ASSOCIATION OF AIR POLLUTION WITH PLACENTAL PATHOLOGY IN PREGNANT WOMEN OF ODISHA, INDIA: A CASE STUDY

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Abstract

Background: The exposure of pregnant women to atmospheric pollutants is related to the period of early development of the fetus, low birth weight, preterm delivery and low placental weight which occurs due to oxidative stress and it may cause placental abnormalities.

Objective: The present cross-sectional study was conducted to know the effects of air pollution on placental pathology by taking 373 women volunteer participants with normal pregnancy from thickly populated, traffic-congested, industrial and remote rural areas of Odisha, India.

Methods: We collected the pollution level of air pollutants of the three study areas during the period from 2015 to 2018 from the Odisha State Pollution Control Board (OSPCB) Bhubaneshwar. The placental tissue was collected immediately after delivery. The effect of air pollutants on the placental pathology was analyzed by Histopathological studies done by using Hematoxyline and Eosin staining methods and photographed by florescent microscope.

Results: The present study showed the level of pollution, percentage of exposure of pregnant mothers to ambient air pollution, preterm delivery, significant increase in preterm birth in heavy traffic congestion areas (Cuttack zone) and industrial areas (Jajpur zone) in comparison to rural non-industrial areas (Nilagiri zone). The histopathological study of placenta showed presence of PM2.5, Neutrophil granules, damaged tissues, desquamated epithelial tissues, thrombus and less number of villi in heavy traffic and industrial areas as compared to non-industrial areas.

Conclusion: Our findings advocate that the exposure of pregnant women to air pollutants may adversely affect the growth and development of placenta and fetus in highly polluted areas. More studies are required for confirmation.

Key words: Placenta, villi, Nacrotic cells, Preterm Birth, Desquamated epithelial tissue

I. Introduction:
Exposure of pregnant women to ambient air pollution (AAP) may influence the growth and development of foetus. Pollutants enter the human body through several routes such as inhalation of contaminated air, consumption of contaminated water and food, exposure to contaminated soil and industrial waste (Bostrom et al., 2002). Ambient air particles vary in size, chemical composition, origin, shape, surface, charge, concentration and toxicity, which make the assessment of exposure difficult (Geller et al., 2005). It was reported that traffic-related particulate pollutants and combustion particles have toxic effect on human beings (Lewtas et al., 2007). The pregnant mother is also affected by exposure to both outdoor (NOx, SO2, O3, CO, hydrocarbons (HC), and particulate matters (PM) of different sizes) and indoor (NOx, SO2, O3, CO, volatile and semi-volatile organic compounds (VOCs), PM, radon, passive smoking and microorganisms) ambient air pollutants. The effect of these air pollutants on human beings depends on their toxicity, concentration and duration of exposure and may vary from person to person. It has been estimated that 2.4 million people die each year due to indoor (Lewtas et al., 2007) and 800,000 for outdoor air pollution. There is extensive evidence from epidemiological studies that long-term and short-term exposure to ambient air pollution increase the risk of lung cancer, respiratory and cardiovascular morbidity in adults, children and neonates living in urban areas (Kunzli et al., 2000; Turner et al., 2011; Brunekreef et al., 2002).
Particulate matters have been reported in both placenta and umbilical cord blood of pregnant mothers exposed to ambient air pollution (Nugent et al., 2015). Fine ambient particulate matter (less than 2.5 mm in aerodynamic diameter, PM$_{2.5}$) is a collection of inorganic pollutants and can penetrate deep into the lung and result in inflammation (Bhatnagar et al., 2006) which is associated with impairments in placenta during pregnancy.

The placental chronic inflammatory lesions are chronic villitis, chorioamnionitis, or intervillositis (Scapellato et al., 2007). The placenta exposed to biomass smoke would demonstrate hypoxic lesions, particularly as CO levels increases in blood (Wylie et al., 2017). Increased level of CO elevates carboxyhemoglobin level by displacing oxygen from hemoglobin and reducing its availability to the fetus (Soothill et al., 1996) which ultimately affects the growth and development of the fetus.

