Novel prognostic Potential of Early Second Trimester and Mid-pregnancy 8-hydroxy-2-deoxyguanosine/Placental growth factor ratio for preeclampsia: A Longitudinal Nested-Case Control Study

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Abstract

Objective The study used both subjective, Suboptimal Health Status (SHS) concept along with objective, biomarkers of oxidative stress (OS): 8-OHdG, 8-epi-PGF2α and total antioxidant capacity (TAC); and angiogenic growth mediators (AGMs): VEGF-A, sFlt-1, PlGF and soluble endoglin (sEng) for predicting early-onset (EO) and late-onset (LO) preeclampsia (PE) Design A hospital-based longitudinal nested case-control study Setting Obstetrics and Gynaecology Department at Komfo Anokye Teaching Hospital, Ghana Population/Sample Singleton normotensive pregnancies (NTN-P) at baseline W1 (10-20th week gestation) (n= 593) of which 498 (197 developed PE) completed the study. Methods: The overall health status of the NTN-P participants was assessed at W1 and categorised as SHS and optimal health status (OHS) using a validated SHS questionnaire-25. Participants were followed at W2 (21-31st week, mid-pregnancy) and 32-42nd week. Samples were collected and analysed for biomarkers of OS and AGMs at the three-time points. Main Outcome Measures Receiver operative characteristics curve analysis was performed for the single and combined W1 and W2 biomarkers of OS and AGMs for predicting PE and its subtypes (EO-PE and LO-PE) Results Compared to single biomarkers of OS and AGMs, their combined ratios particularly, the W2 8-OHdG/PIGF ratio was a potent biomarker for PE [AUC=0.93]. Additionally, 8-OHdG/PIGF ratio best identified SHS-pregnant women who later developed EO-PE [AUC=0.97] and LO-PE [AUC=0.93]. Moreover, 8-OHdG/PIGF ratio best identified OHS-pregnant women who later developed EO-PE [AUC=0.94] and LO-PE (AUC=0.94). Conclusion Combination of biomarkers of OS and AGMs, particularly, mid-pregnancy 8-OHdG/PlGF ratio is a potent biomarker for PE and its subtypes.

Novel prognostic Potential of Early Second Trimester and Mid-pregnancy 8-hydroxy-2-deoxyguanosine/Placental growth factor ratio for preeclampsia: A Longitudinal Nested-Case Control Study

Running Title: Novel Prognostic Markers for Preeclampsia

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Annani-Akollor[4] , Samuel Asamoah Sakyi[4] , Linda Ahenkorah Fondjo[4] , Emmanuel Acheampong[2,4] , Evans Asamoah Adu[4] , Ebenezer Afrifa-Yamoah[5] , Xueqing Wang[2] , Youxin Wang[6] , Wei Wang[2,6] *

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Abstract

Objective

The study used both subjective, Suboptimal Health Status (SHS) concept along with objective, biomarkers of oxidative stress (OS): 8-OHdG, 8-epi-PGF2α and total antioxidant capacity (TAC); and angiogenic growth mediators (AGMs): VEGF-A, sFlt-1, PlGF and soluble endoglin (sEng) for predicting early-onset (EO) and late-onset (LO) preeclampsia (PE)

Design

A Hospital-based longitudinal nested case-control study

Setting

Obstetrics and Gynaecology Department at Komfo Anokye Teaching Hospital, Ghana

Population/Sample

Singleton normotensive pregnancies (NTN-P) at baseline W1 (10-20th week gestation) (n= 593) of which 498 (197 developed PE) completed the study.

Methods:

The overall health status of the NTN-P participants was assessed at W1 and categorised as SHS and optimal health status (OHS) using a validated SHS questionnaire-25. Participants were followed at W2 (21-31st week, mid-pregnancy) and 32-42nd week. Samples were collected and analysed for biomarkers of OS and AGMs at the three-time points.

Main Outcome Measures

Receiver operative characteristics curve analysis was performed for the single and combined W1 and W2 biomarkers of OS and AGMs for predicting PE and its subtypes (EO-PE and LO-PE)

Results

Compared to single biomarkers of OS and AGMs, their combined ratios particularly, the W2 8-OHdG/PIGF ratio was a potent biomarker for PE [AUC=0.93]. Additionally, 8-OHdG/PIGF ratio best identified SHS-pregnant women who later developed EO-PE [AUC=0.97] and LO-PE [AUC=0.93]. Moreover, 8-OHdG/PIGF ratio best identified OHS-pregnant women who later developed EO-PE [AUC=0.94] and LO-PE (AUC=0.94).

Conclusion

Combination of biomarkers of OS and AGMs, particularly, mid-pregnancy 8-OHdG/PlGF ratio is a potent biomarker for PE and its subtypes.

