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Enoch Odame Anto
Edith Cowan University

Peter Roberts
Edith Cowan University

David Anthony Coall
Edith Cowan University

Eric Adua
Edith Cowan University

Cornelius Archer Turpin

See next page for additional authors

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Suboptimal Health Status pregnant women are associated with increased Oxidative Stress and unbalanced pro-and anti-Angiogenic Growth Mediators: a cross-sectional study in a Ghanaian population

[1,3]Enoch Odame Anto, [1]Peter Roberts, [1]David Coall, [1]Eric Adua, [2]Cornelius Archer Turpin, [2]Augustine Tawiah, [4]Youxin Wang, [1,5]Wei Wang*

[1]School of Medical and Health Sciences, Edith Cowan University, Perth, Western Australia

[2]Department of Obstetrics and Gynaecology, Komfo Anokye Teaching Hospital, Kumasi, Ghana, West-Africa

[3]Department of Molecular Medicine, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, West-Africa

[4]Beijing Key Laboratory of Clinical Epidemiology, School of Public Health, Capital Medical University, Beijing, China

[5]School of Public Health, Taishan Medical University, Taian, China

***Address correspondence to:**

1) Professor, Wei Wang, MD, PhD, FFPH, FRSB, FRSM
School of Medical and Health Sciences, Edith Cowan University
270 Joondalup Drive, Perth, WA 6027, Australia
Tel: (61 8) 6304 3717; Fax: (61 8) 6304 2626
E-mail: wei.wang@ecu.edu.au
Orcid: 0000-0002-1430-1360

Abstract

Optimal oxidative stress (OS) is important throughout pregnancy; however, an increased OS may alter placental angiogenesis culminating in an imbalanced of angiogenic growth mediators (AGMs). Suboptimal Health Status (SHS), a physical state between health and disease, may be associated with increased OS and unbalanced AGMs. In this study, we explored the association between SHS, biomarkers of OS (BOS) and AGMs among normotensive pregnant women (NTN-PW) in a Ghanaian Suboptimal Health Cohort Study (GHOACS). This comparative GHOACS recruited 593 NTN-PW from the Komfo Anokye Teaching Hospital, Ghana. SHS was measured using a Suboptimal Health Status Questionnaire-25 (SHSQ-25). Along with the subjective SHS measure, objective BOS: 8-hydroxy-2-deoxyguanosine (8-OHdG), 8-epiprostaglandinF2 alpha (8-epi-PGF2α), total antioxidant capacity (TAC), and AGMs: vascular endothelial growth factor-A (VEGF-A), soluble fms-like tyrosine kinase receptor 1 (sFlt-1), placenta growth factor (PIGF) and soluble endoglin (sEng) were evaluated. Compared to optimal health NTN-PW, levels of PlGF, VEGF-A and TAC were significantly (p<0.05) reduced and negatively associated with SHS whilst sEng, sFlt-1, 8-epiPGF2α, 8-OHdG, and combined ratios of sFlt-1/PlGF, 8-epiPGF2α/PlGF, 8-OHdG/PlGF, and sEng/PlGF were significantly increased and positively associated with SHS. The 1st quartile for PIGF (2.79-fold) and VEGF-A (5.35-fold), and the 4th quartile for sEng (4.31-fold), sFlt-1(1.84-fold), 8-epiPGF2α (2.23-fold), 8-OHdG (1.90-fold) and urinary 8-OHdG (1.95-fold) were independently associated with SHS (p<0.05). SHS is associated with increased OS and unbalanced AGMs. Early identification of SHS-related OS and unbalanced AGMs may inform clinicians of the need for therapeutic options.

Key words: pregnant women, oxidative stress, angiogenic growth mediators, suboptimal health status

Introduction

In recent years, a number of normotensive pregnant mothers, particularly in sub-Saharan African (SSA) countries continue suffer health complaints without diagnosable conditions, and this has led to increased morbidity and mortality rates [1,2]. Particularly, the stressful demands during pregnancy may alter physiological and metabolic functions and lead to health complaints including high-oxygen requirement and high-energy demand [3]. Although these dramatic events occur to sustain the mother and the growing foetus, they may also culminate in oxidative stress and adverse pregnancy outcomes such as stillbirth, intrauterine growth restriction and preterm delivery among others [4,5].

Oxidative stress (OS) is an imbalance between pro-oxidant and anti-oxidant capacity [6]. Meanwhile, an optimal OS and reactive oxygen species (ROS) are essential throughout pregnancy to regulate a successful placental angiogenesis; a process whereby new blood vessels are formed from pre-existing ones during vascular development [7,8]. In an optimal OS state during placental angiogenesis and maternal vascular remodeling, the extravillous cytotrophoblast (EVT) cells of foetal origin invade the maternal uterine spiral arteries [7,9]. The resultant is the formation of a large capacity conduit vessel network which allows adequate exchange of blood and nutrient between the mother and the foetus [10]. During this process, the invasive EVT expresses a number of angiogenic growth mediators (AGMs) including pro-angiogenic growth factors such as VEGF-A and PlGF, and anti-angiogenic factor like sFlt-1 [7]. Both VEGF-A and PlGF are involved in angiogenesis, placental vascular remodeling,

vascular permeability, nitric oxide (NO) production, promoting endothelial cell control and proliferation [7,8].

Meanwhile, increased OS can be detrimental [6-8]. For instance, in an increased OS state, the EVT overexpresses sFlt-1, which antagonises the function of VEGF and PlGF on the endothelial cells leading to endothelial dysfunction [6,7]. Soluble endoglin (sEng), another anti-angiogenic growth factor which is highly expressed on the endothelial cells and cell membrane of the syncytiotrophoblast cells also antagonises the function of transforming growth factor beta 1 (TGFβ1), resulting in a loss of endothelial cell control, vasoconstriction and increased OS [7,9]. The increased OS may be caused by placental hypoxia/ischaemia originating from an incomplete maternal vascular remodeling [7,8].

Several other factors including advanced maternal age, increased inflammatory response, cardiovascular diseases and hormonal changes contribute to increased ROS formation and OS in the circulation [10,11]. Particularly, an altered hormonal function during pregnancy is associated with elevated phospholipid levels/phospholipid accumulation [12]. Subsequently, increased levels of phospholipids at sites where ROS are formed lead to endogenous ROS-induced lipid peroxidation [13]. In addition, increased ROS formation in circulation can cause damage to proteins and DNA and lead to protein oxidation and oxidative DNA damage, respectively [7,8]. Biomarkers of oxidative stress (BOS) 8-epiPGF2α, and 8-OHdG are formed by free radical-catalysed phospholipid peroxidation and are potent markers indicative of *in-vivo* OS and oxidative DNA damage, respectively [13]. A compromised antioxidant system on the other hand, depicts a correspondingly reduced level of TAC [10].

Previous studies have extensively focused on increased levels of OS and imbalance in AGMs among women with complicated pregnancies [5,14] while paying

less attention to these changes in normal pregnancies [15]. In addition to the dearth of data on evaluation of BOS and AGMs together in normal pregnancy, previous studies evaluated these markers in third trimester while paying less attention to these levels in the early trimesters of pregnancy. Early identification of increase OS and unbalanced levels of AGMs would improve diagnosis and treatment. Despite the fact that BOS and AGMs are sensitive and dynamic in both pregnancy and neonatal medicine, they are not used in routine antenatal care because they are expensive, invasive, requires a long turnaround time and expertise, and may not be readily available to women who visit under resourced hospitals. In addition, a longer turnaround time leads to delayed therapeutic interventions. An attempt to overcome this over the past few years has been the need to shift from reactive medical intervention to predictive, preventive and personalised medicine (PPPM) [16-20]. The approach of PPPM has adopted traditional, behavioural and environmental factors for early treatment and prevention of unrecognised diseases [20]. One way to identify participants with preconditions even before the onset of clinical manifestations, is to evaluate their physiological metrics at the preclinical or suboptimal health stage [19].

From the public health perspective, a recent development in the research for a promising suboptimal health status (SHS) evaluation measure that can be used in PPPM, is the development of a 25-question item Suboptimal Health Status Questionnaire (SHSQ-25). It is a subjective and non-invasive health assessment tool which is inexpensive, and requires less expertise and turnaround time. The SHSQ-25 was first created by our team and the term ‘suboptimal health status’ (SHS) was coined to define a physical state between health and disease [21,22]. SHS is recognised as a subclinical, reversible stage of chronic disease and characterised by poor health, low energy or vitality and general body weakness [19,21,22]. SHSQ-25 has since been used to evaluate SHS in

several studies and was found useful for early detection and risk stratification of several symptoms and diseases [19,23-29]. For example, SHS was found to be an independent risk factor for type II diabetes mellitus in an African population [23], arterial stiffness and cardiovascular disease in European population [24], type II diabetes mellitus [25], cardiovascular diseases [26,27], psychosocial stress [28], and telomere length [29] in an Asian population.

Even though previous studies have reported a correlation of SHS with cardiovascular disease and arterial stiffness, which are both risk factors for increased oxidative stress, no study to date has explored together, its relationship with BOS and AGMs in pregnancy. Although OS and imbalance in AGMs are common in complicated pregnancies like preeclampsia, it is possible that SHS may precede its clinical manifestation. Our ongoing cohort study found that SHS is an independent measure for preeclampsia [30]. As a result, there is the need to evaluate if our NTN-PW experiencing suboptimal health exhibit a variation in OS and AGMs levels compared to optimal health status NTN-PW. For the first time in the present study, we explore an association of SHS with BOS and AGMs among normotensive pregnant women at 10-20 weeks gestation in a Ghanaian Suboptimal Health Cohort Study (GHOACS). An increased OS and unbalanced AGMs, if found associated with SHS, would validate the usefulness of SHSQ-25 thereby creating a possibility to inform clinicians the need for early therapeutic options.

