STUDY PROTOCOL

Ambient and Indoor Air Pollution in Pregnancy and the risk of Low birth weight and Ensuing Effects in Infants (APPLE): A cohort study in Bangalore, South India [version 1; peer review: 1 approved with reservations, 2 not approved]

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Abstract

**Background:** Exposure to air pollution (IAP) from the combustion of solid fuels is a significant cause of morbidity and mortality in developing countries. Pregnant women exposed to higher pollutant levels are at higher risk of delivering a low-birth-weight (LBW) baby. There is a lack of standardized data regarding the levels and types of specific pollutants and how they impact LBW. We aim to prospectively assess the association between ambient and indoor air pollution levels in pregnancy and low birth weight and understand the subsequent risk of adiposity in these infants.

**Methods:** We will conduct a prospective cohort study of 516 pregnant women recruited before 18 weeks of gestation in the urban slums of Bangalore, who have voluntarily consented to participate. We will estimate the level of air pollutants including coarse particulate matter 10 ug/m3 (PM10), fine particulate matter 2.5 ug/m3(PM2.5) and carbon monoxide (CO) parts per million (ppm) levels in both indoor and ambient environment. The follow-up of the delivered children will be done at delivery until the infant is two years old. The association between pollutants and LBW will be evaluated using logistic regression adjusting for potential confounders. Further, we will explore the mediation role of LBW in the hypothesized causal chain of air pollution and adiposity. Nested within a larger Maternal Antecedents of Adiposity and Studying the Transgenerational role of Hyperglycemia and Insulin (MAASTHI) cohort, we can estimate the absolute risk of having low birth weight caused by air pollution and other variables.

**Discussion:** Understanding the association between exposures to ambient and indoor air pollution and low birth weight is essential in India. LBW
babies have a higher risk of developing obesity and Non-Communicable Diseases (NCDs) during adulthood. The results from this study can inform the efforts for controlling the air pollution-related chronic diseases in India.

**Keywords**

Ambient air pollution, indoor air pollution, low birth weight, adiposity, Non-communicable disease

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List of Abbreviation

AQI: Air Quality Index
ATP: Adenosine TriPhosphate
BMI: Body Mass Index
CO: Carbon Monoxide
CSV : comma separated values
DNA: Deoxyribose Nucleic Acid
HBsAg: Hepatitis B Surface Antigen
HIV: Human Immunodeficiency Virus
IAP: Indoor Air Pollution
LBW: Low Birth Weight
NCD: Non-Communicable Disease
PM10: Particulate matter 10 micrometre
PM2.5: Particulate matter 2.5micrometer
PPM: parts per million
RH: Relative Humidity
SES: Socio-Economic Status
SO2: Sulphur Dioxide
SD: Secure Digital
T2DM: Type 2 Diabetes Mellitus
US EPA: The United States Environmental Protection Agency
USB: Universal Serial Bus
VDRL: Venereal Disease Research Diagnostic
XLSX: Excel Microsoft Office Open XML Format Spreadsheet file

Introduction
The developmental origins of health and disease (DOHaD) hypothesis deals with exploring the causal role of intrauterine circumstances to the origins of diseases in adults. The initial studies have revealed that undernutrition during pregnancy is an important determinant of adult cardiac and metabolic disorders due to fetal programming. This was hypothesized to be mediated via altering the fetus’ structure, function, and metabolism (Gluckman et al. NEJM 2008). Since then, development of fetal origins of adult disorders has remained an essential focus of researchers in the exploration of causal mechanisms for hypertension, coronary heart disease, and non-insulin-dependent diabetes. The collation of the prospective evidence using the DOHaD approach helps in assessing and modifying the impact of determinants of health from the life-course perspective. While the evidence regarding nutritional pathways of LBW is available, other antecedents such as air pollution and psychosocial stress are less investigated.

Globally, nearly three billion people use traditional biomass fuels as their primary source of energy comprising of wood, charcoal and agricultural wastes. In India, nearly 67% of the population use biomass as a primary source of fuel for cooking. As a result, exposure to indoor air pollution (IAP) from the combustion of these fuels has emerged as an important cause of morbidity and mortality. Air pollution is contributing to the second-highest associated risk factor for mortality and morbidity. Even a low-dose exposure to pollutants in utero can result in disease, disability and death in childhood. There is evidence that air pollution is associated with the burden of several diseases including respiratory infections, chronic obstructive pulmonary disease, cataracts, cardiovascular events, low birth weight and all-cause mortality. Further, meta-analysis by Stieb et al. indicates that the decrease in birth weight is proportional to higher pollutant concentration.

The putative role of exposure to air pollution during pregnancy resulting in LBW has been assessed in several studies. The suggested mechanisms mediating this path include oxidative stress resulting in placental and endothelial dysfunction, and damage in the DNA productivity due to an imbalance between reactive oxygen species. Specifically, exposure to particulate matter during pregnancy induces changes in multiple placental compartments, including the maternal vascular space, fetal capillaries, and surface exchange areas. These alterations in placental function were associated with a higher incidence of LBW among exposed fetuses. The poor nutrition accentuates the propensity of a baby to be LBW and subsequent inadequate development of pancreatic beta cell mass resulting in a higher risk of development of type 2 diabetes in future. These include greater insulin resistance and storage of fat as compared to children with normal weight. Intratherine malnutrition and other fetal constraints induce insulin deficiency (lack of the growth-promoting activities of insulin) and a postnatal state of regulatory insulin resistance, which leads to a rapid postnatal increase of adipose tissue that remains stable throughout life.

