Household air pollution and angiogenic factors in pregnant Nigerian women: A randomized controlled ethanol cookstove intervention

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HIGHLIGHTS

- Effect of household air pollution (HAP) on placental growth markers was investigated
- Plasma placental growth factor (PlGF) and soluble fms-like tyrosine kinase 1 (sFlt-1) were measured in maternal and fetal cord blood samples
- Pregnant women who cooked with firewood/kerosene stove were recruited and randomized to ethanol or continued with their usual stoves
- Placenta from racially similar pregnant women who were presumed to be liquefied petroleum gas-users in Chicago was collected
- Maternal PlGF and cord blood sFlt-1 and PlGF in Nigerian women with varying HAP exposures were significantly higher than Chicago-based women

GRAPHICAL ABSTRACT
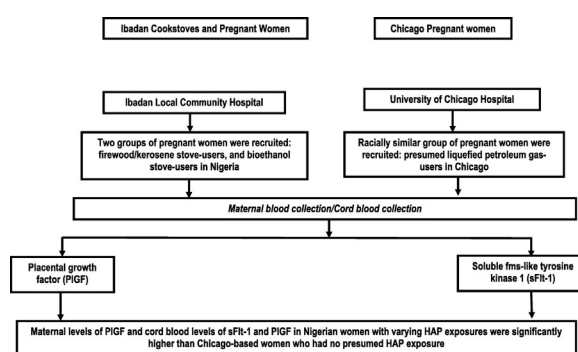
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ABSTRACT

Background: Maternal exposure to ambient air pollution affects placental growth markers.
Objectives: Investigate impact of household air pollution (HAP) on placental growth markers.
Methods: Two groups of pregnant women were identified: firewood/kerosene stove-users (A, n = 33) and bioethanol stove-users (B, n = 44) that participated in a randomized control trial in Ibadan, Nigeria. A third group of non-smoking and presumed liquefied petroleum gas-using Chicago women (C, n = 19) were included in this exploratory pilot to assess for possible differences between similar racial groups. Levels of placental growth factor (PlGF) and soluble fms-like tyrosine kinase 1 (sFlt-1) were measured in maternal and cord plasma using ELISA.

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Results: Maternal and cord blood sFlt-1 and PlGF did not differ significantly between women of groups A and B. Nevertheless, both groups differed significantly from the Chicago group in that group A women had lower maternal sFlt-1 (1372.50 vs. 3194.19) but higher PlGF (1607.87 vs. 442.80), and higher cord blood sFlt-1 (2925.02 vs. 107.53) and PlGF (223.68 vs. 6.92), all $p \leq 0.001$. Group B showed similar trends (all $p \leq 0.002$). Maternal PlGF levels were positively correlated to minutes of HAP exposure when $PM_{2.5}$ concentration was above 100 µg/m^3 in Nigerian women.
Conclusions: Maternal levels of PlGF and cord blood levels of sFlt-1 and PlGF in Nigerian women with varying HAP exposures were significantly higher than Chicago-based women who had no presumed HAP exposure. It suggests that in-utero exposure to HAP influenced levels of angiogenic factors involved in normal placentation and growth and could represent compensation for pollutants exposure to preserve fetal viability.

1. Introduction

Previous work has introduced the concept of early-life stress leading to later illness (Barker, 1990), in which prenatal exposure to environmental contaminants may lead to the development of early childhood diseases like asthma, wheezing, respiratory infections, altered immune defense and chronic diseases like diabetes and heart disease (Barker, 1990). The use of solid fuels such as biomass and kerosene for cooking and household energy by pregnant women exposes the developing fetuses to potentially harmful environmental pollutants (Lim et al., 2012; Lam et al., 2012). Biomass, which is used by nearly three billion people worldwide (Organization WH, 2014), is a major contributor to household air pollution (HAP) that is deleterious to health (Lim et al., 2012), and is the eighth leading risk factor for global disease burden, contributing to 2.9 million premature deaths in 2015 (Forouzanfar et al., 2016). Similarly, the use of kerosene for cooking by >500 million households worldwide, is associated with adverse health outcomes (Lam et al., 2012). Exposure to ambient air pollution and HAP from cooking with biomass and kerosene result in adverse pregnancy outcomes of the fetus (Ballester et al., 2010; Kannan et al., 2006; Morello-Frosch et al., 2010) and neonate (Scheers et al., 2011), stillbirth, fetal growth restriction, preterm birth, preeclampsia and gestational hypertension, small-for-gestational-age babies, low birth weight and neonatal death (Kannan et al., 2006; van den Hooven et al., 2011; Wu et al., 2009; Bonzini et al., 2010; Shah and Balkhair, 2011; Epstein et al., 2013; Aimakhu and Olayemi, 2003).

Maternal exposure to HAP has been hypothesized to affect placentation and placental function (Kannan et al., 2006; Dejmek et al., 1999), and may affect pregnancy outcomes by inducing oxidative stress and systemic inflammation (Brook et al., 2010), resulting in suboptimal placentation or placental inflammation (van den Hooven et al., 2012a) and subsequent development of fetal growth restriction and low birth weight (Kannan et al., 2006). The angiogenic growth factors vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) are important for placental development and angiogenesis, whereas soluble fms-like tyrosine kinase 1 (sFlt-1) binds to these proteins and inhibits their activity (Coolman et al., 2012). Placental angiogenic factors have been implicated in the pathogenesis of placental dysfunction in women whose pregnancies were complicated by intrauterine growth restriction, preeclampsia, and gestational hypertension (Smith et al., 2007).