The effect of exposure to ambient air pollution on birth weight, prematurity and intra-uterine growth restriction (IUGR) can be measured by ultrasound of the foetus during the second and third trimesters (Slama et al., 2009). The exposure of pregnant mother to ambient air pollution showed an increased risk of IUGR in five out of six studies (Maisonet et al., 2004). Expectant mothers who spent more than 2 hours per day outdoors during the second trimester showed significantly reduced birth weight. Significant adverse effect of exposure to ambient air pollution on PTD was recorded in these cases (Lacasana et al., 2005). PTD is a multi-factorial biological process (Sran et al., 2005).

Gestational exposure to PM$_{2.5}$ has correlation with chronic disease risks (e.g., low birth weight, PTD) (Malmqvist et al., 2011; Lamichhane et al., 2015) and adverse child health outcomes (e.g., poorer cognition, asthma) (Basagana et al., 2016). The presence of PM$_{2.5}$ has been reported in both umbilical cord blood, which reflects the state of the foetus and placenta (Nugent et al., 2015). It was reported that the time and duration of PM$_{2.5}$ exposure during pregnancy may be a key factor in triggering a mitochondrial response. Recent studies demonstrate that increased exposure to PM$_{2.5}$ during the third trimester (35–40 weeks gestation) of pregnancy was associated with decreased mitochondrial DNA content in cord blood (Janssen et al., 2012; Lee et al., 2018).

The ultrafine particles (UFP) of urban air may promote inflammation in lungs epithelium, which would increase the concentrations of acute cytokines (van Eeden et al., 2005) like interleukin, in turn cause systemic inflammation. It was reported that the exposure of human beings to PM$_{2.5}$ had shown increased plasma viscosity (Peters et al., 1997) and elevated blood platelet level (Viehmann et al., 2015). The exposure to diesel exhaust particles was associated with enhanced thrombosis susceptibility (Nemmar et al., 2004). It can be concluded that PM$_{2.5}$ may promote thrombosis in capillaries and affect proper functioning of organs. PM$_{2.5}$ induced injury was an important cause for oxidative stress condition (Valavanidis et al., 2008). PM$_{2.5}$ inhalation can exert toxic effects on blood cells and the antioxidant system, stimulating the production of free radicals or reactive oxygen species.

Inhaled PM$_{2.5}$ can easily be transported from lung alveoli to capillaries due to its size. Later on, it is dissolved and circulated into the bloodstream via the pulmonary artery. During blood circulation, components of PM$_{2.5}$ may enter the uteroplacental vascular system and result in placental pathologic changes and decreased transplacental function, with consequent pregnancy complications such as restricted fetal growth. The chorioamnionitis, amnionitis, and vessel thrombus observed in rats exposed to PM$_{2.5}$ are regarded as an intrauterine inflammatory condition (Fielding et al., 2008). They may result in failure of the placenta to nourish the fetus and cause pregnancy complications such as preterm birth (Eder et al., 2009), neonatal cerebral palsy (Fain et al., 2004), and fetal growth restriction (Sun et al., 2005). The exposure to PM$_{2.5}$ and UFPs causes inflammation, amnionitis, chorioamnionitis, intervillositides and squamous epithelial cells (DSE) with loss of papilla. The loss of papilla influences placental capacity and causes reduction in oxygen and nutrient supply to uterus. This could explain the reduced placental growth and high loss of trophoblast cells resulting in damaged tissue (DT) in placenta. Necrosis is a form of cell injury, which results due to premature death of cells in the living tissue by autolysis and the cell is known as necrotic cell. It is the unnatural, unplanned, unprogrammed, immature cell death. Nacrotic cells (NC) appear in placenta due to lack of blood and oxygen supply to tissues (Kloog et al., 2015). Biological disorders induced by traffic-related air pollution and industrial emissions in human beings are not yet completely understood. Therefore our present study concentrates on the effect of exposure of pregnant mother to ambient air pollution on placental pathology.

II. Materials and methods:

: Ethics Statement

The study was approved by the Institutional Ethical Committee on biomedical research on human subjects of SCB Medical College, Cuttack, Odisha, India (213/29.1.16). Written consents were obtained from all women volunteer-participants.

