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Abstract

Background: Pregnant women, particularly in developing countries are facing a huge burden of preeclampsia (PE) leading to high morbidity and mortality rates. This is due to delayed diagnosis and unrecognised early targeted preventive measures. Adapting innovate solutions via shifting from delayed to early diagnosis of PE in the context of predictive diagnosis, targeted prevention and personalisation of medical care (PPPM / 3PM) is essential. The subjective assessment of suboptimal health status (SHS) and objectives biomarkers of oxidative stress (OS) and angiogenic growth mediators (AGMs) could be used as new PPPM approach for PE; however, these factors have only been studied in isolation with no data on their combine assessment. This study profiled early gestational biomarkers of OS and AGMs as 3PM approach to identify SHS pregnant mothers likely to develop PE specifically, early-onset PE (EO-PE) and late-onset PE (LO-PE).

Methods: A prospective cohort of 593 singleton normotensive pregnant (NTN-P) women were recruited at 10-20th (Visit 1) and followed from 21 weeks gestation until the time of PE diagnosis and delivery. At the Visit 1, SHS was assessed using SHS questionnaire-25 (SHSQ-25) and women were classified as SHS and optimal health status (OHS). Biomarkers of OS (8-hydroxy-2-deoxyguanosin [8-OHdG], 8-epi-prostaglansinF2alpha [8-epi-PGF2α] and total antioxidant capacity [TAC]) and AGMs (vascular endothelial growth factor [VEGF-A], soluble Fms-like tyrosine kinase-1 [sFlt-1], placental growth factor [PlGF] and soluble endoglin [sEng]) were measured at the visit 1 and time of PE diagnosis.

Results: Of the 593 mothers, 498 (248 SHS and 250 OHS) returned for delivery and were included in the final analysis. Fifty-six, 97 and 95 of the 248 SHS mothers developed EO-PE, LO-PE and NTN-P respectively, versus 14 EO-PE, 30 LO-PE and 206 NTN-P among the 250 OHS mothers. At the 10-20th week gestation, unbalanced levels of OS and AGMs were observed among SHS women who developed EO-PE than LO-PE compared to NTN-P women (p<0.0001). The combined ratios of OS and AGMs, mainly the levels of 8-OHdG/PIGF ratio at 10-20th week gestation yielded best area under the curve (AUC) and highest relative risk (RR) for predicting SHS-pregnant women who developed EO-PE (AUC=0.93; RR=6.5; p<0.0001) and LO-PE (AUC=0.88, RR=4.4; p<0.0001), as well as, for OHS-pregnant women who developed EO-PE (AUC=0.89, RR=5.6; p<0.0001) and LO-PE (AUC=0.85; RR=5.1; p<0.0001).

Conclusion: Unlike OHS pregnant women, SHS pregnant cohort have high incidence of PE coupled with unbalanced levels of OS and AGMs at 10-20 weeks gestation. Combine early gestational profiling of OS and AGMs created an avenue for early differentiation of PE subtypes in the context of 3PM care for mothers at high-risk of PE.

Keywords: Preeclampsia; suboptimal health status; predictive, preventive and personalised Medicine (3P/PPPM); oxidative stress; angiogenic growth mediators; risk assessment, treatment algorithm, patient stratification, maternal health care, public health education

Introduction

From the early twenty-first century to present, pregnant women around the world are facing the paradoxal epidemic development of non-communicable disease. One of the deadiest among these non-communicable conditions is preeclampsia (PE), a disorder characterised by hypertension and proteinuria after 20 weeks of gestation [1]. PE is known to affect 5-8% of all pregnancies especially in nulliparous, young age and advanced maternal age mothers causing devastating complications for both mothers and babies, from brain and liver injuries in mothers to premature birth [1]. PE is one of the top causes of maternal-foetal mortality worldwide [1]. Early-onset PE (EO-PE) occurring before 34 weeks gestation is known to be associated more with adverse pregnancy risk complications and mortalities unlike the late-onset PE (LO-PE) [2]. This high adverse events and mortality rates may be due to the unmet health needs of pregnant mothers in terms of predictive diagnosis, preventive strategies, personalisation of medical services for those who suffer this condition [3, 4].

While PE is somewhat treatable, as seen in some developed countries, for example in the U.K.; PE is fatal in only one out of every million births [5, 6]. In many other countries, however, particularly those in developing countries, this condition still poses a formidable threat to maternal-foetal health [5, 6]. For example, in Ghana, roughly 18% maternal deaths are due to this condition, which translates to more than 570 deaths per 100,000 livebirths [5]. A cohort study in Ghana reported that 26.4% of all maternal deaths were associated with hypertensive disorders of pregnancy with PE being the leading cause of death [7]. The prevalence of PE was 48.8%, being the highest amongst all the hypertensive disorders of pregnancy evaluated in a cross-sectional study in Ghana [8].

The prevalence rate is gradually increasing in developing countries, particularly in Ghana due to late reporting to antenatal clinic, delayed detection and diagnosis and resource constraints [8].

In most developing countries, resource constraints hamper early detection, targeted prevention and personalisation of medical care. In addition, diagnosis and management of PE for both developed and developing countries is greatly dependent on clinical manifestaion and time of diagnosis [9, 10]. This traditional approach in itself has not yielded successful management outcome but culminate in more severe adverse oucomes [10]. Over the years, the delivery of the placenta and the baby under intensive care has been the dependent remedy that can avert PE onset [10]. This in itself is a delayed targeted preventive approach.

Suboptimal health as an operational era for preventive diagnsosis, targeted prevention and personalisation of medical (PPPM/3PM) care

The paradox, however, is that adverse health effects and/ clinical manifestation of most non-communicable conditions including PE is suboptimal. While suboptimal health conditions can easily be reversed, unfortunately, this unique concept is not recognised in most current healthcare systems [4]. On the contrary, the total dependence on clinical manifestation of diseases or time of diagnosis has been accepted as the only approach to start treatment intervention. The consequencies are that majority of these individuals who show clinical symtoms of PE may progress to more severe and irreversible pathology with collateral complications that affect the liver, brains, kidneys, heart, placenta among other organs.

The suboptimal time-frame is the operational area for predictive diagnosis and identification of persons at risk by innovative screening programmes followed by the most cost-effective personalised treatment possible, namely primary targeted prevention tailored to the person [3, 4]. Assessment of health status at the suboptimal stage as well as follow-up mitigating programmes are essential measures and should be considered a public health priority. Thus, the suboptimal health stage has been proposed as an attractive stage for preventive diagnsosis, targeted prevention and personalisation of medical care (PPPM/3PM)[4]. Therefore the need to shift from reactive medicine to PPPM must be the ideal approach of modern health systems.

PPPM is an integrative concept that enables the prediction of an individual's predisposition before the onset of a disease, to provide targeted preventive measures and create personalised treatment algorithms tailored to an individual [4]. Over the past years,

PPPM has made a significant impact on the prevention and treatment of non-communicable diseases. This is because the PPPM concept adopts an all-inclusive approach to solving health-related problems [3, 4]. There is therefore, the need to shift from reactive medicine to PPPM that allows screening of patients at the preclinical or suboptimal health stage prior to the onset of diseases such as PE.

Suboptimal health status (SHS) evaluation in the context of PPPM identifies poor health in individuals without a diagnosable clinical condition, and create opportunity for reversibility of chronic diseases, patient's surveillance, risk stratifications, prognostics accuracies, and targeted treatment options to the evident profits of patients and general population [11-13]. SHS is defined as the overall physical state between health and disease and charcterised by health complaints, general weakness, and low energy within a period of three months [12, 14]. Several validated questionnaires are in use for SHS assessment. However, the suboptimal health status questionnaire-25 (SHSQ-25), which encompasses five domain namely: fatigue, cardiovascular health, digestive health, immune health and mental health is dynamic its evaluation. Several studies have found a strong association of SHS with N-glycolylation for chronic disease stratification [15], cardiovascular disorders, diabetes mellitus, vaginal dryness, metabolomic, endothelial dysfunction [4], preeclampsia and adverse pregnancy complication [16], oxidative stress and angiogenic growth factor imbalances [17],. The evaluation of SHS, however, is only subjective, and thus, there is a need to evaluate additional objectives or phenotypic measures that allows for multilevel diagnostics. This is beacause PE is a multisytem disorder and as such subjective evaluation is not enough to meet preventive diagnostic, targeted prevention and personalised medical care [1].

Oxidative Stress and Angiogenic Growth Mediators as an attractive preventive diagnostic measures and targeted treatment approach tailored towards SHS mothers at risk of PE

Accumulating evidence, however, indicates that increase oxidative stress (OS), poor placental angiogenesis and incomplete maternal artery remodelling are among the leading contributing factors for PE development [18, 19]. Complete placental angiogenesis and vasculogenesis are key to maternal well-being and growth of the foetus. An incomplete placental vascular development, however, may result in placental hypoxia and ischaemia, which subsequently stimulates an increased reactive oxygen species (ROS) and OS response [19, 20]. In poor placental angiogenesis and incomplete maternal artery remodelling, the syncytiotrophoblast in early-onset preeclampsia (EO-PE) and late-

onset preeclampsia (LO-PE) show altered mitochondrial structure and function resulting in ROS overproduction, OS, and cell damage and death [21]. OS, which is an imbalance between pro- and anti-oxidants, 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 [22].

Increase placental ROS results in tissue OS, which increases the release of anti-angiogenenic molecules such as soluble FMS-tyrosine kinase receptor-1 (sFlt-1) [19]. Increased circulatory sFlt-l antagonises the function of proangiogenic molecules such as placental growth factors (PIGF) and vascular endothelial growth factor (VEGF), which drive high blood pressure and endothelial dysfunction in PE [19]. Reduced levels of PlGF and VEGF-A and increased levels of sEng and sFlt-1 have been reported at the time of PE diagnosis [23] and at 10-20th weeks gestation even before the clinical manifestation of PE [24]. Mediators of vascular dysfunction are multifactorial phenomenon known as the basis for many disorders like hypertensive diosrders of pregnancy and their consequential complications [25]. However, this in the context of PPPM tailored towards individuals with PE has received little attention. Mitochondrial or endothelial dysfunction is the key hallmark of PE because the condition affects multiorgans from brain, to heart, liver, peripheral, guts, nervous, endocrine systems, and kidney, amongst other organs, and as such, vascular dysfunction or mitochondrionopathies have been proposed as an attractive preventive diagnostic and targeted preventive approach to be investigated in any patient with unexplained progressive multisystem disorder [26].