Though there are few studies that have reported association of placental function and impairment with ambient air pollution (van den Hooven et al., 2012b; Latzin et al., 2011), to our knowledge, few published articles have investigated the relationship with HAP (Wylie et al., 2016). Based on their observations, van den Hooden et al. (van den Hooven et al., 2012b) concluded that ambient air pollution might influence markers of placental growth and function. Wylie et al. (Wylie et al., 2016) also observed an association of HAP exposure with placental histopathology. Both investigators suggested that more studies are needed to explore and confirm their findings (van den Hooven et al., 2012b; Wylie et al., 2016).

Hence, this pilot study was undertaken to investigate the underlying mechanisms behind maternal exposure to HAP during pregnancy which may alter the levels of angiogenic factors in maternal and cord blood samples from pregnant Nigerian women. The Nigerian samples were obtained from a subgroup of pregnant women who participated in a large, randomized control trial that investigated the impact of transitioning from firewood or kerosene to ethanol as cooking fuel on pregnancy outcomes. As a supplement to this study, we assessed for possible differences between the Nigerian women and a similar group of pregnant African American women in Chicago. Hence, we obtained maternal and cord blood samples from Chicago women who had uncomplicated delivery at the University of Chicago and were presumed to have no HAP exposure as they cooked with liquefied petroleum gas (LPG). The Chicago subjects (n = 19) were selected based on being African American and racial similarity with the Nigerian women, and had maternal and cord blood samples that were collected during normal vaginal delivery.

2. Methods

2.1. Study design, eligibility criteria and participant recruitment

A randomized control trial (RCT) was conducted in Ibadan, Nigeria between June 2013 and October 2015, which recruited healthy pregnant women who were <18 weeks gestational age. Participant recruitment has been detailed in previous publications (Olopade et al., 2017; Alexander et al., 2017a). Non-smoking women (n = 324) who cooked regularly with firewood/kerosene were enrolled and randomized to receive the CleanCook ethanol stove (FE: previous firewood user randomized to ethanol; KE: kerosene randomized to ethanol) or continue to cook with firewood (FF) or kerosene (KK). Randomization was done between 16 and 18 weeks of gestational age and the pregnant women were then followed to term. The average duration of time in the study was 156 days. Participants gave written informed consent at the time of recruitment. The Institutional Review Board of the University of Chicago and the University of Ibadan approved the study protocol, which was registered as NCT02394574 on ClinicalTrials.gov.

For the current ancillary study, Nigerian women (n = 77) from the parent trial for whom both maternal and cord blood samples were available are included. Chicago subjects (n = 19) were selected based on being African American and racial similarity with the Nigerian women and had maternal and cord blood samples that were collected at delivery. All women in the current study had uncomplicated vaginal delivery.

2.2. Placenta weight and ELISA measurement of PlGF and sFlt-1

Maternal blood samples from Nigerian women were collected at 34 weeks and before delivery from women in Chicago. Corresponding fetal cord blood samples were collected at delivery. The initial processing and storage of samples were performed at the Institute for Medical research and training in Ibadan, Nigeria. Through established MTA agreement, FedEx shipped the samples in dry ice to the University of

Chicago laboratories, where quality control was done on the samples as part of the laboratory routine standard operating procedure prior to storage. The final laboratory studies were all performed at the University of Chicago once all the samples were brought together. Angiogenic factors have been shown to be stable over a long period of time and prior studies have used measurement of these factors in samples stored for over 10 years (Levine et al., 2004). There were no technical issues related to shipment or storage of plasma samples. Concentrations of PlGF and sFlt-1 were measured in EDTA plasma samples by enzyme-linked immunosorbent assay (ELISA) following the manufacturer's (R&D Systems) protocol [Catalog# DPG00 PlGF (Human) ELISA kit; Catalog# DVR100B Human sFlt-1/VEGF receptor 1 ELISA Kit]. Absorbance for all assays was measured at 450 nm in an ELISA reader (Bio-tek Synergy™ HT Multi-Detection Microplate Reader, BioTek® Instruments, Inc., Vermont, USA).

2.3. Placenta weight and placental ratio

Placenta weight and birth weight for each subject was recorded during delivery. Placental ratio was calculated as (placenta weight / birth weight) × 100% (van den Hooven et al., 2012b).

2.4. Personal exposure monitoring of $PM_{2.5}$ for Nigerian women

Personal exposure levels to particulate matter with aerodynamic diameter < 2.5 μm ($PM_{2.5}$) was measured using RTI MicroPEM for three consecutive days in the Nigerian women because 72-h exposure had been shown to correlate with three month exposure (Alnes, 2011). The MicroPEM is a new monitor developed and evaluated for use in settings with high concentrations of $PM_{2.5}$. The MicroPEM had an internal accelerometer that was analyzed with the RTI software as a quality control check to ensure the monitor was worn. Each woman in the study carried the MicroPEM in a small, culturally appropriate bag placed near the breathing zone (Northcross et al., 2016). The parameters measured were: mean $PM_{2.5}$, minimum and maximum levels of $PM_{2.5}$ over 72 h, and the time in minutes spent by the women with $PM_{2.5}$ levels above 100 μg/m^3 were tabulated. These were measured as an a priori choice, as has been reported in our earlier publications (Olopade et al., 2017; Alexander et al., 2017b). For the women from Chicago, data on personal air pollution exposure information was not available.

2.5. Covariates

Information on birth weight, placenta weight, birth length, birth head circumference, and maternal blood pressure was collected during the study. Information from Chicago women was extracted from medical records while personal face-to-face interviews with a structured survey questionnaire were conducted in Nigerian women. Data collection included information on socio-demographics (age, education, habits, family, occupation of the participants, average family income, cooking hours per day, cooking-years, types of fuel used for cooking, home kitchen design, especially the presence or lack of windows), past medical history, dietary details, occupation of the spouse and environmental tobacco smoke (ETS). Questionnaires were presented in the local language (Yoruba) for Nigerian women who did not speak or understand English.