Materials and Methods

Study design and participants

As a part of the on-going Ghanaian Suboptimal Health Cohort Study (GHOACS), this hospital-based comparative cross-sectional study included 593 normotensive pregnant

156 women (NTN-PW) attending regular antenatal care at the Obstetrics and Gynaecology
157 Department of Komfo Anokye Teaching Hospital (KATH), Kumasi Ghana. Both
158 nulliparous and multiparous NTN-PW aged from 18 to 45 years with a singleton
159 pregnancy from 10 to 20 weeks gestation gave written informed consent and were
160 included in the present study. All participants were physically examined by a qualified
161 consultant obstetrician/gynaecologist. The normotensive pregnancy was classified as
162 pregnancy without measurable proteinuria and had normal blood pressure (< 140/90
163 mmHg) on two occasions at least four hours apart and had no history of a clinically
164 diagnosed condition during the three months prior to the start of the present study.
165 Exclusion criteria were women of advanced maternal age (>45 years), those below 18
166 years, multiple pregnancies, previous clinically known conditions such as preeclampsia,
167 gestational diabetes, gestational hypertension, sexually transmitted infections, sickle
168 cell anaemia, obesity and any form of clinically diagnosed cardiovascular condition.
169 Also, those with current or previous history of smoking and alcoholic beverage intake at
170 the time of sampling were excluded.

171 **Ethical consideration**

172 This study was approved by the Committee on Human Research Publication and Ethics
173 (CHRPE) of the School of Medical Science (SMS) /KNUST and Research and
174 Development Unit, Komfo Anokye Teaching Hospital (KATH) (CHRPE/AP/146/17)
175 and the Human Research Ethics Committee (HREC) of Edith Cowan University (ECU)
176 (17509). Written informed consent in the form of signature and fingerprint was obtained
177 from participants and Legally Authorised Representatives before the start of the present
178 study. This study was conducted in accordance with the guidelines of the Helsinki
179 Declaration.

Suboptimal Health Status assessment and sociodemographic, clinical and obstetric data

The overall SHS of NTN-PW was assessed using SHSQ-25. The SHSQ-25 consist of five subclasses namely: fatigue (9 question item), cardiovascular system (3 question item), digestive system (3 question item), immune system (3 question item) and mental health (7 question item) [19,22,31]. These questions were explained to each participant in the native language by the consultant obstetrician/gynaecologist and their response were translated into English. Each pregnant woman was asked to rate her health statement on a 5-point Likert scale: never or almost never (1), occasionally (2), often (3), very often (4) and always (5) based on how often they had experienced a particular health complaint in the past 3 months. The raw scores of 1 to 5 were recoded as 0 to 4 for each participant followed by a summation of the codes for the 25 answered questions. The median of the total score was recorded as the cut-off point and values ≥ the median represented 'SHS' (poor health) and those < indicated 'optimal health status (OHS)' [19,22,31]. In the present study, a score ≥ 19 depicted SHS and <19 depicted OHS. A reliability test was performed on the SHSQ-25 and a Cronbach's alpha coefficient value was found to be 0.95.

Sociodemographic, clinical and obstetric data were obtained from the antenatal folder and participant's record in the database of the KATH. Double measurements of blood pressure (BP) as well as weight, height and body mass index (BMI) were performed by trained personnel and midwives and values were recorded. The last BMI before conception (pre-gestation BMI) was also obtained from participants' records.

Biospecimen collection

Participants provided 10-20 millilitre midstream urine samples in sterile leak-proof containers. Dipstick proteinuria was determined for each participant. Samples were centrifuged at 3000 rpm for 10 minutes at 4 ^{o}C (HERMLE® Z306K, Wehingen, Germany) and the supernatants were aliquoted into two cryovials tubes (1 ml each). One millilitre of the aliquot was used to measure urine creatinine (Cr) concentrations and the rest were stored at -80 ^{o}C (Thermo scientific ultra-low freezer) until further analysis. An overnight fasting venous blood sample (10 millilitres) were collected between 8am and 11am from each of the 593 participants and were dispensed into specialised vacutainer® tubes. The serum and plasma were obtained following centrifugation at 3000 rpm for 10 minutes and were separated into two cryovials each and stored at -80 ^{o}C (Thermo scientific ultra-low freezer) until assay.

Haematobiochemical assay

Plasma fasting blood glucose (FBG), serum triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), total protein (TP), albumin (ALB), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), urea, creatinine (Cr), uric acid (UA), sodium (Na), potassium (K), chloride (Cl-), magnesium (Mg) and calcium (Ca) were measured using an automatic chemistry analyser (Roche Diagnostics, COBAS INTEGRA 400 Plus, USA). Haemoglobin, red blood cell distribution width (RDW) and platelet count (PLT) were analysed using a Mindray Haematology Analyzer BC 2800.

Angiogenic growth mediator (AGMs) assay

Serum concentrations of VEGF-A, sFlt-1, PIGF, and sEng were measured in duplicate using competitive 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 concentrations of each biomarker were derived from standard curves from a known standard concentration of recombinant factors.

Biomarkers of oxidative stress (BOS) assay

Following the manufacturer's instructions, urinary and serum 8-OHdG were analysed in duplicates using highly sensitive and competitive ELISA kits (ab201734, Abcam, China). Serum concentrations were determined by comparison to a standard curve and recorded in ng/L. The inter-and-intra assay coefficients of variation (CV) were 3.5% and 4.5%, respectively. Urinary 8-OHdG concentrations obtained from the standard curves were normalised to creatinine concentrations and recorded as ng/mg Cr.

Serum 8-epi-PGF2α was analysed in duplicate using competitive ELISA kits from ELabscience, China (cat. LogE-EL-0041). The intra-and-inter assay coefficients of variation (CV) were 5.6% and 6.4%, respectively. The absorbance of both 8-epi-PGF2α and 8-OHdG was read at 450nm on a microplate reader (Bio-Tek ELx808 microplate reader, Hayward, CA, USA).

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 ferric reducing ability of plasma (FRAP) and the protocol as described by Benzie and Strain [32]. The absorbance was

used to obtain the concentrations after comparison to standard curves and recorded in µmol/l.

Statistical analysis

Normalisation of the data was performed using Kolmogorov-Smirov test. Data was presented as mean ± SD for parametric continuous variables, median (interquartile ranges) for non-parametric continuous variables and frequency (percentages) for categorical variables. Chi-square test was performed to test associations between categorical variables. The difference in mean variables between SHS and OHS was tested using an independent sample t-test. The difference in median variables between SHS and OHS was tested using the Mann Whitney U-test. A multivariate logistic regression model was performed to test risk factors associated with SHS. Linear regression models were performed to test the associations between SHS, AGMs and OS biomarkers. Data analysis was performed using R version 3.4.3 (R core Team 2017), SPSS version 24 (IBM Corp, NY, USA) and XLSTAT Premium version 2018.1 for windows. *P value* < 0.05 was considered statistically significant.

Results

Sociodemographic characteristics of NTN-PW stratified as SHS and Optimal health status

The average age of the participants was 29.64 years (**Table 1)**. A higher proportion [34.6% (205/593)] of the study participants were aged 25 to 30 years. There was no statistically significant difference between the mean ages of pregnant women with SHS compared to those with OHS (29.44 ± 5.92 vs. 29.77 ± 6.08; $p = 0.5045$). Overall, a higher proportion of the pregnant women had completed secondary education [40.8% (242/593)], were married [84.5% (501/593)], were Akan's by ethnicity [87.2% (517/593)], had an informal occupation [63.2% (375/593)] and earned a low-income per month [38.8%

(230/593)]. However, there was no statistically significant difference in proportion between pregnant women with SHS compared to OHS in terms of level of education (p = 0.7577), marital status (p = 0.7000), ethnicity (p = 0.9140), occupation (p = 0.7913) and basic monthly salary income (p = 0.8384) **(Table 1).**

Table 1 Sociodemographic characteristics of NTN-PW stratified by SHS and OHS

Characteristics	**Total (N=593)**	**SHS** (N=297)	**OHS** (N=296)	Statistics	*p-value*
Age (mean ± SD) (years)	29.64 ± 5.98	29.44 ± 5.92	29.77 ± 6.08	*0.6678*	0.5045
Age (years)					
18-24	130(21.9)	66(22.2)	64(21.6)		
25-30	205(34.6)	110(37.0)	95(32.1)		
31-34	124(20.9)	58(19.5)	66(22.3)		
35-45	134(22.6)	63(21.2)	71(23.9)		
Highest Level of Education				1.180, 3	0.7577
Unschooled	5(0.8)	2(0.7)	3(1.0)		
Primary	203(34.2)	100(33.7)	103(34.8)		
Secondary	242(40.8)	127(21.4)	115(38.9)		
Tertiary	143(24.1)	68(22.9)	75(25.3)		
Marital Status				0.714, 2	0.7000
Never married	86(14.5)	42(14.1)	44(14.9)		
Married	501(84.5)	251(84.5)	250(84.5)		
Cohabiting	6(1.0)	4(1.3)	2(0.7)		
Ethnicity				0.522, 3	0.9140
Akan	517(87.2)	273(91.9)	244(82.4)		
Ga-Adangbe	10(1.7)	6(2.0)	4(1.4)		
Mole Dagbani	49(8.2)	49(16.5)	45(15.2)		
Ewe	8(1.3)	5(1.7)	3(1.0)		
Occupation				0.468, 2	0.7913
Unemployed	63(10.6)	34(11.4)	29(9.8)		
Formal	155(26.1)	78(26.3)	77(26.0)		
Informal	375(63.2)	185(62.3)	190(64.2)		
Basic monthly income (GH₵)				0.846, 3	0.8384
None	63(10.6)	34(11.4)	29(9.8)		
Low (<500.0)	230(38.8)	114(38.4)	116(39.2)		
Middle (500.0-1000.0)	198(33.4)	101(34.0)	97(32.8)		
High (>1000.0)	102(17.2)	48(16.2)	54(18.2)		

