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Household air pollution and chronic hypoxia in the placenta of pregnant Nigerian women: A randomized controlled ethanol Cookstove intervention

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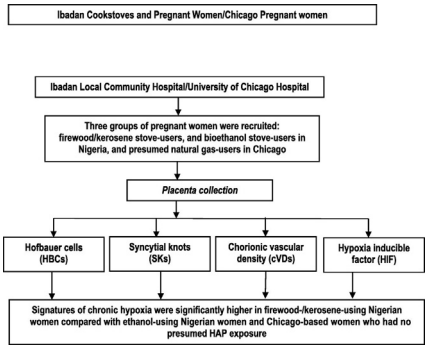
HIGHLIGHTS

- Effect of household air pollution (HAP) on signatures of chronic hypoxia in placenta was investigated
- Hofbauer cells, syncytial knots, chorionic vascular density and hypoxia-inducible factor were measured in placenta samples
- Pregnant firewood/kerosene and bioethanol stove-users in Nigeria, and presumed natural gas-users in Chicago were recruited
- Chronic hypoxia was significantly higher in firewood-/kerosene-users compared with ethanol-users and Chicago-based women

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Household air pollution (HAP) is associated with adverse pregnancy outcomes.
Objectives: Investigate impact of *in-utero* HAP exposure on placental development and chronic hypoxia.
Methods: Markers of chronic placental hypoxia [Hofbauer cells (HBC), syncytial knots (SK), chorionic vascular density (cVD) and hypoxia-inducible factor (HIF)] were stained by hematoxylin-eosin and/or immunohistochemically in placenta samples collected from firewood −/kerosene-users (A,$n = 16$), and ethanol-users (B,$n = 20$) that participated in a randomized controlled intervention trial in Ibadan, Nigeria. A third group of non-smoking and presumed natural gas-using Chicago women (C,$n = 12$) were included in this exploratory pilot to assess for

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Syncytial knots
Chorionic vascular density
Hypoxia inducible factor

possible differences in placenta histology between similar racial groups. All patients had uncomplicated pregnancies and delivered at term.
Results: HBC, SK and cVD were significantly increased among firewood-/kerosene-users compared to ethanol-users and natural gas-using Chicago women (HBC medians 5.5, 3.5, and 2.0, respectively; SK means 55.6, 41.8 and 30.1; cVD means 8.8, 6.2, and 5.2; all $p < 0.01$). HIF expression was significantly higher in Group A compared to B and C (all $p < 0.001$).
Conclusions: In-utero exposure to HAP is associated with pathologic changes and HIF expression consistent with chronic hypoxia in placenta of firewood/kerosene-users compared to ethanol-users with less HAP exposure and Chicago women with no presumed HAP exposure. Presence of chronic hypoxic signature in placenta of women exposed to HAP has implications for adverse pregnancy complications and future growth and development of the young children. Future larger studies need to focus on HAP exposure and placental disorders like preeclampsia and long-term health impact of *in-utero* exposure to HAP.

1. Introduction

Household air pollution (HAP) is the eighth leading risk factor for global disease burden, contributing to 2.9 million yearly premature deaths (Forouzanfar et al., 2016). Nearly 80% of the sub-Saharan population and about 90 million households in Nigeria use biomass as their primary fuel for cooking and energy needs (WHO, 2014), which can adversely impact their health (Olopade et al., 2017; Alexander et al., 2017). Exposure to air pollution has been linked to adverse pregnancy outcomes like stillbirth, preeclampsia, preterm birth, low birth weight, reduced fetal head circumference, miscarriage, and intra-uterine fetal growth retardation (IUGR) (Dadvand et al., 2013; Patelarou and Kelly, 2014; Wylie et al., 2014). Studies on the underlying mechanism that might link exposure to air pollution and pregnancy outcomes are few.

The placenta is the most specialized organ of pregnancy (Gude et al., 2004) and normal pregnancy requires sufficient placentation and functioning, impairment of which may result in maternal and fetal complications (Salafia et al., 2006; Ness and Sibai, 2006). Evidence suggests that *in utero* exposure to particulate matter (PM) with a diameter less than or equal to 2.5 μm ($PM_{2.5}$) affects normal fetal development in humans because of the suboptimal intrauterine environment (Ballester et al., 2010). *In utero* exposure to carbon monoxide (CO) caused severe fetal damage in a 22-year-old pregnant woman who was exposed to carbon monoxide poisoning at 30 weeks of gestation due to a fire in her home (Delomenie et al., 2015). Exposures to CO (Liu et al., 2003; Salam et al., 2005) and sulphur dioxide (Liu et al., 2003; Ha et al., 2001) are positively associated with fetal growth retardation. $PM_{2.5}$, CO, oxides of nitrogen (NOx) and sulphur (SOx), formaldehyde, benzene, benz(a)pyrene (Smith, 2000) and several endocrine disruptors (Wu et al., 2002; Wang et al., 2005) are hazardous pollutants that are components of biomass smoke, which lead to HAP. Perturbations *in utero*-placental exchange of nutrients and oxygen due to these exposures may program the fetus in such a way that the risk of developing cardiovascular disease and diabetes in adult life may increase (Ramadhani et al., 2006; Jansson and Powell, 2007). Placenta forms the interface between fetal and maternal circulation and plays a critical role in the regulation of fetal growth and development through controlled nutrient supply. Systemic inflammation and oxidative stress play important mechanistic roles in mediating the harmful effects of HAP (Dutta et al., 2012) and may play a significant role in the process of regulating fetal growth and development. The hypoxia inducible factor (HIF), a transcription factor that responds to changes in oxygen tension and hypoxia in the placenta, ultimately leading to proper placental development (Fryer and Simon, 2006), may also be a key player. Chronic hypoxia has been shown to impair fetal nutrition and growth by causing hypoperfusion of the placenta (Jakoubek et al., 2008). This may cause critical injury to vital organs (Hutter et al., 2010a) that is causally implicated in fetal growth restriction and preeclampsia (Zamudio et al., 2007) and slows fetal growth (Hutter et al., 2010b). Presence of Hofbauer cells, syncytial knots and chorionic vascular density are signatures of chronic hypoxic placental injury (Stanek, 2012; Stanek, 2013a).