2.6. Blood pressure monitoring

Blood pressure (BP) was measured among participants after they had been seated for at least 10 min. As mentioned in our earlier paper (Alexander et al., 2017b), a trained nurse took the measurements using an automatic BP monitor (Microlife BP 3BM1-3, Switzerland) that measured systolic BP (SBP) and diastolic BP (DBP) in the supported left arm of the seated participant. The BP measurements taken at the time of blood collection were correlated with the levels of angiogenic factors. Hypertension was diagnosed following the Seventh Report of the Joint Committee on the Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC-7, 2003) and 2003 recommendation of the World Health Organization/International Society of Hypertension (Whitworth, 2003). Hypertension was confirmed when SBP rose to 140 mm Hg or more, or DBP elevated to 90 mm Hg or more on two separate occasions with an interval of 48 h, or being on regular anti-hypertensive therapy.

2.7. Statistical analysis

The primary endpoints of this study were concentrations of sFlt-1 and PlGF in the maternal and cord blood samples. Data is presented as mean ± standard deviations or median (quartile 1, quartile 3) for variables not normally distributed. The sample sizes of 33 and 44 in groups A and B, respectively, were found to be large enough to provide over 80% power at a 5% significance level to detect differences of 0.57 standard deviations for each (transformed) primary endpoint. Comparisons between groups were done by using unpaired t-tests or Mann–Whitney U tests as appropriate. Unadjusted and adjusted Pearson's correlation coefficients were generated in order to identify the relationship between two measurable parameters as continuous variables, and the result was expressed as Pearson's correlation coefficient (r) value. The results were statistically analyzed using SAS (SAS 9.3, SAS Institute Inc., Cary, NC) or SPSS statistical software (Statistical Package for Social Sciences for windows, release 10.0, SPSS Inc., Chicago, IL, USA). Statistical significance was assigned at $p < 0.05$.

3. Results

3.1. Sociodemographic characteristics of the participants

Women were divided into two groups: firewood/kerosene stove-users (group A, n = 33), and bioethanol stove-users (group B, n = 44) that participated in a randomized control trial (ClinicalTrials.gov NCT02394574) in Ibadan, Nigeria. Socio-demographic characteristics of the two groups of women are detailed in Table 1. The groups were well matched with respect to their age, parity and gestational age at delivery.

The Chicago women differed from the Nigerian women in body mass index (BMI) and gestational age at sample collection. While maternal blood samples were collected at 34 weeks from Nigerian women, they were collected from Chicago women at about 39 weeks ($p < 0.001$). The BMI for Chicago women ranged between 29.1 and 37.6 kg/m^2 compared with 20.2–30.0 kg/m^2 for Nigerian women ($p < 0.001$). These covariates were adjusted for while exploring any correlations or associations of the plasma angiogenic factors with outcomes like fetal growth measures when both Chicago and Nigerian groups were taken together.

3.2. Plasma levels of angiogenic factors

The data on the levels of maternal and cord blood concentrations of sFlt-1 and PlGF of the different groups are summarized and compared in Table 2. Though the concentration of maternal and cord blood PlGF and sFlt-1 levels varied in the samples collected from firewood/kerosene users (group A) and the ethanol-using group (Group B), no significant differences was observed. Cord blood PlGF levels among kerosene users was significantly higher compared with the firewood users (Table 2; p = 0.03). A similar observation was made in kerosene-using women who were randomized to use ethanol (KE) compared to those who were randomized to ethanol-using group from firewood (FE; Table 2). In the FE group, maternal sFlt-1 was positively correlated to maternal PlGF levels (r = 0.650, p = 0.002). Cord sFlt-1 was positively correlated to cord PlGF levels in the FE (r = 0.646, p = 0.002), FF (r = 0.622, p = 0.004) and KE (r = 0.516, p = 0.02) groups.

Table 1
Demographic characteristics of the parturients.

Variables	Group A (n = 33) Control		Group B (n = 44) Intervention		p-Value
	KK (n = 19)	FF (n = 14)	KE (n = 23)	FE (n = 21)	
Age, *years*	29 (26, 34)	25.5 (23, 30)	26 (24, 30)	26 (25, 33)	0.23
Non-smoker smoking status	19 (100)	14 (100)	23 (100)	21 (100)	–
Normal mode of delivery	19 (100)	14 (100)	23 (100)	21 (100)	–
Parity	1 (1, 1)	1 (1, 1)	1 (1, 1)	1 (1, 1)	0.09
Gestational age at sample collection, *weeks*	34.0 (34.0, 34.0)	34.0 (34.0, 34.0)	34.0 (34.0, 34.0)	34.0 (34.0, 34.0)	1.00
Height, *cm*	158.21 ± 5.6	155.79 ± 5.2	162.22 ± 5.9	157.67 ± 5.6	0.01
Weight, *kg*	65 (55, 75)	56 (50, 61)	60 (56, 67)	55 (49, 64)	0.21
Body mass index, *kg/m²*	25.6 (22.4, 30.0)	23.2 (21.6, 24.6	23.6 (21.1, 26.2)	22.9 (20.2, 25.2)	0.12
Highest systolic blood pressure, *mm Hg*	119 (113, 127)	117 (111, 120)	121 (114, 127)	118 (112, 121)	0.43
Highest diastolic blood pressure, *mm Hg*	79 (75, 85)	72 (68, 80)	77 (70, 81)	73 (70, 77)	0.11
Gestational age at delivery, *weeks*	40.0 (39.0, 40.0)	39.0 (38.0, 40.0)	39.0 (39.0, 41.0)	40.0 (39.0, 40.0)	0.69
Hemoglobin, *g/dl*	10.4 (9.8, 11.5)	10.0 (8.9, 11.0)	10.5 (9.8, 10.8)	10.6 (9.7, 11.0)	0.48
WBC, *×10³/μl*	5.7 (4.4, 7.4)	6.5 (5.4, 8.0)	6.6 (5.7, 7.7)	6.7 (5.8, 7.8)	0.59
Platelets, *×10³/μl*	175.2 ± 55.9	183.3 ± 49.5	209.9 ± 75.6	209.1 ± 57.7	0.21

Values are presented as either mean ± standard deviation or as median (lower quartile, upper quartile); KK: continued using kerosene in Nigeria; FF: continued using firewood in Nigeria; KE: previously used kerosene but randomized to use ethanol during study in Nigeria; FE: previously used firewood but randomized to use ethanol during study in Nigeria.