: Study Design

The present cross-sectional study-design includes 373 female volunteers of three distinctly different areas such as Cuttack zone, Jajpur zone and Nilagiri zone of Odisha, India. The Cuttack zone has reference as a thickly populated and traffic polluted area which includes the urban city of Cuttack, the old capital of Odisha. Kalinganagar, regarded as the steel hub of Asia, is situated in the district of Jajpur. Kalinganagar has more than twenty steel plants and other ancillary industries. The non-industrial areas refer to remote tribal-rural areas of Nilagiri in Balasore district.

Volunteers belong to almost the same socio-economic conditions and pre-pregnancy BMI. All of them delivered their single-ton babies between January 2015 and March 2018.

The questionnaire administered to the participant mothers specifically covers all possible aspects of their exposure to air pollution from the early days of their conception up to delivery. The assessments of pregnancy include physical examinations and opinion of the treating physicians.

: Data Collection

The base line questionnaire was used to collect socio-demographic and lifestyle information by the researcher inside the labour room in the presence of the doctors and nurses. The enrolment was restricted to women volunteers in their singleton pregnancy without any complicity. Women volunteers with pre-medical history of serious chronic diseases were excluded from the study.
Maternal Questionnaire

A maternal questionnaire was prepared with slight modifications and administered to the volunteers at the time of delivery to obtain information about maternal characteristics and other aspects relating to the present study (Polanska et al., 2014). The questionnaire covers socio-demographic characteristics (age, marital status, gravidity, parity (Borton et al., 2013), maternal education and employment), health of parents, environmental exposures (outdoor/indoor), occupational exposures, maternal diets, height, weight, medical treatment, vaccination and duration of sleep during pregnancy. Basic demographic factors such as age, ethnicity, profession, and education of father, annual household income, regular occurrence of menstrual cycle, last menstrual period (LMP), gestational hypertension or gestational diabetes were collected using questionnaires. The study also covers reproductive history, family history of allergies, respiratory and hereditary diseases, place of residence (distance from industries or mines), type and size of residence. The questionnaire also covers whether the expecting mother was exposed to smoke of the cooking fire and burning candles or lamps, passive smoking and alcohol consumption etc.

Outcome Variables:
In addition to the above data pre-pregnancy body mass index (BMI) [weight (kg)/height (m²)] of mothers and neonates were calculated (Polanska et al., 2014).

Sample collection and preparation:
Just after delivery placental tissues were collected from both maternal and fetal side of placenta, and repeatedly washed with 9% saline water and stored at -20°C temperature for further analysis (Basu et al., 2015).

Histopathological studies (Hematoxylene and Eosin staining)

Histopathological study was done by adopting Hematoxylene and Eosin staining procedure (R&D Systems a biotechne brand).

Steps showing protocol for histopathological study

 III. Results:

Sample Characteristics and Level of Air Pollutants in Three Different Areas

In the present study, the level of ambient air pollutants for the year (2015-18) in different study areas of Odisha was collected from The ‘Odisha State Pollution Control Board’ (OSPCB), Bhubaneswar (Figure-1). The air population level of three different study zones was calculated to represent the range of pollutant exposure to heavy traffic congestion areas like Cuttack, industrial areas like Jajpur (Kalinganagar) and rural non-industrial areas like Nilagiri of Balasore districts of Odisha. The sources of air pollution of thickly populated Cuttack zone, industrial areas like Jajpur and rural Nilagiri zones were shown in Table-1(a). The major components of air pollution and the air quality index of three different study zones were shown in Table-1(b).

Percentage of Outdoor and Indoor Exposure to Pregnant Women

The percentage of indoor exposure of pregnant women of Cuttack zone is 87%, Jajpur zone is 90%, and Nilagiri zone is 92%. The outdoor exposure of pregnant women of Cuttack zone is 96%, Jajpur zone is 66% and Nilagiri zone is 35%. The histogram represents the comparison between percentage of outdoor and indoor exposure of pregnant women of three study areas at p <0.05. Significant difference was not found among the indoor exposure of Cuttack, Jajpur and Nilagiri zones. There was significant difference between indoor and outdoor exposure of three study areas at p < 0.05. Significant difference was recorded among outdoor exposure of Cuttack,
Jajpur and Nilagiri zone at p < 0.05. The outdoor exposure of Cuttack zone was significantly higher than that of Jajpur and Nilagiri zone at p < 0.05. The outdoor exposure of Jajpur zone was significantly higher than that of Nilagiri zone at p < 0.05 (Figure-2).