Keywords: Preeclampsia, suboptimal health status, optimal health status, prognostics accuracies, combined biomarkers, oxidative stress, angiogenic growth mediator, discriminating power**Introduction**

Preeclampsia (PE) is a disorder of pregnancy characterised by hypertension and proteinuria, noticeable after 20 weeks gestation.[1] The condition is easily treatable, as seen in developed countries—in the U.K. for example, PE is fatal in only one out of every million birth.[2] In many other countries, however, particularly those in developing countries, this condition still poses a formidable threat to maternal-foetal health.[2, 3] In Ghana, for example, roughly 18 maternal deaths are due to this condition, which translates to more than 570 deaths per 100,000 live births.[3]

In a retrospective study at the Komfo Anokye Teaching Hospital (KATH), Ghana, 26.4% of all maternal deaths were associated with hypertensive disorders of pregnancy.[4] The prevalence of PE was 48.8%, being the highest amongst all the hypertensive disorders of pregnancy evaluated in a recent cross-sectional study at KATH.[5] The prevalence rate is gradually increasing in developing countries, particularly in Ghana due to delayed detection and diagnosis.[5] In most developing countries, resource constraints hamper early detection and effective diagnosis.[6] Aside from the numerous treatment options available for managing PE, the delivery of the placenta and the baby under intensive care is another remedy that can avert PE onset.[7]

Until now, the diagnosis of PE has been based on blood pressure and proteinuria measurements, but these measures have not proven to predict the course and progression of the condition.[6] This is because, PE is multifactorial, leaving the exact aetiology unknown. Researchers have been exploring avenues to understand the exact pathophysiology and develop promising biomarkers for early PE onset, even though the disorder has not been fully elucidated.[8] Accumulating evidence, however, indicates that oxidative stress (OS), poor placental angiogenesis and incomplete maternal artery remodelling are among the leading contributing factors.[9, 10] Thus, evaluating their markers may be useful for understanding the condition.

OS, which is an imbalance between pro-oxidants and antioxidants, has been reported among pregnant women who develop PE with increased levels of 8-epi-PGF2α (a marker of endogenous lipid peroxidation) and 8-OHdG (a marker of oxidative DNA damage) and a correspondingly reduced total antioxidant capacity (TAC) compared to normotensive pregnant women.[11] Aside from the involvement of OS in the pathophysiology of PE, OS plays a central role in placental angiogenesis and vasculogenesis. [9, 10] Also, complete placental angiogenesis and vasculogenesis are key to maternal well-being and growth of the foetus. However, an incomplete placental vascular development may result in placental hypoxia and ischaemia, which subsequently stimulates an increased OS response. [9]The result is an increased release of sFlt-1, an anti-angiogenic factor which antagonizes both PIGF and VEGF-A to drive high blood pressure and endothelial dysfunction in

PE.[12] Reduced levels of PlGF and VEGF-A and increased levels of sEng and sFlt-1 have been reported at the time of PE diagnosis [13] and in early gestational age, even before the clinical manifestation of PE.[14, 15]

Even though the roles of OS and AGMs are synergistic to the development of PE, longitudinal evaluation of both biomarkers throughout pregnancy is not available in Ghana, to the best of our current knowledge. A previous study has evaluated the potential of these markers in isolation.[10] In this study, the suboptimal health status (SHS) concept was introduced to understand the independent role of OS and AGMs in PE. The concept has been previously used to identify pregnancies at increased risk of incidence PE and adverse pregnancy outcomes.[16, 17] Similarly, SHS pregnancies were associated with increased OS, unbalanced pro- and AGMs.[16] This signifies that the SHS concept can be integrated as an additional health assessment approach to identify early signs of OS and high-risk populations of pregnant women likely to develop PE. Thus, combining the concept of SHS and biomarkers (OS and AGMs) identification will be a better approach to predicting and monitoring the progression of PE. This study, therefore, used SHS concept to identify pregnant women though clinical normotensive but having poor health using SHSQ-25 and evaluated the prognostic accuracies o f early second trimester (10-20 weeks gestation) and mid-pregnancy (21-31weeks gestation) of single and combined biomarkers of OS and AGMs for preeclampsia (PE) and its subtypes: early-onset and late-onset PE in a Ghanaian Prospective Cohort birth Study.

Methods

Participants

This hospital-based nested case-control study was based on a longitudinal GHOACS conducted at the Obstetrics and Gynaecology Department of Komfo Anokye Teaching Hospital (KATH). Both nulliparous and multiparous normotensive pregnant women (NTN-PW) aged from 18 to 45 years with a singleton pregnancy at 10-20 weeks gestation, that provided written informed consent, were recruited at baseline.

Sociodemographic data were obtained through a completed questionnaire, and clinical and obstetric data were obtained from the antenatal folder and participant's record in the database of the KATH. The overall health of participants at visit 1 was assessed using the Suboptimal Health Questionnaire-25 (SHSQ-25) and pregnant women were classified as SHS and OHS based on the procedure described in previous studies.[16, 17]

At the start of the study, defined as wave 1 (W1) (10-20 weeks gestation, median, 17 weeks), 593 clinically diagnosed normotensive pregnant women (NTN-PW) were included. Of the 593, 504 returned to participate in the first follow-up, wave 2 (W2) (21-31 weeks gestation, median 27 weeks), whereas 498 completed the study, wave 3 (W3) (32-42 weeks gestation) and were included in the final assessment. At the time of delivery (32-42 weeks gestation), 197 had developed PE and were classified as cases whereas 301 returned as NTN-PW and were classified as controls**.** Of the 498 participants, 248 of them had 'SHS' at baseline of which 153 later developed PE (56 EO-PE and 97 LO-PE) and 95 returned as NTN-PW. Also, 250 had 'optimal health status'(OHS) at baseline of which 44 developed PE (14 EO-PE and 30 LO-PE) and 206 returned as NTN-PW. A total of 95 women were lost to follow-up due to unwillingness to continue (n=32), relocation (n=48), spontaneous abortion (n=4) and self-induced abortion (n=11) **(Figure 1).**

Figure 1. Study participants.