Values are presented as frequency (proportion); mean ± SD (standard deviation); GH₵: Ghana cedi. Statistics is represented as Chi-square value, degree of freedom (X^2, df), and t-test value (italised)

Clinical, obstetrics and routine biochemical profile of NTN-PW stratified as SHS and optimal health status

A higher proportion of pregnant women were nulliparous [39.6% (235/593)], primigravida [46.2% (274/593)], had optimal blood pressure [60.4% (358/593)] and were overweight at both pre-gestational [37.8% (224/593)] and the time of sampling [38.6% (229/593)] (**Table 2)**. There was a statistically significant difference in proportion between pregnant women with SHS compared to OHS in terms of parity (p =0.0311), gravidity (p =0.0309), and BP (p <0.0001). In comparison to pregnant women with OHS, those with SHS had higher proportions in terms of high BP (11.4% vs. 2.0%; p <0.0001), family history of hypertension (23.2% vs. 7.1%; p <0.0001) and history of spontaneous abortion (37.0% vs. 28.0%; p = 0.0282). However, there was no statistically significant difference in proportion between pregnant women with SHS compared to OHS in terms of previous caesarean section (19.5% vs. 21.6%; p = 0.5436). Consequently, there was a statistically significant difference in the mean systolic blood pressure (SBP) between pregnant women with SHS compared to OHS (p =0.0071) but no significant difference in the mean diastolic blood pressure (DBP) (p =0.1574), gestational age (p =0.9515), pre-gestation BMI (p =0.6855) and BMI at the time of sampling (p =0.7658) between groups. There were significantly reduced levels of serum Mg (p <0.0001), Ca (p <0.0001), haemoglobin (p =0.0428) and HDL-c (p =0.0481) but significantly elevated levels of AST (p <0.0001), ALT (p =0.0158), ALP (p =0.0032), GGT (p <0.0001), urea (p=0.0242), creatinine (p =0.0467), uric acid (p =0.0002) and TG (p =0.0007) among participants with SHS compared to those with OHS (**Table 2).**

Table 2. Obstetric, clinical and haematobiochemical characteristics of NTN-PW stratified by SHS and OHS

Characteristics	Total (N=593)	SHS (N=297)	OHS (N=296)	Statistics	*p-value*
Parity				8.870, 2	**0.0311**

Nulliparous (0)	235(39.6)	113(38.0)	122(41.2)		
Primiparous (1)	114(19.2)	64(21.5)	50(16.9)		
Multiparous (2-4)	244(41.2)	120(40.5)	124(41.9)		
Gravidity				6.951, 2	**0.0309**
Primigravida (1)	274(46.2)	153(51.5)	121(40.9)		
Multigravida (2-4)	175(29.5)	81(27.3)	94(31.8)		
Grand multigravida (>5)	144(24.3)	63(21.2)	81(27.4)		
BP (mmHg)				54.65, 2	**<0.0001**
Normal (120-129/80-84)	553(93.3)	263(88.5)	290(98.0)		
High (130-139/85-89)	40(6.7)	34(11.4)	6(2.0)		
FH of HTN (Yes)	90(15.2)	69(23.2)	21(7.1)	33.81, 1	**<0.0001**
H. Spont. Abort. (Yes)	193(32.5)	110(37.0)	83(28.0)	5.083, 1	**0.0282**
Previous CS (Yes)	122(20.6)	58(19.5)	64(21.6)	0.397, 1	0.5436
Protein (<0.3g/g/24hr)	593(100.0)	297(100.0)	276(100.0)		0.9991
GA (weeks)	16.98 ± 2.01	16.97 ± 2.08	16.98 ± 1.98	*0.061*	0.9515
SBP (mmHg)	114.7 ± 10.57	115.8 ± 11.00	113 ± 10.01	*2.703*	**0.0071**
DBP (mmHg)	72.58 ± 9.26	73.12 ± 9.31	72.04 ± 9.20	*1.416*	0.1574
Pre-gest. BMI (Kg/m^2)	27.04 ± 4.83	26.65 ± 4.74	27.12 ± 4.92	*0.405*	0.6855
Gest. BMI (Kg/m^2)	27.33 ± 4.81	27.39 ± 4.74	27.12 ± 4.92	*0.298*	0.7658
Mg (mmol/l)	0.95 ± 0.19	0.91 ± 0.24	0.99 ± 0.13	*5.384*	**<0.0001**
Ca (mmol/l)	2.18 ± 0.35	2.07 ± 0.38	2.29 ± 0.27	*8.431*	**<0.0001**
Na (mmol/l)	136.3 ± 2.00	136.4 ± 1.99	136.2 ± 2.01	*0.958*	0.3384
K (mmol/l)	4.18 ± 0.38	4.21 ± 0.45	4.17 ± 0.33	*1.195*	0.2326
Cl-(mmol/l)	105.6 ± 2.32	105.5 ± 2.31	105.6 ± 2.33	*0.399*	0.6889
LDH (IU/L)	173.3 ± 41.25	176.4 ± 45.14	170.1 ± 36.73	*0.061*	0.0605
AST (IU/L)	15.70(13.70-20.50)	16.10(13.80-26.15)	15.20(13.60-19.30)	3504	**<0.0001**
ALT (IU/L)	11.50(10.30-16.65)	12.60(10.30-18.40)	11.05(10.20-14.50)	3893	**0.0158**
ALP (IU/L)	201.0(168.0-228.0)	205.0(168.0-235.0)	195.0(168.0-218.0)	3781	**0.0032**
GGT (IU/L)	10.40(9.80-13.50)	11.30(10.10-15.40)	10.34(9.70-12.20)	3204	**<0.0001**
Total protein (g/L)	67.98 ± 2.21	68.01 ± 2.21	67.96 ± 2.20	*0.266*	0.7900
Albumin (g/L)	36.85 ± 1.27	36.88 ± 1.26	36.82 ± 1.27	*0.556*	0.5782
Urea (mmol/l)	3.76 ± 1.61	3.92 ± 1.79	3.62 ± 1.39	*2.260*	**0.0242**
Creatinine (µmol/l)	61.19 ± 13.51	62.29 ± 15.17	60.08 ± 11.53	*1.995*	**0.0465**
Uric acid (µmol/l)	290.0 ± 46.10	297.0 ± 42.54	283.0 ± 48.48	*3.748*	**0.0002**
Haemoglobin (g/dL)	11.65 ± 0.60	11.01 ± 0.63	11.69 ± 0.57	*1.646*	**0.0428**
RDW-CV (%)	13.65 ± 1.25	13.66 ± 1.30	13.65 ± 1.19	*0.123*	0.9022
PLT (X10^9 / L)	296.9 ± 86.75	290.8 ± 85.70	303.0 ± 87.51	*1.718*	0.0864
FBG (mmol/L)	5.09 ± 0.74	5.12 ± 0.77	5.08 ± 0.69	*0.635*	0.5085
TC (mmol/L)	4.65 ± 1.18	4.69 ± 1.23	4.61 ± 1.11	*0.827*	0.4088
TG (mmol/L)	1.31 ± 0.68	1.39 ± 0.76	1.24 ± 0.58	*2.706*	**0.0070**
HDL-c (mmol/L)	1.45 ± 0.32	1.40 ± 0.32	1.48 ± 0.34	*1.898*	**0.0481**
LDL-c (mmol/L)	2.79 ± 1.05	2.84 ± 1.12	2.74 ± 0.98	*1.171*	0.2421

Values are presented as frequency (proportion); mean ± SD (standard deviation); median (interquartile range). FH: Family history; PH: Previous history; CS: Caesarean section; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; BMI: body mass index; HTN: hypertension; H. Spont. Abort: history of spontaneous abortion. Statistics is represented as Chi-square value, degree of freedom (X^2, df), Mann-Whitney U-test value (unitalised); t-test value (italised)

Relationship between SHS and biochemical risk factors

As shown in **Table 3**, after adjusting for confounding factors using a multivariate logistic regression model, the association remained significant with high BP [aOR=5.96, 95% CI (2.39-14.85); p<0.0001], low Mg [aOR=4.47, 95% CI (3.16-10.15); p<0.0001], low Ca [aOR=2.19, 95% CI(1.19-5.03), p<0.0001], high LDH [aOR= 2.75(1.60-5.07), p=0.0006], high AST [aOR=2.22(1.68-8.14), p=0.0018], high creatinine [aOR=3.15, 95% CI (1.55-7.04), p=0.0028], anaemia [aOR=1.58, 95% CI (1.11-2.62), p=0.0397] , high TG [aOR=2.14, 95% CI (1.08-4.79), p=0.0206] and low HDL-c [aOR=2.57, 95% CI (1.15-7.05), p=0.0418] as independent risk factors for SHS.