**Percentage of Full-term Birth and Preterm Birth / Preterm Delivery (PTD)**

The percentage of full-term birth of pregnant women of Cuttack zone is 35%, Jajpur zone is 45% and Nilagiri zone is 86%. The preterm delivery of pregnant women of Cuttack zone is 65%, Jajpur zone is 55% and Nilagiri zone is 14%. Significant difference between full term and preterm birth of neonates from Cuttack, Jajpur and Nilagiri zones was recorded at p <0.05. The full-term birth of neonates of Cuttack zone was significantly lower than that of Jajpur and Nilagiri zone at p < 0.05. The full-term birth of neonates of Jajpur zone was significantly lower than that of Nilagiri zone at p < 0.05. The preterm birth of Cuttack zone was significant ly higher than that of Jajpur and Nilagiri zone at p < 0.05 (Figure-3).

Pre-pregnancy BMI of volunteers in Cuttack zone, Jajpur zone and Nilagiri zone was 22.23, 22.14, and 21.0 respectively (Table-1c). The neonatal BMI were 11.62, 13.3, and 14.07 in Cuttack, Jajpur and Nilagiri zone respectively (Table-1c). The percentage of normal mother weight in Cuttack zone was 94%, Jajpur zone 93% and Nilagiri zone 96% (Table-1d). The gravity and parity history of participants was recorded in present study to know the details of the women’s obstetric history (Creinin et al., 2009).

**Histopathological Study of Placental Tissue**

Placenta collected from volunteers of three study regions showed rough and spongy maternal surface. They were dull red in colour and divided into 15-20 cotyledons by septa (Figure-5). Umbilical cord was attached at or near the centre of placenta at fetal surface (Figure-4). Branches of the umbilical vessels were visible beneath the amnion as they radiate from the area of insertion of the umbilical cord to placenta (Figure-4).

It was observed that stem villi sprouts from chorionic plate and characterized by a large number of vessels and micro-vessels. Stem villi branch off to intermediate villi and intermediate to terminal villi (Figure-6). Syncytiotrophoblast is the epithelial covering of the terminal villi and invades the wall of the uterus to establish nutrient circulation between the embryo and the mother. It is multinucleated and has terminally differentiated syncytial knots (Figure-10). Syncytial knot increases with the increase in gestational age and are used to evaluate villous maturity.

The deposition of PM$_{2.5}$ was more in placentas of traffic congestion Cuttack zone than industrial Jajpur and non-industrial Nilagiri zones. The lowest PM$_{2.5}$ deposition was recorded in Nilagiri zone (Figure-7). The thickly populated Cuttack zone showed more Neutrophil granules (NG), Desquamated epithelial tissue (DSET), Damaged tissues (DT), Nacreotic cells (NC), Thrombus (T) than the industrial Jajpur zone and rural Nilagiri zone. Jajpur zone showed more NG (Figure-8), DSET (Figure-11), DT (Figure-14), NC (Figure-15), and T (Figure-16) than Nilagiri zone. The number of villi (V), recorded in thickly populated Cuttack zone was less than that of other zones. Industrial area Jajpur zone showed less number of V (Figure-9) than non-industrial area Nilagiri zone.

The wall of artery and vein of chorionic tissue (Figure-17, 18) and umbilical cord (Figure-19, 20) are composed of three layers. The inner most layer is known as tunica intima, the middle layer is tunica media and the outermost layer is tunica adventitia. Warton’s Jelly (WJ) envelops artery and vein of umbilical cord. Amnion nodosum (AN) of placenta usually appears at the point of intersection of cord (Figure-13). Amniotic membrane consists of epithelial villi, epithelial cells, basement membrane, compact layer, fibroblast layer, spongy layer (Figure-21).