The roles of OS and AGMs even though are synergistic to the development of PE, their biomarkers for PE have previously been studied in isolation notwithstanding the high rates of false positivity associated with measure single markers. Unfortunately, data on the prospective cohort evaluation of both biomarkers of OS and AGMs in pregnancy is not available in Ghana up to date. The present study hypothesises that the combined evaluation or algorithm of OS and AGMs biomarkers would generate a useful approach to PPPM, and monitoring the progression of PE. Again, since suboptimal stage is the most recommended for reversing chronic disease and its associated complications, evaluating algorithm of OS and AGMs biomarkers among suboptimal health pregnant mothers may generate a useful understanding of the pathogenesis of PE and create opportunity for preventive diagnosis, targeted prevention and personalisation of medical care for normotensive mothers at risk of PE.

The present prospective cohort study for the first time in a Ghanaian population, explored the levels of individual and combined ratios of the biomarkers of OS and AGMs, in addition to, the predictive diagnostic potential of these markers measured at 10-20 weeks of gestation among Ghanaian SHS pregnant women for the prediction of PE, specifically, early-onset PE (EO-PE) and late-onset PE (LO-PE). Thus, the present study hypothesized that the combined evaluation of oxidative stress biomarkers and angiogenic growth mediators would create diagnostics screening algorithm and allow for multilevel diagnostics, early stratification of PE subtypes as well as differentiation of high-risk SHS normotensive pregnant mothers with imbalance circulatory levels of pro-and antioxidants and pro-and antiangiogenic growth mediators who may need early targeted combination of antioxidant and proangiogenic treatment. PPPM has over the past few years adopted environmental, traditional and behavioural factors to solving public health conditions, and this would further increase health policy, prognosis, diagnostic screening algorithm, individualised treatment and prevention and public health education in maternal health care.

Materials and Methods

Study participants and selection

This prospective cohort study was based on a Ghanaian Suboptimal Health Status Cohort Study conducted at the Obstetrics and Gynaecology Department of the 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 were recruited. Approvals for the present study were obtained from the Ethical Committees on of the School of Medical Science, KNUST and KATH (CHRPE/AP/146/17) and Edith Cowan University (ECU) (17509), respectively. 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. 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.

Suboptimal health status assessment and outcome

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 procedure as described in the previous studies [16, 17]. Briefly, at visit 1 (10-20 weeks gestation/average gestation of 17 weeks), the overall health status of the 593 NTN-PW was measured using SHSQ-25. Based on how often each pregnant woman had experienced a particular health complaint in the past three (3) months, they were asked to rate a health statement on a 5-point Likert scale: never or almost never (1), occasionally (2), often (3), very often (4) and always (5). These scores were recoded as 0-4 followed by a summation of the codes for the 25 answered items. The median of the total score was recorded as the cut-off point and values ≥ the cut-off represented 'SHS' (Poor health) and those < the cut-off indicates 'optimal health'.

In the present study, a median score ≥ 19 depicted SHS and <19 depicted optimal health status (OHS). Of the 593 NTN-PW, 297 were SHS whereas 296 had OHS. A Cronbach's alpha coefficient value of 0.95 was found after testing the reliability of SHSQ-25.

Participants' Follow-up and outcome

The 593 comprising of the 297 SHS and 296 OHS clinically diagnosed NTN-PW were followed from 21 weeks of gestation until time of PE diagnosis and birth after their clinical, sociodemographic, and obstetric characteristics were assesses at 10-20 weeks of gestation (visit 1). Of the 593, 498 completed the study and were included in the final assessment whereas 95 were lost to follow-up. At the time of diagnosis (32-42 weeks gestation) [visit 2], 197 of the 498 had developed PE and were classified as cases whereas 301 of the 498 had normotensive pregnancies and were classified as controls**.** Among the 197 women who developed PE, 153 were SHS pregnant women whereas 44 were OHS pregnant women when their overall health was assessed at 10-20 weeks of gestation (visit 1). Of the 153 SHS pregnant women who developed PE, 56 had early-onset PE (EO-PE) whereas 97 had late-onset PE (LO-PE). Meanwhiles, of the 44 OHS women who developed PE, 14 had EO-PE whereas 30 had LO-PE. Among the 301 controls, 95 had SHS whereas 206 were OHS as at visit1. 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) **(Fig 1).** The drop out rate was 16.0%.

Physical examination and diagnosis of PE patients

A qualified consultant obstetrician/gynaecologist physically examined all participants. PE was diagnosed 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 occasions at least four hours apart detected after 20 weeks gestation in previously normotensive pregnant women. Alternatively, PE diagnosis was based on high blood pressure (≥ 140/90 mmHg) combined with the presence of multisystemic manifestations such as HELLP syndrome, renal insufficiency, pulmonary oedema, and visual or cerebral disturbances even in the absence of proteinuria according to the definition of International Society for the Study of Hypertension in Pregnancy (ISSHP) [2]. Pregnant women with PE were classified as early-onset PE (EO-PE) and late-onset PE (LO-PE) when PE diagnosis occurred before and at or after 34 weeks gestation, respectively [2].

Laboratory assays

Serum, plasma and urine samples were obtained from all participants up to a total of the two visits, i.e., visit 1 (10-20 weeks gestation) and time of diagnosis. Samples were stored at -80 ºC (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.

Total Antioxidant Capacity (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 KNUST and the Biochemistry and Immunology Department of the KATH, Ghana.

Statistical analyses

The normality of the data was tested using 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 post-hoc 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 and R version 3.4.3 [32].

Results

Demographics, obstetrics, biomarkers of OS and AGMs among SHS and OHS pregnant women at visit 1

At visit 1, women with SHS had significantly increased percentage history of spontaneous abortion (36.7% vs. 19.2%; p=0.0001), nulliparity (41.1% vs. 32.8%; p=0.0202) and primigravidity (62.9% vs. 30.8%; p<0.0001) compared to those with OHS. None of the NTN pregnant women had proteinuria at visit 1. There was a statistically significant

difference between the mean level of SBP among NTN pregnant women with SHS compared to those with OHS (116.0 vs. 113.2; p=0.0036). Meanwhile, there was no statistically significant difference between the mean age, gestational age, DBP, pre-gestational and gestational BMI among SHS_NTN pregnant women compared to those with OHS ($p > 0.05$). At visit 1, SHS_NTN pregnant women had significantly lower levels of P1GF, VEGF-A and TAC but significantly higher levels of sEng, sFlt-1, 8-epiPGF2α, and 8-OHdG compared to OHS-NTN pregnant women ($p < 0.0001$) **(Table 1)**.

Visit 1 (10-20 weeks gestation): Maternal levels of OS biomarkers and AGMs among SHS and OHS who subsequently developed EO-PE and LO-PE compared to NTN-PW

At visit 1, 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 ($p<0.0001$). Similar observations occurred among the OHS group ($p < 0.0001$) 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).**

Visit 2: Obstetrics, delivery outcomes and levels of biomarkers of OS and AGMs among SHS and OHS pregnant women who developed EO-PE and LO-PE compared to NTN-P at the time of diagnosis and delivery

Of the 498 pregnant women who returned for delivery, 248 had SHS and 250 were identified as OHS at visit 1. The incidence of EO-PE, LO-PE and NTN-P among SHS mothers were 56 (11.2%), 97 (19.5%) and 95 (19.0%), respectively whereas the incidence among OHS mothers were 14(2.8%), 30 (6.0%) and 206 (41.4%), respectively. Overall, the incidence of PE was 39.5%. Compared to NTN-PW, a significantly higher proportion of the EO-PE rather than LO-PE were nulliparous, delivered preterm babies, had lower monthly income, had history of spontaneous abortion/miscarriage, had previous caesarean section and had family history of hypertension (all $p<0.05$). The proportions were higher among SHS groups compared to OHS group **(Table 3)**.

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<0.0001). There was a significantly increased systolic blood pressure (SBP), diastolic blood pressure (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<0.0001). 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<0.0001)**.**

Although no statistical significance was observed, the clinically significant difference indicated by the high level of imbalances in SHS group 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=0.0381) **(Table 3)**.

Visit 1: Levels of OS biomarkers and AGMs, and their combined ratios for the prediction of SHS-pregnant women who developed (PWD) early-onset PE (EO-PE) and late onset-PE (LO-PE)

Compared to the individual biomarkers of OS and AGMs, their combined ratios measured at visit 1 yielded the highest discriminating power for predicting SHS-pregnant women who were at risk of developing EO-PE and LO-PE. Particularly, 8-OHdG/PIGF ratio was the best marker for predicting SHS-pregnant women who were at risk of developing EO-PE. At a cut-off value ≥ 0.7, 8-OHdG/PIGF ratio yielded a significantly (p<0.0001) high discriminating power of 93% (AUC=0.93) **(Fig 2c),** a sensitivity of 96.4%, specificity of 100.0%, negative predicted value (NPV) of 100.0%, positive predicted value (PPV) of 75.0%, positive likelihood ratio (LR+) of 12.2 and a negative likelihood ratio LR- of 0.0. SHS pregnant women who had 8-OHdG/PIGF ratio ≥0.7 were at 6.5-fold increased relative risk (RR) of developing EO-PE [RR =6.5, 95%CI (1.4-12.5), p<0.0001] **(Table 4)**. Conversely, three (3) markers including 8-OHdG/PIGF ratio, sFlt-1/PlGF ratio and 8-epiPGF2alpha/PlGF ratio yielded the highest discriminating power of approximately 88% (AUC=0.88) for predicting SHS-pregnant women who at risk of developing LO-PE, with a similar sensitivity of 76.3% even though 8-OHdG/PIGF ratio yielded the highest specificity of 86.8% compared to 83.2% for sFlt-1/PlGF ratio and 8-epiPGF2alpha/PlGF ratio. At a cut-off value of ≥ 0.8 for 8-OHdG/PIGF ratio, ≥7.3 for

sFlt-1/PIGF ratio and ≥4.9 for 8-epiPGF2alpha /PIGF ratio SHS pregnant women were at 4.4-fold, 3.2-fold and 3.0-fold increased relative risk (RR) of developing LO-PE. Except for TAC levels, which did not show significant AUC, all the individual biomarkers and the combined ratios of OS and AGMs yielded a significant (p <0.05) discriminating power and relative risk for predicting the likelihood of SHS pregnant women developing EO-PE and LO-PE **(Table 4).**

Visit 1: Levels of OS biomarkers and AGMs, and their combined ratios for prediction of OHS pregnant women likely to develop EO-PE and LO-PE

Overall, 8-OHdG/PIGF ratio measured at visit 1 was the best predictive marker for both EO-PE and LO-PE compared to the single markers **(Table 5)**. 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) **(Fig 3f)**, 83.3% sensitivity, 90.0% specificity, 54.4% PPV, 97.4% NPV, 20.6 LR+ and 0.0 LR- were observed for predicting OHS pregnant women who developed LO-PE.