Available evidence suggests that several environmental factors induce intra uterine growth retardation (IUGR) and subsequent LBW in newborns. These include diet, diabetes, hormone exposure, air pollution, psychosocial stressors and hypoxia. Our ongoing, MAASTHI birth cohort study characterises the impact of these factors, including exposure to higher glucose levels during pregnancy, in the intrauterine milieu on the fetus, barring the effect of air pollution. A cohort study is an efficient study design to assess the association between prenatal exposure to air pollutants and infant health outcomes.

Objectives
We aim to evaluate the association between prenatal exposure to ambient and indoor air pollutants and low birth weight in newborns (Figure 1 and Figure 2). We will also be exploring the mediation role played by LBW in the causal path between air pollution in pregnancy and adiposity in infancy. (Figure 3 and Figure 4).

1. To explore the association of prenatal exposure to indoor air pollutants in pregnancy and low birth weight at birth.

   Hypothesis 1: Prenatal exposure to indoor air pollution increases the risk of low birth weight

2. To explore the association of prenatal exposure to ambient air pollutants in pregnancy and low birth weight at birth.
Figure 1. Directed acyclic graph displaying the causal pathway of indoor air pollution during pregnancy with low birth weight.

Figure 2. Directed acyclic graph displaying the causal pathway of ambient air pollution during pregnancy with low birth weight.

Figure 3. Directed acyclic graph displaying the causal pathway of showing the mediating effect of low birth weight due to indoor air pollution during pregnancy on adiposity.
Hypothesis 2: Prenatal exposure to ambient air pollution increases the risk of low birth weight.

3. To evaluate the association between prenatal exposure to indoor air pollutants in pregnancy and adiposity in infants, mediated through low birth weight

Hypothesis 3: Low birth weight at birth mediates the effect of indoor air pollution in pregnancy on adiposity in children (Figure 3)

4. To examine the association between prenatal exposure to ambient air pollutants and adiposity in infants, mediated through low birth weight.

Hypothesis 4: Low birth weight mediates the effect of ambient air pollution in pregnancy on adiposity in children (Figure 4)

Methods
Study design
A prospective cohort study is planned in the urban slums of Bangalore. The study duration is three years. In this regard, a pilot study was carried out in September 2017. Subsequently, the recruitment and follow-up visits are scheduled between August 2018 and December 2020. The study population comprises of households with pregnant women. All the pregnant women in the study population will be followed up until delivery, and their infants will be followed up further irrespective of the exposure status. The follow-ups will be performed at delivery, at six months, and eighteen months of age. Infant anthropometry, morbidity, feeding practices, and child developmental milestones will be assessed during each follow-up visit

Setting
The study area is located in the slums of east and west zones of urban Bangalore. The selected areas are Srirampura, Kodandarampura, Shirdi Saibaba Nagar, Subas Nagar from the West Zone, and DJ Halli, Bagalur Layout and Pulikeshinagar from the East Zone (Figure 5). A slum is defined as an area comprising of at least 60-70 households living in poorly built congested tenements along with the neighbouring well-built houses (see data from the Karnataka Slum Development Board).

Participants
The study participants will be selected from the Bruhath Bangalore Mahanagara Palike (BBMP) health centres. Permissions have been obtained from the Chief Health Officer, Bangalore to conduct the study in the selected BBMP urban health centres. The selected centres are, Ramachandrapura Urban family welfare centre (UFWC), Subash Nagar urban health centre (UHC), Shirdi Saibaba UHC, Kodandarampura UHC from the west zone and DJ Halli, Bagalur Layout, Robertsonpet and KG Halli UHCs from the east zones of BBMP. The research staff will screen the eligible respondents in the health centre and in the community. Only eligible respondents will be enrolled in the study after obtaining their informed consent.
Eligibility criteria: Pregnant women aged between 18–45 years with a gestational age of under 18 weeks who reside in the slums and plan to deliver at the study locations are eligible for recruitment in the study. Women with severe co-existing illness and those who plan to move out of the study location during the study period will be excluded. Women who are mentally or physically not capable of voluntarily consenting or participating in the study will be excluded (Figure 6).

Ethical considerations
Institutional Ethics Committee: Ethical clearance for the proposed study has been obtained from the institutional review board (IEC) at Bangalore, IIPH-H (Approval Number IIPHHB/TRCIEC/121/2017 Dated 27 July 2017).

Written informed consent will be obtained from participants before the start of the study. They will be informed in detail about the study and their voluntary agreement to participate in the research will be obtained.

Variables
The list of variables used for exposure, confounder, intermediate and outcome assessment are provided in Table 1.