**IV. Discussion**

The pregnant women of our study areas had normal health as their pre-pregnancy BMI ranged from 21.0 to 22.33 (healthy range is 18.5-25 and 25-30 is overweight). Being too thin or overweight can affect fertility and neonate’s health (Chang T et al., 2017). Exposure of pregnant women to AAP is causally related to adverse pregnancy outcomes. Air pollutants may be absorbed into the body (DT), Nacrotic cells (NC), Thrombus (T) than the industrial areas of our study areas had normal health as their pre-pregnancy BMI ranged from 21.0 to 22.33 (healthy range is 18.5-25 and 25-30 is overweight). Being too thin or overweight can affect fertility and neonate’s health (Chang T et al., 2017). Exposure of pregnant women to AAP is causally related to adverse pregnancy outcomes. Air pollutants may be absorbed into the body through air, water, soil, and food. These pollutants include particulate matter (PM), heavy metals, volatile organic compounds (VOCs), and nitrogen oxides (NOx). Exposure to these pollutants can lead to adverse health effects, including respiratory and cardiovascular problems, neurological damage, and reproductive outcomes. For example, exposure to PM$_{2.5}$, an ambient air particulate composed of nitrates, Black Carbon (BC), Organic Carbon (OC), soot of sulfate, and transition metals. Black carbon is generated directly from vehicular combustion. Organic carbons are emitted both from primary and secondary chemical reactions of gaseous organic precursors e.g. PAH (Vilcassim et al., 2014). Recent studies have demonstrated that vehicular emission particles were more strongly associated with cardiovascular problems than secondary coal-burning particles (Zanobetti et al., 2006; Kloog et al., 2015).

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Vehicular emissions, active and passive cigarette smoking are related to oxidative stress and inflammation in human body. During exposure to household air pollutant specifically when the level of PM$_{2.5}$ was high in atmosphere, the placenta would suffer damage by circulating particulate matters, which cause systemic inflammation. This may result in placental chronic inflammatory lesions such as chronic-villitis, chronic chorioamnionitis, or intervillositis (Katzman et al., 2015). The hypoxic lesions were reported in smoke-exposed placentas particularly when CO levels increase. The level of carboxyhemoglobin increases by displacing oxygen from hemoglobin and reduces oxygen availability to the foetus (Soothill et al., 1996). In an air pollutant exposed placenta, PM$_{2.5}$-mediated inflammation may play a major role for oxidative stress. Due to its small size, PM$_{2.5}$ can reach the alveoli. These small airborne particles can be eliminated by bronchial epithelial cells, but still some amount of PM$_{2.5}$ remain and subsequently pass into the blood stream and reached placental tissue. The placental tissue of pregnant women from Cuttack zone showed more PM$_{2.5}$ deposition than that of Jajpur and Nilagiri zones. Placental tissue of postpartum from Nilagiri zone showed least amount of PM$_{2.5}$ deposition.

PM$_{2.5}$ is recognized as foreign matter for which the local immune response is activated, and pro-inflammatory cytokines are released by NG (Brown et al., 2001). Cytokines causes inflammation in placenta which results in placental chorioamnionitis. Release of NG is the sign of acute infection and inflammation. NG has contribution to atherosclerosis and cardio vascular disease. In the present study more NG were observed in placental tissues of postpartum of thickly populated Cuttack zone than that of Jajpur and Nilagiri zones. The placental tissues of postpartum obtained from rural Nilagiri zone showed least number of NG. The chorioamnionitis is related to preterm birth, cognitive impairment and death and neurodevelopmental impairment in preterm infants (Brown et al., 2001). The preterm delivery of pregnant women of Cuttack, Jajpur, Nilagiri zone is 65%, 55% and 14% respectively. Exposure to PM$_{2.5}$ is known to cause cardiovascular (Shah et al., 2013) and chronic pulmonary diseases (Zanobetti et al., 2009). Epidemiological evidence suggests that ambient air pollution has adverse effect on maternal and fetal development. A population-based cohort study investigated that exposure to high levels of PM$_{2.5}$ during the third trimester of pregnancy was associated with 42% increased risk of stillbirth (De Franco et al., 2015). Cardiopulmonary researchers have demonstrated that inhaled ultrafine particles can enter the placental circulation and cause systemic inflammation (Donaldson et al. 2005; Scapellato et al., 2007). The chorioamnionitis, amnionitis, and vessel thrombus were observed in rats exposed to PM$_{2.5}$. This may occur due to intrauterine inflammatory condition (Conti et al., 2015). Those may be the result of failure of the placenta to nourish the fetus and can cause pregnancy complications such as preterm birth (Pappas et al., 2014), neonatal cerebral palsy and fetal growth restriction (Williams et al., 2000). There are 3 potential mechanisms of PM$_{2.5}$ exposure proposed by the previous studies such as high oxidative stress (Dellinger et al., 2001), hypercoagulability (Pekkanen et al., 2000), and inflammation (Brook et al., 2010). The present study reported that more chronic-villitis, chronic chorioamnionitis were observed in placental tissues of postpartum of Cuttack zone than that of Jajpur and Nilagiri zones. Placentas obtained from Nilagiri zone showed least number of chronic-villitis and chronic chorioamnionitis.

