0.001*
ALT (μ/L)	24.92 ± 0.92	20.99 ± 0.85	<0.001*
AST (μ/L)	30.53 ± 1.00	28.82 ± 0.99	0.0027*
GGT (μ/L)	33.75 ± 0.99	15.20 ± 0.65	<0.001*
Serum albumin (g/L)	37.16 ± 1.04	39.02 ± 1.00	<0.001*
Urea (mg/dL)	23.05 ± 0.95	11.71 ± 0.64	<0.001*
Blood urea nitrogen (mg/dL)	10.76 ± 0.44	5.47 ± 0.30	<0.001*
Serum creatinine (mg/dL)	1.10 ± 0.02	0.54 ± 0.04	<0.001*
Serum uric acid (mg/dL)	5.94 ± 0.21	3.57 ± 0.19	<0.001*
Serum total protein (g/L)	76.31 ± 0.99	57.93 ± 1.98	<0.001*
Urine creatinine (mg/mL)	0.48 ± 0.01	0.56 ± 0.01	0.0001*
Urinary isoprostane (ng/mg creatinine)	2.81 ± 0.14	2.01 ± 0.18	0.0006*
TAP (mM)	1.68 ± 0.05	1.89 ± 0.04	0.0008*

Notes: Values shown as the mean ± standard error of mean. *$P < 0.05$ (pre-eclampsia versus normal pregnancy).

Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; BP, blood pressure; GGT, gamma glutamyl transferase; MAP, mean arterial pressure; RBC, red blood cells; TAP, total antioxidant power; WBC, white blood cells.

Urinary isoprostane and TAP

The pre-eclampsia group had significantly ($P = 0.0006$) higher urinary isoprostane excretion (2.81 ± 0.14 ng/mg creatinine) than the controls (2.01 ± 0.18 ng/mg creatinine). TAP was significantly ($P = 0.0008$) lower in women diagnosed with pre-eclampsia (1.68 ± 0.05 mM) than in the controls (1.89 ± 0.04 mM, see Table 1).

eGFR and isoprostane excretion in pre-eclampsia

eGFR was significantly lower in the pre-eclampsia group than in the control group ($P < 0.001$, Figure 1). Assessment of the relationship between eGFR and isoprostane excretion showed a weak negative correlation (r = –0.219) in the women with pre-eclampsia.

Isoprostane excretion, microalbuminuria, TAP, and MAP in pre-eclampsia

Investigation for a possible association between isoprostane and microalbuminuria showed a strong positive correlation between the two markers (r = 0.510). However, there was a weak positive correlation between urinary isoprostane excretion and MAP in the women with pre-eclampsia (r = 0.261). Urinary isoprostane excretion was also negatively correlated with TAP, but the association was not strong (r = –0.247).

Discussion

In this study, urinary isoprostane excretion was significantly higher in patients with pre-eclampsia than in controls, suggesting increased lipid peroxidation and hence increased oxidative stress in pre-eclampsia. Previous studies have been unable to demonstrate differences in urine isoprostane excretion between women with pre-eclampsia and those with healthy pregnancies.[14–16] Recently, Wikstrom found no significant difference in mean 15-isoprostane-F2t concentration in urine between Swedish women with pre-eclampsia and healthy pregnant controls, although in that study there was a tendency toward higher urinary isoprostane concentrations in

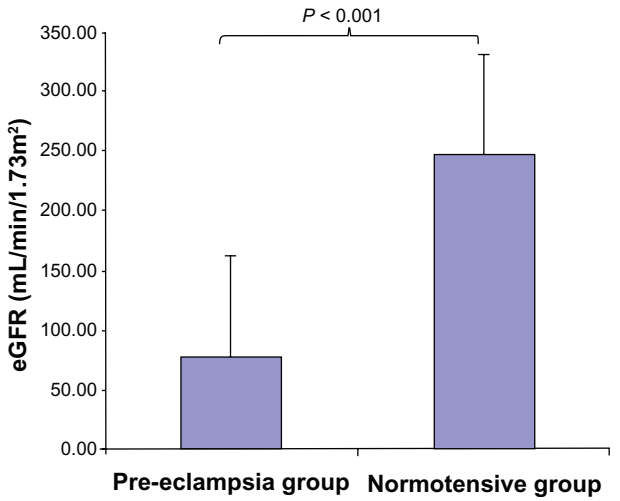

Figure 1 Estimated glomerular filtration rate in patients with pre-eclampsia and normotensive controls.