Fundamental mechanisms by which PM exposure may impair fetal growth and development are poorly understood. Therefore, we sought to undertake this pilot study to investigate whether the presence of the signature markers of chronic placental hypoxia (Hofbauer cells, syncytial knots, chorionic vascular density) and expression of HIF, which is intricately involved in regulating and responding to hypoxia, were higher among the firewood/kerosene stove users who were exposed to higher levels of HAP compared to the ethanol stove-users who had reduced exposure to HAP in Ibadan, Nigeria. 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. Furthermore, as a supplement to this study, we investigated and compared the same endpoints among African American women from Chicago who were selected based on their racial similarity with the Nigerian women. The Chicago women typically cook with natural gas and were presumed to have no HAP exposure. Hence, this pilot study was undertaken to explore if *in-utero* HAP exposure affects placenta function or chronic hypoxia.

2. Methods

2.1. Study design, eligibility criteria and subject recruitment

This study is part of a larger randomized control trial (RCT; registered on ClinicalTrials.gov: NCT02394574) that was conducted in Ibadan, Nigeria from June 2013 to October 2015. Details of subject recruitment are provided in our earlier publications (Olopade et al., 2017; Alexander et al., 2017). Briefly, 324 apparently healthy women who were <18 weeks gestational age were enrolled in the parent study. Parturients who cooked regularly with firewood/kerosene and did not have high-risk pregnancy (multiple gestations, uncontrolled maternal hypertension, maternal age > 35 years for their first delivery, three or more prior miscarriages, or a prior cesarean section) were included. Pregnant women that met entry criteria were recruited and randomized to the ethanol (E) or control (C) arm. Those in the E group were given a CleanCook ethanol stove (CLEANCOOK Sweden AB) and an initial supply of fuel on a home visit between 16 and 18 weeks gestational age (GA). Written consent was obtained from participants at recruitment. The Institutional Review Boards (IRB) of the University of Ibadan and the University of Chicago approved the study protocol.

For the current ancillary study, placenta samples of 36 Nigerian women from the parent trial were studied. African American participants from Chicago ($N = 12$) were selected in order to reflect a similar racial demographic with the Nigerian women. Of the 36 samples from Nigerian women, 16 women used firewood/kerosene for cooking and 20 had been using ethanol CleanCook stoves at the time of sample collection.

Pregnancy and delivery characteristics from the patient's medical record were recorded. All women in the current study had uncomplicated vaginal deliveries. None of them suffered from any medical complications and were not receiving any medications. Gestational diabetes was not present in any participant.

2.2. Material

Utmost care was taken regarding collection, storage and shipment of collected samples from Nigeria to Chicago. Placental tissue that was collected during this randomized controlled trial was safely stored at the University of Ibadan in Nigeria. This bank has also been made available to investigators at the University of Chicago. The morphological analysis for hypoxic study was performed on formalin-fixed paraffin embedded sections of central parts of placentas. Overall quality of tissue was performed on slides stained with hematoxylin and eosin, and vimentin served as a control to monitor quality of tissue fixation and antigen quality. The specimens included formalin-fixed tissues embedded in paraffin, samples placed into RNA-later and frozen in optimal cutting temperature medium (OCT), fresh frozen tissue in OCT, blood from placenta and umbilical cord vessels as well as RNA and protein lysates. All samples were stored at −80 °C and thawed once before analysis.

2.3. Tissue preparation, staining and scoring for placental chronic hypoxia signature

2.3.1. Nigeria samples

All fresh placenta samples were triaged for biorepository purposes immediately after delivery according to the approved IRB protocol. Shortly after, placenta samples were immediately placed into 10% neutral buffered formalin. After fixation, placenta samples were examined grossly according to the standard protocol including trimmed weighting, measurements, evaluation and description of the condition of all parts of the placenta. Then the placenta was serially sectioned (1.0–1.5 cm sections) by cutting all the way through the disc, from maternal surface through the fetal surface to look for infarcts, or other lesions (Fig. 1). Routine sections included umbilical cord, membranes and through-and-through sections of placental tissue, including maternal and fetal surfaces were removed from the center and periphery. Only the central part of the placenta was used for our study. The sections of placental tissue were processed and paraffin-embedded according to routine surgical pathology practices in the department of pathology at the University of Ibadan by the chief technician who was trained at the University of Chicago. Samples were shipped on 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.

2.3.2. Chicago samples

All fresh placenta samples were collected as part of ongoing biorepository according to an approved IRB protocol. Placentas were processed within 30 min of delivery. Five, 18 mm full thickness core biopsies were done from predefined regions of placenta. Each section was divided into two halves, one placed in RNA-later for 24 h and then frozen and other half flash frozen in liquid nitrogen. All samples were stored at −80 °C. Placental samples were paraffin-embedded according to routine surgical pathology practices in the department of pathology at the University of Chicago.

