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Association Of Air Pollution With Serum Lactate Dehydrogenase Level In Placenta Of Pregnant Women Of Odisha, India: A Case Study

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Abstract

Background: Exposure of pregnant women to atmospheric pollutants (AP) in both open and closed environments has an adverse effect upon the early development of the foetus leading to preterm delivery, low placental weight, premature deaths, which occur due to oxidative stress.

Objective: The present cross-sectional study was undertaken to investigate the lactate dehydrogenase (LDH) activity in mother blood, placenta and cord blood. Mothers with normal and singleton pregnancy were selected from thickly populated, traffic-congested, industrial and remote rural areas of Odisha, India.

Methods: The Air Quality Index of three study areas was collected from the Odisha State Pollution Control Board (OSPCB), Bhubaneswar. Maternal venous blood was collected prior to and cord blood, placental tissue was collected immediately after delivery. The effect of air pollution on the placental tissue was analysed by histopathological study and photographed by fluorescent microscope. The estimation of LDH level in serum was done by using Boils 24i automated clinical chemistry analyzer.

Results: Significant increase of LDH level in maternal and cord blood of heavy traffic congestion areas and industrial areas was recorded in comparison to rural non-industrial areas. The histopathological study of placenta showed presence of $PM_{2.5}$ and less number of villi in heavy traffic and industrial areas as compared to non-industrial areas (Das P et al., 2022).

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

Key words: Placenta, villi, Lactate dehydrogenase, Air Pollution

I: Introduction

Human placenta provides a unique opportunity to investigate the exposure of pregnant women to ambient air pollution. The frequently studied air pollutants are sulphur dioxide (SO_2), nitrogen dioxide (NO_2), particulate matter ($PM_{2.5}$, PM_{10}) and polycyclic aromatic hydrocarbons (PAHs). $PM_{2.5}$, is composed of nitrates, Black Carbon (BC), Organic Carbon (OC), soot of sulphate, 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). Particulate pollutants enter into placenta through blood stream. Histology of placenta shows three types of cells such as trophoblast cells, cytotrophoblast cells and syncytiotrophoblast cells. The syncytiotrophoblast is present in the epithelium of chorionic villi. It is the site of oxygen exchange between mother and foetus. It is also the site of excretion of toxic metabolites of foetus. Hence syncytiotrophoblast layer has an intense enzyme activity (Reichrtová et al., 1998). Accumulation of ambient air pollutants in the syncytiotrophoblast, may cause hypoxia and results in changes in enzyme activities of cellular energy metabolism (Niweliński et al., 1990). Hypoxic condition causes oxidative stress in placental tissue.

Ambient air pollutants are responsible for free radical production, mitochondrial dysfunction, inflammation (Kelly et al., 2003; Risom et al., 2005) and lead to hypoxia or oxidative stress condition. Oxidative stress causes adverse health effects such as asthma, chronic obstructive pulmonary disorders, decreased lung function (Kelly et al., 2003). During hypoxia condition glucose is metabolized through anaerobic glycolysis. Anaerobic glycolytic pathway is associated with increased activity of lactate dehydrogenase (LDH) and extracellular appearance of LDH. The extracellular appearance of Lactate dehydrogenase (LDH) signifies cell damage, organ disorder in host body (Lott et al., 1987; Matusiewicz et al., 1993). The risk profile is exacerbated in pregnant women exposed to particulate pollutants.

The aim of this study is to determine the impact of environmental pollutants at the place of residence of pregnant women on their cellular energy metabolism and level of oxidative stress. Oxidative stress is associated with the release of lactate dehydrogenase.

1.1: Oxidative stress and lactate dehydrogenase in serum

Lactate dehydrogenase (LDH) is a cytoplasmatic enzyme and essentially present in all major organ systems. The presence of LDH is used as a marker of cell damage or cell death (Lott JA et al., 1987, Moss DW et al., 1986, Glick JH et al., 1969). After cell death it is released in the peripheral blood vessels (Lott JA et al., 1987, Moss DW et al., 1986).

S-Adenosyl-homocysteine accepts one H_2O molecule in the presence of Adenosyl homocysteine hydrolase and releases one adenosine molecule and becomes Homocysteine. The adenosinekinase (AK) phosphorylates adenosine to adenosine mono phosphate (AMP) and then AMP to adenosine diphosphate (ADP). Pyruvatekinase (PK) converts phosphoenolpyruvate to pyruvate and phospholylates ADP to ATP. Lactate dehydrogenase (LDH) reduces the released pyruvate to lactate, using the cofactor NADH as reducing agent in anaerobic condition.