Exposure assessment. We will assess the level of air pollutants namely, fine particulate matter (PM$_{2.5}$) and coarse particulate matter (PM$_{10}$) and carbon monoxide (CO). All these pollutants will be measured both indoor and in the ambient environment using air quality monitors. Personal sampling monitors will be used to measure the individual exposure level of PM$_{2.5}$, PM$_{10}$ and CO at the household level. The eligible participants will be required to wear a personal sampling device for one entire day during each trimester. The samplers will be kept inside a small fully ventilated, appealing sling bag that they can wear while carrying out the routine household chores (Figure 7). The samplers record the data continuously for 24-hours, and are connected to a portable outlet power source or power bank. The data will be stored in the memory card in the sampler which is then transferred and stored safely in a hard disk later by the research staff. The exposure data will be measured twice during pregnancy, during the second and the third trimester of pregnancy. A tentative schedule for the distribution of the monitors is provided in Appendix 1 (Supplementary Table 1, Supplementary File 1). The ambient air quality monitor will be kept outside the house in a protected environment, within 5 km radius from the respective participant's house to assess live ambient data on all three pollutants (PM$_{2.5}$, PM$_{10}$ and CO) for 24 hours. The device will
Figure 6. Flow diagram depicting the steps in the APPLE cohort study.

Table 1. The exposure, outcome, list of confounders and possible effect modifiers and intermediate factors for the study objective.

<table>
<thead>
<tr>
<th>Objectives</th>
<th>Exposure during pregnancy</th>
<th>Intermediate</th>
<th>Confounder</th>
<th>Effect modifier</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Indoor air pollutants(PM$<em>{2.5}$, PM$</em>{10}$ and CO)</td>
<td>Household/indoor temperature</td>
<td>Season, Maternal smoking(active and passive), Socio-economic status</td>
<td>Maternal Age, Occupational H/o exposure</td>
<td>LBW</td>
</tr>
<tr>
<td>2</td>
<td>Ambient air pollutants(PM$<em>{2.5}$, PM$</em>{10}$ and CO)</td>
<td>Ambient temperature</td>
<td>Season, Heavy traffic roadways</td>
<td>Maternal age, Occupational H/o exposure</td>
<td>LBW</td>
</tr>
<tr>
<td>3</td>
<td>Indoor air pollutants(PM$<em>{2.5}$, PM$</em>{10}$ and CO)</td>
<td>LBW</td>
<td>SES, Physical activity</td>
<td>Infant age, Gender Feeding habits</td>
<td>Infant Adiposity</td>
</tr>
<tr>
<td>4</td>
<td>Ambient air pollutants(PM$<em>{2.5}$, PM$</em>{10}$ and CO)</td>
<td>LBW</td>
<td>SES, Physical activity</td>
<td>Infant age, Gender Feeding habits</td>
<td>Infant Adiposity</td>
</tr>
</tbody>
</table>

PM – particulate matter, CO – Carbon Monoxide, H/o – History of, LBW – low body weight

be about five feet above the ground level provided with a shelter to secure the device from direct sunlight and rain. We will also obtain the readings of the ambient air pollutant levels from the nearby ambient air quality monitoring devices and stations installed by the pollution control board (monitored via Eprolytics).

Together, we will assess the ambient exposure level of that particular area. This data will be considered as a proxy for ambient data for each household in that area.

Covariates. We will assess socioeconomic and demographic variables, current and previous obstetric history, fuel used for cooking and heating, and the location of the kitchen, using pre-tested questionnaires (Supplementary File 1). The information on other sources of pollution such as burning of agarbatti/dhoop (Incense sticks); and use of mosquito repellents and candles will be collected. The duration of exposure to indoor as well as outdoor air pollution will be collected. (Detailed in Supplementary Table 1, Supplementary File 1). Trained research staff will
record the height and weight of the participants by using SECA 213 (seca Precision for health) stadiometer and digital Omron HN-283 (Omron Healthcare Co., Ltd.) weighing scale. The scale will be placed on a level ground, the research staff will check for a ‘zero’ reading, and after ensuring that the respondent has removed heavy outer clothing and shoes, two readings to the nearest 10 grams will be recorded. A portable stadiometer will be used for measuring height to the nearest 0.1 cm; measured with the participant standing straight with her feet together, the head plate of the stadiometer will then be pulled down to ensure that it rests on the crown of the head. Blood pressure will be measured using OMRON HEM-7203 (Omron Healthcare Co., Ltd.) automated blood pressure monitor. We will also measure the fasting and 2-hour post-prandial blood glucose levels (Oral glucose tolerance test) and haemoglobin during the day as a markers of hyperglycaemia and anaemia in pregnant women who complete their 24 weeks of gestation (Table 2). The tests will be conducted by Medall Healthcare Pvt. Ltd. using fully automated analyzer, non-cyanide methods for hemoglobin estimation and glucose will be estimated using Glucose oxidase (GOD)-preoxidase (POD) colorimetric method. The procedure has been detailed further in the laboratory analysis and sample storage section. The field staff will assess for obstetric morbidity and hospitalization during the monitoring visits.