Similarly, at a cut-off value ≥0.7, 8-OHdG/PIGF ratio yielded a significantly (p<0.0001) high discriminating power of 89% (AUC=0.89) **(Fig 3c),** a sensitivity of 92.9%, specificity of 71.8%, negative predicted value (NPV) of 99.3%, positive predicted value (PPV) of 48.3%, positive likelihood ratio (LR+) of 20.6 and a negative likelihood ratio LR- of 0.0 for predicting EO-PE among OHS pregnant women. OHS pregnant women who had 8-OHdG/PIGF ratio ≥ 0.7 were at 5.6-fold increased relative risk (RR) of developing EO-PE [RR =6.5, 95%CI (1.5-11.9) p<0.0001] **(Table 5)**.

Except for visit 1 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 **(Table 5)**.

Discussion

The need to shift from delayed diagnosis to predictive preventive and personalised medical (PPPM) care has become necessary in modern-day health care systems. This is because delayed diagnosis predisposes the individual to more severe and irreversible

pathology with collateral complications. In the case of women with preeclampsia (PE), adverse foeto-martenal complications become prevalent leading to increased fatal rates. The present study, for the first time, explored the predictive potential of the individual biomarkers of oxidative stress (OS) and angiogenic growth mediators (AGMs), and their combined ratios measured at 10-20 weeks gestation for the early identification of SHS and OHS normotensive pregnant women at risk of preeclampsia (PE), specifically, early-onset PE and late-onset PE. Since PE onset is noticeable after 20 weeks of gestation, evaluation of these factors at 10-20 weeks would create an avenue for early 3P medical care for high-risk pregnant women. Overall, our findings indicated that suboptimal health status (SHS) pregnant women rather than their optimal health status (OHS) women were at high risk of developing PE. Compared to the individual biomarkers of OS and AGMs, their combined ratios particularly, the 8-OHdG/PIGF ratio measured at 10-20 weeks gestation yielded the best predictive accuracies for identifying SHS and OHS pregnant women likely to EO-PE and LO-PE. The ability of combined markers of OS and AGMs at early gestation to predict the onset of PE indicates their importance in PPPM.

Incidence of PE in Suboptimal Health Normotensive pregnancy

In the present study, the incidence of PE among SHS pregnant mother was 30.70% versus 8.8% for OHS pregnant women. An incidence rate of 9.03% was reported by Ahenkorah et al. [27] among Ghanaian pregnant women visiting the Komfo Anokye Teaching Hospital, which is similar to the incidence of 8.8% recorded among optimal health status in the present study. Conversely, the incidence rate of PE among SHS pregnant women was unexpectedly high. A cohort study by Husse et al [28] found that 41% of pregnant women developed PE coexisting with HELLP syndrome or Intrauterine growth restriction. The explanation of high incidence rate in the present study may be attributed to the use of smaller sample population and the choice of setting used in the present study. Komfo Anokye Teaching Hospital, which is the present study setting is a referral centre for most PE cases and pregnancies with history of hypertensive disorders.

In a cross-sectional study conducted in a Ghanaian population, imbalance in oxidative stress (OS) biomarkers and angiogenic growth mediators (AGMs) was associated with SHS pregnant women [17], which is consistent with the present study findings **(Table 1),** and may be one of the probable explanations to the high incidence rate of PE among SHS women. A prospective cohort study by Anto et al., [16] found that SHS pregnant women were at high-risk incidence of PE compared to OHS pregnant women, indicating that diagnosis of normotensive pregnancies based on blood pressure

and proteinuria alone is not good enough. This is because some normotensive pregnant women may have subclinical or poor health without visible symptoms i.e., SHS. Another supportive reason for the high incidence of PE among the SHS pregnant mothers was that when we evaluated visit 1 levels of OS biomarkers and AGMs among SHS and OHS pregnant women who went on to develop PE, there was a significant imbalance in biomarkers of OS and AGMs in the SHS group than the OHS group **(Table 2).** This signifies that the SHS can differentiate or identify early signs of increase OS and unbalanced AGMs to explain the embodies of a reduced antioxidant system and poor placental angiogenesis in high-risk pregnant women at risk of PE. Identification of SHS pregnant women in early gestation of pregnancy is a predictive sign of high risk. This would allow prevention of clinical symptoms of PE and its progression to more severe and irreversible pathology with collateral multi-organ dysfunction. The concept of SHS if applied in addition to standard criteria for PE diagnosis, and may be useful in screening pregnant women who are at high-risk of developing PE thereby, creating an opportunity for early predictive diagnosis, targeted prevention and personalised medical services tailored towards the high-risk pregnant mothers. From treatment viewpoint, this may inform clinician of the need to start targeted preventive measures such as antioxidant and proangiogenic medical supplementation and nutrition tailored toward the high-risk pregnant mothers. Thus, incorporating of SHS evaluation as a criterion for the prediction of PE is highly recommended for healthcare management in pregnancy [16]. A combine algorithm of subjective SHS assessment and objective biomarkers of OS is needed in antenatal care screening.

SHS-associated imbalance in Oxidative Stress and Angiogenic Growth Mediators as early risk indications for PE development in the Context of PPPM

Another major finding of the present study was that at the time of PE diagnosis there was an increased OS among SHS more than the OHS pregnant women who developed PE compared to normotensive (NTN) pregnant women. This was depicted by a significantly reduced plasma total antioxidant capacity (TAC) and correspondingly increased levels of serum 8-epiprostaglandin F2-alpha (8-epiPGF2α), 8-hydroxydeoxyguanosine (8-OHdG), and urinary 8-OHdG. The degree of imbalance was among SHS pregnant women who developed early-onset PE (EO-PE) rather than late-onset PE LO-PE compared to NTN pregnancy **(Table 3)**. To the best of our knowledge, this is the first time such a finding has been reported. This finding is indicative of high endogenous peroxidation, oxidative DNA damage and a compromised antioxidant

system, which is common in SHS pregnant women who develop PE rather than OHS pregnant women. In PE women, increased OS may originate from placental hypoxia and ischaemia, high demands of the foetus and the pregnancy that further induce systemic reactive oxygen species (ROS) production and cause damage to lipids, DNA and proteins. Increase OS and ROS production are also key hallmarks of mitochondrial and endothelial dysfunction, and in view of this, mitochondrionopathies have been proposed as an attractive preventive diagnostic and targeted preventive approach to be investigated in any patient with unexplained progressive multisystem disorder [26]. Thus, the present study findings indicating high incidence of PE among SHS and correspondingly significant imbalance in OS biomarkers at visit 1 among those who went on to develop PE signifies the usefulness of SHS screening and the need to integrate in healthcare management of suboptimal health individuals.

Furthermore, at the time of diagnosis, a significantly reduced PIGF and VEGF-A, and correspondingly increased levels of sEng, sFlt-1, sFlt-1/PlGF ratio, and sEng/PlGF ratio among SHS pregnant women who developed EO-PE than those who had LO-PE, when both cases were compared to NTN pregnancies **(Table 3)**. Several longitudinal cohort studies [24, 28, 29] have also reported that imbalances in AGMs can occur before 20 weeks gestation to the clinical manifestation of PE, which is consistent to the present study findings. In the present study, an imbalance in AGMs was observed at 10-20 weeks gestation (visit 1) among SHS than OHS pregnant women prior to the development of EO-PE and LO-PE compared to NTN pregnant women**.** This suggests that SHS mothers are at high risk of defective angiogenesis and vasculogenesis. Vascular dysregulation is a multifactorial phenomenon known as the basis for many disorders and their consequential complications. The mechanism may operate via shallow extravillous trophoblast invasion and subsequent poor maternal artery remodelling resulting in placental underperfusion and hypoxia [30]. This further stimulates the antagonistic activity of sFlt-1 to impair the physiological function of PIGF and VEGF-A [30]. In addition, the anti-AGM, sEng interferes with transforming growth factor beta 1, which is important in nitric oxide synthesis. Inadequate availability of nitric oxide may cause vasoconstriction and subsequently endothelial dysfunction and clinical manifestation of PE [25, 31, 32]. SHS evaluation can be used as an early risk stratification tool for pregnant mothers with poor placental angiogenesis, while informing clinicians the need for pro-angiogenic supplementation targeted at the high-risk pregnant mother. The observed significant imbalance in AGMs before the clinical manifestation of PE further indicates

that these markers may be potential predictive and targeted preventive markers of PE, and may be useful for personalisation of medical care. This from the perspective of PPPM will promote health education, policy making, risk stratification, adequate patient surveillance, predictive diagnosis, and prediction of adverse drug-to-drug interactions [4].