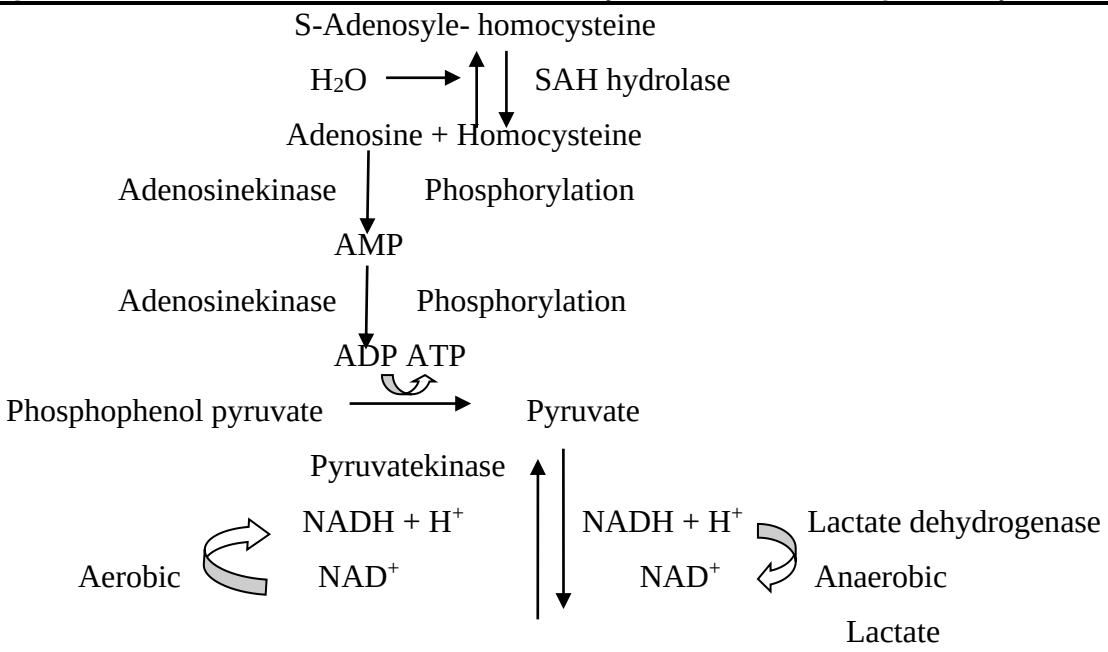
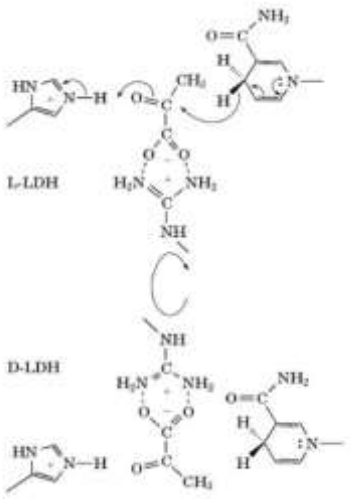


Figure-8: Schematic representation of relationship between homocysteine and LDH.

Lactate dehydrogenase (LDH) exists in two forms such as NAD (P)-dependent L-lactate dehydrogenase and cytochrome-c dependent D-lactate dehydrogenase. It catalyses the conversion of lactate to pyruvate and converts NAD^+ to NADH + H^+ in aerobic condition. It also catalyses the backward reaction i.e. conversion of pyruvate to lactate and $NADH+H^+$ to NAD^+ in anaerobic condition. LDH is an enzyme that transfers a hydride from one molecule to another. Aerobic condition occurs in liver and anaerobic condition in muscle cells.



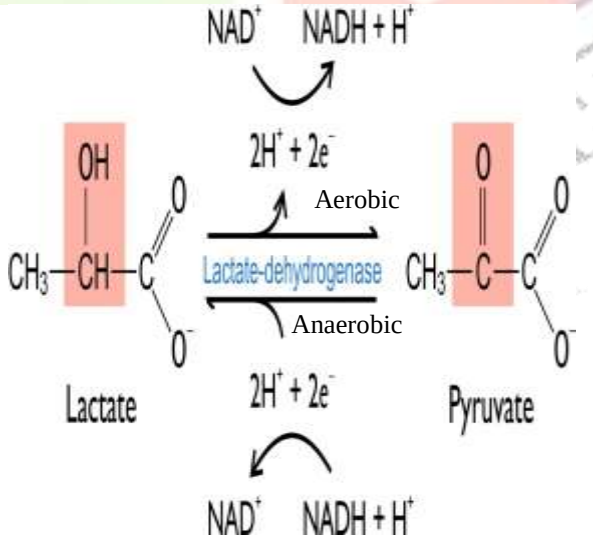


Figure-9: Structure of LDH

Figure-10: Reaction catalysed by LDH

Courtesy: Introduction to Practical Biochemistry by György Hegyi, József Kardos, Mihály Kovács, András Málnási-Csizmadia, László Nyitray, Gábor Pál, László Radnai, Attila Reményi, István Venekei, Chapter-7

In liver two lactate molecules are converted to two pyruvate molecules in the presence of LDH in aerobic condition. Two pyruvate molecules enter into gluconeogenesis along with six ATP molecules and syntheses one glucose molecule. Glucose is carried to muscle cells through blood vessels and enter into

glycolysis pathway. One glucose molecule is broken down to two pyruvate molecules and releases two ATP molecules. LDH catalyses the conversion of two pyruvate molecules to two lactate molecules in anaerobic condition. (Roth et al., 1981) Lactate molecules are carried to liver through blood vessels. In this way lactate shows a cyclic metabolic pathway. LDH exists as 5 isoenzymes as LDH1, LDH2, LDH3, LDH4, LDH5. Isoenzymes or isozymes are multiple forms of same enzyme that catalyses the same chemical reaction. They show different chemical and physical properties as Electrophoretic mobility, kinetic properties, amino acid sequence, amino acid composition and immunological characters. They can be separated from each other by electrophoresis, ion exchange chromatography and immunological methods. Each isoenzyme consists of 4 polypeptide chains which are of 2 sub units: M (muscle-chromosome 11 basic) and H (heart-chromosome 12 acidic). LDH1 is released due to damage in kidney and heart, LDH2 from kidney, heart, brain and RBC, LDH3 from brain, lungs and RBC, LDH4 from lungs and skeletal muscle, LDH5 from skeletal muscle and liver.