The final laboratory studies were all performed at the University of Chicago once all the samples were brought together. Paraffin specimens were cut into 4 μm sections and mounted on positively charged slides at the University of Chicago. The slides were stained with Hematoxylin and Eosin (H&E) and were evaluated for morphological quality by two trained pathologists independently. Immunohistochemical staining with anti-vimentin primary antibody (V9) served as a control to monitor antigen quality of tissue (Battifora, 1991). Specimens with negative vimentin staining were excluded in the final analysis. Paraffin blocks with inadequate or questionable material were excluded. The agreement between readers was 98%. All the discrepancies were resolved after jointly reviewing the slides using a multi-headed microscope. Immunohistochemical assays were performed using a DAKO immunostainer with Vimentin, CD68 as monocyte-macrophage marker (Dako) antibody and antigen unmasking as detailed in previous publications (Forouzanfar et al., 2016; WHO, 2014; Olopade et al., 2017).

A. Hofbauer cells

Immunohistochemical (IHC) assays were performed with CD68 as monocyte-macrophage marker (DAKO) antibodies as detailed in earlier publications (Khramtsova et al., 2009; Khramtsova et al., 2015). Slides were incubated in 3% hydrogen peroxide for 10 min to block endogenous peroxidase activity, followed by incubation for 30 min in a protein-blocking solution (Background Sniper, Blocking Reagent from BioCare) to reduce nonspecific background. MACH 3 (BioCare) was used as a detection system. Slides were then treated with Betazoid DAB Chromogen, counterstained with hematoxylin, and cover-slipped. A histological study of H&E and IHC sections from placental tissues was performed for semi-quantitative determination of macrophage (Hofbauer cells) concentration per villus and their basic morphology as previously described by Grigoriadis et al. (Grigoriadis et al., 2013). At least 60 villi per case were examined. Two independent observers who were blinded to the subjects' Group Assignment determined the density of Hofbauer cells per villus. Hofbauer density was graded as focal (+; 1–3 Hofbauer cells/villus), intermediate (++; 3–6 Hofbauer cells/villus), or diffuse (+++; >6 Hofbauer cells/villus).

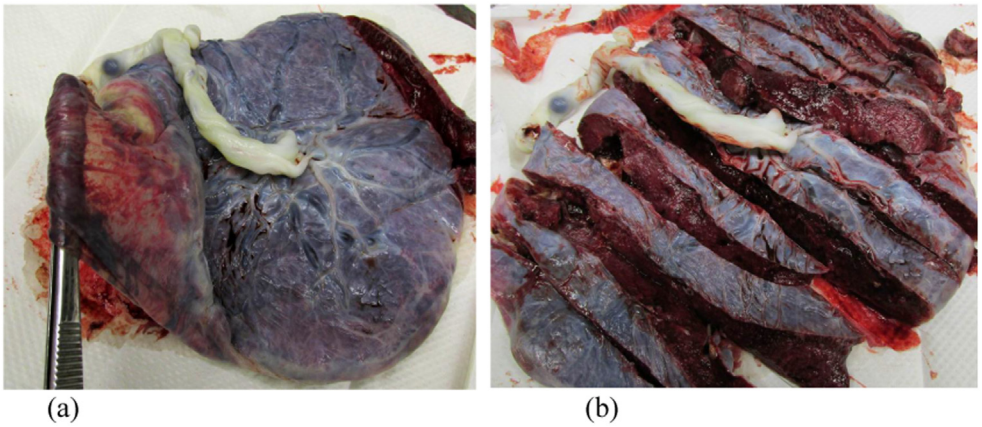

Fig. 1. Photographs showing the intact placenta (a) and full-thickness sections of the placental disc (b). The intact placenta (a) was serially sectioned (1.0–1.5 cm sections) by cutting all the way through the disc (b) from maternal surface through the fetal surface to look for infarcts, or other lesions. The section from central part of each placental disc was submitted for hypoxic study.

B. Syncytial knots and chorionic vascular density

The number of syncytial knots was counted using a 200× magnification in 15 fields of view for each experimental condition. A syncytial knot was defined as a multi-layered aggregation of at least 10 syncytiotrophoblast nuclei protruding from the villous surface not in contact with adjacent villi (Cantle et al., 1987). We counted the syncytial knots in which the nuclei were rich in heterochromatin, partially pyknotic, clustered, and the surrounding syncytioplasm showed marked degenerative changes. Data were normalized to give a measure of the number of syncytial knots per 1 mm^2 of villus. Villous vascularity was assessed manually using a hematoxylin-eosin stain. Chorangiosis, which is an important placental sign for perinatal morbidity like premature birth and low birth weight, was diagnosed if ten vessels per villus were visible under 10× objective, each with 10 or more vascular channels as laid down by Altshuler (Altshuler, 1984).

C. Hypoxia inducible factor (HIF) expression

Immunohistochemical (IHC) staining for studying HIF expression was done using anti-HIF-1α (Abcam, ab51608) with a dilution of 1/100. The percentages of placenta cells with nuclear HIF-1α expression and percentages of placenta cells with cytoplasmic HIF-1α expression were assessed separately in all scored fields. Using these scores, the overall percentage of cells with nuclear and cytoplasmic HIF-1α expression were calculated and considered as: 0–25% low, 25–75% moderate, and 75–100% high.