On comparing the Nigerian groups with the Chicago group, the maternal PlGF levels and cord PlGF and sFlt-1 levels were significantly higher and maternal sFlt-1 level was significantly lower among group A (all p = 0.001) and group B (p = 0.01) compared to the Chicago group (maternal: PlGF 442.8 pg/ml and sFlt-1 3194.2 pg/ml; cord: PlGF 6.92 pg/ml and sFlt-1107.5 pg/ml).

3.3. Outcome characteristics

The birth outcome characteristics among the study participants are shown in Table 3. There was no significant difference between groups A and B for the length of the newborn. Placental ratio between the two groups was not markedly different. The two groups were similar with regard to head circumference of the newborns (p > 0.05). The Nigeria and Chicago groups differed significantly in the birth length of

the newborns (p < 0.05). Babies born to the Chicago group were taller compared to A and B.

3.4. Angiogenic factors and fetal growth measures

In the Pearson's correlation analysis, maternal sFlt-1 was negatively correlated to birthweight in the FF (r = −0.75, p = 0.01) group. It reached near significance and was positively correlated to birthweight in the FE (r = 0.44, p = 0.05) group. Maternal sFlt-1 was positively correlated to head circumference of baby with an r of 0.30 (p = 0.01) for all groups taken together. In the KE group, maternal sFlt-1 was positively correlated to placenta weight (r = 0.62, p = 0.005). Since the women from Nigeria and Chicago differed significantly in their BMI and gestational age for sample collection, these factors were adjusted for in the Pearson's correlation analysis. Maternal PlGF was positively correlated

Table 2
Angiogenic profile comparing intervention and control patients in Nigeria.

Angiogenic biomarkers	Group A (n = 33) Control		p-Value	Group B (n = 44) Intervention		p-Value	p-Value	p-Value	p-Value
	KK	FF	KK vs FF	KE	FE	KE vs FE	KK vs KE	FF vs FE	A vs B
Maternal angiogenic profile									
sFlt1, *pg/mL*	1372.5 (830.7, 1876.5)	1686.3 (1257.8, 2427.8)	0.26	1579.2 (870.0, 2655.7)	1417.5 (908.3, 2016.4)	0.61	0.29	0.47	0.73
PlGF, *pg/mL*	1607.9 (1236.8, 1984.9)	1183.4 (672.5, 2356.8)	0.73	1229.9 (703.1, 1806.9)	1269.8 (879.4, 1685.2)	0.91	0.19	1.00	0.28
sFlt1/PlGF	1.0 (0.6, 1.3)	1.0 (0.8, 2.2)	0.42	1.8 (0.7, 2.9)	1.0 (0.7, 1.4)	0.38	0.23	0.49	0.67
Cord blood angiogenic profile									
sFlt1, *pg/mL*	2925.0 (425.1, 62,169.1)	784.4 (283.0, 3522.3)	0.21	1850.1 (201.8, 49,767.6)	1432.0 (501.7, 3987.7)	0.73	0.70	0.70	0.92
PlGF, *pg/mL*	223.7 (35.1, 435.7)	33.2 (14.1, 78.0)	0.03	118.4 (20.0, 390.6)	38.9 (12.0, 217.0)	0.05	0.60	0.89	0.58
sFlt1/PlGF	45.2 (8.3, 127.9)	32.4 (17.4, 109.0)	0.96	75.7 (8.7, 187.9)	83.9 (13.6, 171.6)	0.71	0.62	0.47	0.40

Values are presented as median (lower quartile, upper quartile); KK: continued using kerosene in Nigeria; FF: continued using firewood in Nigeria; KE: previously used kerosene but randomized to use ethanol during study in Nigeria; FE: previously used firewood but randomized to use ethanol during study in Nigeria.

Table 3
Comparison of birth outcome characteristics among the participants.

Parameters	Group A (N = 33)	Group B (N = 44)	Chicago group (N = 19)
Birth length (cm)	46 (32–74)	45.5 (35–52)	50.8 (34.5–57.2)[*,#,^]
Birth head circumference (cm)	34 (24–42)	35 (25–44)	33 (29–51)
Placental ratio (%)	18.8 (13.7–21.6)	19.2 (14.3–29.4)	19.8 (14.6–27.1)
Fetal weight (g)	3000 (2200–4000)	3200 (2000–4300)	3195 (2100–4180)
Placental weight (g)	700 (600–1000)	600 (500–1000)	625 (370–1050)

Values are presented as the median (range). p-Value as in Mann-Whitney U test; birth length was significant at $p < 0.001$ when Chicago group was compared with group A only [*], with group B only [#] and with groups A and B taken together [^]; Data missing for: placental ratio and placental weight (n = 2 for group A; n = 1 for group B; n = 1 for Chicago group). [*,#,^] = $p < 0.001$.

to birthweight (r = 0.248, p = 0.04) when all groups were taken together (groups A, B and C).