Table 3. Univariate and multivariate logistic regression model of clinical and haematobiochemical profile as risks factors for SHS

			Model 1		Model 2	
Characteristics	**SHS**	**OHS**	cOR (95% CI)	*P* value	aOR (95% CI)	*P* value
BP (mmHg)						
Optimal Normal	263(88.6)	290(98.0)	1.00		1.00	
High	34(11.4)	6(2.0)	6.6(2.74-15.96)	< 0.0001	5.96(2.39-14.85)	**< 0.0001**
Mg (mmol/l)						
Low	50(16.8)	8(2.7)	5.28(3.37-11.67)	<0.0001	4.47(3.16-10.15)	**<0.0001**
Normal	247(83.2)	288(97.3)	1.00		1.00	
Alb. Adj. Ca (mmol/l)						
Low	159(53.5)	70(23.6)	2.72(1.61-5.29)	<0.0001	2.19(1.19-5.03)	**<0.0001**
Normal	138(46.5)	226(76.4)	1.00		1.00	
LDH (IU/L)						
High	50(16.8)	20(6.8)	2.79(1.62-4.82)	0.0002	2.75(1.60-5.07)	**0.0006**
Normal	247(83.2)	276(93.2)	1.00		1.00	
AST (IU/L)						
High	24(8.1)	6(2.0)	2.25(1.71-9.56)	0.0011	2.22(1.68-8.14)	**0.0018**
Normal	273(91.9)	290(98.0)	1.00		1.00	
ALP (IU/L)						
High	82(27.6)	79(26.7)	1.04(0.72-1.50)	0.8536	1.08(0.78-1.93)	0.8054
Normal	215(72.4)	217(73.3)	1.00		1.00	
Urea (IU/L)						
High	12(4.0)	4(1.4)	3.07(0.97-9.64)	0.0729	3.03(0.73-10.51)	0.0910
Normal	285(96.0)	292(98.6)	1.00		1.00	
Creatinine (IU/L)						
High	32(10.8)	11(3.7)	3.12(1.54-6.33)	0.0013	3.15(1.55-7.04)	**0.0028**
Normal	265(89.2)	285(96.3)	1.00		1.00	
Uric acid (µmol/l)						
High	10(3.4)	8(2.7)	1.25(0.48-3.22)	0.8117	1.18(0.41-3.88)	0.8531
Normal	287(96.6)	288(97.3)	1.00		1.00	
Hb (g/dl)						
Anemia	80(26.9)	57(19.3)	1.55(1.05-2.27)	0.0319	1.58(1.11-2.62)	**0.0397**
Non-anemia	217(73.1)	239(80.7)	1.00		1.00	
FBS (mmol/L)						
High Normal	27(9.1)	16(5.4)	1.75(0.92-3.32)	0.1124	1.85(0.81-3.85)	0.1068
Normal	270(90.9)	280(94.6)	1.00		1.00	
TC (mmol/L)						
High	91(30.6)	76(25.7)	1.27(0.89-1.83)	0.2013	1.30(0.94-2.03)	0.2750
Desirable	206(69.4)	220(74.3)	1.00		1.00	
TG (mmol/L)						
High	30(10.1)	14(4.7)	2.26(1.17-4.36)	0.0179	2.14(1.08-4.79)	**0.0206**
Normal	267(89.8)	282(95.3)	1.00		1.00	
HDL-c (mmol/L)						
Low	18(6.1)	7(2.4)	2.66(1.09-6.47)	0.0390	2.57(1.15-7.05)	**0.0418**
Normal	279(93.9)	289(97.6)	1.00		1.00	
LDL-c (mmol/L)						
High	54(18.2)	37(12.5)	1.55(0.98-2.44)	0.0679	1.38(0.689-2.67)	0.0890
Normal	243(81.8)	259(87.5)	1.00		1.00	

cOR: Crude odds ratio; aOR: adjusted odds ratio; CI: confidence interval; 1.00: reference category; Model 1:unadjusted odds ratio; Model 2 adjusted for maternal age, gestational age, parity, gravidity, family history of hypertension , maternal BP, history of spontaneous abortion, pre-gestational BMI. Alb. Adj. Ca: albumin adjusted calcium

Biomarkers of Oxidative stress and angiogenic growth mediators of NTN-PW stratified by SHS and Optimal health status

As shown in **Figure 1,** there were statistically significantly increased urinary 8-OHdG (p <0.0001) and serum levels of sEng (p <0.0001), sFlt-1 (p <0.0001), 8-isoPGF2α (p <0.0001), 8-OHdG (p <0.0001), sFlt-1: PlGF ratio (p <0.0001), sEng: PlGF ratio (p <0.0001), 8-isoPGF2α: PlGF ratio (p <0.0001) and 8-OHdG: PlGF ratio (p <0.0001) among pregnant women with SHS compared to those with OHS. Conversely, there were statistically significant low serum levels of PlGF (p <0.0001) and VEGF-A (p <0.0001) among pregnant women with SHS compared to those with OHS. However, the serum levels of TAC were low in pregnant women with SHS compared to those with OHS although there was no statistically significant difference (p=0.0860) (**Figure 1)**.

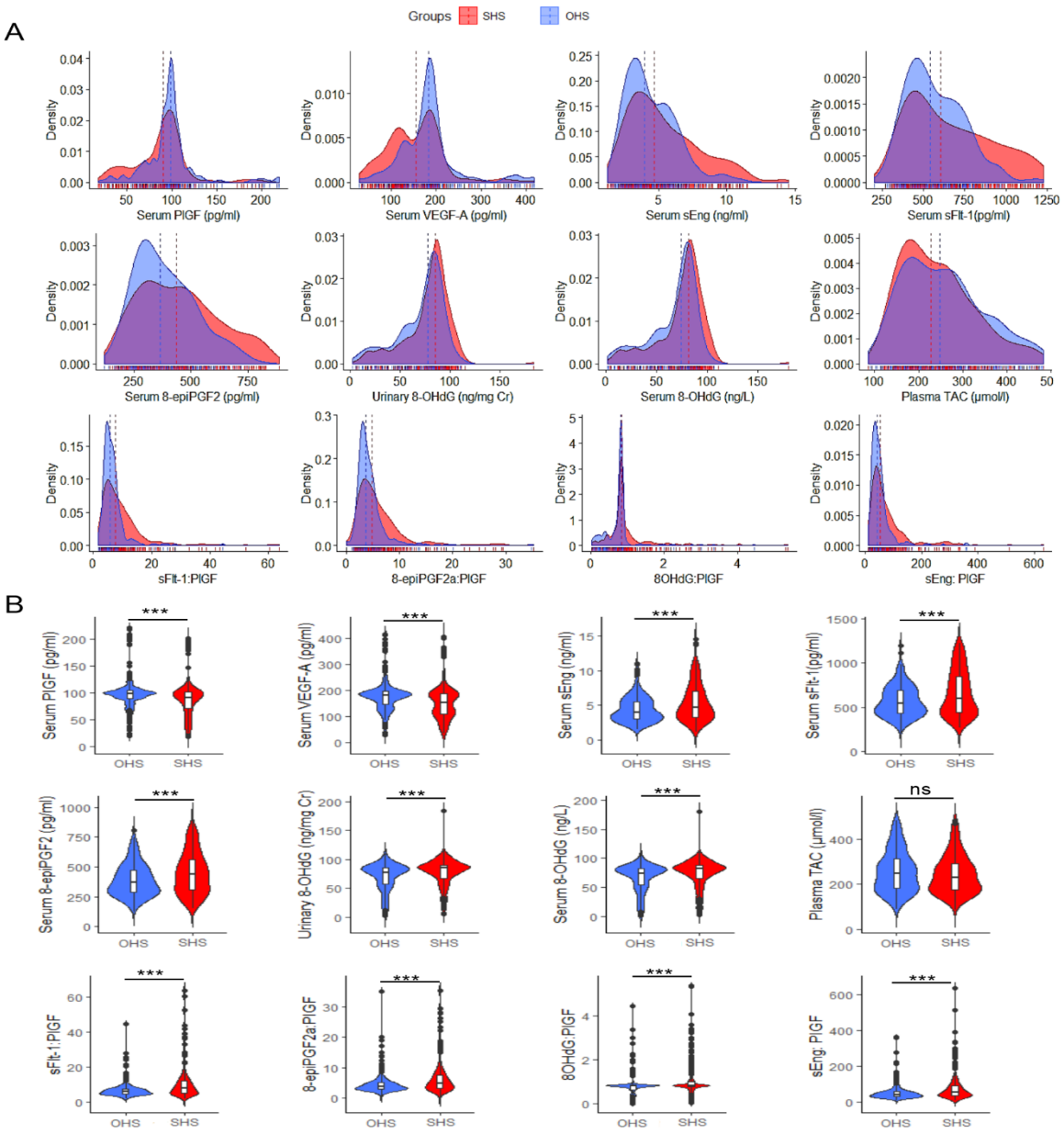


Figure 1. Density (A) and violin (B) plots of individual and combined levels of AGMs and BOS levels stratified by SHS and OHS NTN-PW

PIGF: placental growth factor; VEGF-A: Vascular endothelial growth factor-A; sEng: soluble endoglin; sFlt-1: soluble fms-like tyrosine kinase-1; 8-epiPGF2 α: 8-epiprostaglandin F2 alpha; 8-OHdG: 8-hydroxy-2-deoxyguanosine; TAC: Total antioxidant capacity. *** represents p <0.0001.