Combined Algorithm of Oxidative Stress and Angiogenic Growth Mediators markers as early predictive markers and preventive treatment for SHS mother at risk of PE and its subtypes

While measuring individual markers may be associated with false positivity, a combine evaluation of markers is mostly ideal because it yields high discriminatory power and specificity, and also allows for multi-level testing and diagnostics. This promotes algorithm of targeted preventive medical services for patients and high-risk populations in the healthcare systems. Our findings indicated that unlike the individual biomarkers of OS and AGMs, the combined ratios including sFlt-1/PlGF, 8-epiPGF2α/PlGF, 8-OHdG/PlGF and sEng/PlGF yielded a better discriminatory power or area under the curve (AUC), sensitivity, specificity, positive predicted values (PPV), negative predictive values (NPV), highest positive likelihood ratio (LR+) and lowest negative likelihood ratio (LR). On the whole, the 8-OHdG/PlGF ratio levels measured at 10-20 weeks of gestation (visit 1) yielded the best discriminatory power and significantly increased adjusted relative risk ratios for predicting early-onset PE and late-onset PE **(Tables 4 and 5)**. The strength of the 8-OHdG/PIGF ratio is that when the cut-off value was applied in a logistic regression model, the SHS mothers were at increased relative risk of developing PE, indicating that its prognostic potential is independent of any confounding factors. This finding is novel. The inclusion of the SHS concept from the PPPM perspective for pregnancy stratification and identification of combine effect of OS and AGMs in the pathogenesis of PE embodied individuals has increased knowledge of new preventive and targeted treatment strategies. The present study, therefore, hypothesises that 8-OHdG/PlGF ratio is the ideal predictive diagnostic marker that gives a comprehensive understanding of the pathogenesis of PE. The abnormally increased 8-OHdG/PIGF ratio reflects the imbalance between both OS and AGMs indicating that the increased oxidative DNA damage has created disequilibrium in pro-angiogenic function culminating in the clinical manifestation of PE. By measuring both OS biomarkers and AGMs, the synergistic physiology of both factors may be known. Thus, combined evaluation of oxidative stress biomarkers and angiogenic growth mediators would create

diagnostics screening algorithm and allow for multilevel diagnostics, early stratification of PE subtypes as well as differentiation of high-risk SHS normotensive pregnant mothers with imbalance circulatory levels of pro-and antioxidants and pro-and antiangiogenic growth mediators who may need early targeted combination of antioxidant and proangiogenic treatment. Early gestational identification of increased 8-OHdG/PIGF ratio among SHS mothers will inform clinicians of the need for a targeted preventive combined prophylaxis of antioxidant and proangiogenic supplementation. This combined therapy is likely to reduce the circulatory ischaemic/hypoxic insult while enhancing placental angiogenesis as well as growth of foetus in the context of PPPM.

Strengths and Limitations

One of the strengths of the present study is the application of the both subjective concepts, SHS and objective biomarkers of OS and AGMs for differentiating the high-risk normotensive pregnant women who may develop PE. SHS is a subjective, cost-efficient and non-invasive approach to identify poor health at the preclinical stage of diseases, and thus must be highly recommended. Another strength is our ability to identify for the first time the novel combined biomarker ratio of OS and AGMs, i.e., 8-OHdG/PlGF ratio for the prediction the subtypes of PE namely early and late-onset PE. Despite the novel findings, our study has a few limitations. 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. Moreover, the high Area Under the ROC curve generated may be due to the high incidence rate of PE and may not necessarily be due to the fact that the combined biomarkers are accurate.

Conclusions and Expert Recommendation

The incidence of preeclampsia is high in suboptimal heath than in optimal health normotensive pregnancies. An imbalance in the levels of oxidative stress (OS) as well as angiogenic growth factors (AGMs) at 10-20 weeks gestation occurs more among suboptimal health normotensive who are likely to develop early-onset preeclampsia than late-onset preeclampsia. The detection of imbalance in OS and AGMs early in pregnancy and their association with suboptimal health status prior to the onset of preeclampsia has increased knowledge of the predictive biomarkers of preeclampsia while informing the need for a paradigm shift from reactive medicine to predictive, preventive and

personalised (PPPM/3P) medicine. The combined ratio of OS and AGMs yields a better discriminating power or predictive accuracies for prediction of suboptimal health normotensive pregnant women likely to develop early-onset preeclampsia and late-onset preeclampsia unlike using the single markers. On the whole, the combined ratio of OS and AGMs biomarkers, particularly the 8-hydroxydeoxyguanosine-to-placental growth factor (8-OHdG/PlGF) ratio measured at early gestation (10-20) weeks gestation is the best marker for predicting preeclampsia.

Expert recommendations to personalisation of medical approaches in the management of suboptimal health mothers at risk of preeclampsia

Suboptimal health status (SHS) evaluation is subjective, cost-effective and non-invasion, and thus, integration of SHS screening in both early antenatal care and monitoring would allow early detection of increase oxidative stress and poor placental angiogenesis while creating an opportunity for PPPM policies such as early screening programmes, education, risk assessment, stratifications, preventive diagnosis, targeted prevention and personalisation of medical care. The idea of SHS profile that highly differentiated between physiological and phenotypic risk factors, suggest though usually perceived as 'subjective', we can still feel the internal pathologies of the condition. This is very essential for self-education and policy making, suggesting a potential for combine 'subjective and objective' as a model of PPPM approach in preeclampsia prognosis.

The role of suboptimal health status in the context of PPPM/3P medicine is to force innovative analytical approaches which would allow for distinguishing between pregnancy outcomes under circumstance of poor health, increased oxidative stress and poor placental angiogenesis at early gestation prior to the clinical manifestations of preeclampsia. Thus, individualised patient profiling, patient stratification, screening programmes focused on suboptimal health pregnancies, non-invasive prediction by integration of SHS criterion, which is a cost-effective targeted prevention are instrumental for the paradigm shift from reactive medicine to PPPM. Against this background, incorporating of SHS evaluation as a criterion for the prediction of preeclampsia is highly recommended for healthcare management and treatment algorithm tailored high-risk pregnancies.

Furthermore, individualised phenotyping is important for screening programmes focused on individuals with reversible or suboptimal damage associated with systemic ischemic-reperfusion effects clearly predisposed to mitochondrial and vascular dysfunction and associated complications [3]. Individual and combined evaluation of oxidative stress

biomarkers and angiogenic growth mediators in suboptimal health pregnancies is instrumental for multi-level testing in the context of PPPM. SHS evaluation for the differential screening of increase OS and imbalance AGMs may not only benefit individual from developed countries but can recommended in emergency situations as a substitute prescreening health measure in low-resourced clinical laboratory, field and community health centres in developing countries. Further studies are needed to replicate the present study and to validate the finding that the novel biomarker, 8-OHdG/PlGF ratio is the best predictive marker for PE and its subtypes.

Finally, while oxidative stress and angiogenic growth mediators have synergistic pathways in the pathogenesis of preeclampsia, combined treatment options are supportive for the effective treatments considering the hallmark of endothelial dysfunction and individual predisposition to adverse maternal and perinatal complications [19]. From therapeutic standpoint, mitigating measures against increased oxidative stress and unbalanced angiogenic mediators in preeclampsia include application of combined antioxidant supplements with pro-angiogenic molecules, and individualised lifestyle recommendations [33]. Normal body weight but borderline body mass index might be optimal for one person but apparently suboptimal for others depending on the genetic predisposition factors, geographic location, cultural and nutritional habits and relevant lifestyle parameters. Furthers studies may focus on suboptimal and optimal body weight and its impact on the development of adverse pregnancy outcome like preeclampsia by stratifying the individual patient profile based on these factors.

Abbreviations

sEng: soluble endoglin, sFlt-1: soluble Fms-like tyrosine kinase-1, 8-epi-PGF2α: 8-epi-prostaglansinF2alpha, 8-OHdG: 8-hydroxy-2-deoxyguanosin, AGMs: Angiogenic growth Mediators, AUC: Area under the curve, CS: Caesarean section, EO-PE: Early-onset PE, GHOACS: Ghanaian Suboptimal Health Cohort Study, KATH: Komfo Anokye Teaching Hospital, LO-PE: Late-onset PE, NPV: Negative predicted value, NTN-P: Normotensive pregnancy, NTN-PW: Normotensive pregnant women, OHS: Optimal health status, OS: Oxidative Stress, PE: Preeclampsia, PlGF: Placental growth factor, PPV: Positive predicted value, PWLD: Pregnant women who later developed, RR: Relative risk, SHS: Suboptimal health status, SHSQ-25: Suboptimal Health Status questionnaire, TAC: total antioxidant capacity, VEGF-A: Vascular endothelium growth factor-A.

Declarations

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Availability of Data and Material: The data used in the analysis is available upon request from the corresponding author.

Authors' contribution:

EOA, DAC and WW conceived the study. EOA, AT OAM, CO, YAW, WKBAO, BAO and YW performed the investigation and collected the data. EOA, SO, EAA, BKD and EA performed the statistical analysis. EOA, EAA, HH, MEAA, AT, EA and XW wrote the initial draft paper. All authors revised, read and approved the final manuscript.

Consent for publication: Not Applicable

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Declaration. Written informed consent in the form of a signature and fingerprint was obtained from all participants and legally authorized representatives after the protocol of the study was explained to them in plain English language and native Ghanaian language where appropriate.

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Table 1. Visit 1: sociodemographic, obstetrics, clinical and biomarker characteristics of normotensive pregnant women who completed the study stratified by SHS and OHS