Oxidative stress results in desquamated epithelial tissues (DSET) in amniotic membrane. More DSET were reported in AM of placenta obtained from heavy traffic area Cuttack zone than Jajpur and Nilagiri zones. The remote non-industrial area Nilagiri zone showed least DSET in amniotic membrane of placental tissue. Amnion nodosum (AN) of placenta usually appears at the point of intersection of cord. It is composed of striated epithelial cells. The exact cause of AN of placenta is not known. It may occur due to deficiency of amniotic fluid. The presence of AN may lead to incomplete development of fetal lungs, fetal renal agencies (absence of one or both kidneys) and still birth. In the present study AN were clearly visible. Placenta exposed to PM$_{2.5}$ showed amnionitis with loss of papilae, resulting in decreases of surface area. This may influence placental transport capacity and cause reduction of oxygen and nutrient supply to uterus. This could explain the high loss of trophoblast cells and results in damaged tissue (DT) in placenta. More DT was reported in placental tissues obtained from thickly populated Cuttack zone than Jajpur and Nilagiri zones. Placental tissues of postpartum from Jajpur zone showed more DT than that of Nilagiri zone. PM$_{2.5}$-exposed placenta showed amnionitis with loss of papilae/villi, which decreases the surface area. It may influence placental transport capacity and reduces oxygen and nutrient supply to trophoblast cells of placenta. It consequently results in the high loss of trophoblast cells and causes premature death of cells or cell necrosis. Large numbers of necrotic cells (NC) were observed in thickly polluted Cuttack zone. Jajpur zone showed more necrotic cells than that of rural Nilagiri zone. The increase of 10µg m$^{-3}$ PM$_{2.5}$ exposure was associated with short-term, 6.98% increase in long-term and 0.63% increase in deep vein thrombosis (DVT) (Kloog et al., 2015). Hypercoagulable state was found in our study. High platelet count may affect blood coagulation. The circulating PM$_{2.5}$ may cause inflammation and consequently blood coagulation. PM$_{2.5}$ exposure resulted in thrombosis in capillaries of placental tissue. Thrombi were seen in most of the placenta of post-partum from heavy traffic congestion area Cuttack zone. Less number of thrombus were reported in capillaries of placenta of postpartum from non-industrial Nilagiri zone than that of industrial Jajpur zone. The presence of thrombus/vascular block reduces the surface area for the transfer of oxygen from mother to fetus and may contribute to placental dysfunction. Such changes in placental morphology and function may lead to reduced fetal growth. Chorionic fluid, amniotic fluid and fetal vessels transport nutrient and oxygen to fetus from mother (Toda et al., 2007). The thickness of AM ranges from 0.02 to 0.5 mm. It consists of three main histological layers such as the epithelial layer, the thick basement membrane and the vascular mesenchymal tissue (Benirschke et al., 2006). Microvilli are present at the apical surface of the amniotic epithelial cells. Epithelial microvilli probably have an active secretory and intra and trans-cellular transport functions (Pollard et al., 1976). In the present study cellular layers and microvilli of AM were clearly visible. AM surrounds the embryo/fetus and delimits the amniotic cavity, which is filled by amniotic fluid (Herendael et al., 1978). The placenta acts as a bridge between mother and fetus. Fetal nutrient and oxygen supply, waste removal, and protection from xenobiotics rely occurs through maternal-fetal circulation. Placental inflammation may also play an important role in placental impairment that leads to short-term and long-term complications in pregnancy.
V. Conclusion:
The present study provides evidence that the exposure of pregnant women to ambient air particulate pollutants adversely affects the growth and development of the fetus. Exposure to particulate pollutants like PAH may induce oxidative stress in the pregnant mother, reduced birth weight or low birth weight. It also showed reduced body mass index, placental weight and head circumference of the newborn baby, which may be due to the significantly increased lipid peroxidation by production of reactive oxygen species. Ambient air exposure is associated with an increased serum homocysteine concentration in maternal and cord blood as a biomarker of exposure effects. Thus, exposure of air pollutants during pregnancy should be avoided for substantial health benefits of newborns and their subsequent neural development.