NTN-PW: normotensive pregnant women; BOS: biomarkers of oxidative stress; AGMs: angiogenic growth mediators; O&G: obstetrics and gynaecology; W1, wave 1 or visit 1, W2, wave 2 or visit 2; W3, wave 3 or visit 3

A qualified consultant obstetrician/gynaecologist physically examined all participants. PE was defined as systolic blood pressure (SBP) /diastolic blood pressure (DBP) greater than or equal to 140/90mmHg with visible proteinuria ([?]1+ dipstick) or 24-hour proteinuria of [?]300mg/day on two (2) occasions at least four (4) hours apart detected after 20 weeks gestation in previously normotensive pregnant women. Early-onset

PE (EO-PE) and late-onset PE (LO-PE) were defined as PE that occurred before and at or after 34 weeks gestation, respectively (American College of Obstetricians and Gynecologists & Task Force on Hypertension in Pregnancy, 2013; Raymond & Peterson, 2011)

Laboratory assays

Serum, plasma and urine samples were obtained from all participants up to a total of 3 visits or waves, at 10-week intervals throughout gestation (median 17, 27, and 37 weeks). Samples were stored at -80°C (Thermo Scientific Ultra-Low Freezer) until the biomarkers of OS and AGMs were analysed.

Following the manufacturer's instructions, urinary and serum 8-OHdG were analysed in duplicate using highly sensitive and competitive ELISA kits (ab201734, Abcam, China). Serum concentrations of 8-OHdG were measured immediately after sample collection to avoid autoxidation during long storage. The inter-and intra- assay coefficients of variation (CV) were 3.5% and 4.5%, respectively. Urinary 8-OHdG concentrations were normalised to creatinine (Cr) concentrations and recorded as ng/mg Cr. Serum 8-epi-PGF2α was analysed in duplicate using competitive ELISA kits from ELabscience, China (cat. log E-EL-0041). The intra-and-inter assay coefficients of variation (CV) were 5.6% and 6.4%, respectively.

TAC reagents were obtained from Sigma-Aldrich (Hong Kong, China). Plasma samples were thawed to measure TAC spectrophotometrically at 593 nm using Mindray BA-88A, China. The estimation of TAC was based on the Ferric Reducing Ability of Plasma (FRAP) and the protocol as described by Benzie and Strain (1996). The absorbance was used to obtain the concentrations after comparison to standard curves and recorded in μmol/l.

AGMs including serum concentrations of VEGF-A, sFlt-1, PlGF, and sEng were measured in duplicate using competitive Quantikine ELISA kits from R&D System Inc. (Minneapolis, MN USA). Absorbance was measured at 450 nm wavelength using a microplate ELISA reader (Bio-Tek ELx808 microplate reader, Hayward, CA, USA). The inter-and intra- assay coefficient of variation obtained in our laboratory was 1.1 and 1.3 for VEGF-A, 1.5 and 3.8 for sFlt-1, 4.6 and 3.3 for PlGF and 2.8 and 5.2 for sEng, respectively.

All laboratory assays were performed at the Molecular Medicine Laboratory of the Kwame Nkrumah University of Science and Technology and the Biochemistry and Immunology Department of the Komfo Anokye Teaching Hospital, Ghana.

Statistical analyses

The normality of the data was tested using the Kolmogorov-Smirnov test. Data were presented as median (interquartile ranges) for non-parametric continuous variables and frequency (percentages) for categorical variables. A Chi-square test was performed to test associations between the proportions of variables among the study groups. Median comparisons between more than two independent variables were performed using Kruskal-Wallis one-way ANOVA followed by a Bonferroni posthoc multiple comparison test and adjusted p-values were recorded. A receiver operating characteristic (ROC) curve and area under the curve (AUC) were generated to evaluate the diagnostic performance of the model. $P< 0.05$ was considered statistically significant. Data were analysed using SPSS version 24 (IBM Corp, NY, USA), XLSTAT Premium version 2018.1 and R version 3.4.3 (R core Team 2017).