Relationship between the individual SHS-specific domains score and biomarkers of oxidative stress and angiogenic growth mediators

As summarised in **Table 4**, the individual SHS domains such as fatigue, cardiovascular system, immune system and mental health were significant ($p < 0.05$) and negatively associated with PlGF and VEGF-A, but positively associated with sEng, sFlt-1, 8-epiPGF2α, 8-OHdG, sFlt-1/PlGF ratio 8-epiPGF2α/PlGF ratio, 8-OHdG/PlGF ratio and sEng/PlGF ratio. The SHS-specific domain, 'digestive system' showed the same pattern of results except that there was no significant association with 8-OHdG. TAC was non-significant but negatively associated the individual SHS domains except for the SHS-specific domain, 'immune system', which showed a significant association. The multivariate model showed that 14.0% variation in SHS was explained when all the significant independent markers were included in the model (**Table 4)**.

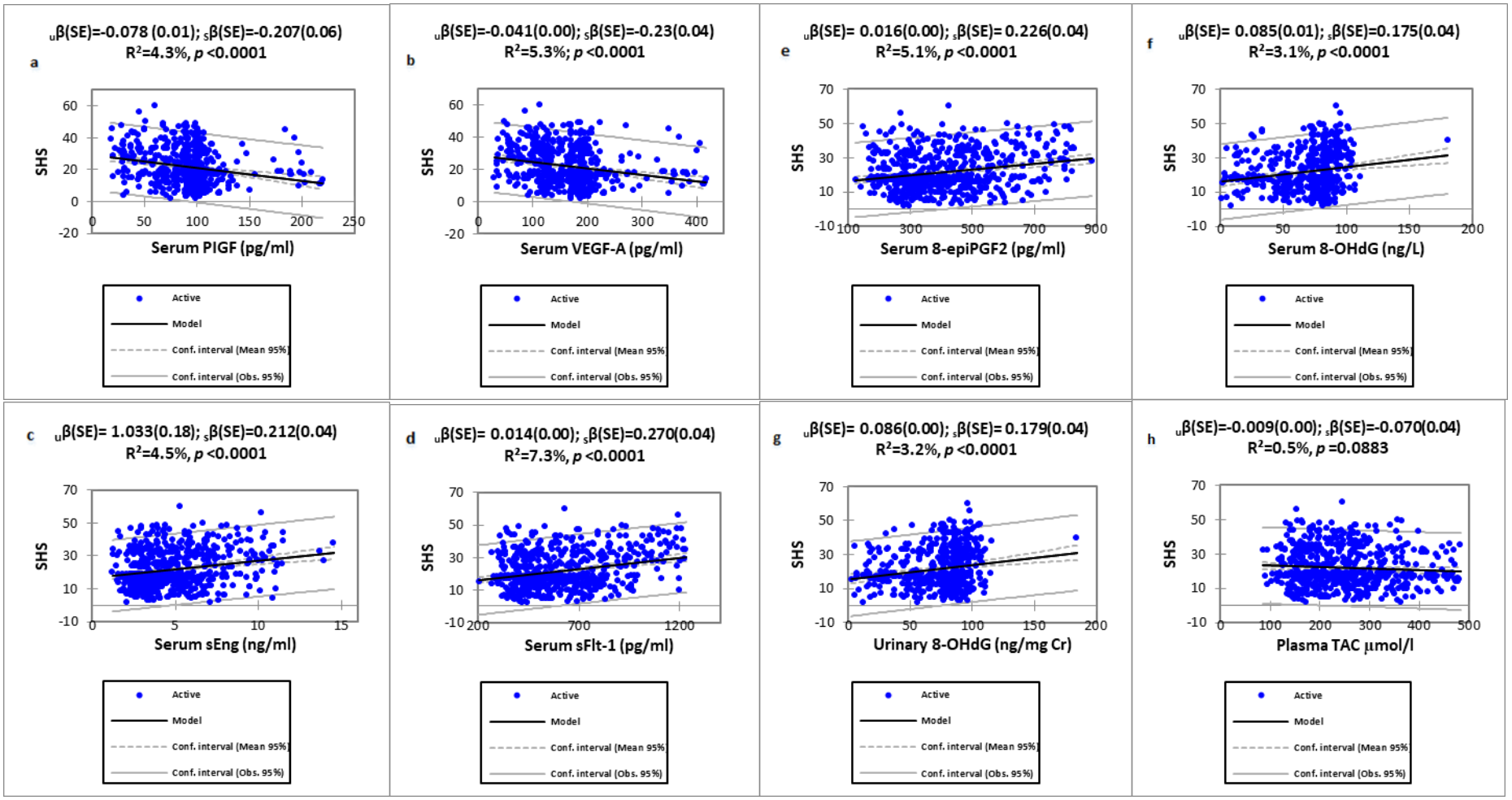
Table 4. Univariate and multivariate linear regression model for individual domain of SHS score in association with obstetric-related factors, clinical, AGMs and BOS

Parameters (N=593)	Fatigue $_s\beta$ (R^2)	*p-Value*	Cardiovascular System $_s\beta$ (R^2)	*p-Value*	Digestive System $_s\beta$ (R^2)	*p-Value*	Immune System $_s\beta$ (R^2)	*p-Value*	Mental Health $_s\beta$ (R^2)	*p-Value*	Overall SHS $_s\beta$ (R^2)	*p-Value*
Model 1												
Age (years)	-0.007(0.0%)	0.8521	-0.014 (0.0%)	0.7369	-0.020 (0.0%)	0.6239	-0.003 (0.0%)	0.9422	-0.068 (0.5%)	0.0982	-0.034(0.2%)	0.4560
parity	-0.009(0.0%)	0.3593	-0.072 (0.5%)	0.0788	-0.015 (0.0%)	0.7144	0.006 (0.0%)	0.8443	-0.028 (0.0%)	0.4969	-0.045(0.2%)	0.2771
gravidity	-0.003(0.0%)	0.8113	-0.079 (0.6%)	0.0526	-0.026 (0.0%)	0.5232	-0.028 (0.0%)	0.4949	-0.067 (0.5%)	0.1027	-0.051(0.3%)	0.2120
Gest. Age (Weeks)	-0.001(0.0%)	0.9683	-0.001 (0.0%)	0.9879	-0.022 (0.0%)	0.5896	0.014 (0.0%)	0.7394	-0.007 (0.0%)	0.8623	-0.005(0.1%)	0.9040
SBP (mmHg)	0.077(0.6%)	0.0616	0.090 (0.8%)	**0.0281**	0.058 (0.3%)	0.1579	0.130 (1.7%)	**0.0015**	0.148 (2.2%)	**0.0003**	**0.144(2.1%)**	**0.0004**
DBP (mmHg)	-0.009(0.0%)	0.8285	0.036 (0.1%)	0.3814	-0.001 (0.0%)	0.9781	0.067 (0.5%)	0.1042	0.183 (3.3%)	**< 0.0001**	**0.086(0.6%)**	**0.0375**
Gestational BMI	-0.004(0.0%)	0.8980	0.084 (0.7%)	**0.0400**	0.060 (0.4%)	0.1442	0.017 (0.0%)	0.6834	0.038 (0.1%)	0.3499	0.038(0.1%)	0.3608
Pre-gestation BMI	-0.029(0.1%)	0.4745	0.186 (0.5%)	0.0867	0.046 (0.2%)	0.2673	0.001 (0.0%)	0.9685	0.022 (0.0%)	0.5990	0.010(0.0%)	0.8064
PIGF (pg/mL)	-0.123(1.5%)	**0.0028**	-0.114 (1.3%)	**0.0054**	-0.150 (2.3%)	**0.0002**	-0.167 (2.8%)	**< 0.0001**	-0.175 (3.1%)	**< 0.0001**	**-0.207(4.3%)**	**<0.0001**
VEGF-A(pg/mL)	-0.142(2.0%)	**0.0005**	-0.143 (2.0%)	**0.0005**	-0.140 (2.0%)	**0.0006**	-0.164 (2.7%)	**< 0.0001**	-0.202 (4.1%)	**< 0.0001**	**-0.230(5.3%)**	**<0.0001**
sEng (ng/mL)	0.186(3.5%)	**< 0.0001**	0.101 (1.0%)	**0.0137**	0.087 (0.8%)	**0.0333**	0.097 (1.0%)	**0.0177**	0.155 (2.4%)	**0.0002**	**0.212(4.5%)**	**<0.0001**
sFlt-1 (pg/ml)	0.182(3.3%)	**< 0.0001**	0.155 (2.4%)	**0.0001**	0.162 (2.6%)	**< 0.0001**	0.209 (4.4%)	**< 0.0001**	0.208 (4.3%)	**< 0.0001**	**0.270(7.3%)**	**<0.0001**
8-epiPGF2α(pg/ml)	0.139(1.9%)	**0.0007**	0.138 (1.9%)	**0.0008**	0.136 (1.8%)	**0.0009**	0.148 (2.2%)	**0.0003**	0.206 (4.3%)	**< 0.0001**	**0.225(5.1%)**	**<0.0001**
8-OHdG(ng/mgCr)	0.119(1.4%)	**0.0037**	0.110 (1.2%)	**0.0073**	0.058 (0.3%)	0.1683	0.128 (1.6%)	**0.0019**	0.158 (2.5%)	**0.0001**	**0.175(3.1%)**	**<0.0001**
U8-OHdG(ng/ml)	0.125(1.6%)	**0.0023**	0.101 (1.0%)	**0.0134**	0.069 (0.5%)	0.0956	0.140 (2.0%)	**0.0006**	0.151 (2.3%)	**0.0002**	**0.178(3.2%)**	**<0.0001**
TAC (µmol/L)	-0.062(0.4%)	0.1301	-0.002 (0.0%)	0.9560	-0.003 (0.0%)	0.9345	-0.100 (1.0%)	**0.0178**	-0.045 (0.2%)	0.2775	-0.072(0.5%)	0.0883
sFlt-1: PIGF ratio	0.177(3.2%)	**< 0.0001**	0.205 (4.2%)	**< 0.0001**	0.167 (2.8%)	**< 0.0001**	0.248 (6.1%)	**< 0.0001**	0.233 (5.4%)	**< 0.0001**	**0.292(8.5%)**	**<0.0001**
sEng: PIGF ratio	0.160(2.6%)	**< 0.0001**	0.149 (2.2%)	**0.0003**	0.140 (2.0%)	**0.0006**	0.194 (3.8%)	**< 0.0001**	0.193 (3.7%)	**< 0.0001**	**0.244(6.0%)**	**<0.0001**
8-epiPGF2α: PIGF	0.146(2.1%)	**0.0004**	0.187 (3.5%)	**< 0.0001**	0.163 (2.7%)	**< 0.0001**	0.223 (5.0%)	**< 0.0001**	0.223 (5.0%)	**< 0.0001**	**0.262(6.9%)**	**<0.0001**
8-OHdG: PIGF	0.141(2.0%)	**0.0006**	0.167 (2.8%)	**< 0.0001**	0.159 (2.5%)	**< 0.0001**	0.219 (4.8%)	**< 0.0001**	0.211 (4.4%)	**< 0.0001**	**0.250(6.3%)**	**<0.0001**
Model 2												
R^2	7.2%		5.1%		5.0%		10.7%		11.4%		14.0%	
Adjusted R^2	5.5%		2.9%		3.5%		8.8%		9.4%		12.2%	
Constant p-value	<0.0001		0.0042		0.0004		<0.0001		<0.0001		<0.0001	