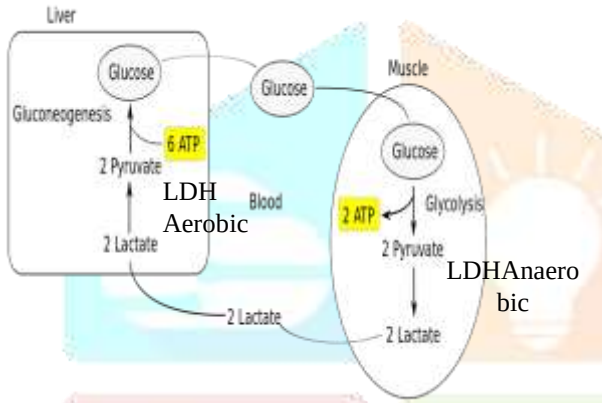


Figure-11: Cyclic metabolic pathway of lactate

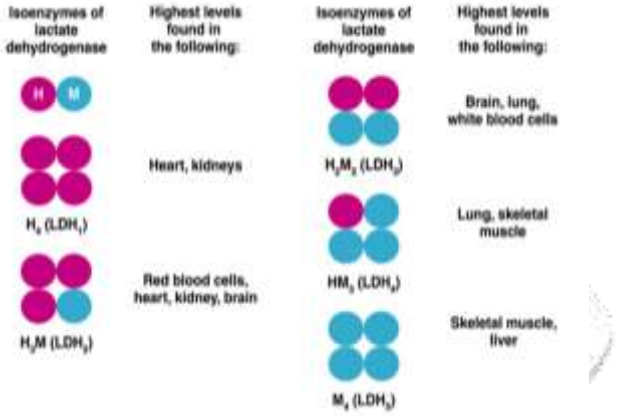


Figure-12: Isoenzymes of LDH

Courtesy: Eventoff et al., 1977

During anaerobic glycolytic pathway pyruvate is converted to lactate in the presence of lactate dehydrogenase (LDH). Air pollution at the place of residence of pregnant mother causes anaerobic condition in placenta due to hypoxia.

II. Martials and Methods:

II.1: Ethics statement

The design and procedure of the experiment were approved by the Institutional Ethical Committee on biomedical research on human subjects of SCB Medical College, Cuttack, Odisha, India in compliance with the guidelines of Indian Council of Medical Research (ICMR) (213/29.1.16). Written consents were obtained from all women volunteer participants.

II.2: Study design

The present cross-sectional study-design includes 270 female volunteers of three distinctly different areas such as Group-1 (Cuttack zone), Group-2 (Jajpur zone) and Group-3 (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 and its peripheral villages that depend upon S.C.B. Medical College, Cuttack. Kalinganagar, regarded as the steel hub of Asia, is situated in the district of Jajpur, Odisha.

Kalinganagar has more than seventeen different steel and other ancillary industries. The non-industrial areas refer to remote tribal-rural areas of Nilagiri in Balasore district of Odisha.

II.3: Selection of study population

Volunteers belong to almost the same socio-economic class. The questionnaires administered to the participant mothers specifically cover all possible aspects of their exposure to air pollution from the early days of their conception to delivery. The assessments of pregnancy include physical examinations and opinion of the treating physicians. The data were collected by the researcher adopting the questionnaire method. The measurements of new born babies like length, weight, head and chest circumference, weight of placenta of postpartum, blood sample from mother and cord blood etc. were personally taken 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 complicacy. Women volunteers those have pre-medical history of serious chronic diseases were excluded from the study.

II.4: Collection and processing of biological samples

II.4 (a): Blood Sample Collection and Preparation

Venous blood (5 ml) was collected from the expected mother just before delivery. Immediately after delivery umbilical cord arterial blood (5 ml) was collected from neonates, after cord separation. Then the blood samples collected in 4ml gel tube (gel and clot activator, 13x75 mm) were put in incubation for a period of 15 minutes at the room temperature (RT-37^{0}C). After 15 minutes the blood samples were centrifuged at 4000 rpm for 5 minutes and serum was collected and stored at -20^{0}C until taken for clinical analysis (Guttormsen et. al., 1994).

II.4 (b): Collection of gestational history by 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, parity, maternal education and employment) and health of parents, environmental exposures (outdoor and indoor), occupational exposures, maternal diets, height, weight, medical treatment, vaccination and duration of sleep during pregnancy etc. The study also covers reproductive history, family history of allergies, respiratory and hereditary diseases, educational qualifications, place of residence (distance from plants or mines), type and size of residences. 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.

II.4 (c): Estimation of Lactate dehydrogenase in blood

Detection Method:

Serum Lactate dehydrogenase level was measured using Boils 24i automated clinical chemistry analyzer.

Clinical Significance:

Lactate dehydrogenase (LDH) is an enzyme with wide tissue distribution in the body. The higher concentrations of LDH are found in liver, heart, kidney, skeletal muscle and erythrocytes. Increased level

of the enzymes is found in serum in case of liver disease, myocardial infarction, renal disease, muscular dystrophy and anaemia (Pesce et al., 1984; Burtis et al., 1999; Tietz et al., 1995).