3.5. Exposure and angiogenic factors

There was a significant difference in the measured exposure parameters across the Nigerian groups: $p = 0.02$ for mean $PM_{2.5}$ over 72 h [median (lower quartile, upper quartile): 148.1 (48.2, 241.2) for FE; 67.6 (47.0, 102.9) for FF; 43.9 (34.5, 55.0) for KE; 39.1 (24.5, 75.3) for KK groups], $p = 0.01$ for maximum level of $PM_{2.5}$ over 72 h [median (lower quartile, upper quartile): 1516.9 (716.0, 3597.6) for FE; 5709.1 (3451.0, 6667.6) for FF; 1722.3 (566.8, 2337.8) for KE; 1665.5 (752.0, 3449.0) for KK groups], and $p = 0.01$ for the time in minutes spent by the women when $PM_{2.5}$ levels were above 100 µg/m³ [median (lower quartile, upper quartile): 53 (23, 1333) for FE; 152 (50, 533) for FF; 21 (12, 54) for KE; 74 (22, 188) for KK groups]. However, when Pearson's correlation test was done, only maternal PlGF was positively correlated to the duration of exposure (minutes) to HAP when the concentrations of $PM_{2.5}$ were above 100 µg/m³. The Pearson's correlation coefficient, r was 0.38 (p = 0.001). When the correlation was examined separately for each group, the duration of exposure to smoke when the concentration of $PM_{2.5}$ were above 100 µg/m³ was found to be positively correlated to maternal PlGF in the FE group (r = 0.456, p = 0.04), and to cord blood PlGF (r = 0.604, p = 0.01) in the KE group.

3.6. Blood pressure and angiogenic factors

All patients in this analysis were normotensive. As shown in Table 1, blood pressure was not significantly different between groups A and B (SBP: p = 0.43; DBP: p = 0.11). Correlation between SBP and DBP with maternal and cord blood PlGF and sFlt-1 were also non-significant (Table 4).

4. Discussion

This is the first study to explore and report that variation in maternal exposure to HAP does alter levels of markers of placental growth and function, as evidenced by higher levels of angiogenic factors in maternal and cord plasma compared to what have been cited in literature as normal levels (Sundrani et al., 2013) and comparing them to women in Chicago. Though preliminary in nature, this study is important because to date, no study has explored the association between angiogenic factors and personal HAP exposure. Furthermore, the underlying mechanisms by which air pollution exposure may induce adverse fetal health effects are poorly understood. The novelty of the current study is that it provides initial explanation for the poorly understood mechanism of air pollution on adverse fetal health. No significant difference was found between the firewood/kerosene users and ethanol users for any of the angiogenic factors. The levels were, however, higher in the firewood/kerosene group.

Under normal conditions, maternal PlGF levels remain low and sFlt-1 levels are slightly higher at delivery compared to the entire gestational period (Sundrani et al., 2013), while cord blood levels of these factors generally remain very low (Sundrani et al., 2013). In this study, we observed that maternal PlGF and cord blood levels of PlGF and sFlt-1 in the Nigerian cohort (groups A and B) were higher compared with the typical values described in Sundrani et al. (Sundrani et al., 2013). Though higher levels of sFlt-1 and lower levels of PlGF have been associated with several pregnancy complications (Smith et al., 2007; van den Hooven et al., 2012b), there are conflicting results (Jacobs et al., 2011; Widmer et al., 2007). In our study, maternal PlGF was positively correlated to birthweight. This shows that PlGF is beneficial to fetal development. This finding is consistent with previous observations that PlGF levels are positively associated with increases in infant birth weight (James-Todd et al., 2016). Maternal PlGF was also positively correlated to the duration of exposure to HAP when the concentration of $PM_{2.5}$ was above 100 µg/m³. PlGF is known to play a crucial role in modulating inflammation (De Falco, 2012) and has a major role in vasculogenesis and angiogenesis in human placenta (Vrachnis et al., 2013). Hence, we posit that PlGF levels may have been increased as compensation for the high levels of exposure to HAP.

The placental ratio, a marker of placental function, is indicative of the capacity to transport oxygen and nutrients and high placental ratio is also indicative of good placental function (Salafia et al., 2006). Comparatively higher placental ratio was observed among the ethanol-users. The ethanol group had lower HAP exposure as shown by the significant difference in measured $PM_{2.5}$ exposure parameters. Hence, we suggest that HAP exposure from use of firewood and kerosene during pregnancy may be deleterious for fetal development and growth.

van den Hooven et al. (2012b) found maternal PM_{10} exposure to be associated with higher fetal cord blood sFlt-1 and lower PlGF levels in fetal cord blood suggesting that air pollution may alter angiogenesis and impair placental function. Compared to normal levels cited in literature (Sundrani et al., 2013), we observed comparatively higher levels of angiogenic factors among pregnant Nigerian women who cooked with firewood and kerosene and were exposed to the highest concentrations of HAP. Similar to observations of van den Hooven et al. (2012b), we also did not find any significant differences in maternal levels of angiogenic factors PlGF and sFlt-1 between the Nigerian groups. There are a few notable differences between our study and van den Hooven et al. (2012b). In our study, fetal cord blood PlGF and sFlt-1 levels from Nigeria births were higher compared to what was observed by van den Hooven et al. who reported higher sFlt-1 and lower PlGF in fetal cord blood (van den Hooven et al., 2012b). This may be due to the difference in maternal gestational age when the blood samples were obtained (34-weeks in the Nigerian women) while van den

Table 4
Pearson correlation between actual blood pressure values and levels of angiogenic factors among the Nigerian women.

Parameters	Number of subjects	SBP (mm Hg) r (p value)	DBP (mm Hg) r (p value)
Maternal PlGF (pg/ml)	77	−0.1 (0.22)	−0.01 (0.42)
Maternal sFlt-1 (pg/ml)	77	−0.07 (0.52)	−0.07 (0.52)
Cord PlGF (pg/ml)	77	0.1 (0.40)	0.06 (0.62)
Cord sFlt-1 (pg/ml)	77	0.07 (0.57)	0.07 (0.54)

Results are expressed as Pearson's correlation coefficient, r and p-values within parentheses. SBP, systolic blood pressure; DBP, diastolic blood pressure.