Results

Unlike OHS groups, there was a statistically significant difference between the median maternal ages between SHS pregnant group who developed PE compared to those who did not (p <0.001). There was a significantly increased SBP, DBP, sEng, sFlt-1, 8-epiPGF2α, serum 8-OHdG, urinary 8-OHdG and combined ratios of sFlt-1/PlGF ratio, 8-epiPGF2alpha/PlGF ratio, 8-OHdG/PlGF ratio and sEng/PlGF ratio, and correspondingly reduced PIGF, VEGF-A and TAC among PE groups compared to NTN-PW group (p <0.001). Unlike the OHS groups, the degree of imbalance in biomarkers of OS and AGMs was higher in SHS who developed

EO-PE followed by LO-PE compared to NTN-PW (p <0.001)**.** Although no statistical significance was observed, the clinically significant difference indicated by the high level of imbalances in favour of SHS rather than the OHS group was observed in biomarkers of OS and AGMs. Meanwhile, there was a significant difference in median SBP between SHS-associated NTN-pregnancy and OHS- associated NTN-pregnancy (p =0.038)**(Table 1)** .

Overall, SBP, DBP, and biomarkers of OS and AGMs increased from baseline to mid-pregnancy among SHS women who later developed PE and NTN-pregnancies rather than OHS pregnant women who later developed PE and NTN-pregnancies. At both early 2nd trimester (10-20 weeks gestation, W1) and mid-pregnancy (21-31 weeks gestation, W2), the median maternal serum levels of PlGF, VEGF-A, and plasma TAC were significantly decreased whereas those of sEng, sFlt-1, 8-epiPGF2α, 8-OHdG, urinary 8-OHdG and the ratios: sFlt-1/PlGF, 8-epiPGF2α/PlGF, 8-OHdG/PlGF and sEng/PlGF were significantly increased among the SHS who later developed EO-PE followed by LO-PE compared to NTN-PW (p <0.001). Similar observations occurred among the OHS group (p <0.001) even though the trend of imbalance was higher among the SHS group. There was a clinically significant difference between the SHS group and the OHS who later developed PE and those who did not **(Table 2).**

Within each visit, there was no difference in gestational age across the groups. Meanwhile, there was a significant difference in SBP and DBP at both visit 1 or W1 and visit 2 or W2 across the study groups (p<0.05) **(Table 2).**

Compared to the individual biomarkers at visit 1 or W1 and visit 2 or W2, the combined biomarkers of OS and AGMs, particularly the mid-pregnancy (W2) 8-OHdG/PIGF ratio yielded the highest discriminating power or AUC (0.93, p <0.001) **(Figure 2c)** with the best sensitivity (85.6%), specificity (92.4%), positive predictive value (PPV) (86.6%), negative predictive value (NPV) (85.2%), positive likelihood ratio (LR+) (9.9) and negative likelihood ratio (LR-) (0.1) at a cut-off value [?]0.80. At the cut-off value for 8-OHdG/PIGF ratio, NTN-PW had 4.8-fold increased odds of developing PE (adjusted odds ratio (aOR) =4.8 95%CI (1.5-11.5), p <0.001).

Except for W1 TAC levels, all the single and combined biomarkers of OS and AGMs yielded a significant (all p <0.05) discriminating power and adjusted odds ratios for predicting PE**(Table S1)** .

Figure 2: The area under the ROC curves for the single and a combination of biomarkers of OS and AGMs measured at 10-20 weeks gestation (W1) and 21-31 weeks gestation (W2) for the prediction of PE (all cases).

The best predictive marker for SHS pregnant women likely to develop EO-PE was the mid-pregnancy 8-OHdG/PIGF ratio with a significantly high discriminating power or AUC (0.97, p <0.0001)**(Figure 3c),** sensitivity (96.4%), specificity (81.1%), NPV (75.5%) and PPV (97.5%), at a cut-off value [?]0.8. At this cut-off value, SHS-PW were at 6.5-fold increased odds of developing EO-PE [aOR =6.5, 95%CI (1.4-12.5), p <0.001] **(Table S2)** .

Similarly, the mid-pregnancy (W2) 8-OHdG/PIGF ratio and sFlt-1/PlGF ratio yielded the best and same AUC (0.93, p <0.001) for predicting SHS pregnant women likely to develop LO-PE **(Figure 3f)** . A cut-off value [?]0.8 for the 8-OHdG/PIGF ratio yielded a sensitivity and specificity (97.8% and 92.7%, respectively), PPV (92.4%), NPV (78.7%) and 4.5-fold increased odds (aOR =4.5 95%CI (1.5-10.2), p <0.001) of SHS pregnant women developing LO-PE **(Table S3).**

Except for W1 TAC levels, all the single and combined biomarkers of OS and AGMs yielded a significant (all p <0.05) discriminating power and adjusted odds ratios for predicting SHS pregnant women likely to develop EO-PE and LO-PE, however, the combined biomarkers yielded a highest predictive accuracy **(Tables S2 and 3)** .

Figure 3: The area under the ROC curves for the single biomarkers of OS and AGMs, and

their combinations at early pregnancy [W1] and mid-pregnancy [W2] for the prediction of SHS-PWLD EO-PE (Figure 3a-c) and LO-PE (Figure 3d-f)

Overall, the mid-pregnancy (W2) 8-OHdG/PIGF ratio was the best prognostic marker for both EO-PE **(Table S4)** and LO-PE**(Table S5)** compared to the single markers. At a cut-off value [?]9.0 for the 8-OHdG/PIGF ratio, OHS pregnant women were at 5.6-fold increased odds [aOR =5.6, 95%CI (1.5-11.9),p <0.001] of developing EO-PE with a significantly higher discriminating power or AUC of 0.94 (p <0.001)

(Figure 4c).