Principle:

Lactate dehydrogenase (LDH) catalyses the reduction of pyruvate by NADH, according the following reaction:

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{Lactate dehydrogenase}} \text{L-lactate} + NAD^+$$

The rate of decrease in concentration of NADPH, measured photo metrically, is proportional to the catalytic concentration of LDH present in the sample-1.

Reagents

Each LDH kit contains Reagent 1 Buffer Imidazol Pyruvate 65 mmol/L 0.6 mmol/L and Reagent 2 substrate NADH 0.18 mmol/L

Storage Instructions and Reagent Stability

All the components of the kit were used before the expiry date. The kit was stored tightly-closed at 2-8ºC. The kit was protected from light and contaminations during their use.

Reagent Preparation

Working reagent (WR) was prepared by adding 4 vol. (R1) Buffer with 1 vol. (R2) Substrate. The working solution was kept for 15 days at 2-8ºC or 5 days at 15-25ºC to maintain the stability.

Materials required

Materials required for the assay are Spectrophotometer or colorimeter (to measuring at 340 nm), Thermostatic bath (at 25O C, 30O C, 37O C (+0.1OC), Matched Cuvettes 1.0 cm light path and general laboratory equipment.

Specimens

Serum was collected from both maternal and cord blood. Haemolysed samples were not used. Stability of the test serum can be obtained by keeping serum at 2-8ºC for 2 days.

Assay Procedure

Assay conditions: Assay was done by taking 1ml samples in 2ml. cuvette, having 1 cm width and measuring optical density (OD) by spectrophotometer at 340 nm wave length at visible spectrum of light. Constant temperature (25ºC /30ºC / 37ºC) was maintained. First the instrument was adjusted to zero by taking 1ml of with distilled water in cuvette. The blank reading was taken. Then sample was pipetted into the cuvette. Sample was kept for 1minute and allowed to mix. The absorbance (A) of the sample was taken at 1-minute intervals thereafter for 3 minutes. The difference was calculated taking the absorbance and the average absorbance difference per minute (AA/ min.).

Calculation

37ºC A/min x 10756 = U/L LDH

Units: One international unit (IU) is the amount of enzyme that transforms 1 Dmol of substrate per minute, in standard conditions. The concentration is expressed in units per litre of sample (U/L).

Statistics: The results were statistically evaluated by using SPSS-20 software and analysis of variance at a significance level $\alpha = 0.05$

III. Result:

Sample characteristics and Level of Air pollutants in three different areas

The study included data from 270 pregnancies (volunteer participants) out of 365 pregnant mothers between 2023 and 2025, the period for which complete pollution information was available.

In the present study, the level of ambient air pollutants for the year (2023-25) in different study areas of Odisha was collected from 'The Odisha State Pollution Control Board' (OSPCB), Bhubaneswar. Air population level of three different study zones was selected to represent the range of pollutant exposure (heavy traffic congestion areas like Cuttack, industrial areas like Jajpur (Kalinganagar) and rural non-industrial areas like Nilagiri of Balasore districts of Odisha). The major components of air pollution and the air quality index of three different study zones were shown in Table-3. The sources of air pollution of thickly populated Cuttack zone, industrial areas like Jaipur and rural Nilagiri zones were shown in Table-1 and percentage of outdoor and indoor exposure were given in Table-2.

III.1: Serum Lactate dehydrogenase (LDH) level

Serum lactate dehydrogenase level of maternal blood (MB) and cord blood (CB) of participants from Cuttack, Jajpur and Nilagiri zones showed positive correlation at the level ($p<0.01$). Serum LDH level of both maternal and cord blood of participants significantly increased in heavy traffic area like Cuttack zone (MB was 432.40 ± 32.37& CB was 784.65 ± 14.89) and industrial areas like Jajpur zone (MB, 412.18 ± 31.65& CB, 754.35 ± 31.01) in comparison to non-industrial areas like Nilagiri zone (MB, 368.18 ± 10.15& CB, 734.98 ± 26.05). A positive correlation was observed between ambient air particulate pollutants and augmented serum LDH level of maternal blood before delivery and umbilical cord blood of participants from Cuttack, Jajpur and Nilagiri zones at the level ($p < 0.01$).

III.2: Serum Lactate dehydrogenase (LDH) level in mother blood

The LDH level in mother serum of participants of Cuttack zone was significantly higher than that of Jajpur and Nilagiri zone at ($p<0.05$). The LDH level in mother serum of participants of Jajpur zone was significantly higher than that of Nilagiri zone at ($p< 0.05$). The histogram represents LDH level in mother serum of participants of three study zones.(Figure-3).

III.3: Serum Lactate dehydrogenase (LDH) level in cord blood

The LDH level in cord serum of participants of Cuttack zone was significantly higher than that of Jajpur and Nilagiri zone ($p<0.05$). The LDH level in cord serum of participants of Jajpur zone was significantly higher than that of Nilagiri zone ($p<0.05$). The histogram represents LDH level in cord serum of participants of three study areas. (Figure-4).

III.4: Comparison of Lactate dehydrogenase level between mother blood and cord blood

Serum lactate dehydrogenase level of maternal blood (MB) was significantly lower than that of cord blood (CB) of participants from Cuttack, Jajpur and Nilagiri zones at ($p<0.01$). The histogram represents LDH level of mother serum and cord serum of participants of three study areas. The LDH level in mother serum of participants of Cuttack zone was significantly higher than that of Jajpur and Nilagiri zone ($p<0.05$). The LDH level in mother serum of participants of Jajpur zone was significantly higher than that of Nilagiri

zone ($p<0.05$). The LDH level in cord serum of participants of Cuttack zone was significantly higher than that of Jajpur and Nilagiri zone ($p<0.05$). The LDH level in cord serum of participants of Jajpur zone was significantly higher than that of Nilagiri zone ($p<0.05$)(Figure-5).