	Normotensive pregnant women (NTN-PW)				
Variable	**Total** (N=498)	**SHS** (N=248)	**OHS (**N=250)	Statistics	p-value
Age (years)	29.64 ± 5.98	29.42 ± 5.92	29.60 ± 6.08	0.667	0.5049
Gestational age (weeks)	16.98 ± 2.01	16.97 ± 2.08	17.04 ± 1.98	0.060	0.9586
SBP (mmHg)	114.7 ± 10.57	116.0 ± 11.00	113 .2 ± 10.01	2.703	**0.0036**
DBP (mmHg)	72.58 ± 9.26	73.0 ± 8.78	71.8 ± 8.42	1.618	0.1341
Pre-gestational BMI (Kg/m^2)	27.04 ± 4.83	26.86 ± 4.74	27.07 ± 4.92	0.405	0.6887
Gestational BMI (Kg/m2)	27.33 ± 4.81	27.32 ± 4.74	27.2 ± 4.92	0.298	0.7658
Marital Status				0.207, 2	0.9018
Never married	78(15.7)	37(14.9)	41(16.4)		
Married	416(83.5)	209(84.3)	207(82.8)		
De-facto	4(0.8)	2(0.8)	2(0.8)		
Ethnicity				2.768, 3	0.4288
Akan	407(81.7)	196(79.0)	211(84.4)		
Ga-Adangbe	9(1.8)	6(2.4)	3(1.2)		
Mole Dagbani	75(15.1)	42(16.9)	33(13.2)		
Ewe	7(1.4)	4(1.6)	3(1.2)		
Highest Level of Education				1.794, 3	0.6163
Unschooled	3(0.4)	1(0.4)	2(0.8)		
Primary	168(33.7)	82(33.1)	86(34.4)		
Secondary	208(41.8)	110(44.4)	98(39.2)		
Tertiary	119(23.9)	55(22.2)	64(25.6)		
Basic income (GH₵)				2.777, 3	0.4273
None	47(9.4)	28(11.3)	19(7.6)		
Low (<500.0)	191(38.4)	92(37.1)	99(39.6)		
Middle (500.0-1000.0)	170(34.1)	87(35.1)	83(33.2)		
High (>1000.0)	90(18.1)	41(16.5)	49(19.6)		
Parity				7.706, 2	**0.0212**
Nulliparous	184(36.9)	102(41.1)	82(32.8)		
Primiparous	135(27.1)	54(21.7)	81(32.4)		
multiparous	179(36.0)	92(37.1)	87(34.8)		
Gravidity				51.54, 1	**<0.0001**
Primigravida	233(46.8)	156(62.9)	77(30.8)		
Multigravida	265(53.2)	92(37.1)	173(69.2)		
FH of Hypertension (Yes)	110(22.1)	57(23.0)	53(21.2)	0.230,1	0.6314
History of SA (Yes)	139(27.9)	91(36.7)	48(19.2)	5.083, 1	**0.0001**
Previous CS (Yes)	99(19.9)	48(19.4)	51(20.4)	0.085, 1	0.7701
No proteinuria (<0.3g/g/24hr)	498(100.0)	248(100.0)	250(100.0)		0.9999
Biomarkers of AGMs					
Serum PlGF (pg/ml)	97.3(80.3-102.9)	90.5(70.4-100.8)	98.6(89.6-105.5)	23521	**<0.0001**
Serum VEGF-A (pg/ml)	171.5(124.1-191.4)	138.7(106.4-189.1)	185.1(148.7-199.2)	20856	**<0.0001**
Serum sEng (ng/mL)	4.9(3.4-6.5)	5.2(3.9-7.4)	4.1(3.3-5.8)	23016	**<0.0001**
Serum sFlt-1 (pg/ml)	611.2(462.9-797.1)	673.8(486.5-906.5)	562.4(446.9-717.5)	23020	**<0.0001**
OS biomarkers					
Serum 8-epiPGF2α (pg/ml)	421.0(318.0-545.1)	466.2(337.3-600.1)	387.5(300.4-496.1)	23151	**<0.0001**
Serum 8-OHdG (ng/L)	79.9(61.8-84.3)	82.5(70.9-89.1)	74.7(53.6-83.0)	22819	**<0.0001**
Plasma TAC (µmol/l)	237.5(178.6-305.6)	228.3(176.1-289.9)	246.5(180.0-314.8)	28116	**0.0371**

Values are presented as mean ± SD, frequency (percentage), BMI: body mass index; CS: caesarean section; GH₵: Ghana cedi; SBP: systolic blood pressure; DBP: diastolic blood pressure; SHS: Suboptimal Health Status; OHS: optimal health status; SA: spontaneous abortion; sFlt-1: soluble fms-like tyrosine kinase-1; PIGF: placental growth factor; VEGF-A: vascular endothelial growth factor-A; sEng: soluble endoglin; 8-OHdG: 8-hydroxydeoxyguanosine; 8-epi-PGF2α; 8-epiprostaglandinF2-alpha; TAC: total antioxidant capacity.

Table 2. Visit 1: Maternal levels of OS biomarkers and AGMs among SHS and OHS pregnant women who went on to developed PE

	SHS (N=248)				OHS (N=250)			
Parameter	SHS-PWLD EO-PE (N=56)	SHS-PWLD LO-PE (N=97)	SHS-PWLD NTN-P (N=95)	*p*-value	OHS-PWLD EO-PE (N=14)	OHS-PWLD LO-PE (N=30)	OHS-PWLD NTN-P (N=206)	*p*-value
10-20 weeks gestation								
GA (weeks)	17.0(15.3-18.0)	17.0(16.0-18.0)	18.0(15.0-19.0)	0.6930	17.0(15.8-18.0)	18.0(16.0-18.0)	17.0(16.0-18.0)	0.7259
SBP (mmHg)	123.0(120.0-128.8) *	117.0(108.0-125.5) *	113.0(105.0-120.0)	**<0.0001**	108.5(102.3-121.5)	119.5(108.8-122.5)	114.0(106.0-120.0)	0.1504
DBP (mmHg)	77.0(69.0-84.0) *	76.0(67.0-82.5) *	69.0(64.0-76.0)	**<0.0001**	70.5(67.0-78.0)	72.0(66.8-82.0)	70.0(66.0-78.0)	0.6262
Serum PlGF (pg/mL)	81.3(50.2-95.0) *	87.3(68.5-95.3) *	99.4(91.1-107.4)	**<0.0001**	85.6(59.2-97.3) ǂ	93.9(58.8-100.5) ǂ	100.5(90.5-109.4)	**<0.0001**
Serum VEGF-A (pg/mL)	117.8(95.8-150.9) *	121.0(92.1-170.2) *	187.3 (170.1-204.2)	**<0.0001**	120.6(90.2-147.4) ǂ	161.5(103.8-184.9) ǂ	187.5 (170.1-196.3)	**<0.0001**
Serum sEng (ng/mL)	7.0(5.8-9.3) *	5.9(4.3-8.5) *	4.1(3.2-5.1)	**<0.0001**	6.4(4.3-8.2) ǂ	5.5(3.1-8.03) ǂ	4.1(3.1-5.5)	**<0.0001**
Serum sFlt-1 (pg/mL)	897.5(624.9-1100) *	770.2(582.9-966.4) *	543.0(433.3-682.4)	**<0.0001**	794.1(553.9-945.0) ǂ	616.3(506.4-752.6) ǂ	533.9(434.0-660.9)	**<0.0001**
Serum 8-epiPGF2α (pg/mL)	600.0(499.2-667.6) *	506.8(391.9-668.7) *	371.9 (291.7-465.8)	**<0.0001**	535.8(364.9-641.2) ǂ	399.1(350.0-506.5) ǂ	360.1(297.8-451.7)	**<0.0001**
Urinary 8-OHdG (ng/mg Cr)	87.75(81.6-99.4) *	86.6(77.6-96.1) *	75.20(51.3-86.1)	**<0.0001**	87.5(79.6-88.8) ǂ	86.5(83.5-95.5) ǂ	76.50(54.4-85.5)	**<0.0001**
Serum 8-OHdG (ng/L)	88.0(81.2-96.1) *	83.3(74.8-92.8) *	73.1(48.9-82.2)	**<0.0001**	84.0(79.3-93.5) ǂ	81.6(71.4-85.9) ǂ	73.1(50.9-82.1)	**<0.0001**
Plasma TAC (µmol/L)	147.6(94.6-259.3) *	220.0(170.1-275.6) *	234.6(180.3-317.2)	**<0.0001**	220.5(196.7-282.7) ǂ	235.2(175.4-330.2) ǂ	249.2(179.0-315.3)	**<0.0001**
sFlt-1/PlGF ratio	10.1(7.7-13.4) *	9.8(6.8-13.5) *	4.4(3.5-5.9)	**<0.0001**	8.8(6.4-12.9) ǂ	8.2(5.7-10.4) ǂ	4.1(2.9-5.3)	**<0.0001**
8-epiPGF2alpha/PlGF ratio	7.4(5.6-9.4) *	6.9(4.9-9.6) *	3.4(2.7-4.5)	**<0.0001**	5.9(4.3-10.5) ǂ	5.8(3.8-6.9) ǂ	3.7(2.8-4.8)	**<0.0001**
8-OHdG/PlGF ratio	1.1(0.8-1.6) *	0.9(0.8-1.5) *	0.8(0.4-0.8)	**<0.0001**	0.9(0.8-1.1) ǂ	0.8(0.7-1.5) ǂ	0.7(0.4-0.8)	**<0.0001**
sEng/PlGF ratio	88.1(67.1-127.5) *	80.8(50.7-131.4) *	41.7(29.7-51.6)	**<0.0001**	71.9(40.2-100.8) ǂ	69.8(47.2-116.9) ǂ	41.8(30.0-54.8)	**<0.0001**

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, SBP: systolic blood pressure; DBP: diastolic blood pressure; sFlt-1: soluble fms-like tyrosine kinase-1; PIGF: placental growth factor; VEGF-A: vascular endothelial growth factor-A; sEng: soluble endoglin; 8-OHdG: 8-hydroxydeoxyguanosine; 8-epi-PGF2α; 8-epiprostaglandinF2-alpha; TAC: total antioxidant capacity.

* indicates significance compared to SHS-PWD NTN-PW; ǂ 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 3. Visit 2 obstetrics characteristics, and biomarkers of OS and AGMs among SHS and OHS pregnant women who developed EO-PE and LO-PE compared to NTN-P at the time of diagnosis