Abbreviation: eGFR, estimated glomerular filtration rate.

women with pre-eclampsia.[17] The differences in isoprostane excretion between that study and ours could have arisen through differences in the populations sampled which may have affected isoprostane excretion. Another study found that urinary 15-isoprostane-F2t excretion is depressed during pregnancy in women with pre-eclampsia,[7] and suggested that depressed excretion in pre-eclamptic women is likely to reflect more global impairment of renal function, although in that study they did not perform renal function tests. The role of oxidative stress in pre-eclampsia has been investigated in some African populations,[11,17] but no study has considered isoprostane excretion and TAP as indices of oxidative stress in pre-eclampsia or the effect of renal function when assessing oxidative stress.

Pregnancies with renal complications are associated with a high rate of obstetric complications.[18] The most accurate measurement of GFR requires complex technology, which is not routinely available. Therefore, alternative estimates of GFR have been developed to assess renal function, such as the Modification of Diet in Renal Disease formula,[13] the Cockcroft-Gault equation,[19] and measurement of creatinine clearance.[19–21] In this study, the Modification of Diet in Renal Disease formula, which requires age, gender, and serum creatinine only,[22] was used to estimate renal function. eGFR was significantly decreased in the women with pre-eclampsia, implying impaired renal function in this group. Moreover, the negative correlation between eGFR and isoprostane excretion in the women with pre-eclampsia suggests that the greater the degree of renal impairment, the higher the isoprostane excretion. Pathologically, glomerular endotheliosis decreases GFR, and reduction in the density and size of the endothelial fenestrae dimensions and subendothelial accumulation of fibrinoid deposits markedly decrease the glomerular hydraulic permeability. Interposition of mesangial cells also decreases the surface area available for filtration, resulting in cumulative depression of the filtration coefficient that is proportional to the GFR.[23] However, it is unclear if change in size of the glomerular pore accounted for the increased urinary isoprostane excretion in the pre-eclampsia group because this was not analyzed histologically.

Under pathologic conditions, the integrity of the glomerular barrier basement membrane, which is normally lined with negatively charged proteoglycans, may be compromised, leading to increased excretion of charged molecules. Given that free isoprostanes are negatively charged compounds of low molecular weight,[24,25] they might be cleared in the absence of proteoglycans, leading to decreased GFR and increased urinary excretion of isoprostanes in the pre-eclampsia group. A recent study in black South Africans showed increased urinary excretion of heparin sulfate and chondroitin sulfate proteoglycans in women with pre-eclampsia compared with normal pregnant women.[26] It is likely that there was destruction of the anionic barrier by reactive oxygen species in the Ghanaian pre-eclampsia group. Loss of glomerular charge may induce structural changes in the filtration barrier which can lead to increased excretion of negatively charged proteins such as albumin. It may also be the major mechanism accounting for proteinuria in pre-eclampsia.[22]

For most healthy nulliparous women, the parameter that differentiates pre-eclampsia from other hypertensive disorders of pregnancy is concomitant proteinuria, defined as 0.3 g protein in a 24-hour urine sample. When daily urine collection is not feasible, two random urine samples taken at least 4–6 hours apart are usually acceptable. Under these sampling conditions, proteinuria is defined as 0.3 g/L protein or 1+ on a dipstick test strip. Albumin accounts for most of the protein in the urine due to glomerular injury. As a result, urinary albumin is often measured as an index of proteinuria. While investigators generally quantify proteinuria by measuring urinary albumin levels either on 24-hour excretion or on spot measurements, some prefer to use the spot urinary protein/creatinine ratio or the urinary albumin/creatinine ratio measurement to define protein excretion in patients with pre-eclampsia. However, concerns have been raised about the correlation between the two methods. Some recent studies have shown consensus between random protein concentration or urinary dipstick measurements and 24-hour protein excretion in women with pre-eclampsia.[19,27,28] In this study, analysis of spot urine samples showed increased albuminuria in pre-eclamptic patients, in agreement with their diagnosis.