A positive correlation was recorded between the lactate dehydrogenase levels between mother blood and $PM_{2.5}$. (Table-4), umbilical cord blood and $PM_{2.5}$. (Table-5) also between maternal blood and cord blood (Table-6).

IV: Discussion

Most of environmental toxicants are female reproductive toxicants and able to cross placental and blood brain barrier (Karwacka et al., 2017). Female reproductive toxicants can alter structure and function of many reproductive organs such as ovary, uterus, breast and brain during embryogenesis, puberty and reproductive cycle. As a result of which prenatal development is susceptible to environmental toxic exposure (ETE) (Ingram et al., 1986). Increased environmental toxic smoke (ETS) induces oxidative stress. Increased oxidative stress elevates lactate dehydrogenase level in serum (Abarikwu et al.,2018). Its presence in serum indicates tissue/cellular damage due to breakdown of mitochondria. Normal range of LDH in serum is 105 to 333 IU/L (international units per litre). Tissue damage leads to various diseases, circulatory problems, respiratory disorder, cancer etc. A particular type of LDH isoenzyme conforms a specific organ's tissues damage. Medical professionals diagnose the type of damaged tissue and organ by LDH secretion. Venous lactate concentration increases with gestational age (Soothill et al., 1986). Advancing gestational age causes increase of lactate concentration in arterial and venous umbilical cord plasma. Under normal conditions, the foetus maintains a steady state of pyruvate and lactate, higher than that of causes various disorders in the mother and foetus (Rooth et al., 1988; Suidan et al., 1984). Vaginal delivery induces a foetal catecholamine surge (Hagnevik et al., 1984). Positive correlation was reported between catecholamine surge and lactate levels in normal vaginal deliveries (Nordstrom et al., 1996). Exposure to ambient PM induces systemic and local release of catecholamine (Li et al., 2017). Oxidative stress, caused due to exposure of PM induces catecholamine liberation. Catecholamine liberation increases anaerobic metabolism (Larivée et al., 1990). Conversion of pyruvate to lactate occurs in anaerobic condition in presence of LDH. Hence enzyme activities (LDH) and oxidative stress parameters show significant correlation with each other.

A particular type of LDH isoenzyme conforms a specific type of tissues damage. LDH is a biomarker of oxidative stress. Health condition of patient can be assessed by measuring type and level or concentration of LDH. Measure of LDH can be useful in future studies aiming to identify various environmental pollutants. LDH is abundant in red blood cells and can function as a marker for haemolysis (Bergen et al., 2012; Prinzinger et al., 2010). The blood of chicks from the polluted environment shows high LDH concentration. The heavily polluted industrial areas showed an increased content of lead and mercury in the placental samples (Reichrtová et al., 1995). It was hypothesized that fine metal particles deposited in the placental tissue might be phagocytised by the syncytiotrophoblast, contributing to the decreased oxygen level in placental tissue. The phagocytic activity of syncytiotrophoblast is associated with a high oxygen demand. Decreased oxygen levels in placental tissue may affect placental glucose metabolism

leading to increased LDH activity. Lower oxygen tensions are associated with higher lactate dehydrogenase activity in placental villous tissue (Kay et al., 1997). Deterioration of oxidative phosphorylation may lead to a shift towards anaerobic glycolysis, linked to increased LDH activity (Kay et al., 1997). The results of our study suggest that the type of environmental pollution at the place of residence of pregnant women and their smoking habits affect LDH activity in the placenta. Increased LDH activity might reflect a shift from oxidative phosphorylation to anaerobic glycolysis as an adaptation to hypoxic conditions in placental tissue resulting from accumulation of heavy metals and toxic compounds of tobacco smoke.

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). The deposition of $PM_{2.5}$ in placental tissues causes oxidative stress. Increased oxidative stress elevates lactate dehydrogenase level in serum (Abarikwu et al.,2018). The deposition of $PM_{2.5}$ was more in placental tissues of traffic congestion Cuttack zone than industrial Jajpur and non-industrial Nilagiri zones. The lowest $PM_{2.5}$ deposition was recorded in Nilagiri zone (Das P, Das L, Pari M et al.,2022). More desquamated epithelial tissues (DSET) were reported in amniotic membrane (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. More desquamated epithelial tissues (DSET) were recorded in amniotic membrane of placenta obtained from Cuttack Zone due to more deposition of $PM_{2.5}$ and oxidative stress (Das P, Das L, Pari M et al.,2022). It was recorded that cigarette smoke is associated with the development of mitochondrial cardiomyopathy, deterioration of oxidative phosphorylation and mitochondrial respiratory chain in rabbits (Gvozdjáková et al. 1999). Decrease of oxidative phosphorylation may lead to anaerobic glycolysis, which is linked to increased LDH activity (Prinz and Schuhmann 1980, Kay et al. 1997).