Hooven et al. collected samples during the first- (median, 13.2 weeks; 95% range: 9.5–17.5) and second- (median, 20.4 weeks; 95% range: 18.5–23.5) trimesters of pregnancy. Also, racial and ethnic differences between the studies may explain some of the differences, as ethnicity has been shown to play a vital role in PlGF concentrations. PlGF levels have been shown to be substantially higher in sub-Saharan African population compared to Caucasians or Afro-Caribbeans (Browne et al., 2016). A population-based study involving subjects of white, Hispanic and black origin found that black participants had higher levels of placental growth factor in both cases and controls when compared to whites and Hispanics (Yang et al., 2016). Only one group has studied levels of sFlt-1 in an ethnically diverse group of Malaysian patients (Jeevaratnam et al., 2010). The sFlt-1 levels of the Nigerian women in our study were higher than those of Malaysian women. Extending our study to compare differences between women of different origins would be interesting (Fig. 1).

The small sample size in our exploratory analysis is an important limitation of our study. However, this innovative study related sFlt-1 and PlGF concentrations obtained from maternal and cord blood samples to HAP exposure levels and pregnancy outcome and made significant observations. As mentioned earlier, the sample sizes were found to be large enough to provide over 80% power at a 5% significance level to detect differences of 0.57 standard deviations for each (transformed) primary endpoint. Utmost care was taken regarding collection, storage and shipment of collected samples from Nigeria to Chicago.

In order to assess for possible differences between similar racial groups, we also compared the angiogenic factors in African-American women who delivered at the University of Chicago and were presumably LPG-users. Concentrations of maternal PlGF, cord blood PlGF and cord blood sFlt-1 were significantly higher among Nigerian firewood/kerosene users compared to their Chicago counterparts who were presumed not to be exposed to any type of HAP from cooking indoors. The maternal sFlt-1 levels were, however, higher among Chicago women. This is most likely related to advanced gestational age at the time of sample collection in the Chicago group (39 weeks in Chicago women vs. 34 weeks in the Nigerian women). Though no exposure data was available on the Chicago women, we presumed they had lower exposure to air pollution than the Nigerian group. The global interactive map of the World Health Organization (WHO) (WHO, 2016) shows that the annual mean ambient air pollution in Ibadan, Nigeria is nearly 6- to 7-times higher than that in Chicago, USA. The observed changes in the levels of angiogenic factors in this study suggest that HAP exposure from use of firewood and kerosene during pregnancy may be deleterious for fetal development and growth.

In summary, HAP exposure from burning of firewood and kerosene did not seem to have influenced the levels of angiogenic factors that are involved in normal placentation and growth. One reason for this might be that the Nigerian women in the intervention and control groups were exposed to higher background ambient air pollution, which might explain why the effect of the intervention was not as dramatic. However, when Nigerian women were compared to the Chicago women with no smoking history or known HAP exposure, the difference in the altered levels of angiogenic factors was more obvious. Though exploratory, it appears that HAP exposure plays a role in influencing the levels of these factors that are involved in normal placentation and growth. Exposure of pregnant women to air pollution has also been hypothesized to affect placentation and placental function (Kannan et al., 2006; Dejmek et al., 1999), but associations between HAP exposures and markers of placental growth and function has never been studied. Hence, our study provides a basis for expanded investigation into the underlying mechanisms that influence angiogenesis, placentation and placenta function during pregnancy and its impact on pregnancy outcomes. As previously described by Barker (Barker, 1990), in utero exposure to air pollution may result in later-life diseases. It is in the public's interest given that a large, vulnerable population is exposed to HAP, such that policies and measures should
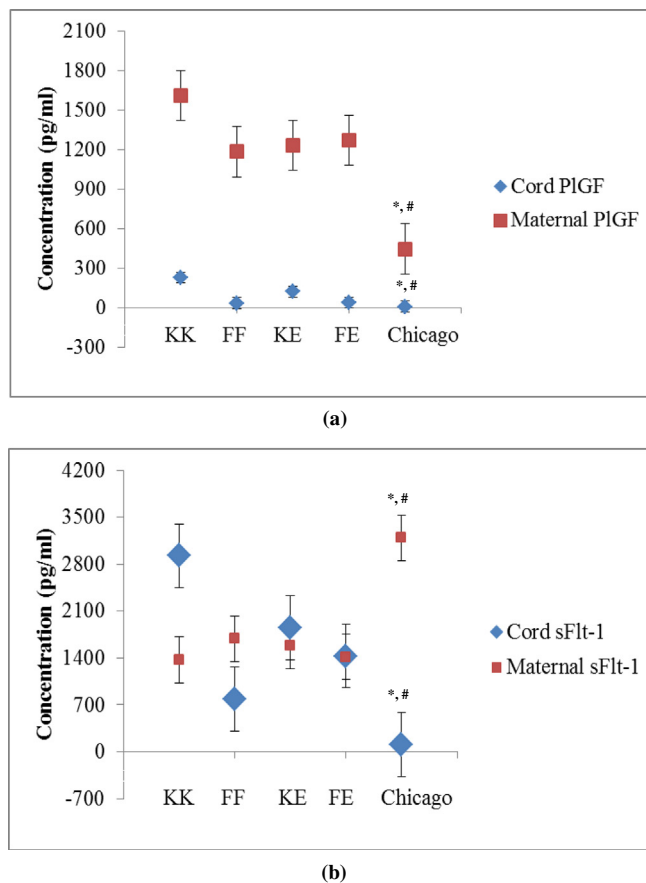