	SHS (N=248)				**OHS (N=250)**			
Parameter	SHS-PWD EO-PE (N=56)	SHS-PWD LO-PE (N=97)	SHS-PWD NTN-PW (N=95)	*p*-value	OHS-PWD EO-PE (N=14)	OHS-PWD LO-PE (N=30)	OHS-PWD NTN-PW (N=206)	*p*-value
Nulliparous	38(67.9) *	24(24.7) *	26(27.4) †	**<0.0001**	9(64.3) ǂ	8(26.7) ǂ	3(1.5)	**<0.0001**
Family history of HTN	14(25.0) *¥	18(18.6) *‡	8(12.6) †	**0.0197**	6(42.9) ǂ	9(30.0) ǂ	4(1.9)	**<0.0001**
History of miscarriage	18(32.1) *	12(12.3) *	3(3.2) †	**<0.0001**	3(21.4) ǂ	6(20.0) ǂ	2(0.9)	**<0.0001**
Previous caesarean section	22(39.2) *	15(15.5) *	8(8.4) †	**<0.0001**	5(35.7) ǂ	3(10.0) ǂ	5(2.4)	**<0.0001**
Preterm delivery	39(69.6) *	20(20.6) *‡	5(5.3) †	**<0.0001**	8(57.1) ǂ	15(50.0) ǂ	8(3.9)	**<0.0001**
Low monthly income	27(48.2) *	13(13.4) *	6(6.3) †	**<0.0001**	7(50.0) ǂ	5(16.7) ǂ	28(13.6)	**0.0015**
Maternal age (years)	34.0(21.8-38.8) * ¥	28(24-33) *	30.0(27.0-34.0)	**<0.0001**	29.0(26.0-33.0)	28.5(22-33.5)	30.0(26.0-34.0)	**0.0731**
SBP (mmHg)	180.0(168-189.0) *	160.0(156.0-180.0) *‡	120.0(114.0-122.0) †	**<0.0001**	164.0(160.0-184.0) ǂ	148.0(159.0-173.0) ǂ	116.0(114.0-121.0)	**<0.0001**
DBP (mmHg)	105.0(100-113.0) *	104.0(100.0-110.0) *	78.0(70.0-80.0)	**<0.0001**	103.0(100.0-113.0) ǂ	101.0(100.0-110.0) ǂ	76.0(69.0-80.0)	**<0.0001**
GA at delivery	32.0(32.0-33.0) *	37.0(35.0-38.0) *	38.0(37.0-39.0)	**<0.0001**	33.0(32.8-33) ǂ	36.0(34.0-37.0) ǂ	38.0(37.0-39.0)	**<0.0001**
Serum PlGF (pg/ml)	71.5(45.2-95.2) *	73.8(42.4-95.6) *	104.6(97.5-109.9)	**<0.0001**	90.1(62.2-102) ǂ	83.3(51.4-96.9) ǂ	103.5(96.8-110.1)	**<0.0001**
Serum VEGF-A (pg/ml)	114.1(71.2-137.1)	110.8(82.4-172.2) *	200.6 (182-212)	**<0.0001**	143.1(93.0-186.1) ǂ	126.7(110.5-189.2) ǂ	203.4 (182.0-232.0)	**<0.0001**
Serum sEng (ng/mL)	11.9(10.5-14.3) *	10.7(8.9-13.5) *	8.7(7.8-9.9)	**<0.0001**	11.3(9.0-13.9) ǂ	10.1(7.7-12.9) ǂ	8.5(7.6-10.2)	**<0.0001**
Serum sFlt-1 (pg/ml)	1290(898.1-1581) *	1107(837-1389) *	787.5(623.9-980)	**<0.0001**	1142(796-1358) ǂ	886(727-1082) ǂ	761.1(623-950)	**<0.0001**
Serum 8-epiPGF2α (pg/ml)	2560(2057-3115) *	2187(1599-2882) *	1472 (1185-1894)	**<0.0001**	2223(1489-2636) ǂ	1625(1428-675.8) ǂ	1466(1215-1839)	**<0.0001**
Urinary 8-OHdG (ng/mg Cr)	281.1(249.5-312.3) *	259.3(235.7-296.2) *	178.4(127.4-251.9)	**<0.0001**	258.7(251.1-303.4) ǂ	255.3(234.8-261.2) ǂ	176.5(135.1-238.8)	**<0.0001**
Serum 8-OHdG (ng/L)	142.6(131.6-155.7) *	136.1(124.0-151.2) *	118.4(79.2-133.2)	**<0.0001**	136.4(132.2-151.4) ǂ	132.1(115.6-141.9) ǂ	114.5(82.3-132.0)	**<0.0001**
Plasma TAC (µmol/l)	131.4(109.9-192.3) *	180.9(119.9-249.5) *	373.8(268.5-472.9)	**<0.0001**	160.9(112.4-225.2) ǂ	199.6(118.8-243.4) ǂ	351.6(261.9-475.8)	**<0.0001**
sFlt-1: PlGF ratio	17.5(11.8-31.1) *	15.0(11.0-23.5) *	7.4(5.7-9.5)	**<0.0001**	11.9(8.2-14.0) ǂ	12.9(9.5-18.3) ǂ	6.7(5.4-9.3)	**<0.0001**
8-epiPGF2alpha: PlGF ratio	34.4(26.1-64.8) *	29.5(20.8-48.6) *	14.7(11.3-18.7)	**<0.0001**	21.8(15.0-26.7) ǂ	24.7(15.6-35.8) ǂ	13.4(10.4-17.8)	**<0.0001**
8-OHdG: PlGF ratio	1.9(1.4-3.5) *	1.5(1.2-2.9) *	1.2(0.7-1.3)	**<0.0001**	1.5(1.2-2.2) ǂ	1.3(0.8-1.3) ǂ	1.1(0.7-1.3)	**<0.0001**
sEng: PlGF ratio	173.2(130.8-287.3) *	150.5(109.2-257.1) *	84.6(72.6-101.6)	**<0.0001**	127.8(104.0-173.2) ǂ	126.4(84.5-181.7) ǂ	84.1(72.6-100.8)	**<0.0001**

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; SBP: systolic blood pressure; DBP: diastolic blood pressure; sFlt-1: soluble fms-like tyrosine kinase-1; PIGF: placental growth factor; VEGF-A: vascular endothelial growth factor-A; sEng: soluble endoglin; 8-OHdG: 8-hydroxydeoxyguanosine; 8-epi-PGF2α; 8-epiprostaglandinF2-alpha; TAC: total antioxidant capacity

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

* Indicates significance compared to SHS-PWD NTN-PW; ǂ 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 4. Predictive accuracy of visit 1 levels of OS and AGMs, and their combined ratios for the prediction of SHS-pregnant women likely to develop early-onset PE (EO-PE) and late onset-PE (LO-PE)

	Cut-off value	Sensitivity(95%CI)	Specificity(95%CI)	PPV	NPV	LR+	LR-	RR (95%CI)	*p*-value
		Predicting SHS-PWD EO-PE							
10-20 weeks gestation									
Serum PlGF (pg/mL)	≤98.3	87.5(75.9-94.0)	80.0(70.7-86.8)	72.1	91.6	4.4	0.1	3.3(1.3-7.9)	**<0.0001**
Serum VEGF-A (pg/mL)	≤133.6	87.5(75.9-94.0)	78.9(69.6-85.9)	71.0	91.5	4.2	0.2	2.8(1.1-9.6)	**0.0022**
Serum sEng (ng/L)	≥5.7	78.6(65.9-87.4)	84.2(75.4-90.3)	74.6	86.9	4.9	0.3	3.4(1.2-11.0)	**<0.0001**
Serum sFlt-1 (pg/mL)	≥822.3	67.8(54.7-78.6)	93.7(86.6-97.3)	86.4	83.2	10.7	0.3	4.0(1.1-11.2)	**<0.0001**
Serum 8-epiPGF2α (pg/mL)	≥499.1	80.4(67.9-88.8)	87.4(78.9-92.7)	78.9	88.3	6.4	0.2	4.7(1.1-9.8)	**<0.0001**
Urinary 8-OHdG (ng/mg Cr)	≥78.4	83.9(71.9-91.5)	62.1(52.0-71.2)	56.6	86.8	2.2	0.3	3.8(1.1-7.0)	**<0.0001**
Serum 8-OHdG (ng/L)	≥83.8	66.1(52.9-77.0)	90.5(82.7-95.1)	80.4	81.9	7.0	0.3	3.2(1.2-9.1)	**0.0010**
Plasma TAC (µmol/L)	≤297.7	87.5(75.9-94.0)	65.3(55.2-74.1)	59.8	89.9	2.5	0.2	2.4(1.1-11.0)	**0.0183**
sFlt-1/PlGF ratio	≥8.2	76.8(64.0-85.9)	89.5(81.5-94.3)	81.1	86.7	7.2	0.3	4.8(1.4-12.1)	**<0.0001**
8-epiPGF2alpha/PlGF ratio	≥5.5	76.8(64.0-85.9)	93.7(86.6-97.3)	87.8	87.3	5.5	0.2	4.6(1.3-11.5)	**<0.0001**
8-OHdG/PlGF ratio	**≥0.7**	**96.4(87.0-99.6)**	**100.0(77.9-100.0)**	**75.0**	**100.0**	**12.2**	**0.0**	**6.5(1.4-12.5)**	**<0.0001**
sEng/PlGF ratio	≥66.4	92.9(82.4-97.6)	76.8(67.3-84.2)	70.3	94.8	4.1	0.1	6.3(1.3-9.7)	**<0.0001**
		Predicting SHS-PWD LO-PE							
10-20 weeks gestation									
Serum PlGF (pg/mL)	≤84.6	53.6(43.7-63.2)	89.5(81.4-94.3)	83.9	65.4	5.1	0.5	1.5(1.1-3.4)	**0.0309**
Serum VEGF-A (pg/mL)	≤137.2	69.1(59.2-77.4)	86.3(77.8-91.9)	83.8	73.2	5.0	0.4	1.8(1.1-4.0)	**0.0208**
Serum sEng (ng/L)	≥5.3	61.9(51.9-70.9)	81.1(71.9-87.7)	76.9	67.5	3.3	0.5	2.1(1.1-6.3)	**0.0051**
Serum sFlt-1 (pg/mL)	≥727.4	55.7(45.8-65.1)	88.4(80.2-93.5)	83.1	66.1	4.8	0.5	2.3(1.1-7.8)	**0.0063**
Serum 8-epiPGF2α (pg/mL)	≥446.55	69.1(59.2-77.4)	73.7(63.9-81.5)	72.8	70.0	2.6	0.4	2.0(1.1-5.4)	**0.0174**
Urinary 8-OHdG (ng/mg Cr)	≥77.3	78.4(69.1-85.4)	60.8(49.9-69.3)	66.7	73.1	1.9	0.4	1.8(1.1-3.0)	**0.0294**
Serum 8-OHdG (ng/L)	≥82.9	63.9(53.9-72.8)	80.0(70.7-86.9)	76.5	68.5	3.2	0.5	2.8(1.1-7.1)	**<0.0001**
Plasma TAC (µmol/L)	≤268.1	70.1(60.3-78.3)	40.0(30.7-50.1)	54.4	56.7	1.2	0.7	0.8(0.4-6.2)	0.3810
sFlt-1/PlGF ratio	≥7.3	76.3(66.8-83.7)	83.2(74.2-89.4)	82.2	77.5	4.5	0.3	3.2(1.5-10.7)	**<0.0001**
8-epiPGF2alpha/PlGF ratio	≥4.9	76.3(66.8-83.7)	83.2(74.2-89.4)	82.2	77.5	4.5	0.3	3.0(1.1-7.3)	**<0.0001**
8-OHdG/PlGF ratio	**≥0.8**	**76.3(67.6-84.6)**	**86.8(90.6-99.3)**	**95.5**	**97.6**	**20.9**	**0.3**	**4.4(1.3-11.6)**	**<0.0001**
sEng/PlGF ratio	≥67.7	65.9(56.1-74.6)	92.6(85.3-96.6)	90.1	72.7	8.9	0.4	4.1(1.7-10.3)	**<0.0001**

sFlt-1: soluble fms-like tyrosine kinase-1; PIGF: placental growth factor; VEGF-A: vascular endothelial growth factor-A; sEng: soluble endoglin; 8-OHdG: 8-hydroxydeoxyguanosine; 8-epi-PGF2α; 8-epiprostaglandinF2-alpha; TAC: total antioxidant capacity; AUC: area under the ROC curve; CI: confidence interval; PPV: positive predicted value; NPV: negative predicted value; LR+: positive likelihood ratio; LR-: negative likelihood ratio; aOR: adjusted odds ratio. aOR for maternal age, preterm delivery, family history of hypertension, low-income salary, nulliparity and birthweight; SHS-PWLD EO-PE: Suboptimal health status pregnant women who developed early-onset PE. $p<0.05$ in bold values represents statistical significance. Row values with bold face indicate the best predictive marker.