In the present study, it was also observed that cord blood serum contains more LDH concentration than maternal blood serum (Figure-5). Higher LDH activity was reported in serum of pregnant women from Cuttack zone in comparison with the pregnant women from Jajpur and Nilagiri zone. The pregnant women from Jajpur zone showed significantly higher LDH level than that of Nilagiri zone (Figure-3&4). The present study showed a positive correlation between ambient air particulate pollutants ($PM_{2.5}$) and augmented LDH level in mother blood (0.207) and cord blood (0.176). Again, a positive correlation was recorded between serum LDH level in mother blood and cord blood (0.481).

V: Conclusion

The results of our study suggest that environmental pollution at the place of residence of pregnant women affect LDH activity in the placenta. Oxidative stress in placenta caused increased LDH activity, which might reflect a shift from oxidative phosphorylation to anaerobic glycolysis. It is an adaptation to hypoxic conditions in placental tissue resulting from accumulation particulate maters. Ambient air exposure is associated with an increased serum LDH concentration in maternal and cord blood as a biomarker of exposure effects. Hence exposure of pregnant women to air pollutants should be avoided for substantial health benefits of newborns and their subsequent neural development.

VI: Acknowledgements

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VII. Conflict of Interest

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

Reference:

Abarikwu SO, Njoku RC, Onuah CL. Aged coconut oil with a high peroxide induces oxidative stress and tissue damage in mercury-treated rats. J Basic Clin Physiol Pharmacol. 2018; pii 2016-0138.

Bergen NE, Jaddoe VW, Timmermans S, Hofman A, Lindemans J, Russcher H. Homocysteine outcomes: the Generation R Study. BJOG. 2012;119(6):739-51.

Das P, Lucy D, Manoroma P. Association of air pollution with placental pathology in pregnant women. IJCRT. 2022; Oct 10. vol-10: d142-166.

Eventoff W, Rossmann MG, Taylor SS, Torff HF, Meyer H, Keil W, Kiltz HH. Stractural adaptation of lactate dehydrogenase isozymes. Proc Natl Acad Sci USA.1977;74(7): 2677-81.

Glick JH. Serum lactate dehydrogenase isoenzyme and total lactate dehydrogenase values in health and disease, and clincal evaluation of these tests by means of dis criminant analysis. Am J Clin Pathol 1969; 52: 320–328.

Guttormsen AB, Schneede J, Fiskerstand T, Ueland PM, Refsum HM. Plasma concentration of homocysteine and other aminothiol compounds are related to food intake in healthy human subjects. J Nutr. 1994;24(10):1934-41.

Gvozdjáková A, Imko F, Kucharská J, Braunová Z., Penek P, Kyselovič J. Captopril increased mitochondrial coenzyme Q10 level, improved respiratory chain function and energy production in the left ventricle in rabbits with smoke mitochondrial cardiomyopathy. BioFactors 10: 61-65, 1999.

Hagnevik K, Faxelius G, Irestedt L, Lagercrantz H, Lundell B, Persson B. Catecholamine surge and metabolic adaptation in newborn after vaginal delivery and caesarean section. Acta Paediatr Scand.1984;73(5):602-9.

Ingram DD, Makuc D, Kleinman JC. National and state trends in the use of prenatal care, 1970-83. Am J Public Health.1986; 76(4):415-23.

Karwacka A, Zamkowska D, Radwan M, Jurewicz J. Exposure to modern, widespread environmental endocrine disrupting chemicals and their effects on the reproductive potential of women: an overview of current epidemiological evidence. Hum Fertil (Camb). 2017; 1-24.

Kay HH, Robinette B, Shin YY, Siew P, Shellhaas CS, Tyrey L. Placental villous glucose metabolism and hormone release respond to varying oxygen tensions. J Soc Gynecol Investing. 1997; 4(5):241-6.

Kelly FJ. Oxidative stress: its role in air pollution and adverse health effects. Occup Environ Med. 2003 60(8):612-6.

Kloog I, Zanobetti A, Nordio F, Coull BA, Baccarelli AA, Schwartz J. Effects of airborne fine

particles ($PM_{2.5}$) on deep vein thrombosis admissions in the northeastern United States. J ThrombHaemost. 2015;13(5):768-74.

Larivée P, Cantin A, Dufresne A, Begin R. Enzyme activities of lung lavage in silicosis. Lung. 1990; 168(3):151-8.

Li H, Cai J, Chen R, Zhao Z, Ying Z, Wang L, Chen J, Hao K, Kinney PL, Chen H. Particulate Matter Exposure and Stress Hormone Levels: A Randomized, Double Blind, Crossover Trial of Air Purification. Circulation. 2017; 136(7): 618-627.

Lott JA, Nemensanszky E. Lactate dehydrogenase. In: Lott JA, Wolf PL, eds. Clinical Enzymology, a Case oriented Approach. 1987; pp. 213–244.

Matusiewicz SP, Williamson IJ, Sime PJ Plasma lactate dehydrogenase: a marker of disease activity in cytoplasmic fibrosing alveolitis and extrinsic allergic alveolitis Eur Repair J1993;6:1282-6.

Moss DW, Henderson AR. Enzymes. In: Burtis CA, Ashwood ER, eds. Tietz Textbook of Clinical Chemistry. 2nd edn. Philadelphia, Saunders Co., 1986; pp. 735–896.

Niweliński J, Zamorska L, Kaczmarski F, Pawlicki R: Enzyme histochemistry and microstructure of the human placenta as indicators of environmental pollution. ArchiwumOchronyrodowiska 3:53-59, 1990.