(a)

(b)

Fig. 1. Median (range) levels of placental growth factor (PlGF; a) and soluble fms-like tyrosine kinase-1 (sFlt-1; b) in the maternal and cord plasma of different fuel-using groups of the Nigerian cohort and the Chicago cohort. The maternal levels of sFlt-1 in the Chicago group was significantly higher and the levels of maternal PlGF and cord PlGF and sFlt-1 were significantly lower when compared with either KK or FF or KK + FF; p = 0.001*. Similar trend was observed when those levels in the Chicago group were compared with either KE or FE or KE + FE; p < 0.01#. KK = continued using kerosene in Nigeria; FF = continued using firewood in Nigeria; KE = previously used kerosene but randomized to use ethanol during study in Nigeria; FE = previously used firewood but randomized to use ethanol during study in Nigeria; UCM: Chicago group of women.

be implemented to stop usage of these harmful fuels and undertake measures to shift them to a relatively cleaner fuel. Larger studies on this matter are needed to confirm our findings.

Declaration of competing financial interests

We declare no competing financial interests.

with securing gift of ethanol stoves from the National Biotechnology Development Agency, Abuja, Nigeria and to Shell exploration for the provision of ethanol for the intervention group.

References

Aimakhu, C.O., Olayemi, O., 2003. Maternal haematocrit and pregnancy outcome in Nigerian women. West Afr. J. Med. 22 (1), 18–21.

Alexander, D.N.A., Wilson, N., Dutta, A., Pandya, R., Ibigbami, T., et al., 2017a. Randomized controlled ethanol cookstove intervention and blood pressure in pregnant Nigerian women. Am J Resp Crit Care Med http://dx.doi.org/10.1164/rccm.201606-1177OC (Epub ahead of print).

Alexander, D., Northcross, A., Wilson, N., Dutta, A., Pandya, R., Ibigbami, T., et al., 2017b. Randomized controlled ethanol cookstove intervention and blood pressure in pregnant Nigerian women. Am. J. Respir. Crit. Care Med.

Alnes, L., 2011. How Many Days of Measurement Adequately Predicts Long-term Indoor Concentration Means?

Ballester, F., Estarlich, M., Iniguez, C., Llop, S., Ramon, R., Esplugues, A., et al., 2010. Air pollution exposure during pregnancy and reduced birth size: a prospective birth cohort study in Valencia, Spain. Environ Health. 9, 6.

Barker, D.J., 1990. The fetal and infant origins of adult disease. BMJ (Clinical research ed) 301 (6761), 1111.

Bonzini, M., Carugno, M., Grillo, P., Mensi, C., Bertazzi, P.A., Pesatori, A.C., 2010. Impact of ambient air pollution on birth outcomes: systematic review of the current evidences. La Medicina del lavoro 101 (5), 341–363.

Brook, R.D., Rajagopalan, S., Pope, C.A., Brook, J.R., Bhatnagar, A., Diez-Roux, A.V., et al., 2010. Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association. Circulation 121.

Browne, J.L., Klipstein-Grobusch, K., Koster, M.P., Ramamoorthy, D., Antwi, E., Belmouden, I., et al., 2016. Pregnancy associated plasma protein-a and placental growth factor in a sub-Saharan African population: a nested cross-sectional study. PLoS One 11 (8), e0159592.

Coolman, M., Timmermans, S., de Groot, C.J., Russcher, H., Lindemans, J., Hofman, A., et al., 2012. Angiogenic and fibrinolytic factors in blood during the first half of pregnancy and adverse pregnancy outcomes. Obstet. Gynecol. 119 (6), 1190–1200.

De Falco, S., 2012. The discovery of placenta growth factor and its biological activity. Exp. Mol. Med. 44, 1–9.

Dejmek, J., Selevan, S.G., Benes, I., Solansky, I., Sram, R.J., 1999. Fetal growth and maternal exposure to particulate matter during pregnancy. Environ. Health Perspect. 107 (6), 475–480.

Epstein, M.B., Bates, M.N., Arora, N.K., Balakrishnan, K., Jack, D.W., Smith, K.R., 2013. Household fuels, low birth weight, and neonatal death in India: the separate impacts of biomass, kerosene, and coal. Int. J. Hyg. Environ. Health 216 (5), 523–532.

Forouzanfar, M.H., Afshin, A., Alexander, L.T., Anderson, H.R., Bhutta, Z.A., et al., 2016. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet 388 (10053), 1659–1724.

van den Hooven, E.H., de Kluizenaar, Y., Pierik, F.H., Hofman, A., van Ratingen, S.W., Zandveld, P.Y., et al., 2011. Air pollution, blood pressure, and the risk of hypertensive complications during pregnancy: the generation R study. Hypertension 57 (3), 406–412.

van den Hooven, E.H., de Kluizenaar, Y., Pierik, F.H., Hofman, A., van Ratingen, S.W., Zandveld, P.Y., et al., 2012a. Chronic air pollution exposure during pregnancy and maternal and fetal C-reactive protein levels: the Generation R Study. Environ. Health Perspect. 120 (5), 746–751.

van den Hooven, E.H., Pierik, F.H., de Kluizenaar, Y., Hofman, A., van Ratingen, S.W., Zandveld, P.Y., et al., 2012b. Air pollution exposure and markers of placental growth and function: the generation R study. Environ. Health Perspect. 120 (12), 1753–1759.

Jacobs, M., Nassar, N., Roberts, C.L., Hadfield, R., Morris, J.M., Ashton, A.W., 2011. Levels of soluble fms-like tyrosine kinase one in first trimester and outcomes of pregnancy: a systematic review. Reproductive biology and endocrinology 9, 77.