Household temperature and the status of anaemia will be considered as an intermediate factor between indoor and ambient air pollution and its association with low birth weight\(^{23,24}\). A list of known effect modifiers will be made and collected using a structured questionnaire (Supplementary File 1) and later considered during analysis. (Eg; the age of the respondent, occupational exposure\(^{25}\). Season, socioeconomic status, smoking history and exposure to second-hand smoke, traffic roadways, maternal stress and social support have been considered as confounders\(^{26-31}\). As we cannot identify the relative contribution of tobacco smoke to the indoor or ambient air pollution, we will obtain data using the structured questionnaire. Psychosocial stress data will be collected using the standard Edinburgh Postnatal Depression Scale (EPDS), a widely used self-reporting questionnaire explicitly developed to screen women for perinatal depression\(^{32}\). EPDS has been validated by Fernandes et al. for prenatal depression in South India at a cut-off of \(\varepsilon13\) (sensitivity = 100%, specificity = 84.90%, and area under the curve = 0.95)\(^{33}\). Social support will be measured using a questionnaire that has been used and developed by St. John’s Research Institute to evaluate a broad range of social support (i.e., emotional, instrumental, informational, and appraisal)\(^{34}\). This questionnaire has a total of 12 items, and each item is scored between 0 (definitely not enough) to 3 (definitely enough). The highest score being 36 means excellent social support and 0 meaning very low social support.

**Outcome variables.** Birth weight, length and skinfold thickness of the baby at birth, 6 and 18 months are the outcomes of interest. Feeding practices, morbidity, and child development milestone will be assessed during the follow-up visits. (Table 2). The Trivandrum Developmental Scale will be used to measure the developmental milestones\(^{35}\). The 51-items of Trivandrum Developmental Screening Chart for children of 0-6 y [TDSC (0–6 y)] is a simple, reliable and valid screening tool in the community to identify children between 0–6 years with developmental delay.

For assessment of weight, the baby will be placed naked on the digital SECA 354 weighing scale and readings will be taken to the nearest 0.5g. For measuring infant length, the baby’s head will be held against the end of the head plate of the SECA 417 infantometer, and the foot plate will be bought up to the heels ensuring that the feet and knees are flat.
Table 2. Proposed measurements at baseline and follow up in the APPLE study.

<table>
<thead>
<tr>
<th>Participants</th>
<th>Measurement/tests</th>
<th>Frequency</th>
<th>N</th>
<th>Time points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant women</td>
<td>PM_{2.5}, PM_{10} and CO concentration of both indoor and ambient level.</td>
<td>Two times</td>
<td>516</td>
<td>Once in 2^{nd} and 3^{rd} trimester</td>
</tr>
<tr>
<td>Pregnant women</td>
<td>1. Blood glucose estimation</td>
<td>Once</td>
<td>516</td>
<td>During 24–32 weeks of gestation</td>
</tr>
<tr>
<td></td>
<td>2. Haemoglobin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. Height and weight, Blood Pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4. Socio-demographic characteristics, current obstetric history, psychosocial stress, social support</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newborn</td>
<td>Anthropometry of the child (Skinfold thickness (Biceps, triceps and subscapular) and circumferences (Head, chest, waist, hip and mid-arm circumference)</td>
<td>Once</td>
<td>516</td>
<td>At birth</td>
</tr>
<tr>
<td>Infants</td>
<td>Skinfold thickness (Biceps, triceps and subscapular) and circumferences (Mid-upper arm, Head, chest, waist and hip circumference), morbidity and feeding practices, child developmental milestones</td>
<td>Two times</td>
<td>516</td>
<td>6^{th} month 18^{th} month</td>
</tr>
</tbody>
</table>

PM – particulate matter

Chasmors body circumference tape will be used to measure the circumferences. Mid-upper-arm (MUAC) will be recorded with the arm bent, allowing the measurement to be taken with the baby in its natural position. An ink mark will be made on the anterior and posterior side of the arm to locate the point for biceps and triceps estimation. Head circumference will be measured with the baby’s head on the side. The tape will be placed on the forehead. Waist circumference will be taken by placing the tape around the abdomen immediately above the umbilicus at the end of expiration. Chest circumference will be measured by placing the tape around the chest at the end of expiration. Skinfold thickness will be measured on the left side of the body using the Holtain Calipers. Three readings to the nearest 0.2mm will be taken. For triceps skinfold thickness, the tape will be placed around the upper arm at the level of the mark done at the posterior side while measuring mid-upper arm circumference (MUAC). With the tape in position, a horizontal line will be drawn on the skin at the level of the mark. The point at which the fold is to be measured will then be marked; the skin will be lifted over the posterior surface of the triceps muscle, above the marked point and the calipers will be applied below the fingers. For subscapular skinfold thickness, the inferior angle of the scapula will be identified, and the skin will be marked immediately below the angle. The skinfold will be picked up above the mark, and the caliper jaws will be applied below the fingers, at the apex of the fold

Adiposity

We define adiposity as the sum of the skinfold thickness namely biceps, triceps and subscapular measuring above the 70^{th} percentiles are classified as at risk to obese and more than 85^{th} percentiles are classified as obese.