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, which has been successfully used in settings with high concentrations of $PM_{2.5}$ for three consecutive days in the Nigerian women (Dutta et al., 2017). 72-hour exposure was used because earlier studies had indicated that such exposure duration correlates with a three month exposure (Alnes, 2011). The MicroPEM has 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, 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 $\mu g/m^3$ 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., 2017). For the women from Chicago, data on personal air pollution exposure information was not available.

2.5. Statistical analysis

Given the exploratory nature of this study, a convenience sample of individuals from the parent study was used. No formal power calculation was performed. The results were statistically analyzed using SPSS statistical software (Statistical Package for Social Sciences for windows, release 10.0, SPSS Inc., Chicago, IL, USA) or SAS 9.3 (SAS Institute Inc., Cary, NC). Baseline characteristics, number of Hofbauer cells, syncytial knots and chorionic vascular density are reported as mean ± standard deviation, median (interquartile range [IQR]) or n (%) depending on the type and distribution. Normality was assessed with the Shapiro Wilk test. Differences in continuous baseline characteristics (*e.g.* age, height) were assessed with the use of the Kruskal Wallis test or analysis of variance (ANOVA), depending on the distribution of the data. Differences in categorical variables – including cytoplasmic and nuclear HIF expression – were assessed with a chi-square test. When looking only at two groups differences were assessed with an unpaired *t*-test or Wilcoxon Mann-Whitney test, as appropriate. In order to assess $PM_{2.5}$ levels, 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. Statistical significance was assigned for two-sided *p*-values < 0.05.

3. Results

3.1. Socio-demographic characteristics of the participants

The participating Nigerian women were divided into two groups: firewood/kerosene stove-users (Group A, $n = 16$), and bioethanol stove-users (Group B, $n = 20$). Socio-demographic characteristics of the two groups of women are shown in Table 1. The women who belonged to these groups had no differences with respect to their age, parity, gestational age at delivery, height, and systolic and diastolic blood pressure at delivery.

Women in Chicago were used an additional control. The Chicago women had a higher body mass index (BMI) at enrollment. The BMI interquartile range for Chicago women was between 29.0 and 33.0 kg/m^2 with a median of 30.4 kg/m^2 compared with 20.3 to 26.3 kg/m^2 for Nigerian women ($p = 0.001$). This covariate was adjusted for while exploring any correlations or associations of the placental signatures when both Chicago and Nigerian groups were taken together.

3.2. Comparison of placental pathology between three groups

The number of Hofbauer cells was higher in Group A (median 5.5; IQR: 4.0, 6.0) compared to Group B (3.5; IQR: 3.0, 4.0; $p = 0.01$), as depicted in Fig. 2a. A similar trend in means was observed for chorionic vascular density (Fig. 2a; $p < 0.0001$ for both comparisons to Group A) and syncytial knots (Fig. 2b; $p < 0.0001$ for all comparisons). The means ± standard deviations for chorionic vascular density were 8.8 ± 1.4 for Group A and 6.2 ± 1.6 for Group B, while for syncytial knots, the means were 55.6 ± 7.3 and 41.8 ± 6.9, respectively.

When comparing the Nigerian Group A with the Chicago group, the number of Hofbauer cells was significantly lower in the Chicago group (median 2.0; IQR: 1.0, 2.0; $p = 0.0001$). Similarly, both syncytial knots (30.1 ± 6.8; $p < 0.0001$) and chorionic vascular density (5.2 ± 1.5; $p < 0.0001$) were found to be significantly lower in the Chicago placenta samples compared to Nigerian placenta Group A samples. The number of Hofbauer cells in Group B were significantly higher compared to the Chicago group ($p = 0.001$) but the difference in chorionic vascular density between these two groups was not significant ($p = 0.08$). The images in Figs. 3, 4 and 5 show the Hofbauer cells, chorionic vascular density and syncytial knots, respectively, observed in the Nigerian groups A and B and the Chicago group.

3.3. Expression of hypoxia inducible factor (HIF)

HIF-1α was predominantly expressed in syncytiotrophoblast cells and trophoblastic villus cells. Cytoplasmic staining of HIF-1α was detected in both groups, with an increase in both nuclear and cytoplasmic HIF-1α levels in Group A. No difference was observed between Groups B and C for either nuclear or cytoplasmic HIF-1α levels (*p*-values 0.67 and 0.82, respectively). Table 2 shows the percentage of sections exhibiting low, moderate and high HIF expression while Fig. 6 depicts the expression in images.

3.4. Exposure to particulate air pollution in Nigerian women

The women using firewood/kerosene in Nigeria were exposed to higher levels of particulate air pollution compared to the women who had transitioned to ethanol-burning improved cookstoves, as has been reported in our earlier publications (Olopade et al., 2017; Alexander et al., 2017). Visibly the markers of chronic hypoxia were expressed significantly higher among the firewood/kerosene users compared to the

Table 1
Comparison of demographic characteristics of the participants.