Similarly, at a cut-off value [?] 0.80 for the 8-OHdG/PIGF ratio a significantly higher discriminating power or AUC of 0.94 (p <0.0001) **(Figure 4f)** , 83.3% sensitivity, 90.0% specificity were observed for predicting OHS pregnant women who developed LO-PE. At the cut-off value generated for predicting OHS who are likely to develop LO-PE, OHS-PW were at 5.1-fold increased odds (aOR=5.1, 95%CI (1.5-13.3), p <0.001) **(Table S5).**

Except for W1 TAC levels, all the individual and combined biomarkers of OS and AGMs yielded a significant (all p <0.05) discriminating power and adjusted odds ratios for predicting OHS pregnant women likely to develop EO-PE and LO-PE, however, the combined biomarkers yielded a highest predictive accuracy

Figure 4: The area under the ROC curves for the single biomarkers of OS and AGMs, and their combinations in early pregnancy [W1] and mid-pregnancy [W2] for the prediction of OHS PWLD EO-PE (Figure 4a-c) and LO-PE (Figure 4d-f).

Discussion

Main findings

In line with several other studies [14, 16, 18-22], this study observed an imbalance in OS and AGMs markers in both OHS and SHS pregnant women, who were likely to develop PE. But the effect was more pronounced in the SHS group compared with the OHS group**.** When we compared the prognostic accuracies of biomarkers of OS and AGMs at Wave 1 and Wave 2 to predict SHS and OHS pregnant women who developed PE and its subtypes (EO-PE and LO-PE), the single biomarkers of OS and AGMs yielded fair predictive accuracies. However, the combined biomarkers of OS and AGMs including sFlt-1/PlGF, 8-epiPGF2α/PlGF, 8-OHdG/PlGF and sEng/PlGF yielded very good predictive accuracies. Particularly, mid-pregnancy 8-OHdG/PlGF improved PE (all cases) prognosis by yielding the best discriminating power and best predictive accuracy at a cut-off value [?]0.80. Also, sFlt-1/PlGF and 8-epiPGF2α/PlGF ratio both yielded very good discriminating powers.

Strengths and limitations

A previous longitudinal study by Kusanovic *et al.,*[15] among pregnant Chilean women found the "mid-trimester" as the gestation time for best predicting PE. This finding is consistent with the pattern of results in the present study. To the best of current knowledge, this is the first and largest longitudinal nested case-control study among a Ghanaian population reporting the prognostic potential of both single and combined biomarkers of OS and AGMs for PE. Besides, the present study is the first to identify the 8-OHdG/PlGF ratio as a promising prognostic marker for PE. Furthermore, our ability to explore the prognostic accuracies of the different subtypes of PE (early and late-onset PE) by incorporating the public health concept of SHS is the first of its kind. Despite these strengths, some limitations need to be improved upon for future studies. Firstly, the study was undertaken in a single hospital, which means that the present study may not have sampled representative participants across the entire Ghanaian populace, therefore, ethnic bias may have occurred. Secondly, the present study identified the best biomarkers for the prediction of the onset of PE at

mid-pregnancy (21-31 weeks, median 27 weeks gestation) which is relatively late in the disease progression. Finally, the high AUC generated may be due to the high incidence rate of PE and may not necessarily be because the combined biomarkers are accurate. Therefore, it is recommended that future studies should replicate the present study to validate the finding that the 8-OHdG/PlGF ratio is a potent prognostic marker for PE.

Interpretation

The findings of our study demonstrate that OS and AGMs play a significant role in the development of PE, and SHS mothers are at high risk of defective angiogenesis and vasculogenesis. According to Pratt*et al.,* [23], these mechanisms may operate via shallow extravillous trophoblast invasion and subsequent poor maternal artery remodelling, resulting in placental under-perfusion and hypoxia. The effect of this is stimulating the antagonistic activity of sFlt-1 to impair the physiological function of PIGF and VEGF-A.[23] Also, sEng, an anti-AGM, interferes with transforming growth factor-beta, which results in impaired nitric oxide synthesis, consequent vasoconstriction, endothelial dysfunction and clinical manifestation of PE.[24, 25] Thus, the combined concept of SHS and synergetic effect of OS and AGMs becomes important and useful in pregnancy, especially among mothers from resourced limited countries.

The finding that 8-OHdG/PlGF improved PE prediction is novel according to the currently available information. The synergistic role of both OS and abnormal placental angiogenesis in the pathophysiology of PE, suggest that both factors may share a common pathway.[9, 10] The strength of the 8-OHdG/PIGF ratio is that when the cut-off value was applied in a logistic regression model, the mid-pregnancy mothers were at 4.8-fold increased adjusted odds of developing PE, indicating that its prognostic potential is independent of many confounding factors. Similarly, to other combined ratios, there was significantly increased levels of 8-OHdG/PlGF in both early 2ndtrimester (10-20 weeks) and mid-pregnancy (21-31 weeks) of pregnant women who later developed PE compared to NTN-PW, suggesting that these markers are of prognostic importance**.** The sFlt-1/PlGF ratio is reported as the best marker by some studies [13, 19, 21, 26], even though another study by Park *et al.,*[27] found that its predictive accuracy is, however, comparable to the single AGMs.