The skinfold thickness equation for body fat composition measurement by Holtain Caliper is correlated and validated against dual-energy x-ray absorptiometry (DEXA) which is a standard gold method for body fat estimation and reported a reasonable validation with DEXA. The list of exposure, outcome and potential confounders will be explained in detail below (Table 1)

Laboratory analysis and sample storage

Medall Healthcare Pvt. Ltd (MEDALL), a centralized and nationally accredited laboratory will be engaged to carry out all the tests including haemoglobin and oral glucose tolerance test (OGTT). The pregnant women will be advised by the research staff to visit the hospital after overnight fasting state for at least 7–8 hours. A trained phlebotomist will draw the fasting and postprandial sample 2 hours following a 75 g oral load of glucose. The blood from the fasting sample will be centrifuged within 30 minutes of collection at 3500rpm for about 5 minutes in a REMI Medico centrifuge C-854/6 portable centrifuge. The sample will be transferred into a cold box at 2–8°C and will be transferred to the central laboratory, where assays will be carried out. An aliquot of plasma will be made by centrifuging the fasting Sodium Flouride tube and haemoglobin processed EDTA samples for 3500rpm for 5 min. The remaining sediment of packed red cells will be centrifuged at high speed at 4500rpm for 5–10 min to extract the buffy coat samples. The plain tube containing 6ml of blood will be made to stand for 40 minutes to clot then centrifuged at a speed of 4500rpm for 5–10minutes. Totally, six aliquots of serum will be separated from 6ml of a plain blood sample each holding 0.5ml of serum. 4 aliquots of plasma of 0.5ml each, 3 aliquots of buffy coats (0.1 – 0.5ml) will be stored for the future analysis. The aliquots will be stored in a bio-repository at the research centre (Indian Institute of Public Health, Public Health Foundation of India-Bangalore), wherein the samples will be stored in a stepwise manner (2 –8 °C, -20 °C deep freezer and -80 °C deep freezer) for future analysis. The samples are planned to be stored long term will be moved to a -80 °C, ultra low deep freezer.

Sample size

A research study by Kalpana Balakrishna et al. showed that a population-weighted mean of annual PM_{2.5} exposure in India
has increased from 59.8 μg/m³ to 79.9 μg/m³ (1990–2016). The study also reported that more than 80% of the Indian population were exposed to PM₁₀ of more than 40μg/m³. Delhi has the highest populated weighted mean of PM₂.⁵ (>150 μg/m³) followed by Uttar Pradesh, Bihar and Haryana. We plan to recruit 516 pregnant women over a period of one year with a hypothesized (based on the evidence in India) 20% of LBW in this population⁹, accounting for the design effect (DEFF) of (for cluster surveys) 1.5, for 95% confidence interval, the sample size required is 369. Further, due to the transient nature of pregnant women from the slum population, the sample size required is 516 accounting for 40% loss to follow-up⁹.

The sample size has been estimated using a formula, n=Z²P(1−P)/d²; where n is the sample size, Z is the statistic corresponding to the confidence interval, P is the expected prevalence and d is the precision. The sample size of 246 has been estimated using 20% of expected prevalence of LBW at 95% confidence interval and 5% precision. The two zones of the area considered as a cluster and accounting for the design effect of 1.5 the sample size came out to be 369. Further, due to the transient nature of pregnant women from the slum population, the sample size required is 516 accounting for 40% loss to follow-up.

Data sources and measurements
The study instruments used in this study have been developed by research team using previous research findings. The questionnaire has been piloted and validated before administration in the field (Supplementary File 1). The research staff will administer the questionnaire, conduct blood investigation, and record the anthropometric measurements at the health centre. The air monitoring device with power bank will be distributed to pregnant women by the field staff of the respective area and taken back after the 24 hours estimation is done. The post-monitoring assessment will be performed using a structured questionnaire assessing the cooking practices and activity of the participants during the monitoring period. The enrolled pregnant women due for delivery will be tracked through periodic phone calls by tracking respondent based on their estimated due date (EDD) and scheduling follow-up visits.

Ambient and indoor air quality monitor
We will use the VAYUSESNE (later renamed as VAYUCARE) device for assessing the outdoor and indoor air pollutants. This is designed by Ambience Monitoring India private limited based in Delhi marketed through their selling partner “I love Clean Air”. It is a portable device that can operate for 24 hours with 10,000mAh external power bank (Figure 7). The devices provide real-time monitoring of particulate pollution PM₁₀, PM₂.⁵ and CO. It has air sensors to produce details on air quality. The data will be saved in an SD card. The downloaded data can be viewed in comma-separated values (CSV) and Excel Microsoft Office Open XML Format Spreadsheet file (XLSX) format. The monitors are calibrated, tested and then installed⁹. Upon installation, the monitor will assess the indoor air quality data automatically. The particulate matter is measured in μg/m³, and CO is measured in ppm. The temperature is measured in degree Celsius, and relative humidity (RH) is expressed as a percentage.

Bias
Specific attention will be paid to limit bias by controlling for confounding, minimizing selection bias and measurement errors. In order to prevent any differential misclassification of outcome, the data from laboratory investigations and air pollutant estimation is accessed only by the key research staff who are not involved in data collection. The field team will be trained and certified on anthropometric measurements by St. John’s Research Institute and any possible measurement error will be minimized. Accuracy and inter-observer reliability of their measurements will be assessed at the outset and subsequently for every six month. To control for confounding, information about the potential confounders (Table 1) will be obtained and controlled during the analysis stage.