Parameters	Group A $N = 16$	Group B $N = 20$	Group C $N = 12$	*P* value
Age, years	24.13 ± 5.03	25.85 ± 4.63	23.50 ± 3.15	0.30
Gestational age at delivery, weeks	40.0 (38.5, 40.0)	39.0 (30.0, 40.0)	39.6 (35.9, 40.3)	0.98
Parity	1 (1,1)	1 (1,2)	1 (1,2)	0.31
Height, cm	155.0 (153.0, 158.5)	161.0 (156.0, 164.5)	157.5 (154.9, 165.1)	0.08
Weight, kg	55.5 (49.0, 64.5)	58.5 (54.0, 65.5)	76.3 (72.1, 86.7)	0.001
Body mass index, kg/m^2	23.7 (19.9, 26.4)	22.5 (20.5, 25.5)	30.4 (29.0, 33.0)	0.0003
Systolic blood pressure, mm Hg	114.6 ± 13.3	113.2 ± 8.9	114.9 ± 10.9	0.89
Diastolic blood pressure, mm Hg	72.4 ± 12.5	70.0 ± 5.8	65.9 ± 11.1	0.23

Values are presented as mean ± standard deviation or median (quartile 1, quartile 3) depending on the distribution.

women randomized to the ethanol stove-group. However, no significant correlation or association was found between $PM_{2.5}$ levels and the signatures of chronic hypoxia (Hofbauer cells, syncytial knots, chorionic vascular density and HIF expression).

4. Discussion

In this pilot study, we assessed the placental pathology with regards to the presence and expression of markers of a chronic hypoxic signature and observed that relative to the ethanol-using women, there were significant changes, consistent with chronic hypoxia in the placenta of women exposed to HAP from burning of kerosene and firewood. These observations provide a plausible mechanism for adverse pregnancy outcomes related to *in utero* exposure to HAP. Specifically, we observed a significantly higher number of Hofbauer cells, syncytial knots and chorionic vascular density, and a higher expression of HIF1α in the placentas of firewood/kerosene-using Nigeria women who were exposed to higher levels of HAP compared to the women who used the relatively cleaner ethanol in the improved CLEANCOOK cookstoves. The placental pathology conditions of the Nigerian ethanol users with less $PM_{2.5}$ exposures were comparable to the presumed natural gas users from Chicago who were presumed not to be exposed to HAP. Though Wylie and colleagues (Wylie et al., 2017) have reported on placental histopathological lesions in relation to HAP exposure, ours is the first study to investigate the relationship between *in utero* HAP exposure and hypoxic changes in the placenta.

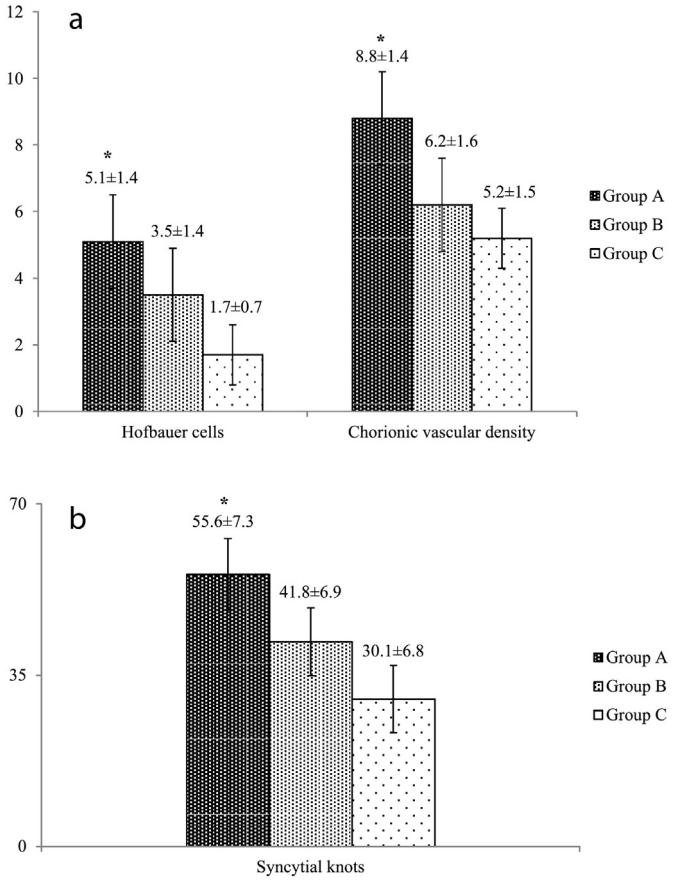

Fig. 2. a. Histograms (mean ± standard deviation) showing the number of Hofbauer cells and chorionic vascular density in the placenta from firewood/kerosene users (Group A), ethanol users (Group B) and the Chicago women (Group C). *, the number of Hofbauer cells and chorionic vascular density were significantly higher in the Group A compared to groups B and C. b. Histograms (mean ± standard deviation) showing the number of syncytial knots in the placenta from firewood/kerosene users (Group A), ethanol users (Group B) and the Chicago women (Group C). *, the number of syncytial knots was significantly higher in the Group A compared to groups B and C.

HAP causes about 2.9 million premature deaths every year (Forouzanfar et al., 2016) from pneumonias, non-communicable diseases (NCDs) including stroke, ischemic heart disease, chronic obstructive pulmonary disease and lung cancer (Smith et al., 2014). Nigeria is among 15 countries that account for most of the premature mortality linked to air pollution with 89 deaths per 100,000 (Lelieveld et al., 2015). *In utero* hypoxic conditions causes perinatal morbidity and mortality (Stanek, 2013b) and this might be triggered by HAP. No exploratory study on this issue has been done to date however. Some of the important factors in the hypoxic pathway are Hofbauer cells. They are placental macrophages (Tang et al., 2011) that synthesize proteins to stimulate villous proliferation in response to hypoxia (Anteby et al., 2005) and play an important role in placental development (Anteby et al., 2005). They are present in placental villi during pregnancy but reduce in numbers progressively (Grigoriadis et al., 2013) and are generally absent or scanty at term (Ingman et al., 2010). The presence of Hofbauer cells towards the end of pregnancy is unwelcomed as the presence of high numbers of Hofbauer cells has been associated with pregnancy complications like pre-eclampsia, intrauterine growth retardation (Grigoriadis et al., 2013), and histological chorioamnionitis (HCA) (Toti et al., 2011). Hence, our study shows that shifting to a cleaner fuel like ethanol is associated with fewer numbers or an absence of Hofbauer cells towards term. This suggests that reductions in HAP exposure may result in fewer pregnancy complications. There is a need to undertake future larger studies among women with abnormal pregnancy outcomes to confirm this hypothesis.