The present study hypothesises that 8-OHdG/PlGF is an ideal prognostic marker that gives a comprehensive understanding of the pathogenesis of PE, unlike sFlt-1/PIGF, which is limited to detecting angiogenesis alone. The abnormally increased 8-OHdG/PIGF ratio reflects the imbalance between OS and AGMs, indicating that the increased oxidative DNA damage has created disequilibrium in pro-angiogenic function. Hence, by measuring this marker, the synergistic physiology of both OS and AGMs may be known. Early detection of increased 8-OHdG/PIGF ratio will inform clinicians of the need for antioxidant supplementation, which is likely to reduce the circulatory ischaemic/hypoxic insult and enhance placental angiogenesis. From a therapeutic standpoint, a combined antioxidant supplement plus pro-angiogenic molecules could be the best therapeutic approach for the management and prevention of PE.[10]

Early-onset PE (EO-PE) is the most severe subtype of PE and is mostly linked with biochemical derangement and adverse perinatal outcomes as opposed to late-onset PE (LO-PE).[20, 28] In the present study, compared to NTN-PW, a marked imbalance of both biomarkers of OS and AGMs was observed at both early 2nd trimester (10-20 weeks) and mid-pregnancy (21-31 weeks) in SHS rather than OHS pregnant women who developed EO-PE, followed by those who developed LO-PE. When the ROC curve analysis of biomarkers of OS and AGMs at early 2nd trimester and mid-pregnancy were tested to predict SHS and OHS pregnant women who developed EO-PE and LO-PE, the mid-pregnancy 8-epiPGF2α/PlGF ratio yielded the best prognostic accuracies. Particularly, discriminating powers were 97.0% for SHS-PWLD EO-PE, 93.0% for SHS-PWLD LO-PE and 94.0% for OHS-PWLD both EO-PE and LO-PE**.** All these findings support the hypothesis that combined biomarkers of OS and AGMs are a new approach to predicting and diagnosing PE and its subtypes.

Conclusions

The incidence of PE was high among SHS compared to OHS pregnant women. There is an imbalance in biomarkers of OS as well as AGMs at both early 2nd trimester and mid-pregnancy among SHS rather than OHS NTN-PW who later developed PE. The single biomarkers of OS and AGMs yielded fair discriminating power or prognostic accuracies, but their combined biomarkers perform better as a prognostic marker for PE. The combination of biomarkers of OS and AGMs, particularly the mid-pregnancy 8-OHdG/PlGF ratio is the best marker for predicting PE and its subtypes. The combination of biomarkers of OS and AGMs will increase our knowledge of the new prognostic markers and inform clinicians of the need for a combined antioxidant plus pro-angiogenic supplementation for women who develop PE. The concept of SHS allows early stratification and intervention for NTN-PW, who are at high risk of developing PE. Thus, it should be recommended as an additional health assessment tool for antenatal care in resource-limited communities.

Disclosure of interests

None

Contribution to authorship

"Conceptualization, E.O.A, D.A.C. and W.W.; Methodology, E.O.A, D.A.C., C.A.T, A.T. and W.W.; Formal Analysis, E.O.A.; Investigation, E.O.A, C.A.T., A.T., C.O., M.E.A-A., L.A.F. and S.A.S.; Resources, C.A.T., A.T., C.O., M.E.A-A., L.A.F., and S.A.S.; Data Curation, E.O.A. D.A.C. and W.W.; Writing–Original Draft Preparation, E.O.A., D.A.C and W.W.; Writing – Review & Editing, E.O.A., D.A.C., YAW, OAM, W.K.B.A.O., C.O., EA, M.E.A-A., S.A.S., L.A.F., EA., EAA., EAY., XW., Y.W. and W.W.; Supervision, D.A.C., C.A.T., and W.W.; Project Administration, E.O.A, D.A.C., C.A.T., and W.W.; Funding Acquisition, Y.W. and W.W.

Details of ethics approval

Approval for the present study was obtained from the Committee on Human Research Publication and Ethics (CHRPE) of the School of Medical Science (SMS), KNUST and Komfo Anokye Teaching Hospital (KATH) (CHRPE/AP/146/17) and the Human Research Ethics Committee of Edith Cowan University (ECU) (17509). This study was conducted following the guidelines of the Helsinki Declaration. Written informed consent in the form of a signature and fingerprint was obtained from all participants and Legally Authorised Representatives after the protocol of the study was explained to them in plain English language and/or native Ghanaian language where appropriate.