Statistical methods
Descriptive statistical estimates of the individual exposure will be reported. Frequencies and percentages will be reported for all the variables. The exposure data distribution will be checked for normality. The mean and standard deviation will be reported for quantitative variables. The minimum and maximum level of exposure will be reported. The prevalence of low birth weight will be calculated. The fasting and postprandial glucose will be categorized based on the WHO recommended cut off levels (fasting glucose of ≥92mg/dl and 2-hr values of ≥153mg/dl) are considered as hyperglycemia⁹. The anaemia status will be categorized as normal when the Hemoglobin (HB) is >11gm/dl, mild anaemia when Haemoglobin 10 – 10.9gm/dl, moderate anaemia when Hemoglobin 7– 9.9gm/dl and severe when the Hemoglobin level is less than 7gm/dl⁸. The outcomes (birth weight and adiposity) will be modeled both as a continuous and binary indicator variable. The exposure of interest (PM₁₀, PM₂.⁵ and CO) will be taken as a continuous scale measure for analysis. The exposure value will then be categorized in terms of quartiles and the lower quartile of the exposure will be taken as the reference category for each pollutant for further analysis. The odds ratio with confidence interval will be reported. We will also report the dose-response relationship between maternal exposure to indoor and ambient air pollutants (PM₁₀, PM₂.⁵ and CO) and birth weight will be assessed through sensitivity analysis⁹. Mediation analysis will be used to test the hypothesized causal chain for third and fourth objectives; involving low birth weight as an intermediate in the association between air pollution and adiposity.

Dissemination of information
The findings of the study will assist the efforts of the Government to counter climate change. The data from the study will pave way for future research and policy-making agendas of the government. We have engaged key stakeholders like health officers and Slum board official to increase their sense of awareness towards the impact of air pollution on health.
Through our membership in National Advisory Group and periodic inputs, we will ensure the wider dissemination of the study findings. The findings will be disseminated to fellow researchers through conference presentations, publications in the peer reviewed journals and through reports to health authorities.

**Study status**
The baseline assessments have been started at two zones of the study area. The study tool has been piloted in the field and made necessary changes. We are currently calibrating the air quality devices before we use them in the field. The calibration process includes passing a known concentration of air pollutant (CO) in a controlled environment with the aim of obtaining measurements from the devices within the acceptable range of ±10 ppm.

**Discussion**
There is a lack of standardized data for the confluence of risk factors including the levels and roles of specific pollutants and how they are associated with low birth weight and adiposity in India. By collecting high-quality prospective data on exposures in pregnant women, this study can provide insights into the environmental causes of low birth weight and obesity in childhood. The results from our study may provide evidence regarding the adverse effects of air pollution in pregnancy, and thereby can help in improving the neonatal and child health outcomes. The results can inform policy regarding limiting air pollution and designing interventions for use in future studies.

**Supplementary material**
Supplementary File 1: File contain the study questionnaire along with the following Supplementary Tables:
Click here to access the data
Supplementary Table 1: Tentative schedule for using the air pollution monitoring devices for exposure assessment
Supplementary Table 2: Description on variables

**References**

5. INDIA PROFILE. In.: Institute for Health Metrics and Evaluation; 2016.


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Julian D. Marshall
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Thank you for this opportunity to review the study protocol, “Ambient and Indoor Air Pollution in Pregnancy and the risk of Low birth weight and Ensuing Effects in Infants (APPLE): A cohort study in Bangalore, South India [version 1]”. In addition, I have read two peer review reports included with the manuscript: one by Kalpana Balakrishnan (Balakrishnan, 2018) and one by Ryan Allen (Allen, 2019).

The proposed study focuses on the impact of air pollution on adverse birth outcomes. Air pollution concentrations for PM2.5, PM10, and CO will be measured indoors and in nearby (within 5km) ambient air, using the VAYUCARE low-cost sensor; measurements as 24-hour averages will be conducted twice per subject: once each during the second and the third trimesters. Health parameters will be investigated for the mother and the infant, focusing on two outcomes: adiposity and birth weight.

The research topics investigated here are very important. A successful investigation would meaningfully add to the literature.

To my knowledge, the health measurements and the plans for epidemiological analyses are robust. (Those aspects are not my area of expertise.) Comments below focus on the air pollution measurements. I am concerned that the proposal’s current plan for air pollution measurement is insufficient; the level of accuracy and precision in those measurements may be too low, and as a result, exposure misclassification may overwhelm the statistical signal the authors seek to quantify.

I agree with the review comments from Professors Balakrishnan and Allen regarding air pollution measurement. I would not want to see the important work of this project move forward, and then later the authors find out that the study is unsuccessful or inconclusive solely because of uncertainties in air pollution measurement.

To help shore up this aspect of the investigation, I suggest the authors conduct two exercises: first, a series of pilot studies (e.g., first without people, then on 5 people, then on 20 people, and then on 100 people, before expanding to the full ~500-person cohort). During this pilot study, the researchers should investigate the robustness of the air pollution measurements for use in their study. Strong attention is
needed for issues such as error, bias, device reliability/failure rate, reproducibility, within- and between-instrument variability, within- and between-participant variability, interference (e.g., dependency on relatively humidity), general spatial and temporal variability, and the range of concentrations observed. This information will usefully inform the study design (e.g., which device to use, how many measurements to make). Running multiple versions of the same device side-by-side will inform reproducibility; running them next to a reference monitor will inform accuracy. Second, a review on devices used to measure air pollution exposures for epidemiology, with special attention to issues of using low-cost, unproven sensors and to approaches that have been used in India.