Table 5 Predictive accuracy of visit 1 levels of OS and AGMs, and their combined ratios for the prediction of OHS-pregnant women likely to develop early-onset PE (EO-PE) and late onset-PE (LO-PE)

OHS-PWLD EO-PE	Cut-off value	Sensitivity(95%CI)	Specificity(95%CI)	PPV	NPV	LR+	LR-	RR (95%CI)	*p*-value
		Predicting OHS-PWD EO-PE							
10-20 weeks gestation									
Serum PlGF (pg/mL)	≤99.6	100(74.4-100.0)	67.9(61.3-73.9)	17.5	100	3.1	0.0	2.8(1.1-9.5)	**0.0006**
Serum VEGF-A (pg/mL)	≤170	85.7(58.5-96.9)	78.2(71.9-83.3)	21.1	98.8	3.9	0.2	3.0(1.1-9.1)	**<0.0001**
Serum sEng (ng/L)	≥7.0	50.0(26.9-73.1)	97.1(93.6-98.8)	53.8	96.6	11.2	0.5	3.2(1.3-11.0)	**<0.0001**
Serum sFlt-1 (pg/mL)	≥499	85.7(58.6-96.9)	41.8(35.2-48.6)	9.1	97.7	1.5	0.3	3.8(1.2-10.0)	**<0.0001**
Serum 8-epiPGF2α (pg/mL)	≥335.6	92.9(66.1-100)	40.3(33.8-47.1)	9.6	98.8	1.6	0.2	3.6(1.1-9.3)	**<0.0001**
Urinary 8-OHdG (ng/mg Cr)	≥86.5	64.3(38.6-83.6)	83.6(77.8-87.9)	20.9	97.2	3.9	0.4	3.1(1.1-7.1)	**<0.0001**
Serum 8-OHdG (ng/L)	≥83.0	50.0(26.9-73.1)	81.1(75.1-85.8)	15.2	95.9	2.6	0.6	3.5(1.3-6.3)	**<0.0001**
Plasma TAC (µmol/L)	≤286.7	100(74.4-100.0)	55.3(48.5-61.9)	13.2	100	2.2	0.0	3.0(1.5-9.4)	**<0.0001**
sFlt-1/PlGF ratio	≥8.6	64.3(38.6-83.6)	94.2(89.9-96.7)	42.9	97.5	11.0	0.4	5.0(1.3-10.0)	**<0.0001**
8-epiPGF2alpha/PlGF ratio	≥5.8	92.9(66.1-100.0)	62.6(55.8-68.9)	34.4	99.2	2.5	0.1	4.8(1.8-14.6)	**<0.0001**
8-OHdG/PlGF ratio	**≥0.7**	**92.9(66.1-100.0)**	**71.8(65.3-77.5)**	**48.3**	**99.3**	**20.6**	**0.0**	**5.6(1.5-11.9)**	**<0.0001**
sEng/PlGF ratio	≥70.9	64.3(38.6-83.6)	94.7(90.6-97.1)	45.0	97.5	12.0	0.4	4.5(1.2-7.2)	**<0.0001**
		Predicting OHS-PWD LO-PE							
10-20 weeks gestation									
PlGF (pg/mL)	≤63.1	93.3(77.4-99.1)	62.6(55.8-68.9)	26.7	98.5	2.5	0.1	1.9(1.1-7.3)	**0.0144**
VEGF-A (pg/mL)	≤376.2	73.3(55.3-85.9)	65.5(58.8 -71.7)	23.7	94.4	2.1	0.4	2.2(1.1-8.5)	**0.0086**
sEng (ng/L)	≥6.2	63.3(45.4-78.1)	89.8(84.8-93.3)	47.5	94.5	6.2	0.4	1.8(1.1-7.7)	**0.0308**
sFlt-1 (pg/mL)	≥757	60.0(42.3-75.4)	88.4(83.2-92.1)	42.9	93.8	5.2	0.5	1.6(1.2-5.8)	**0.0311**
8-epiPGF2alpha (pg/mL)	≥501.6	66.7(48.6-80.8)	82.5(76.7-87.1)	35.1	94.4	3.8	0.4	1.8(1.1-5.3)	**0.0281**
Urinary 8-OHdG (ng/mg Cr)	≥85.6	73.3(55.3-85.9)	76.2(69.9-81.5)	30.9	95.2	3.1	0.3	1.7(1.1-6.7)	**0.0432**
Serum 8-OHdG (ng/L)	≥83.6	60.0(42.3-75.4)	85.4(79.9-89.6)	37.5	93.6	4.1	0.4	3.3(1.5-9.2)	**<0.0001**
Plasma TAC (µmol/L)	≤147.8	80.0(62.2-90.7)	78.2(71.9-83.3)	34.8	96.4	3.7	0.3	1.5(1.1-5.0)	**0.0226**
sFlt-1/PlGF ratio	≥7.3	70.0(51.9-83.4)	90.8(85.9-94.1)	52.5	95.4	7.6	0.3	3.8(1.1-7.5)	**<0.0001**
8-epiPGF2alpha/PlGF ratio	≥5.0	73.3(55.3-85.9)	86.9(81.5-90.9)	44.9	95.7	5.6	0.3	3.5(1.1-9.1)	**<0.0001**
8-OHdG/PlGF ratio	**≥0.8**	**83.3(65.8-93.0)**	**90.0(84.8-93.3)**	**54.4**	**97.4**	**8.2**	**0.2**	**5.1(1.1-10.1)**	**<0.0001**
sEng/PlGF ratio	≥63.1	80.0(62.2-90.7)	79.6(73.5-84.6)	36.4	96.5	3.9	0.3	3.5(1.1-8.6)	**<0.0001**

sFlt-1: soluble fms-like tyrosine kinase-1; PIGF: placental growth factor; VEGF-A: vascular endothelial growth factor-A; sEng: soluble endoglin; 8-OHdG: 8-hydroxydeoxyguanosine; 8-epi-PGF2α; 8-epiprostaglandinF2-alpha; TAC: total antioxidant capacity; AUC: area under the ROC curve; CI: confidence interval; PPV: positive predicted value; NPV: negative predicted value; LR+: positive likelihood ratio; LR-: negative likelihood ratio; aOR: adjusted odds ratio. aOR for maternal age, preterm delivery, family history of hypertension, low-income salary, nulliparity and birthweight; OHS-PWLD EO-PE: optimal health status pregnant women who developed early-onset PE. $p<0.05$ in bold values represents statistical significance. Row values with bold face indicate the best predictive marker.