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Supporting Information

Table S1 shows the potential of single biomarkers of OS and AGMs and their combinations at early (10-20weeks) and mid-pregnancy (21-31 weeks gestation) for the prediction of all cases of preeclampsia (PE)

Table S2 shows the potential of single biomarkers of OS and AGMs, and their combined ratios at 10-20 weeks and 21-31 weeks gestation for the prediction of SHS-PWLD early-onset PE (EO-PE)

Table S3 demonstrates the potential of the single biomarkers of OS and AGMs, and their combination ratios at 10-20weeks and 21-31weeks gestation, for the prediction of SHS-PWLD late-onset PE (LO-PE)

Table S4 shows the potential of the single biomarkers of OS and AGMs, and their combinations at 10-20 weeks and 21-31 weeks gestation for the prediction of OHS-PWLD early-onset PE (EO-PE)

Table S5 demonstrate the potential of the single biomarkers of OS and AGMs, and their ratios in 10-20 weeks and 21-31 weeks gestation for the prediction of OHS-PWLD late-onset PE (LO-PE)

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Table 1. Demographics, obstetrics, single biomarkers of OS and AGMs and their combination among SHS and OHS pregnant women who developed EO-PE and LO-PE compared to NTN-P

	SHS (N=248)	**SHS (N=248)**	**SHS (N=248)**
Parameter	SHS-PWD EO-PE (N=56)	SHS-PWD LO-PE (N=97)	SHS-PWD NTN-PW (N=95)
Nulliparous	38(67.9) *	24(24.7) *	26(27.4) +
Family history of HTN	14(25.0) *¥	18(18.6) *++	8(12.6) +
History of miscarriage	18(32.1) *	12(12.3) *	3(3.2) +
Previous caesarean section	22(39.2) *	15(15.5) *	8(8.4) +
Preterm delivery	39(69.6) *	20(20.6) *++	5(5.3) +
Low monthly income	27(48.2) *	13(13.4) *	6(6.3) +
Maternal age (years)	34.0(21.8-38.8) * ¥	28(24-33) *	30.0(27.0-34.0)
SBP (mmHg)	180.0(168-189.0) *	160.0(156.0-180.0) *++	120.0(114.0-122.0) +
DBP (mmHg)	105.0(100-113.0) *	104.0(100.0-110.0) *	78.0(70.0-80.0)
GA at delivery	32.0(32.0-33.0) *	37.0(35.0-38.0) *	38.0(37.0-39.0)
Serum PlGF (pg/ml)	71.5(45.2-95.2) *	73.8(42.4-95.6) *	104.6(97.5-109.9)
Serum VEGF-A (pg/ml)	114.1(71.2-137.1)	110.8(82.4-172.2) *	200.6 (182-212)
Serum sEng (ng/mL)	11.9(10.5-14.3) *	10.7(8.9-13.5) *	8.7(7.8-9.9)
Serum sFlt-1 (pg/ml)	1290(898.1-1581) *	1107(837-1389) *	787.5(623.9-980)
Serum 8-epiPGF2α (pg/ml)	2560(2057-3115) *	2187(1599-2882) *	1472 (1185-1894)
Urinary 8-OHdG (ng/mg Cr)	281.1(249.5-312.3) *	259.3(235.7-296.2) *	178.4(127.4-251.9)
Serum 8-OHdG (ng/L)	142.6(131.6-155.7) *	136.1(124.0-151.2) *	118.4(79.2-133.2)
Plasma TAC (μmol/l)	131.4(109.9-192.3) *	180.9(119.9-249.5) *	373.8(268.5-472.9)
sFlt-1: PlGF ratio	17.5(11.8-31.1) *	15.0(11.0-23.5) *	7.4(5.7-9.5)
8-epiPGF2alpha: PlGF ratio	34.4(26.1-64.8) *	29.5(20.8-48.6) *	14.7(11.3-18.7)
8-OHdG: PlGF ratio	1.9(1.4-3.5) *	1.5(1.2-2.9) *	1.2(0.7-1.3)
sEng: PlGF ratio	173.2(130.8-287.3) *	150.5(109.2-257.1) *	84.6(72.6-101.6)

Values are presented as median (interquartile ranges); % (n/N). Proportion (sample population/total population). SHS: suboptimal health status; OHS: optimal health status; PWD: pregnant women who developed; EO-PE: early-onset PE; LO-PE: late-onset PE; NTN-PW: normotensive pregnancy women; GA: gestational age.

P< 0.05 and in bold value indicates a statistically significant difference.

* indicates significance compared to SHS-PWD NTN-PW

indicates significant compared to OHS-PWD NTN-PW

¥ indicates significant between SHS-PWLD EO-PE and OHS-PWLD EO-PE

++ indicates significant between SHS-PWLD LO-PE and OHS-PWLD LO-PE

+ indicates significant between SHS-PWLD NTN-PW and OHS-PWLD NTN-PW

Table 2: Maternal levels of single biomarkers of OS and AGMs and their combination at visit 1 (10-20 weeks, early 2nd trimester) and visit 2 (21-31 weeks, mid-pregnancy) among SHS and OHS pregnant women who later developed PE