$_s\beta$ (R^2): Standardised regression coefficient (Coefficient of determination); SBP: systolic blood pressure; DBP: diastolic blood pressure, PIGF: placental growth factor; VEGF-A: Vascular endothelial growth factor-A; sEng: soluble endoglin; sFlt-1: soluble fms-like tyrosine kinase-1; 8-epiPGF2 α: 8-epiprostaglandin F2 alpha; 8-OHdG: 8-hydroxy-2-deoxyguanosine; TAC: Total antioxidant capacity. Univariate (Model 1); Multivariate (Model 2): included all significant parameters in the model

Relationship between the overall SHS score and individual biomarkers of oxidative stress and angiogenic growth mediators

There was a significantly negative association between SHS and serum PlGF ($_s\beta$ = -0.207; R^2=4.3%; p <0.0001) and VEGF-A ($_s\beta$ = -0.230; R^2=5.3%; p <0.0001) but a significantly positive association with sEng ($_s\beta$ = 0.212; R^2= 4.5%; p <0.0001), and sFlt-1 ($_s\beta$ = 0.270; R^2=7.3%; p <0.0001) There was a significantly positive association between SHS and serum 8-epiPGF2α ($_s\beta$ = 0.225; R^2=5.1%; p <0.0001), serum 8-OHdG ($_s\beta$ = 0.175; R^2= 3.1%; p <0.0001), and urinary 8-OHdG ($_s\beta$ = 0.179; R^2=3.2%; p <0.0001) but a negative relationship between SHS and TAC ($_s\beta$ = -0.720; R^2= 0.5%; p =0.0883) **(Figure 2)**.



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407 **Figure 2. Linear regression model of SHS score in association with levels of AGMs and BOS among NTN-PW**

408 uβ: unstandardised co-efficient; sβ: standardised co-efficient; SE: standard error; R^2: coefficient of determination. Significant negative association between SHS and serum PlGF (sβ = -0.207;
409 p<0.0001) (Figure **2a**); and VEGF-A (sβ = -0.230; p <0.0001) (Figure **2b**). Significant positive association between SHS and sEng (sβ = 0.212; p <0.0001) (Figure **2c**); sFlt-1 (sβ = 0.270; p
410 <0.0001) (Figure **2d**); serum 8-epiPGF2α (sβ = 0.225; p <0.0001) (Figure **2e**); serum 8-OHdG (sβ = 0.175; p <0.0001) (Figure 2**f)** and urinary 8-OHdG (sβ = 0.179; p <0.0001) (Figure **2g).**
411 Non-significant negative relationship between SHS and TAC (sβ = -0.720; R^2= 0.5%; p =0.0883) (Figure **2h**)

Relationship between the overall SHS score and combined biomarkers of oxidative stress and angiogenic growth mediators

As shown in **Figure 3**, there was a significantly positive relationship between SHS and sFlt-1: PlGF ratio ($_s\beta$ = 0.292; R^2=8.5%; p <0.0001), 8-epiPGF2α: PlGF ratio ($_s\beta$ = 0.262; R^2=6.9%; p <0.0001), 8-OHdG: PlGF ratio ($_s\beta$ = 0.250; R^2=6.3%; p <0.0001), sEng: PlGF ratio ($_s\beta$ = 0.244; R^2=6.0%; p <0.0001), SBP ($_s\beta$ = 0.144; R^2=2.1%; p =0.0004) and DBP ($_s\beta$ = 0.086; R^2=0.7%; p =0.0375) (**Figure 3)**.

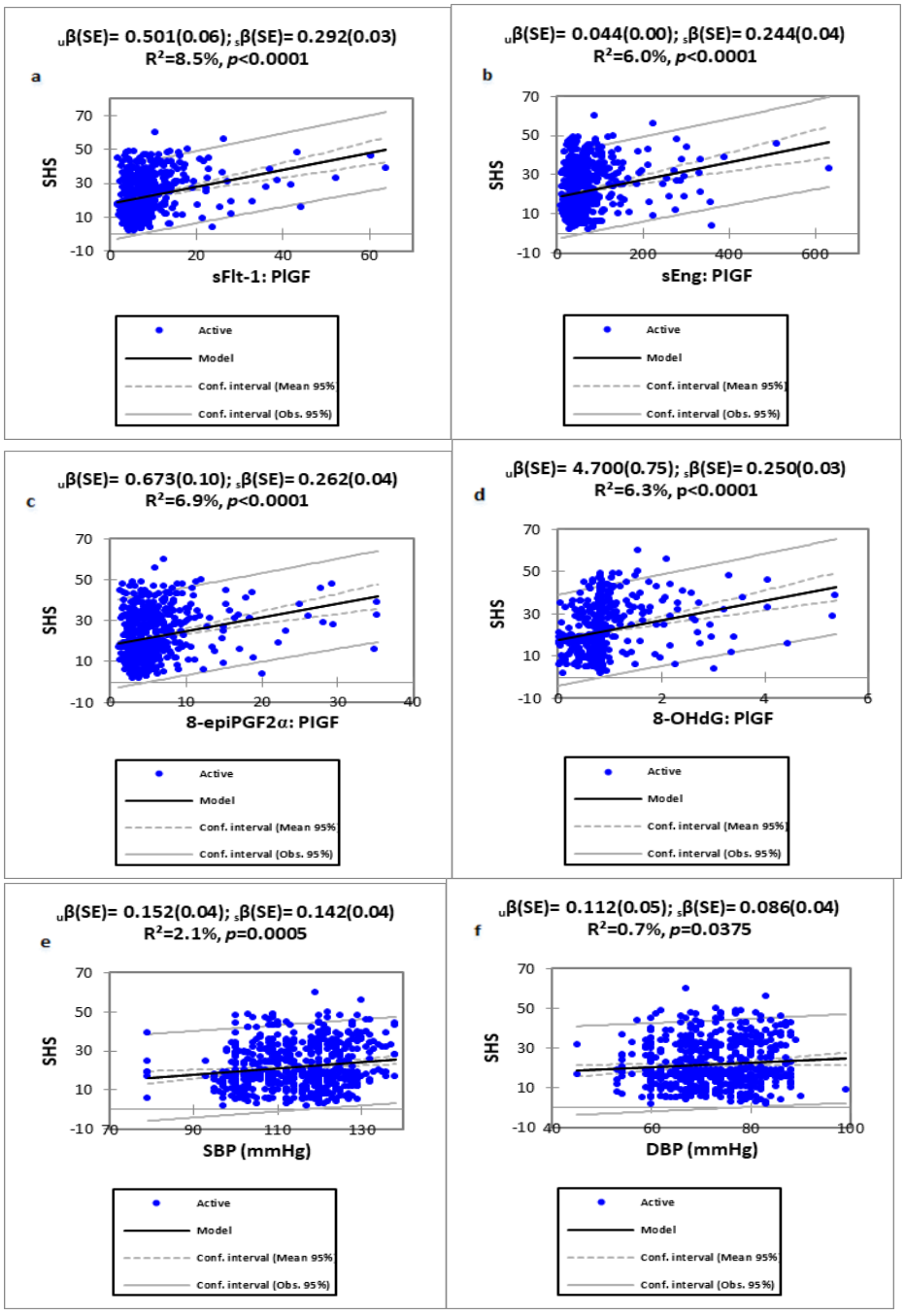

Figure 3. Linear regression model of SHS score in association with ratios of AGMs and BOS and BP among NTN-PW

uβ: unstandardised co-efficient; sβ: standardised co-efficient; SE: standard error; R^2: coefficient of determination. Significant positive association between SHS and sFlt-1: PlGF ratio (sβ = 0.292; p <0.0001) (Figure **3a**); sEng: PlGF ratio (sβ = 0.244; p <0.0001) (Figure **3b**), 8-epiPGF2α: PlGF ratio (sβ = 0.262; p <0.0001) (Figure **3c**), 8-OHdG: PlGF ratio (sβ = 0.250; p <0.0001) (Figure **3d**), , SBP (sβ = 0.144; p =0.0004) (Figure **3e**) and DBP (sβ = 0.086; p =0.0375) (Figure **3f**).