The research would benefit from greater knowledge of expected concentrations and concentration gradients; such information would inform the sample-size/power calculations. Earlier measurements in Bangalore reported relatively high concentrations (e.g., Both et al., 2011); I believe current measurements in Bangalore by ILK and CSTEP, using a reference-grade monitor (Beta Attenuation Monitor [BAM]), suggest cleaner concentrations.

Minor typo:

The abstract contains the text “We will estimate the level of air pollutants including coarse particulate matter 10 ug/m3 (PM10), fine particulate matter 2.5 ug/m3(PM2.5) and carbon monoxide (CO) parts per million (ppm) levels in both indoor and ambient environment.” Both uses of the phrase “ug/m3” should be “μm” (micrometers), reflecting the size of the particles (<10 μm, <2.5 μm) rather than a concentration measurement (mass per volume: μg/m3).

References

Is the rationale for, and objectives of, the study clearly described?
Yes

Is the study design appropriate for the research question?
Partly

Are sufficient details of the methods provided to allow replication by others?
Yes

Are the datasets clearly presented in a useable and accessible format?
Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Air pollution monitoring; exposure assessment
I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 06 June 2019

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Ryan W. Allen
Simon Fraser University, Burnaby, BC, Canada

This paper describes a birth cohort study to evaluate associations between air pollution exposure and low birth weight (LBW) in areas near Bangalore. The investigators also intend to evaluate the mediating role of LBW in associations between prenatal air pollution exposure and adiposity in early childhood. This is an ambitious study focused on important areas of inquiry. The scientific evidence linking air pollution with fetal growth is expanding rapidly, while the research on prenatal exposure and adiposity/overweight/obesity is relatively limited and results are mixed.

I have several concerns with the study as described:

1. Exposure assessment:
   - The measurement plan is difficult to understand, due in part to inconsistent use of terminology. Under “exposure assessment”, the authors describe plans for outdoor, indoor, and personal sampling. Then under “Data sources and measurements” the authors describe only ambient and indoor monitoring, with no mention of personal monitoring. The paper states that “personal sampling monitors will be used to measure…individual exposure…at the household level.” Are the terms “personal” and “indoor” being used interchangeably? Or will each participant undergo both personal and indoor sampling? If so, will these be conducted at the same time?
   - The investigators plan to use the VAYUCARE device for measuring air pollutants, but the paper does not provide sufficient detail on this instrument or a rationale for choosing it. Has the device been validated against other, more established monitors? I recommend that the authors carefully consider their choice and provide a clear rationale for choosing this particular instrument. As interest in relatively low-cost monitors has increased, there have been more evaluations of various monitors and their strengths and weaknesses, and the authors might consider reviewing some of these recent evaluations such as:
     - [http://www.aqmd.gov/aq-spec/evaluations](http://www.aqmd.gov/aq-spec/evaluations)
     - [https://www.epa.gov/air-sensor-toolbox/evaluation-emerging-air-pollution-sensor-performance](https://www.epa.gov/air-sensor-toolbox/evaluation-emerging-air-pollution-sensor-performance)
     - Morawska et al., 2018.
   - The authors should describe their plans for deriving exposure estimates from the measurements. For example, personal monitoring is planned during two 24-hour periods in pregnancy. These will need to be temporally adjusted and combined in some way to estimate long-term exposure during pregnancy. In addition, it is not clear if the investigators have plans to estimate exposure prior to enrolment in the study (the period from conception to enrolment).
• The investigators plan to place outdoor monitors within 5 km of participants’ residences. This may be too far to assess concentrations at the residence location. I suggest that the team consider models for interpolating outdoor measurements.

2. Power calculations:
• The study’s power to detect associations will depend on the exposure gradient, but it is not clear what gradient the investigators assumed for their power calculations. Moreover, the ambiguity in the exposure assessment makes the power calculation difficult to interpret; it is unclear whether the exposure of interest is the pregnancy averaged concentration, trimester-specific concentrations, or concentrations over some other averaging time.
• The investigators note the transient nature of this population in assuming a loss of 40% of the cohort. It is not clear if the investigators also accounted for pregnancy losses in their anticipated loss to follow-up. One might expect that ≥10% of these pregnancies will result in spontaneous abortion or stillbirth (depending on gestational age at enrolment).

3. Analysis plan:
• Hypotheses 3 and 4 assume an association between air pollution during pregnancy and adiposity in childhood. But the literature on this question is mixed, so it is possible that air pollution will not be associated with adiposity in this cohort. I recommend that the authors revise their hypotheses to formally test the air pollution – adiposity association before moving to the mediation analysis.
• For continuous outcome variables in childhood, will each time point be modelled separately or are there plans to take advantage of the study’s longitudinal design to evaluate growth trajectories?
• The literature suggests that rapid “catch-up” growth after birth is strongly tied to poor cardiometabolic health later in life. Thus, in addition to the mediation analysis of LBW on the exposure-adiposity pathway, I suggest that the authors consider also explicitly evaluating catch-up growth as an outcome.
• The paper does not describe how the biomarkers (haemoglobin, oral glucose) will be used. These variables are not listed as potential confounders or possible effect modifiers (in Figure 3 or Table 1), nor are they outcome variables. Collection of these samples will pose a burden to participants, so the use of these samples needs to be justified.