Syncytial knots, which are aggregates of nuclei in the syncytiotrophoblast, are often widely referred to as Tenney-Parker changes (Tenney Jr and Parker Jr, 1940). Syncytial knots have been linked to hypoxia and pregnancy complications like preeclampsia and IUGR (Heazell et al., 2007; Tomas et al., 2011). These syncytial knots or Tenney-Parker changes are generally associated with placental

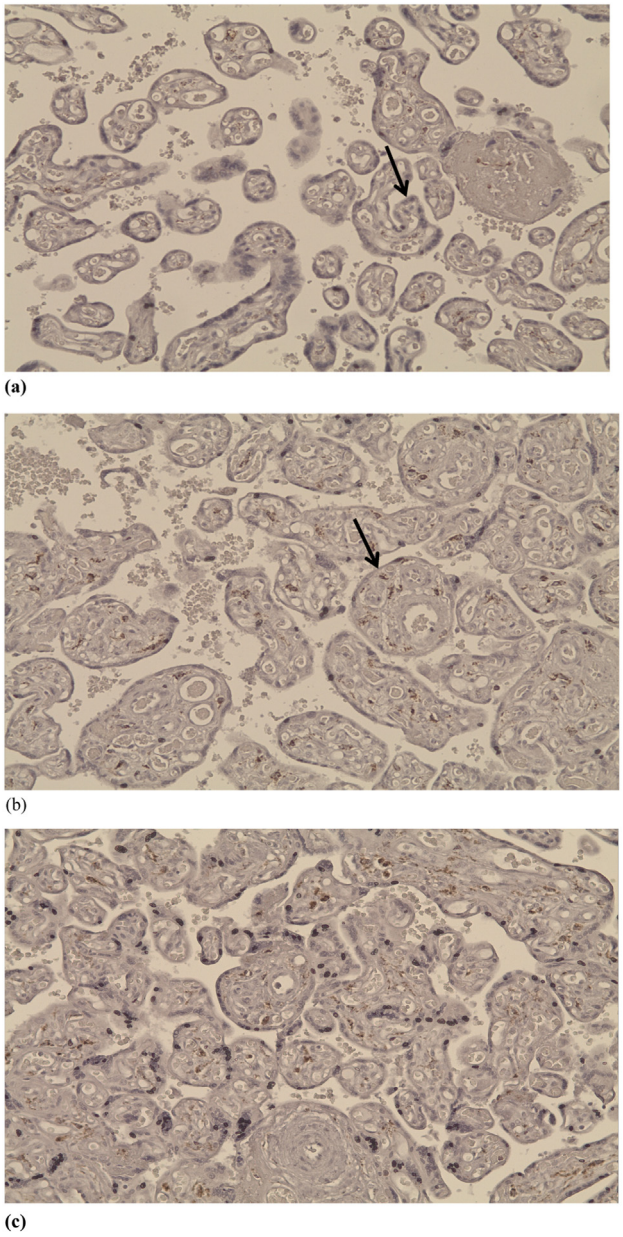

Fig. 3. Immunohistochemical staining of formalin-fixed and paraffin-embedded (FFPE) sections of term placenta with monoclonal antibody (mAb) CD68 against Hofbauer cells (HBCs) showing focal (a), intermediate (b) and diffuse (c); original magnification × 200. Arrow shows HBC. Sections exhibiting diffuse HBCs were noted mostly in the placenta of firewood/kerosene users, intermediate in the ethanol users and focal in the Chicago women.

pathology and is an indication of inadequate internal perfusion (impairment of nutrient and gas exchange) and oxidative damage (Fogarty et al., 2013). Increases in the number of syncytial knots may result from exposure to oxidative stress (Heazell et al., 2007), which has always been associated with HAP (Dutta et al., 2012). In this study, we observed more syncytial knots in the placenta of firewood/kerosene users (Group A) compared with the ethanol users (Group B). We postulate that oxidative stress resulting from HAP exposure may have played an important role in the development of syncytial knots, and this, in turn, might predispose women using firewood and kerosene to adverse pregnancy outcomes. Future studies on women with abnormal pregnancy outcomes could confirm this possibility.