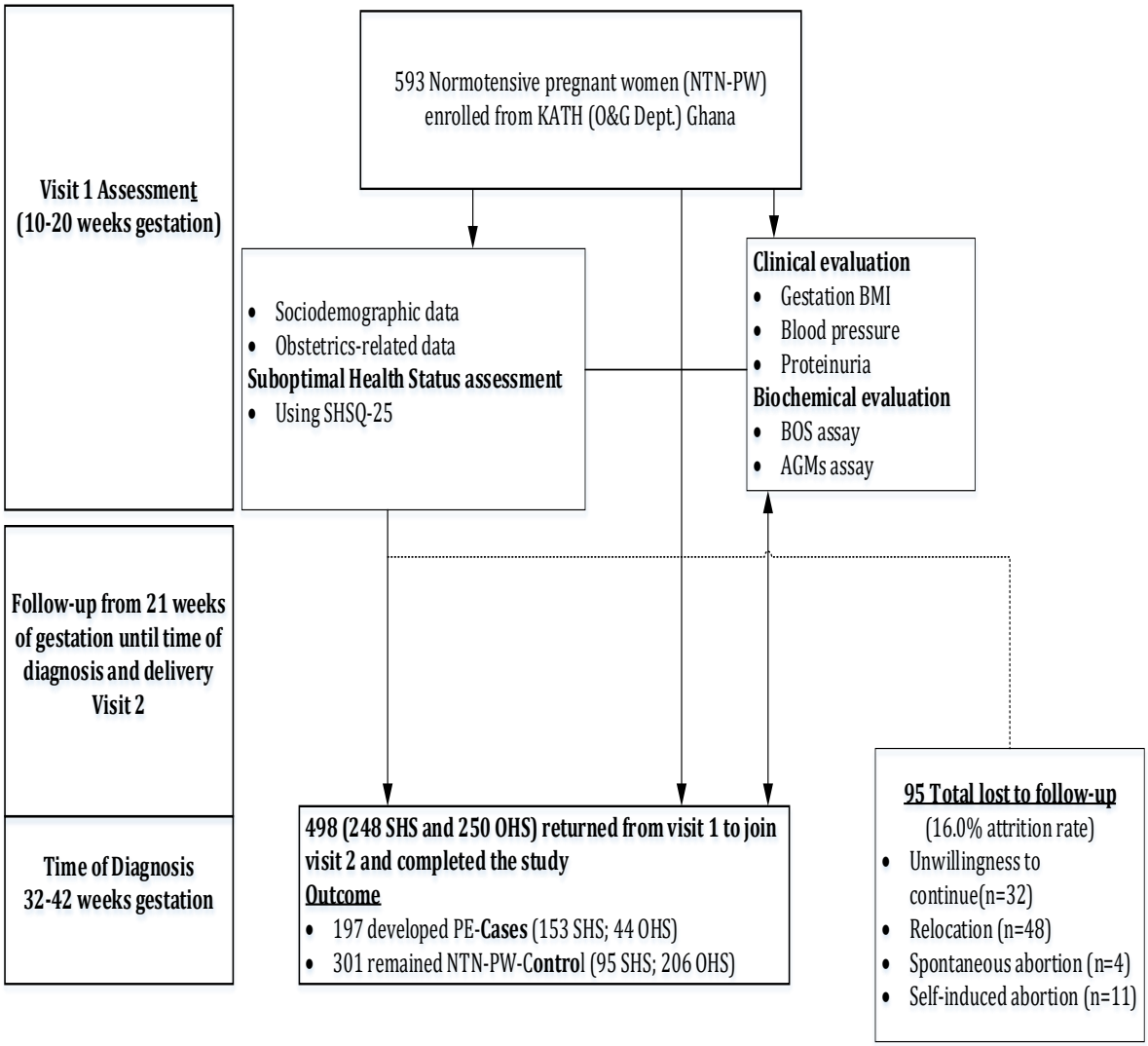

Fig 1. Flow chart of the participants recruitment.

NTN-PW: normotensive pregnant women; BOS: biomarkers of oxidative stress; AGMs: angiogenic growth mediators; O&G: obstetrics and gynaecology

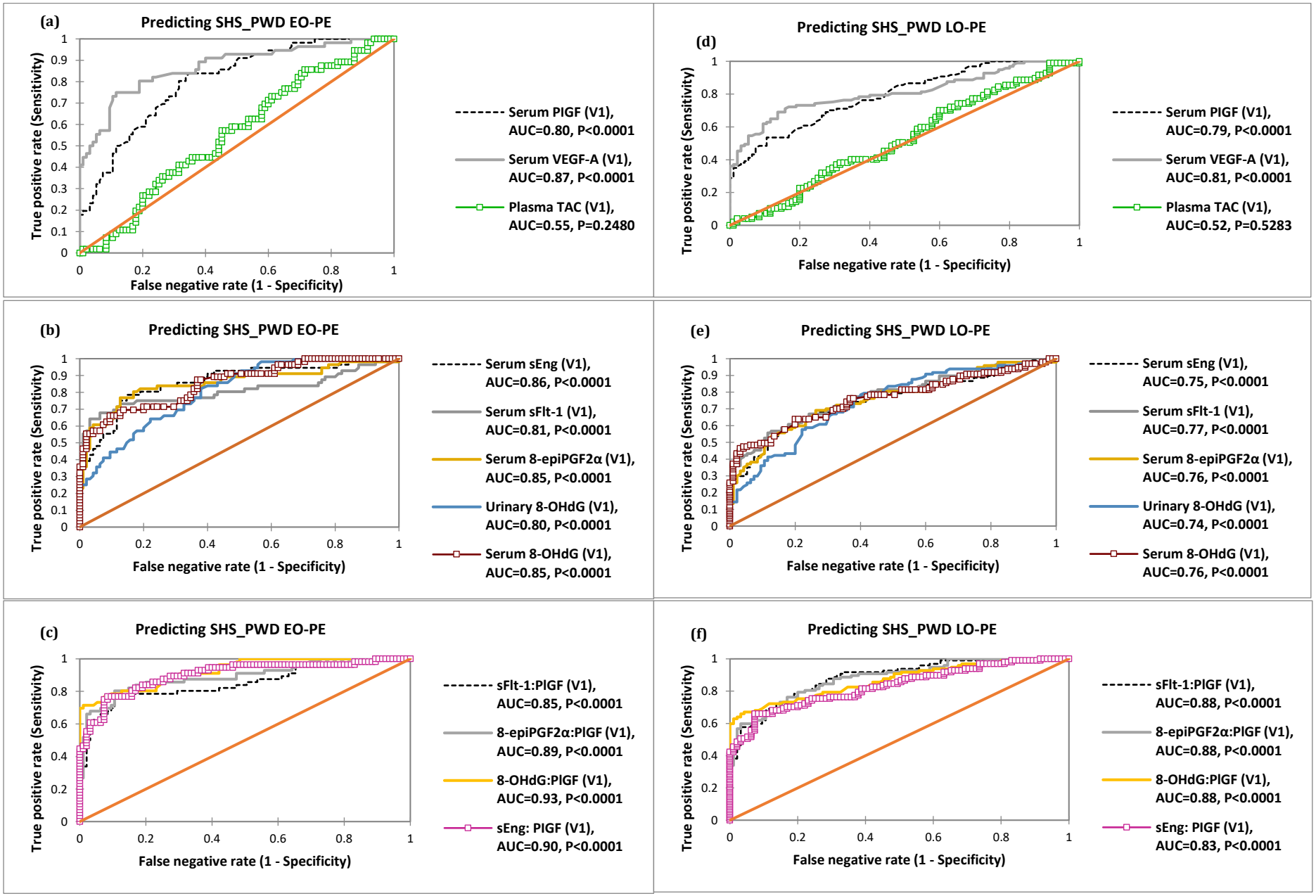

Fig 2. The AUCs of Visit 1 biomarkers of OS and AGMs for the prediction of SHS-pregnant women who developed (PWD) EO-PE **(a-c)** and LO-PE **(d-f).** sFlt-1: soluble fms-like tyrosine kinase-1; PlGF: placental growth factor; VEGF-A: vascular endothelial growth factor-A; sEng: soluble endoglin; 8-OHdG: 8-hydroxydeoxyguanosine; 8-epi-PGF2α; 8-epiprostaglandinF2-alpha; TAC: total antioxidant capacity

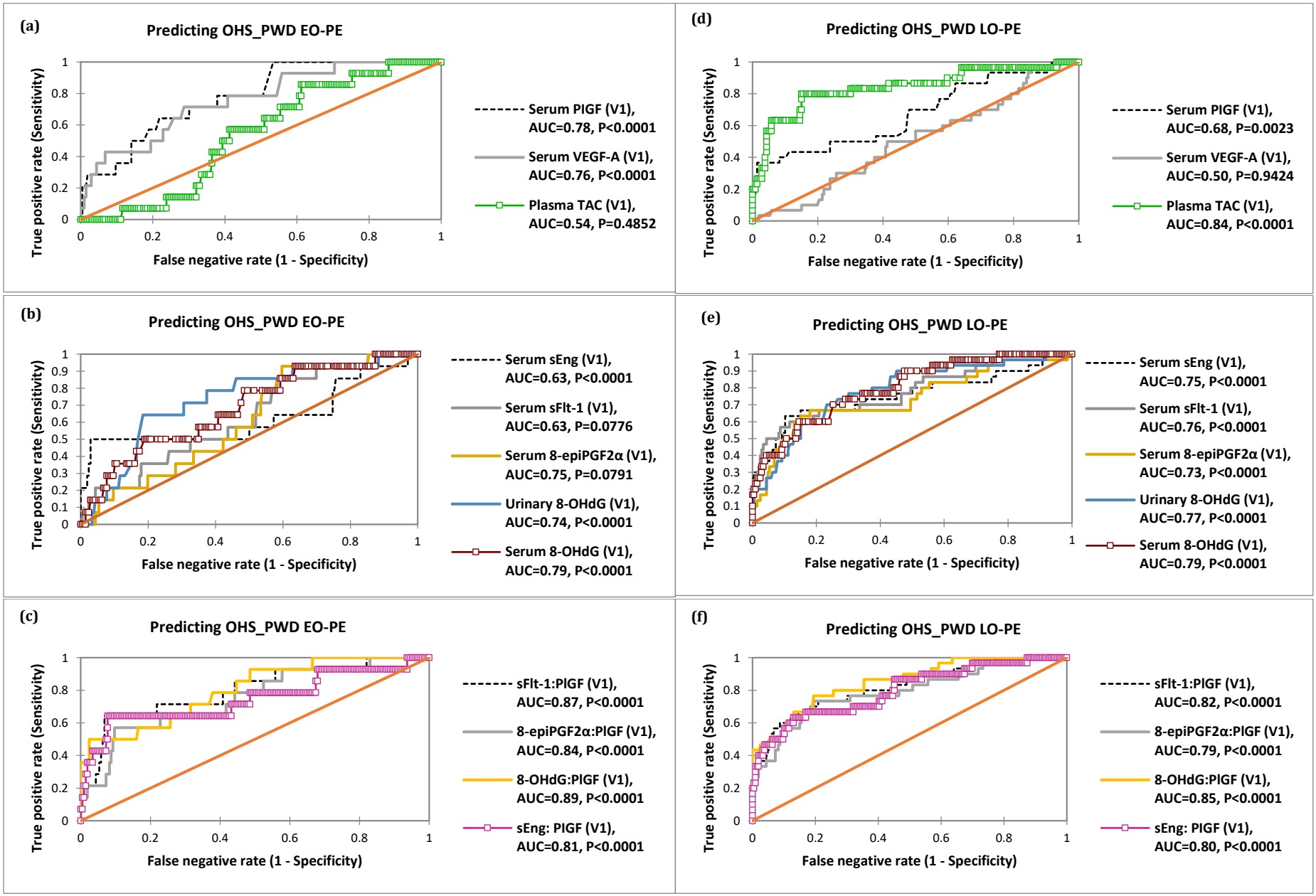

Fig 3. The AUCs of Visit 1 biomarkers of OS biomarkers and AGMs for the prediction of OHS-pregnant women who developed (PWD) EO-PE **(a-c)** and LO-PE **(d-f).** sFlt-1: soluble fms-like tyrosine kinase-1; PlGF: placental growth factor; VEGF-A: vascular endothelial growth factor-A; sEng: soluble endoglin; 8-OHdG: 8-hydroxydeoxyguanosine; 8-epi-PGF2α; 8-epiprostaglandinF2-alpha; TAC: total antioxidant capacity.