Predictive odds ratios of the individual biomarkers of oxidative stress and angiogenic growth mediators in association with SHS

As shown in **Table 5** the 1st quartiles for serum PIGF [aOR=2.79; 95% CI (1.43 to 3.28); p = 0.0002] and VEGF-A [aOR =5.35; 95%CI (2.85 to 10.01); p <0.0001], the 2nd quartile for PIGF [aOR =2.48; 95%CI (1.28 to 5.29)]; p =0.0154) and the 4th quartiles for sEng [aOR =4.31; 95% CI (2.37 to 7.81); p <0.0001], sFlt-1[aOR =1.84; 95% CI (1.15 to 2.83); p = 0.0013], 8-epiPGF2α [aOR =2.23; 95% CI (1.41 to 3.46); p = 0.0001], serum 8-OHdG [aOR =1.90; 95% CI (1.28 to 2.83); p =0.0018] and urinary 8-OHdG [aOR =1.95; 95% CI (1.30 to 2.90); p = 0.0004] were independently associated with SHS with only few variations in the odds ratios after adjusting for confounding factors (**Table 5)**.

Table 5. Crude and adjusted odds ratios of quartile for AGMs and BOS associated with SHS

Parameters	SHS (N=297)	OHS (N=296)	Crudes odds ratio (95% CI)	*p*-value	adjusted odds ratio (95% CI)	*p*-value
Serum PIGF (pg/ml)						
Q1 (<80.10)	91(30.6)	56(18.9)	2.12(1.39 to 3.24)	0.0005	**2.79(1.43 to 3.28)**	**0.0002**
Q2 (80.10-89.10)	27(9.1)	14(4.7)	2.52(1.26 to 5.05)	0.0104	**2.48(1.28 to 5.29)**	**0.0154**
Q3 (89.11-99.10)	78(26.3)	94(31.8)	1.08(0.73 to 1.61)	0.7615	1.13(0.81 to 1.77)	0.4382
Q4 (>99.11)	101(34.0)	132(44.6)	1.00		1.00	
Serum VEGF-A (pg/ml)						
Q1 (<124.4)	108(36.4)	40(13.5)	5.31(2.98 to 9.43)	< 0.0001	**5.35(2.85 to 10.01)**	**< 0.0001**
Q2 (124.4-163.4)	45(15.1)	50(16.9)	1.76(0.96 to 3.22)	0.0704	1.63(0.91 to 3.25)	0.0816
Q3 (163.5-203.4)	115(38.7)	149(50.3)	1.51(0.91 to 2.52)	0.1299	1.39(0.60 to 2.81)	0.3014
Q4 (>203.5)	29(9.8)	57(19.3)	1.00		1.00	
Serum sEng (ng/ml)						
Q1 (<3.194)	65(21.9)	83(28.0)	1.00		1.00	
Q2 (3.194-5.194)	106(35.7)	114(23.6)	1.19(0.78 to 1.81)	0.4563	1.15(0.76 to 1.82)	0.5035
Q3 (5.195-7.195)	59(19.9)	79(26.7)	0.95(0.59 to 1.52)	0.9051	1.07(0.64 to 1.69)	0.9713
Q4 (>7.196)	67(22.6)	20(6.8)	4.28(2.35 to 7.76)	< 0.0001	**4.31(2.37 to 7.81)**	**< 0.0001**
Serum sFlt-1 (pg/ml)						
Q1 (<441.3)	69(23.2)	78(26.4)	1.00		1.00	
Q2 (441.3-561.3)	62(20.9)	83(28.0)	0.84(0.53 to 1.34)	0.4827	0.88(0.51 to 1.40)	0.3016
Q3 (561.4-681.4)	40(13.5)	57(19.3)	0.79(0.47 to 1.33)	0.4305	0.80(0.48 to 1.35)	0.3580
Q4 (>681.5)	126(42.4)	78(26.4)	1.83(1.18 to 2.81)	0.0066	**1.84(1.15 to 2.83)**	**0.0013**
Serum 8-epiPGF2α (pg/ml)						
Q1 (<295.0)	64(21.5)	84(28.4)	1.00		1.00	
Q2(295.0-394.0)	64(21.5)	81(27.4)	1.04(0.65 to 1.64)	0.9066	1.13(0.58 to 1.66)	0.9801
Q3 (395.0-494.0)	60(20.2)	66(22.3)	1.19(0.74 to 1.92)	0.5427	1.16(0.70 to 1.97)	0.4911
Q4 (>495.0)	109(36.7)	65(21.9)	2.20(1.40 to 3.44)	0.0005	**2.23(1.41 to 3.46)**	**0.0001**
Serum 8-OHdG (ng/L)						
Q1(<61.40)	61(20.5)	87(29.4)	1.00		1.00	
Q2(61.40-71.40)	21(7.1)	24(8.1)	1.24(0.63 to 2.44)	0.6059	1.21(0.67 to 2.53)	0.5473
Q3(71.50-81.50)	51(17.2)	61(20.6)	1.19(0.73 to 1.96)	0.528	1.15(0.69 to 1.98)	0.6014
Q4(>81.60)	164(55.2)	124(41.9)	1.89(1.26 to 2.82)	0.0024	**1.90(1.28 to 2.83)**	**0.0018**
Urinary 8-OHdG (ng/mg Cr)						
Q1 (<59.95)	61(20.5)	87(29.4)	1.00		1.00	
Q2 (59.95-69.95)	21(7.1)	21(7.1)	1.43(0.72 to 2.83)	0.3779	1.42(0.71 to 2.85)	0.3506
Q3 (69.96-79.96)	52(17.5)	67(22.6)	1.11(0.68 to 1.80)	0.7097	1.18(0.64 to 1.81)	0.6937
Q4(>79.97)	163(54.9)	121(40.9)	1.92(1.28 to 2.87)	0.0016	**1.95(1.30 to 2.90)**	**0.0004**
Plasma TAC (µmol/L)						
Q1 (<178.9)	78(26.3)	70(23.6)	1.20(0.82 to 1.77)	0.3749	1.48(0.99 to 1.76)	0.0504
Q2 (178.9-198.9)	37(12.5)	28(9.5)	1.43(0.84 to 2.44)	0.2228	1.41(0.81 to 1.71)	0.3001
Q3 (199.9-219.9)	21(7.1)	24(8.1)	0.95(0.51 to 1.76)	0.8753	0.98(0.51 to 1.78)	0.7937
Q4 (>220.9)	161(54.2)	174(58.8)	1.00		1.00	

Values are presented as frequency (proportion); odds ratio (95% confidence intervals). 1.00 (reference category). Q: quartile. PIGF: placental growth factor; VEGF-A: Vascular endothelial growth factor-A; sEng: soluble endoglin; sFlt-1: soluble fms-like tyrosine kinase-1; 8-epiPGF2 α: 8-epiprostaglandin F2 alpha; 8-OHdG: 8-hydroxy-2-deoxyguanosine; TAC: Total antioxidant capacity. Covariate of adjusted model include maternal age, parity, gravidity, high BP, family history of hypertension, history of spontaneous abortion, pre-gestational BMI, high TG, AST, LDH, creatinine, and low Hb, low HDL, low Mg and low Ca.

Discussion

Using the subjective and non-invasive SHSQ-25, we stratified the health status of NTN-PW into SHS and optimal health status (OHS), compared the levels of OS and AGMs in these SHS and OHS groups and further tested the association between SHS and these biomarkers by performing a linear regression and multivariate logistic regression model. Overall, our novel findings indicated that the higher the SHS score, the more deranged the levels of BOS and AGMs, and this was further confirmed by a significant independent association between them after adjusting for confounding factors: maternal age, parity, gravidity, high BP, family history of hypertension, history of spontaneous abortion, pre-gestational BMI, high TG, AST, LDH, creatinine, and low Hb, HDL, Mg and Ca.