Efficiency of placental function is affected by altered chorionic vascular structure and density (Yampolsky et al., 2009). Both low and high chorionic vascular density are undesirable and are associated with

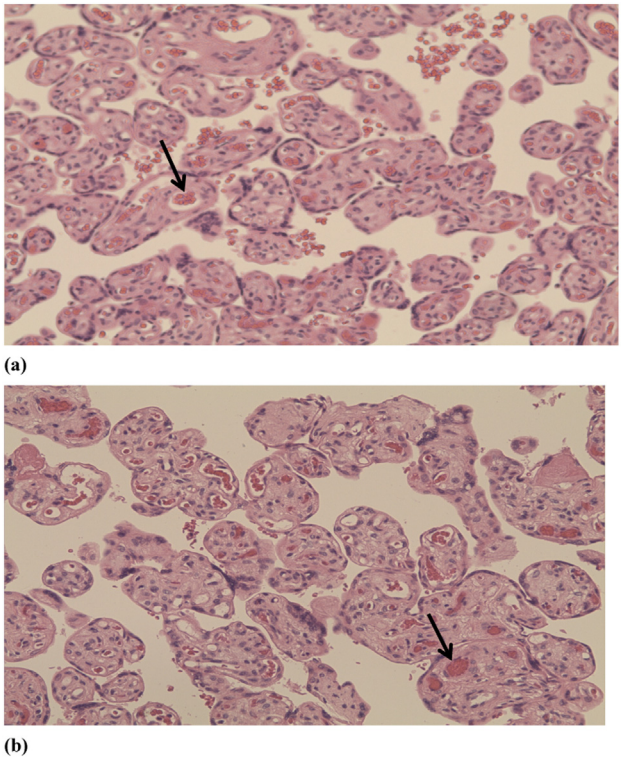

Fig. 4. Hematoxylin and eosin stained section of formalin-fixed and paraffin-embedded (FFPE) sections of term placenta showing high (a) and low (b) chorionic vascular density; original magnification × 200. Arrow shows chorionic vasculature. High chorionic vascular density was noted among placenta of firewood/kerosene users, moderate intensity in ethanol users while placenta from Chicago women showed low-density changes.

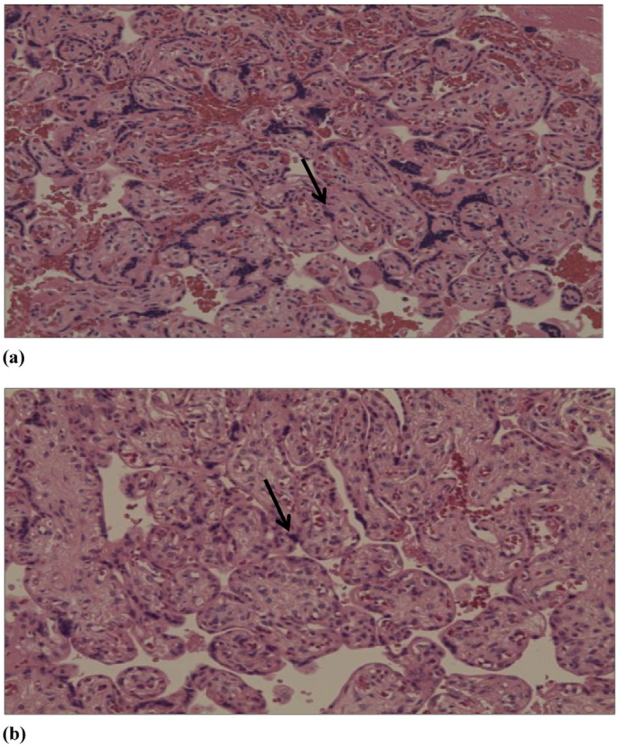

Fig. 5. Hematoxylin and eosin stained section of formalin-fixed and paraffin-embedded (FFPE) sections of term placenta showing high (a) and low (b) density of syncytial knots; original magnification × 200. Arrow shows syncytial knot. High numbers of syncytial knots were present in the firewood/kerosene users, moderate numbers in the ethanol users and low numbers were present in the placenta taken form the Chicago women.

Table 2
HIF expression in different groups.

	Group A N = 16	Group B N = 20	Group C N = 12	P value A *vs.* B	P value B *vs.* C	P value A *vs.* C
Nuclear HIF expression						
High	9 (56.25%)	4 (20.00%)	1 (8.33%)	0.01	0.67	0.001
Moderate	6 (37.50%)	5 (25.00%)	2 (16.67%)			
Low	1 (6.25%)	11 (55.00%)	9 (75.00%)			
Cytoplasmic HIF expression						
High	7 (43.75%)	2 (10.00%)	0 (0.0%)	0.001	0.82	0.0003
Moderate	7 (43.75%)	3 (15.00%)	2 (16.67%)			
Low	2 (12.50%)	15 (75.00%)	10 (83.33%)			

Results are expressed as numbers of individuals (percentage); HIF: hypoxia-inducible factor.

placental hypoxic conditions (26). Embryonic death has been associated with impaired chorionic vascular density (Meegdes et al., 1988). In our study, the number of chorionic vascular density were much higher in the placenta of firewood/kerosene users (Group A) compared with the ethanol users (Group B).