Nordstrom L, Marcus C, Persson B, Shimojo N, Westgren M, Lactate in cord blood and its relationship to pH and catecholamines in spontaneous vaginal deliveries. Early Hum Dev. 1996; 46(1-2):97-104.

Polanska K, Dettbarn G, Jurewicz J, Sobala W, Magnus P, Seidel A. Effect of prenatal polycyclic aromatic hydrocarbons exposures on birth outcome the Polish mother and children cohort study. Biomed Res Int. 2014;214-39.

Prinzinger R, Misovic A. Age-correlation of blood values in the rock pigeon (Columba livia). Comp BiochemPhysiolA Mol Integr Physiol. 2010; 156(3):351-6.

Prinz W, Schuhmann RA. Veränderungenplazentarer Enzyme unterSauerstoffmangel. ZentralblGynäkol 102: 542-549, 1980.

Reichrtová E, Bencko V. Exposure and impact assessment of emission from mercury recycling using domestic rabbits. Cent Eur J Public Health. 1995;3(1):42-7.

Reichrtová E, Dorociak F, Palkovičová Ľ. Sites of lead and nickel accumulation in the placental tissue. Hum Exp Toxicol. 1998; 17: 176-181.

Risom L, Moller P, Loft S. Oxidative stress-induced DNA damage by particulate air pollution. Mutat Res. 2005; 592(1-2): 119-37.

Rooth P, Dawidson I, Clothier N, Diller K. In vivo fluorescence microscopy of kidney subcapsular blood flow in mice. Effects of cyclosporine, (NVA2)-cyclosporine, and isradipine, a new calcium antagonist. Transplantation. 1988; 46(4):566-9.

Suidan JS, Wasserman JF, Young BK. Placental contribution to lactate production by the human fetoplacental unit. Am J Perinatol. 1984;1(4): 306-9.

Soothill PW, Morafa W, Ayida GA, Rodeck CH. Maternal smoking and fetal carboxyhaemoblobin and blood gas levels. Br J Obste Gynaecol.1986; 103(1):78-82.

Vilcassim MJ, Thurston GD, Peltier RE, Gordon T. Black carbon and particulate matter (PM2.5)

concentrations in New York City's subway stations. Environ Sci Technol. 2014 Dec 16;48(24):14738-45.

Zanobetti A, Schwartz J. Air pollution and emergency admission in Boston, MA. J Epidermiol Community Health 2006;60 :890-895.

Zanobetti A, Schwartz J. The effect of fine and coarse particulate air pollution on mortality: A national analysis. Evron Health Prospect.2009; 117:898-903.

Tables:

Table-1: Sources of air pollution of different study areas in Odisha

AREA	Sample size	Source of pollution
Cuttack zone	100	Thickly populated, Heavy traffic congestion, Indoor pollution (fire wood)
Jajpur zone (Kalinganagar)	80	Industrial area, Steel hub of Asia, Indoor pollution (fire wood)
Nilagiri zone	90	Remote rural area, Indoor pollution (fire wood)

Table-2: Percentage of outdoor and indoor exposure

Area	Outdoor Exposure	Indoor Exposure
Cuttack Zone	98	65
Jajpur Zone	77	70
Nilagiri Zone	40	80

Table - 3: Types of air pollutants measured during the year 2023-2025 in different areas (AQI in $\mu g/m^3$)

Area	SO_2	NO_X	$PM_{2.5}$	PM_{10}
Cuttack zone	5.5	38	150	180
Jajpur zone	4.0	30	145	165
Nilagiri zone	3	18	84	95

Table – 4: Correlations between LDH-MB and $PM_{2.5}$

Correlations between LDH-MB and $PM_{2.5}$			
		LDH-MB	PM2.5
LDH-MB	Pearson Correlation	1	.207
	Sig. (2-tailed)		.058
	N	270	270
$PM_{2.5}$	Pearson Correlation	.207	1
	Sig. (2-tailed)	.058	
	N	270	270

**. Correlation is significant at the 0.01 level (2-tailed).

Table – 5: Correlations between LDH-CB and $PM_{2.5}$

Correlation between LDH-CB and $PM_{2.5}$			
		LDH-CB	$PM_{2.5}$
LDH-CB	Pearson Correlation	1	.176
	Sig. (2-tailed)		.106
	N	270	270
$PM_{2.5}$	Pearson Correlation	.176	1
	Sig. (2-tailed)	.106	
	N	270	270

**. Correlation is significant at the 0.01 level (2-tailed).

Table – 6: Correlations between LDH-MB and LDH-CB

Correlation between LDH-MB and LDH-CB			
		LDH-MB	LDH-CB
LDH-MB	Pearson Correlation	1	.481**
	Sig. (2-tailed)		.000
	N	373	373
LDH-CB	Pearson Correlation	.481**	1
	Sig. (2-tailed)	.000	
	N	373	373

**. Correlation is significant at the 0.01 level (2-tailed).

Figures:

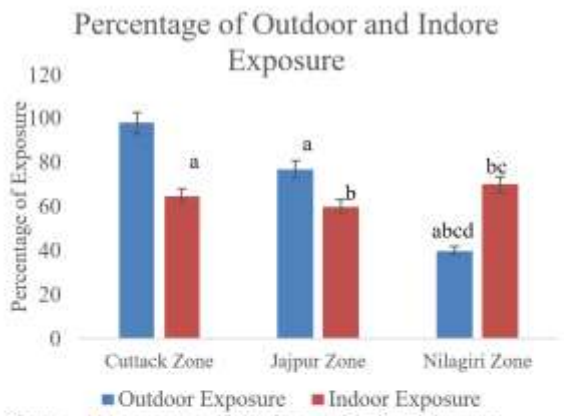

Figure – 1: Percentage of Outdoor and Indore Exposure

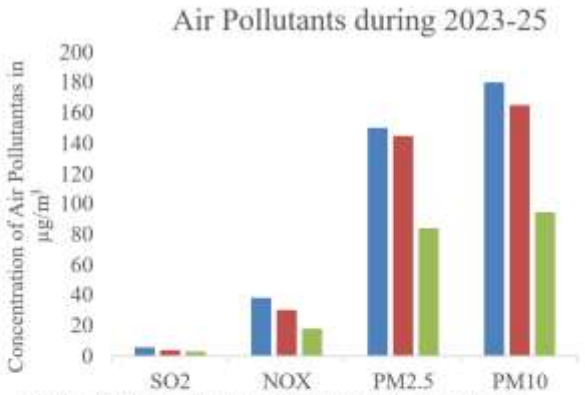

Figure-2: Types of air pollutants measured during the year 2023- 2025 in different areas (AQI in $\mu g/m^3$)

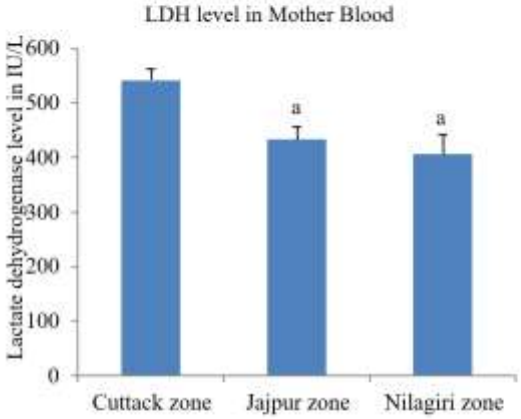

Figure-3: Histogram represents LDH level in mother serum of participants of three study zones (mean ± SEM). LDH level in mother serum of participants of Cuttack zone (a).($p < 0.05$)

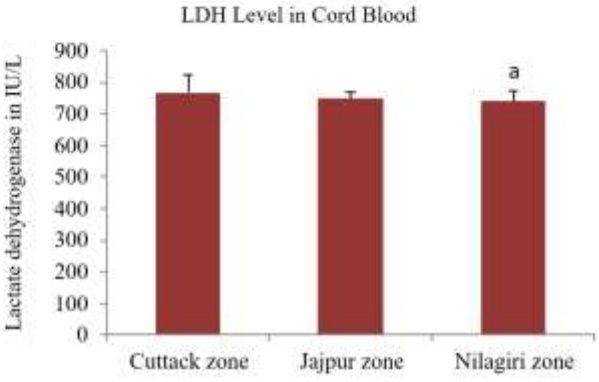

Figure- 4: Histogram represents LDH level in cord serum of participants of three study areas (mean ± SEM). LDH level in cord serum of participants of Cuttack zone (a). ($p < 0.05$)

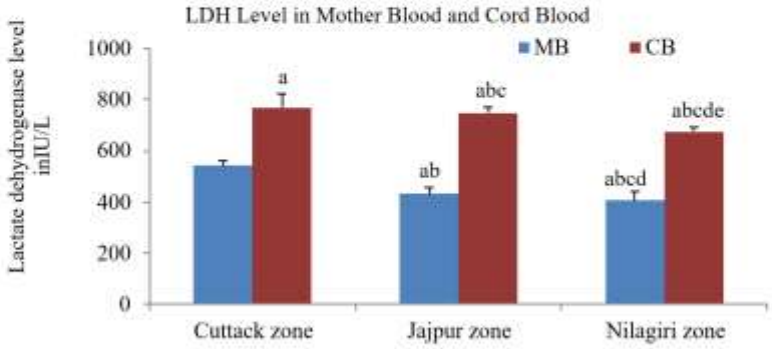

Figure-5: Histogram represents Lactate dehydrogenase (LDH) level of mother serum and cord serum of participants of three study areas (mean ± SEM). LDH level of mother blood (a) and cord blood (b) of Cuttack zone. LDH level of mother blood (c) and cord blood (d) of Jajpur zone. LDH level of mother blood (e) of Nilagiri zone. In each ($p < 0.05$).

Figure Legend

Figure-1: 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-2: Histogram represents different air pollutants level of three distinct areas as Cuttack zone, Jajpur zone and Nilagiri zone during (2023 - 2025). Air Quality Index (AQI) in $\mu g/m^3$.

Figure-3: Histogram represents LDH level in mother serum of participants of three study zones (mean ± SEM). LDH level in mother serum of participants of Cuttack zone (a).($p < 0.05$)

Figure- 4: Histogram represents LDH level in cord serum of participants of three study areas (mean ± SEM).
LDH level in cord serum of participants of Cuttack zone (a). ($p < 0.05$)

Figure-5: Histogram represents Lactate dehydrogenase (LDH) level of mother serum and cord serum of participants of three study areas (mean ± SEM). LDH level of mother blood (a) and cord blood (b) of Cuttack zone. LDH level of mother blood (c) and cord blood (d) of Jajpur zone. LDH level of mother blood (e) of Nilagiri zone. In each ($p < 0.05$).