Endothelial dysfunction or impairment which is prevalent in pre-eclamptic women has been linked to oxidative stress.[29–32] In our study, excretion of albumin was directly proportional to urinary isoprostane excretion and inversely correlated with TAP, suggesting that oxidative stress in the pre-eclamptic patients may contribute to endothelial dysfunction. However, further studies are needed to ascertain whether oxidative stress plays a role in the destruction of the proteoglycan layer in pre-eclampsia and whether isoprostanes in particular contribute to the loss of charge selectivity on the glomerular basement membrane and subsequent renal impairment in pre-eclampsia.

Antioxidant activity in pre-eclampsia is generally low but not uniformly so.[33–35] Chappell et al compared antioxidant concentrations in high-risk and low-risk women, and showed

that vitamin C was lower and uric acid was higher in high-risk women, with no difference in α-tocopherol levels.[34] Similarly, in our study, serum uric acid was higher whilst serum albumin was lower in pre-eclamptic patients compared with the low-risk normotensive pregnant controls. This suggests that measurement of a single marker in biological fluid or tissue in pregnancy may not adequately reflect the balance between pro-oxidant and antioxidant forces. Determination of TAP in pre-eclampsia using urine samples has not been carried out previously. TAP was significantly lower in patients with pre-eclampsia than in the controls, similar to the findings of Scholl et al, although in their study TAP was determined in plasma.[9] Low TAP in patients with pre-eclampsia may signify either deficient antioxidant production or exhaustion of antioxidant reserves in response to lipid peroxidation.[36] In effect, the higher urinary excretion of isoprostane and lower TAP in the urine samples from patients with pre-eclampsia point to increased oxidative stress in pregnant Ghanaian women diagnosed with pre-eclampsia. In addition, we observed decreased urinary isoprostane excretion and lower TAP with increasing gestational age in patients with pre-eclampsia. A recent study that measured urine isoprostane excretion in pregnant women before diagnosis of pre-eclampsia reported that the risk of pre-eclampsia was increased five-fold with higher isoprostane excretion.[9] It is likely that placental factors may induce more oxidative stress in early-onset pre-eclampsia compared with late-onset pre-eclampsia, contributing to the higher severity of early-onset pre-eclampsia. Increased isoprostane excretion and hence oxidative stress may be more important in early-onset than in late-onset pre-eclampsia. The TAP assay used in this study gives a better assessment of maternal antioxidant status because it takes into consideration the ability of all antioxidants (including exogenous or endogenous sources, water soluble, enzymatic, or nonenzymatic) in the maternal system to reduce copper^{2+} to Cu^{+} ions as a measure of global antioxidant capacity. However, it is unclear from our study which of the antioxidants measured was responsible for the lower TAP observed in the pre-eclampsia group.

Overall, we have shown that oxidative stress characterized by higher levels of urinary isoprostane excretion and lower TAP is a phenomenon associated with pre-eclampsia in Ghanaian women. The inverse correlation between decreased eGFR and increased urinary excretion of isoprostanes in the patients with pre-eclampsia seems to suggest that impaired renal function can affect isoprostane excretion. Further, renal impairment should be taken into consideration when using urinary isoprostane as a marker of oxidative stress in pre-eclampsia and other diseases that affect renal function. With better insight into the mechanisms regulating the metabolism of reactive oxygen species, it should be possible to target therapies more effectively so that the detrimental effects of vascular oxygen free radicals can be reduced and the beneficial effects of antioxidants enhanced. Such therapies would be useful in the prevention and management of pre-eclampsia and other forms of pregnancy-induced hypertension.

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Disclosure

The authors report no conflicts of interest in this work.

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