HIF-1α is an important factor required for proper placentation (Adelman et al., 2000) and plays a key role in maintaining optimum developmental conditions during periods of normal oxygen tension and hypoxia in the placenta (Tal, 2012) and works through all stages of placental development (Patel et al., 2010). This study shows that HIF-1α expression was significantly higher in the placenta of the firewood/kerosene users with higher exposure to HAP and lower in the placenta of ethanol-users and presumed natural gas users in Chicago women. We know that exposure to high levels of HAP results in increased oxidative stress (Dutta et al., 2012) and HIF is an important regulator of oxygen homeostasis that increases delivery of oxygen and nutrients to tissues with inadequate oxygen supply (Park et al., 2010). Hence, we suspect that increased HIF expression may be associated with higher HAP exposure in an effort to adapt and maintain homeostasis that is a prerequisite for normal developmental process. The elevated HIF expression may promote angiogenesis (Shimoda and Semenza, 2011), towards ensuring adequate tissue oxygenation to oxygen-starved cells and thus, help in minimizing or mitigating hypoxia-related adverse fetal outcomes. HIF has been shown to play a protective role in the pathophysiology of myocardial ischemia, limb ischemia due to peripheral vascular disease, wound healing, pressure-overload heart failure (Semenza, 2014) and intestinal graft *versus* host disease cases (Feinman et al., 2014) and we assume that it might play a similar role in the placenta too. This might be one of the reasons that in spite of HIF expression being very high among the firewood/kerosene users, we did not find any obvious adverse pregnancy outcomes. We had no access to placenta from women who had adverse pregnancy outcomes such as preeclampsia and intrauterine growth restriction. It is unclear if high HIF expression is always beneficial or is a protective response to HAP exposure during pregnancy.

In order to assess for possible differences between similar racial groups, we also compared the placental physiology in African-American women who delivered at the University of Chicago and were presumably natural gas users. The number of Hofbauer cells, syncytial knots and chorionic vascular density were significantly lower in Chicago women compared to the Nigerian group. HIF expression was also lower in the Chicago group. We were unable to determine personal exposure monitoring for the Chicago women, but we presumed they had lower exposure to air pollution than the Nigerian Group based on data from the global interactive map of the World Health Organization (WHO) (WHO, 2016), which showed that the annual mean ambient air pollution in Ibadan, Nigeria is nearly six to seven-times higher than that in Chicago, USA. Furthermore, we were not able to measures other potential sources of confounding between groups, such as secondhand smoke, which could contribute to the results we observed. Despite this, the observed differences in the numbers and expression of the signatures of chronic hypoxia in this study suggest that HAP exposure from use of firewood and kerosene during pregnancy may be deleterious for fetal development and growth.

An important limitation of our current study is the relatively small number of participants and that the analysis included only pregnant women who had normal deliveries and in whom placenta was available. However, this innovative pilot study related placental pathological findings to difference in HAP exposure levels and made significant observations, which could pave the way for larger studies that could estimate the role of other members of the HIF family and downstream HIF targets and correlate birth outcomes with HIF levels.

Placental dysfunction has been implicated in the development of a variety of pregnancy complications and also in impaired perinatal growth in children into adulthood (Barker, 2007). Therefore, we consider our observations very important as millions of pregnant women are exposed to HAP from burning biomass fuels and kerosene and that *in-utero* exposure to hypoxia could affect fetal growth and development and eventually the cognitive development of the growing child. Given

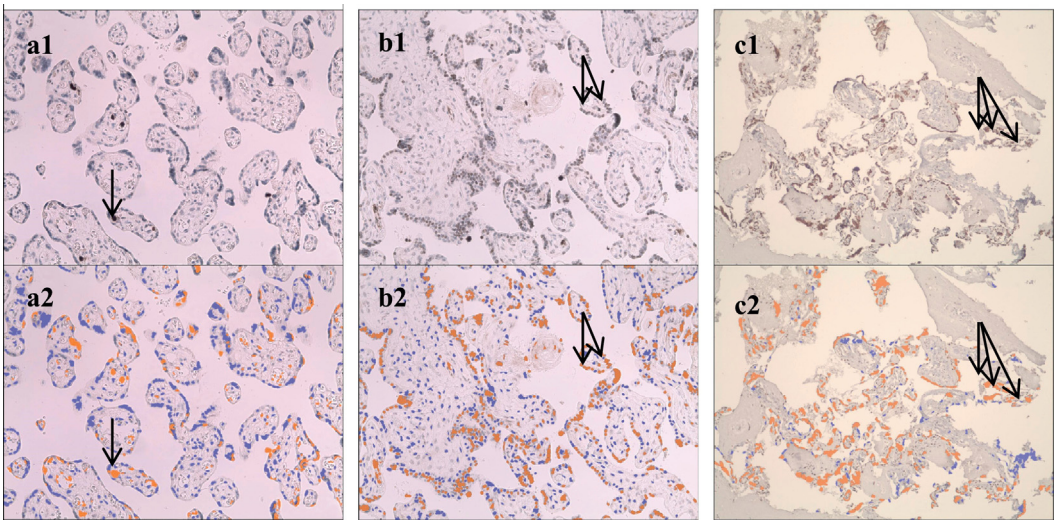

Fig. 6. Immunohistochemical staining of section of formalin-fixed and paraffin-embedded (FFPE) sections of term placenta with anti-HIF-1α antibody against HIF-1 showing low (a), moderate (b) and high (c) HIF-1 expression using microscope (a1, b1, c1) and image software (a2, b2, c2) (http://153.1.200.58:8080/immunoratio/); original magnification × 200. Arrow shows HIF expression. Higher HIF-1 expression was observed in the firewood/kerosene users, moderate expression was noticed in the ethanol group and low expression was present in the placenta from Chicago women.

that adverse impacts of HAP is of great importance from public health perspectives, stringent guidelines and policy measures should be established to eradicate the use of unclean fuels like biomass and kerosene and encourage use of relatively cleaner fuels like ethanol, natural gas and electricity. We suggest that more studies be undertaken to investigate the impact of early *in utero* exposure to HAP on adverse pregnancy outcomes including fetal growth and development of neonatal and childhood diseases.

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Declaration of competing financial interests

We declare no competing financial interests.

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