	SHS (N=248)	**SHS (N=248)**	**SHS (N=248)**
Parameter	SHS-PWLD EO-PE (N=56)	SHS-PWLD LO-PE (N=97)	SHS-PWLD NTN-P (N=95
10-20 weeks gestation (W1)			
GA (weeks)	17.0(15.3-18.0)	17.0(16.0-18.0)	18.0(15.0-19.0)
SBP (mmHg)	123.0(120.0-128.8) *	117.0(108.0-125.5) *	113.0(105.0-120.0)
DBP (mmHg)	77.0(69.0-84.0) *	76.0(67.0-82.5) *	69.0(64.0-76.0)
Serum PlGF (pg/mL)	81.3(50.2-95.0) *	87.3(68.5-95.3) *	99.4(91.1-107.4)
Serum VEGF-A (pg/mL)	117.8(95.8-150.9) *	121.0(92.1-170.2) *	187.3 (170.1-204.2)
Serum sEng (ng/mL)	7.0(5.8-9.3) *	5.9(4.3-8.5) *	4.1(3.2-5.1)
Serum sFlt-1 (pg/mL)	897.5(624.9-1100) *	770.2(582.9-966.4) *	543.0(433.3-682.4)
Serum 8-epiPGF2α (pg/mL)	600.0(499.2-667.6) *	506.8(391.9-668.7) *	371.9 (291.7-465.8)
Urinary 8-OHdG (ng/mg Cr)	87.75(81.6-99.4) *	86.6(77.6-96.1) *	75.20(51.3-86.1)
Serum 8-OHdG (ng/L)	88.0(81.2-96.1) *	83.3(74.8-92.8) *	73.1(48.9-82.2)
Plasma TAC (μmol/L)	147.6(94.6-259.3) *	220.0(170.1-275.6) *	234.6(180.3-317.2)
sFlt-1/PlGF ratio	10.1(7.7-13.4) *	9.8(6.8-13.5) *	4.4(3.5-5.9)
8-epiPGF2alpha/PlGF ratio	7.4(5.6-9.4) *	6.9(4.9-9.6) *	3.4(2.7-4.5)
8-OHdG/PlGF ratio	1.1(0.8-1.6) *	0.9(0.8-1.5) *	0.8(0.4-0.8)
sEng/PlGF ratio	88.1(67.1-127.5) *	80.8(50.7-131.4) *	41.7(29.7-51.6)
21-31 weeks gestation (W2)			
GA (weeks)	27.5(26.0-28.0)	28.0(26.0-28.0)	28.0(26.0-28.0)
SBP (mmHg)	134.5(121.5-138.3) *¥	128.5(109.5-130.0) *++	119.5(106.5-122.5)
DBP (mmHg)	88.5(70.5-85.5) *	78(68.5-84.0) *	73(65.5-77.5)
Serum PlGF (pg/mL)	98.3(61.2-111.7) *	106.0(82.8-111.3) *	132.3(117.9-181.5)
Serum VEGF-A (pg/mL)	152.6(109.1-210.0) *	157.6(111.3-215.5) *	280.3 (229.8-421.4)
Serum sEng (ng/mL)	8.6(7.2-11.1) *	7.5(5.7-10.3) *	5.5(4.6-6.6)
Serum sFlt-1 (pg/mL)	1032(718.6-1265) *	885.7(670.3-1111) *	624.5(499.0-784.7)
Serum 8-epiPGF2α (pg/mL)	963.7(803.9-1145) *	826.8(625.2-1079) *	593.3 (465.3-743.0)
Urinary 8-OHdG (ng/mg Cr)	105.7(97.6-121.7) *	103.3(92.4-114.7) *	90.0(61.8-101.6)
Serum 8-OHdG (ng/L)	98.6(91.0-107.6) *	94.1(85.7-104.6) *	81.9(54.8-92.1)
Plasma TAC (μmol/L)	178.0(127.8-212.3) *	205.7(146.4-253.0) *	258.1(196.4-348.9)
sFlt-1/PlGF ratio	10.8(8.3-13.9) *	10.3(7.2-14.2) *	5.5(4.19-6.9)
8-epiPGF2alpha/PlGF ratio	9.7(7.5-12.2) *	9.1(6.4-13.6) *	3.9(2.8-5.0)
8-OHdG/PlGF ratio	1.0(0.7-1.4) *	0.9(0.7-1.4) *	0.5(0.4-0.7)
sEng/PlGF ratio	89.6(70.2-124.0) *	89.2(57.1-133.7) *	37.1(27.5-51.9)

Values are presented as median (interquartile ranges). *P*- value < 0.05 indicates statistically significant difference. SHS: suboptimal health status; OHS: optimal health status; PWD: pregnant women who developed; EO-PE: early-onset PE; LO-PE: late-onset PE; NTN-PW: normotensive pregnancy women; GA: gestational age.

* indicates significance compared to SHS-PWD NTN-PW; indicates significant compared to OHS-PWD

NTN-PW

¥ indicates significant between SHS-PWLD EO-PE and OHS-PWLD EO-PE; ++ indicates significant between SHS-PWLD LO-PE and OHS-PWLD LO-PE

+ indicates significant between SHS-PWLD NTN-PW and OHS-PWLD NTN-PW