In addition, I have the following minor comments/suggestions:
• The summary of previous studies on air pollution and fetal growth could be updated and expanded to include, for example, some of the papers listed at the end of this review.
• Figure 6 would be more informative as a timeline showing key activities at different stages of pregnancy/childhood.
• The questionnaire (supplementary file 1) needs refinement. Some questions are vague/subjective and may not provide useful information (e.g. “Is that a dusty occupation?”, “Please describe the weather”).

References


**Is the rationale for, and objectives of, the study clearly described?**
Partly

**Is the study design appropriate for the research question?**
Partly

**Are sufficient details of the methods provided to allow replication by others?**
No

**Are the datasets clearly presented in a useable and accessible format?**
Not applicable

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Air pollution exposure assessment and epidemiology.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 13 May 2019

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Kalpana Balakrishnan
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The proposal focuses on a theme that is being increasingly recognized as an important health outcome associated with air pollution exposures. This is especially pertinent in low-middle income countries where the prevalence of health damaging exposures and low birth weight are both high. The investigators have previously been engaged with cohort studies and thus have experience in launching such studies. The health outcomes section of the proposal is quite strong.

The proposal unfortunately does not provide a correct framing for addressing air pollution exposures and uses terminologies rather loosely making it difficult to interpret the rationale, objectives and the study methods proposed. A detailed listing of the issues and suggestions are provided below for possible consideration by the investigators and the funders prior to a final decision:

1. The term "indoor air pollution" is not defined correctly. The investigators seem to want to address air pollution exposures from solid fuel use and term it as indoor air pollution. Air pollution resulting from solid fuel combustion is defined by WHO as household air pollution and contributes to both indoor and ambient air pollution. However in the study population chosen (urban slums near Bangalore) the contributions from solid fuel use to indoor and ambient air pollution is likely to be negligible. In the event it is not so, the investigators should provide supporting information to make a case for prevalence of solid fuel use in the study population. The all India average of 67% solid fuel use is dated and currently stands at 59%. In large metropolitan cities such as Bangalore, even in slums the prevalence of solid fuel use is not likely to be greater than 5%. Indoor air pollution in this setting therefore will not represent contributions from solid fuel use.

Ambient air pollution in the setting proposed (based on recent source apportionment studies) is primarily from transport-related emissions and is likely to be responsible for bulk of the indoor exposures, especially in slums. In the absence of other prominent indoor sources (as is likely in urban slums) ambient/indoor ratios for most pollutants will be close to 1.

The study design and methods thus cannot examine the independent association of indoor vs ambient air pollution with birth weight in this setting, nor can it address household air pollution from solid fuel use.

2. The investigators have not used the most recent or pertinent literature for the proposal. See several citations that have been added that provide a more comprehensive background on the topic. In particular recent studies in Chennai have documented the impacts on birthweight in a rural-urban cohort that address the dual risk of household and ambient air pollution through longitudinal household area measurements. A similar such study (DAPHNE) is underway in Delhi that focuses on personal exposures for urban pregnant women.

3. The sample size is not well informed by the expected exposure variability in the setting. The average exposure used in the cited reference (Balakrishnan et al., 2018) is a national average for ambient air pollution. This does not address expected variability in personal exposures in an urban slum. It is also not clear if the effects estimate they have used is informed by the recent Chennai cohort study or meta-analytical estimates from other LMIC studies. Further performing two
measurements may not adequately capture temporal variability (assuming that indeed women are able to consistently participate in a third trimester personal measurement). A 10000mAh power bank can be quite cumbersome to carry and may pose some safety/personal concerns for pregnant women.

4. The investigators do not provide any validation/citations/specifications for the instrument to be used for personal exposure monitoring. It is not clear if this is a new generation low cost sensor and if so, appropriate validations against reference monitors would be needed to develop quantitative exposure-response relationships. CO exposure is unlikely to be of concern in an urban population (given the expected low prevalence of solid fuel use). CO in the ambient environment is unlikely to be detected at good enough resolution in low cost sensors.

5. Numerous infant outcomes are being measured including growth and development milestones for which there exists a stronger pool of evidence for air pollution-related impacts but yet only mediations through pre-natal exposures on adiposity is being examined. It would be immensely useful to measure post-birth so that each of the outcomes could be independently studied in addition to mediation effects. The sample size requirement will however likely be much higher.

6. Finally, the model specifications need to be elaborated further. Given the spatio-temporal variability and the inability to characterize this fully with just two measurements during pregnancy, there will presumably be a need for mixed effects models to estimate long-term (pregnancy period) exposures. Planned sensitivity analyses should be described in greater detail.

The exposure assessment components of the proposal need to be totally re-structured to focus only on ambient air pollution, using alternative exposure measurement approaches, in a larger cohort and using well validated instrumentation. A wealth of high quality health information is being collected but without a similar rigour in exposure measurements, the ability to develop quantitative exposure-response would not be realized.

Much has advanced in terms of using alternative exposure assessment methodologies in large cohorts. These should find a place in this cohort as well.

References


**Is the rationale for, and objectives of, the study clearly described?**
No

**Is the study design appropriate for the research question?**
Partly

**Are sufficient details of the methods provided to allow replication by others?**
Yes

**Are the datasets clearly presented in a useable and accessible format?**
Not applicable

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Exposure assessment, air pollution and health effects, environmental epidemiology, bio-monitoring, environmental policy

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.