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VII. Conflict of Interest
The authors declare that they have no conflict of interests regarding the publication of this paper.

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Figures:

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Figure-20: Hematoxylene and eosin (H&E) stain of vein of umbilical cord (VC). C.S. of the vein of umbilical cord. L – Lumen, TI – Tunica intima, TM – Tunica media, TA – Tunica adventitia, WJ – Wartson’s Jelly

Figure-21: Hematoxylene and eosin (H&E) stain of Amniotic membrane showing EV – Epithelial Villi, E – Epithelial cells, BM – Basement membrane, CL – Compact Layer, FbL – Fibroblast Layer, SpL – Spongy Layer.

**Figure Legend**

Figure-1: Histogram represents different air pollutants level of three distinct areas as Cuttack zone, Jajpur zone and Nilagiri zone during (2015 - 2018).

Figure-2: Histogram represents percentage of outdoor and indoor exposure of three study areas (mean ± SEM). Indoor (a) and outdoor (b) exposure of Cuttack zone, indoor (c) outdoor (d) exposure of Jajpur zone, indoor exposure of Nilagiri zone (e) was compared.( p < 0.05).

Figure-3: Histogram represents percentage of full term and preterm birth of three study areas (mean ± SEM). Full term (a) and preterm baby (b) of Cuttack zone, full term (c) and preterm baby (d) of Jajpur zone and full term baby (e) of Nilagiri zone were compared (p < 0.05).

Figure-4: Foetal surface of placenta showing A-Artery, V-Vein

Figure-5: Maternal surface of placenta showing A-Artery, V-Vein

Figure-6: Hematoxylene and eosin (H&E) stain of placental tissue showing Ch P – Chorionic plate, IVS – Intervillous space, SV –
Figure-7: Hematoxyline and eosin (H&E) stained of placental tissue showing more PM$_{2.5}$ deposition in Cuttack zone (A) than Jajpur (B) and Nilagiri zone (C). Nilagiri zone showed least deposition. Magnification at 40x.

Figure-8: Hematoxyline and eosin (H&E) stain of placental tissue. (A) C.S. of placental tissue from Cuttack zone showed more Neutrophil granules (NG) than (B) and (C) (C.S. of placent of Jajpur zone and Nilagiri zone respectively).

Figure-9: Hematoxyline and eosin (H&E) stain of placental tissue. (A) C.S. of placental tissue from Cuttack zone showing comparatively less no. of villi (V) than (B) and (C). (B) C.S. of placent of Jajpur zone, showed less no. of villi than (C) Nilagiri zone.

Figure-10: Hematoxyline and eosin (H&E) stain of placental tissue. C.S. of placental tissue Showing SK-Syncitial knots, S-Syncytotrophoblast, C- Cytotrophoblast

Figure-11: Hematoxyline and eosin (H&E) stain of placental tissue. (A) C.S. of placental tissue from Cuttack zone, showing comparatively highest desquamated epithelial tissue (DSET). (B) C.S. of placent of Jajpur zone, showed more desquamated epithelial tissue than (C) Nilagiri zone

Figure-12: Hematoxyline and eosin (H&E) stained placental tissue showed Chorionic plate (Ch P), Amniotic membrane (AM) and Lumen of artery (LA).

Figure-13: Hematoxyline and eosin (H&E) stain of placental tissue of Cuttack zone showing Amnion nodosum (AN).

Figure-14: Hematoxyline and eosin (H&E) stain of placental tissue. (A) C.S. of placental tissue from Cuttack zone, comparatively highest damaged tissue (DT). (B) C.S. of placent of Jajpur zone, showed more damaged tissue than (C) Nilagiri zone.