James-Todd, T., Cohen, A., Wenger, J., Brown, F., 2016. Time-specific placental growth factor (PlGF) across pregnancy and infant birth weight in women with preexisting diabetes. Hypertens Pregnancy 35 (3), 436–446.

Jeevaratnam, K., Nadarajah, V.D., Judson, J.P., Nalliah, S., Abdullah, M.F., 2010. Periodic assessment of plasma sFlt-1 and PlGF concentrations and its association with placental morphometry in gestational hypertension (GH) - a prospective follow-up study. BMC Pregnancy and Childbirth 10 (1), 58.

JNC-7, 2003. Seventh Report of the Joint Committee on the Prevention, Detection, Evaluation and Treatment of High Blood Pressure.

Kannan, S., Misra, D.P., Dvonch, J.T., Krishnakumar, A., 2006. Exposures to airborne particulate matter and adverse perinatal outcomes: a biologically plausible mechanistic framework for exploring potential effect modification by nutrition. Environ. Health Perspect. 114 (11), 1636–1642.

Lam, N.L., Smith, K.R., Gauthier, A., Bates, M.N., 2012. Kerosene: a review of household uses and their hazards in low- and middle-income countries. Journal of toxicology and environmental health Part B, Critical reviews 15 (6), 396–432.

Latzin, P., Frey, U., Armann, J., Kieninger, E., Fuchs, O., Roosli, M., et al., 2011. Exposure to moderate air pollution during late pregnancy and cord blood cytokine secretion in healthy neonates. PLoS One 6 (8), e23130.

Levine, R.J., Maynard, S.E., Qian, C., Lim, K.H., England, L.J., Yu, K.F., et al., 2004. Circulating angiogenic factors and the risk of preeclampsia. N. Engl. J. Med. 350 (7), 672–683.

Lim, S.S., Vos, T., Flaxman, A.D., Danaei, G., Shibuya, K., et al., 2012. A comparative risk assessment of burden of disease and injury attributable to 67 risk factor and risk factor clusters in 21 regions, 1990-2010: systematic analysis for the Global Burden of Disease Study 2010. Lancet 380, 2224–2260.

Morello-Frosch, R., Jesdale, B.M., Sadd, J.L., Pastor, M., 2010. Ambient air pollution exposure and full-term birth weight in California. Environ. Health 9, 44.

Northcross, A., Shupler, M., Alexander, D., Olamijulo, J., Ibigbami, T., Ana, G., et al., 2016. Sustained usage of bioethanol cookstoves shown in an urban Nigerian city via new SUMs algorithm. Energy for Sustainable Development 35, 35–40.

Olopade, C.O., Frank, E., Bartlett, E., Alexander, D., Dutta, A., Ibigbami, T., et al., 2017. Effect of a clean stove intervention on inflammatory biomarkers in pregnant women in Ibadan, Nigeria: a randomized controlled study. Environ. Int. 98, 181–190.

Organization WH, 2014. Fact Sheet No 292: Household Air Pollution and Health.

Salafia, C.M., Charles, A.K., Maas, E.M., 2006. Placenta and fetal growth restriction. Clin. Obstet. Gynecol. 49 (2), 236–256.

Scheers, H., Mwalili, S.M., Faes, C., Fierens, F., Nemery, B., Nawrot, T.S., 2011. Does air pollution trigger infant mortality in Western Europe? A case-crossover study. Environ. Health Perspect. 119.

Shah, P.S., Balkhair, T., 2011. Air pollution and birth outcomes: a systematic review. Environ. Int. 37 (2), 498–516.

Smith, G.C., Crossley, J.A., Aitken, D.A., Jenkins, N., Lyall, F., Cameron, A.D., et al., 2007. Circulating angiogenic factors in early pregnancy and the risk of preeclampsia, intrauterine growth restriction, spontaneous preterm birth, and stillbirth. Obstet. Gynecol. 109 (6), 1316–1324.

Sundrani, D., Khot, V., Pisal, H., Mehendale, S., Wagh, G., Joshi, A., et al., 2013. Gestation dependant changes in angiogenic factors and their associations with fetal growth measures in normotensive pregnancy. PLoS One 8 (1), e54153.

Vrachnis, N., Kalampokas, E., Sifakis, S., Vitoratos, N., Kalampokas, T., Botsis, D., et al., 2013. Placental growth factor (PlGF): a key to optimizing fetal growth. J. Matern. Fetal Neonatal Med. 26 (10), 995–1002.

Whitworth, J.A., 2003. 2003 World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension. J. Hypertens. 21 (11), 1983–1992.

WHO, 2016. Global Interactive Map.

Widmer, M., Villar, J., Benigni, A., Conde-Agudelo, A., Karumanchi, S.A., Lindheimer, M., 2007. Mapping the theories of preeclampsia and the role of angiogenic factors: a systematic review. Obstet. Gynecol. 109 (1), 168–180.

Wu, J., Ren, C., Delfino, R.J., Chung, J., Wilhelm, M., Ritz, B., 2009. Association between local traffic-generated air pollution and preeclampsia and preterm delivery in the south coast air basin of California. Environ. Health Perspect. 117 (11), 1773–1779.

Wylie, B.J., Matechi, E., Kishashu, Y., Fawzi, W., Premji, Z., Coull, B.A., et al., 2016. Placental pathology associated with household air pollution in a cohort of pregnant women from Dar es Salaam, Tanzania. Environ Health Perspect. 125, 134–140.

Yang, J., Pearl, M., DeLorenze, G.N., Romero, R., Dong, Z., Jelliffe-Pawlowski, L., et al., 2016. Racial-ethnic differences in midtrimester maternal serum levels of angiogenic and antiangiogenic factors. Am J Obstet Gynecol. 215 (3), 359.e1-9.