Particularly, NTN-PW with SHS had significantly increased OS biomarkers as depicted by increased levels of the pro-oxidants (8-OHdG, 8-epiPGF2α) and a fairly reduced antioxidant (TAC) **(Figure 1).** This was confirmed by a significant positive association between SHS and 8-OHdG and 8-epiPGF2α but a negative association with TAC, which means that SHS increases with increasing pro-oxidant activity and a reduced anti-oxidant system. This finding might be indicative of increased oxidative DNA damage, endogenous oxidative stress and a compromised anti-oxidant system [8]. Previous studies have also explained that an incomplete maternal vascular artery remodeling results in placental hypoxia/ischaemia, which eventually leads to OS [6,8]. Cardiovascular risk factors including dyslipidaemia and hypertension have also been linked to OS [33,34]. Hormonal imbalances are commonly associated with pregnancy and it is reported to contribute to increased phospholipid accumulation [12]. In the present study, cardiovascular indicators such as high triglyceride, low HDL-c and high BP were found to be independent risk factors for SHS (Table 3). The association was confirmed by positive correlation between the SHS-specific domain, ‘cardiovascular system’ and unbalanced BOS (Table 4). This finding is consistent

with a cross-sectional study conducted in a Chinese population which observed an association between SHS and ideal cardiovascular health metrics [26]. The observed increased OS among SHS individuals may further be explained by these cardiovascular risk factors associated with SHS. Hormonal imbalances are commonly associated with pregnancy and it is reported to contribute to increased phospholipid accumulation [12]. Increased lipids at sites where ROS are formed can result in endogenous lipid peroxidation [12]. Hence, the observed increased OS among SHS NTN-PW may possibly be due to ROS-induced lipid peroxidation [12]. In order to understand the strength of the association between OS and SHS, we adjusted for the significant haematobiochemical, clinical and obstetrics factors associated with SHS. Interestingly, the association between SHS and OS biomarkers still remained significant with slight variations in the odds ratios, indicating that the association is independent of confounding factors. Particularly as shown in **Table 5**, the fourth quartile for serum 8-epiPGF2α, serum 8-OHdG and urinary 8-OHdG showed a 2.23-fold, 1.90-fold and 1.95-fold increased adjusted odds of SHS compared to the first quartile levels. This finding makes SHS an independent risk factor for OS. We therefore hypothesize that SHS is associated with increased OS and poor maternal vascular remodeling compared to pregnant women with optimal health. This would inform clinicians the need for a combined antioxidant supplement and pro-angiogenic molecules. Evaluation of SHS criterion can create an opportunity for predictive, preventive and personalised medicine.

Increased OS during normotensive pregnancy, although not well understood may also be attributed to several mechanisms. For instance, maternal anaemia is reported as one major risk factor that contributes significantly to increase OS [3]. In the present study, the significant association between maternal low haemoglobin levels and SHS may be a contributing factor for the observed increased OS among SHS compared to optimal health NTN-PW; however, the relationship between OS and SHS was independent of anaemia.

Also, psychosocial stress which is a health complaint commonly among pregnant has been linked with OS [4]. A cross-sectional study among normal pregnant women reported that an increased OS may be associated with maternal psychosocial stress [4]. Another cross-sectional study among an adult Chinese population also found a significant relationship between SHS and psychosocial stress [28]. In the present study, 'fatigue', which is an index of psychosocial stress, and also one of the SHS domains was associated with increased OS and a compromised antioxidant system **(Table 4)**. The observed OS among SHS participants may be somewhat due to its association with the SHS domain, 'fatigue'. Increased OS has also been associated with dietary magnesium (Mg) and calcium (Ca) deficiencies [35,36], even though the associations are still debateable. In the present study, low Mg and Ca levels were significantly associated with SHS. Decreased Mg and Ca levels stimulate increased release of catecholamine, which can further increase the production and formation of ROS and result into OS [37]. In addition, Mg deficiency may induce ROS formation and lead to OS via activation of the renin-angiotensin-aldosterone system (RAAS) [37]. Mg deficiency is also reported as an early marker of endothelial dysfunction, which is also a complication of OS [35,36]. The relationship between SHS and increased OS observed in the present study may partly be due to the hypomagnesaemia and hypocalcaemia observed among SHS participants. Thus, early identification of SHS along with low Mg and Ca levels can inform clinicians of the pregnant women who stand the risk of increased OS, thus allowing the need to administer magnesium and calcium supplementations to prevent OS and possible adverse perinatal outcome.

Another major novel finding in the present study was the significantly reduced PlGF and VEGF-A levels and a correspondingly increased sFlt-1 and sEng among SHS compared to optimal health NTN-PW **(Figure 1)**. This finding signifies that SHS NTN-PW may have suffered an overexpression of anti-angiogenic growth mediators which has in turn interfered

with the pro-angiogenic function. The imbalance in AGMs observed in the present study was further confirmed by a significantly negative association of SHS with PIGF and VEGF-A, but a positive association with sFlt-1 and sEng **(Figure 2).** These imbalances could possibly be explained as a local placental ischaemia originating from incomplete maternal vascular remodeling which has increased systemic OS culminating in a shift in function in favour of sFlt-1 [7]. Increased OS is reported to stimulate the antagonistic activity of sFlt-1, which in turn neutralises the function of VEGF-A and PlGF [7,8]. The increased OS and unbalanced AGMs among SHS NTN-PW is a clear indication of a compromised immune health, as both factors play important role in the immune response of pregnancy. Our present study found an association between the SHS-specific domain, 'immune system', and increased OS and imbalance in pro-and anti-AGMs **(Table 4).**

Also, the reduced PIGF and VEGF-A concentration and increased anti-AGMs (sFlt-1 and sEng) observed among SHS NTN-PW in the present study can be linked to an event of endothelial dysfunction. While VEGF-A is an essential factor for regulating the endothelium, sEng may interfere with endothelial control by inhibiting the function of TGFβ1, which plays a central role in nitric oxide (NO) production and vasodilation [8]. The relationship between SHS and an imbalance in AGMs may be explained by the increased OS observed among SHS participants. A cross-sectional study found a significant association between SHS and endothelial dysfunction in an adult Russian population [24]. Endothelial dysfunction, although mostly associated with preeclamptic pregnancies can also be associated with uncomplicated pregnancies due to physiological adaptations [38]. The first quartile for VEGF-A and PIGF and the fourth quartile for sFlt-1 and sEng were independently associated with SHS. The first quartile for VEGF-A and PIGF were 5.35 and 2.79 times, and sFlt-1 and sEng were 1.84 and 4.31 times increased adjusted odds of SHS, respectively **(Table 5)**. This supports our findings that SHS is associated with an imbalance in AGMs in pregnancy and

563 thus, incorporating SHSQ-25 as a tool in early antenatal health screening can be used as a
564 risk stratification for abnormal maternal vascular remodeling and placental angiogenesis.
565 This can create an opportunity for clinicians to detect early and administer appropriate
566 medicinal intervention such as angiogenic molecules to SHS pregnant women to prevent
567 likely adverse pregnancy outcomes.

568 Previous studies have reported that an algorithm of markers explain and predict better
569 the physiological variation in a condition compared using the individual markers [8,39]. In
570 the present study, we created a novel combined OS/AGMs ratio: 8-epiPGF2/PlGF and 8-
571 OHdG/PIGF in addition to the previously known ratios: sFlt-1/PlGF and sEng/PIGF. There
572 were significantly increased levels of sFlt-1/PlGF, 8-epiPGF2/PlGF, 8-OHdG/PIGF and
573 sEng/PIGF ratios among SHS compared to OHS NTN-PW **(Figure 1).** Based on this finding,
574 we performed a linear regression model and found a significantly positive association
575 between these ratios and SHS. A higher percentage coefficient of variation in SHS was
576 explained by these combined markers compared to using the individual markers **(Figure 3)**.
577 Increased levels of these combined markers among SHS NTN-PW support our present study
578 findings that an imbalance in AGMs and increased OS are associated with SHS. Hence, we
579 hypothesize that these combined panel markers can be used as a potential diagnostic tool for
580 OS-induced abnormal placental angiogenesis and are likely to be useful generic markers of
581 adverse pregnancy outcomes. The observed association signifies that SHS, oxidative stress
582 and placental angiogenesis may exhibit a synergistic physiological function.

583 While the findings in the present study are novel, there were some limitations.
584 Firstly, because the present study is a cross-sectional hospital-based study, our results
585 cannot be generalised for the entire population. Nevertheless, this study is the baseline
586 of an ongoing prospective GHOACS. Aside from these limitations, there were some
587 strengths to highlight. This is the first cross-sectional study which sought to ascertain if

SHS is associated with increased OS and unbalanced AGMs among normotensive pregnant women in a Ghanaian. Another strength of the present study finding was that the association remained significant after adjusting for confounding factors, indicating that SHS is an independent risk factor of increased OS and unbalanced AGMs.

Conclusion

In summary, increase oxidative stress and imbalances in pro and-anti-angiogenic growth mediators are independently associated with SHS. This was supported by an association of OS and AGMs with the individual SHS-specific domains. SHSQ-25 evaluation, which is a subjective non-invasive assessment for SHS can be used to identify increased OS and poor maternal vascular remodeling and thus inform clinicians of the need for antioxidant supplementation. Evaluation of SHSQ-25 may be an effective and time-efficient tool that can augment other point-of-care testing especially in resource-limited facility in sub-Saharan African to improve poor health among normotensive pregnant women who suffer adverse health complaints without a diagnosable condition.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Data availability statement

Data set for this paper is part of a bigger data set from an ongoing Cohort study and is currently stored on internal storage systems of the corresponding author. We are able to provide data specific to this paper on request, once the purpose for the request fits into the ethics approval we received for the work. Request for the data set specific to this paper may be made through the corresponding author. Authors are still be working on the bigger data set to answer other questions and objectives of the bigger study so are unable to make it available to others as at now.

Authors Contribution

Conceptualization, E.O.A, P.R, D.C, E.A, C.A.T, A.T, Y.W, and W.W.; Methodology, E.O.A, C.A.T and A.T.; Formal Analysis, E.O.A, P.R, D.C, E.A, C.A.T, A.T, Y.W, and W.W.; Investigation, E.O.A, C.A.T and A.T.; Data Curation, EOA; Writing – Original Draft Preparation, E.O.A; Writing – Review & Editing, E.O.A, P.R, D.C, E.A, C.A.T, A.T, Y.W, and W.W.; Supervision, P.R, D.C, C.A.T and W.W; Project Administration, E.O.A, P.R,

D.C, C.A.T and W.W.; Funding Acquisition, Y.W and W.W.", All authors read and approved the final manuscript.

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