Figure-15: Hematoxyline and eosin (H&E) stain of placental tissue. (A) C.S. of placental tissue from Cuttack zone showed more necrotic cells (NC) than (B) and (C) C.S. of placent of Jajpur zone and Nilagiri zone respectively.

Figure-16: Hematoxyline and eosin (H&E) stain. (A) C.S. of placental tissue from Cuttack zone showed more no. of Thrombus (T) than (B) and (C) (C.S. of placent of Jajpur zone and Nilagiri zone respectively).

Figure-17: Hematoxyline and eosin (H&E) stain of placental tissue of Chorionic plate (Ch P). C.S. of artery. LA – Lumen of artery, TI – Tunica intima, TM – Tunica media, TA – Tunica adventitia.

Figure-18: Hematoxyline and eosin (H&E) stain of placental tissue of Chorionic plate (Ch P). C.S. of Vein. LV – Lumen of vein, TI – Tunica intima, TM – Tunica media, TA – Tunica adventitia.


Figure-21: Hematoxyline and eosin (H&E) stain of Amniotic membrane showing EV – Epithelial Villi, E – Epithelial cells, BM – Basement membrane, CL – Compact Layer, FbL – Fibroblast Layer, SpL – Spongy Layer.
Table:

**Table-1(a): Sources of air pollution of different study areas in Odisha**

<table>
<thead>
<tr>
<th>AREA</th>
<th>Sample size</th>
<th>Source of pollution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cuttack zone</td>
<td>127</td>
<td>Thickly populated, Heavy traffic congestion, Indoor pollution (fire wood)</td>
</tr>
<tr>
<td>Jajpur zone (Kalinganagar)</td>
<td>122</td>
<td>Industrial area, Steel hub of Asia, Indoor pollution (fire wood)</td>
</tr>
<tr>
<td>Nilagiri zone</td>
<td>124</td>
<td>Remote rural area, Indoor pollution (fire wood)</td>
</tr>
</tbody>
</table>

**Table-1 (b): Types of pollutants measured during January 2015 to March 2018**

<table>
<thead>
<tr>
<th>Area</th>
<th>SO₂ (µg/m²)</th>
<th>NOₓ (µg/m²)</th>
<th>PM₂.₅ (µg/m²)</th>
<th>PM₁₀ (µg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cuttack zone</td>
<td>4.16</td>
<td>31</td>
<td>94</td>
<td>114.33</td>
</tr>
<tr>
<td>Jajpur zone</td>
<td>3.4</td>
<td>20</td>
<td>71</td>
<td>101.66</td>
</tr>
<tr>
<td>Nilagiri zone</td>
<td>2.5</td>
<td>12</td>
<td>52</td>
<td>83</td>
</tr>
</tbody>
</table>

**Table-1 (c): Maternal characteristics with neonatal BMI**

<table>
<thead>
<tr>
<th>Index</th>
<th>Cuttack zone</th>
<th>Jajpur zone</th>
<th>Nilagiri zone</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age</td>
<td>25.72±1.05</td>
<td>24.41±0.75</td>
<td>22.70±0.78</td>
<td>3.0</td>
<td>0.53</td>
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<tr>
<td>Pre-pregnancy BMI (kg/m²)</td>
<td>22.23±1.17</td>
<td>22.14±0.59</td>
<td>21. ±0.38</td>
<td>0.05</td>
<td>0.95</td>
</tr>
<tr>
<td>Neonatal BMI (kg/m²)</td>
<td>11.62 ± 0.31</td>
<td>13.35 ± 0.41</td>
<td>14.07 ± 0.56</td>
<td>12.54</td>
<td>0.06</td>
</tr>
</tbody>
</table>

**Table-1 (d): Percentage of Mother Weight**

<table>
<thead>
<tr>
<th>Area</th>
<th>Age</th>
<th>Under weight</th>
<th>Normal weight</th>
<th>Over weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cuttack zone</td>
<td>16-39</td>
<td>2%</td>
<td>94%</td>
<td>4%</td>
</tr>
<tr>
<td>Jajpur zone</td>
<td>19-37</td>
<td>4%</td>
<td>93%</td>
<td>3%</td>
</tr>
<tr>
<td>Nilagiri zone</td>
<td>19-37</td>
<td>2%</td>
<td>96%</td>
<td>2%</td>
</tr>
</tbody>
</table>