Article

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Oxidative Stress in the Early Neonatal Period as a Possible Effect of BMI, Smoking Habits, and Level of Urbanization of the Mother

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Introduction: Oxidative stress (OS) can play a negative role in perinatal outcomes, but the underlying pathophysiology remains mostly unknown. This is a cross sectional study aiming to evaluate how the living environment, combined with some important maternal risk factors (i.e., smoke, overweight), could influence OS and inflammation markers during pregnancy and in newborns.
Methods: Mothers and newborns were recruited at the Sant'Anna Gynecological Hospital (Turin, Italy). Environmental and lifestyle information was obtained through a standardized questionnaire (PRAMS). OS and inflammation markers (Isoprostane, IL (interleukin)-1, and IL-6) were analyzed in urine samples.
Results: Overall, 126 mother-newborn couples were recruited. Oxidative stress and inflammation levels of mothers and infants have been shown to be significantly associated with each other (Spearman $p < 0.01$). Active and passive tobacco smoke during pregnancy (Spearman $p < 0.01$ and < 0.02, respectively), traffic exposure (Spearman $p < 0.02$), and higher BMI (body mass index) (Spearman $p < 0.05$) shown a positive role in this relationship.
Conclusions: Our preliminary findings suggest that neonatal OS and inflammation are positively influenced by the three maternal risk factors analyzed: Tobacco smoke exposure, high urbanization levels, and high BMI. Further analysis are mandatory to better understand the biological mechanisms underlying such relationships. Nevertheless, correct management and monitoring of these factors must be considered by preventive Public Health strategies, to improve maternal and neonatal health and outcomes.

Keywords: neonatal oxidative stress; pregnancy; risk factors; maternal BMI; public health strategies

Introduction

Oxidative stress (OS), defined as an imbalance between pro-oxidant and antioxidant factors, is known to play a central role in impaired pregnancy and neonatal outcomes [1,2]. Several diseases have been linked to OS, both in pregnancy (eclampsia, miscarriage, preterm labor, and intrauterine growth restriction) and newborns (bronchopulmonary dysplasia, chronic lung disease, retinopathy, enterocolitis, periventricular leukomalacia, and patent ductus arteriosus), but the mechanisms underlying such relationship are yet to be elucidated [1]. Reactive sxygen species (ROS) represents the molecular basis of OS. ROS are molecules with one or more unpaired electrons, extremely unstable and highly reactive. When produced in excess in the body, they become mediators of cell and tissue damage, resulting in OS [3].

Several risk factors have been associated with OS development in pregnancy and newborns, among which maternal obesity seems to be strongly correlated [4–6]. Obesity is associated with elevated inflammatory levels, ROS generation, lipid peroxidation, decreased antioxidant levels, and subsequent OS in non-pregnant and pregnant women as too [7]. Likewise, during pregnancy, pro-oxidants plasmatic concentration increases whereas enzymatic antioxidants activity decreases [8], probably as a result of gestational metabolic and oxygen demands. Obesity throughout pregnancy may lead to a pro-oxidant/antioxidant imbalance both in mother and fetus, with greater lipid accumulation in the placenta, because of its high metabolic activity [9]. Furthermore, according to maternal adiposity, ROS generation in placentas increases [10], and fetuses of obese mothers show increased oxidative stress (OS) levels and production of pro-inflammatory adipocytokines, such as leptin, as well as increased insulin resistance [6]. In turn, these factors are associated with a greater risk of developing chronic diseases in later stages of life, including hypertension, type 2 diabetes mellitus (T2DM), and obesity [11].

Journal of
BIOLOGICAL REGULATORS
and Homeostatic Agents

Tobacco smoking is another factor strongly related to OS [12–14] and a risk factor during pregnancy known for a long time as such. In fact, cigarette smoking during pregnancy is one of the leading environmental factors that can adversely affect the health of mother and newborn. In this vulnerable phase, active and passive exposure to tobacco smoke have been shown to have deleterious effects on the development process and may result in permanent damage [15,16]. Maternal smoking during pregnancy strongly increases risk of preterm birth, intrauterine growth retardation, placental abruption, abortion, placenta previa and several other pregnancy and neonatal complications [17–19].

Urbanization and traffic-air pollution represents one of the main environmental and public health challenges nowadays. In this regard, pregnancy and neonatal age is a particularly important window of susceptibility. Traffic-air exposure can affect both mother and the developing fetus. Growing evidence links exposure traffic-air pollutants during early life to adverse pregnancy outcomes, including preterm birth [20], reduced lung function, low birth weight, intrauterine growth retardation, impaired neurodevelopment and susceptibility to later metabolic diseases [21–23]. Moreover, exposure to air pollutants in early life can show up later consequences in childhood or in adulthood with chronic or lifelong conditions. The precise molecular mechanism that led up to air pollution-associated health outcomes has not been clearly elucidated but several studies attribute adverse birth and health outcomes to OS.

Pro-inflammatory cytokines, such as interleukin-1 beta (IL-1β) and interleukin-6 (IL-6), are directly correlated with OS levels, playing a central role in human pathology, including COVID-19, neurological, kidney, cardiac, infectious and several other diseases [24–28]. At the same manner, Isoprostanes are well known to be OS markers and mediators [29,30]. In these perspectives, the aim of the present study was to identify maternal and environmental risk factors directly associated with OS and inflammation in both pregnancy and newborns, through pro-inflammatory biomarkers (urinary Isoprostane and interleukins) measurements in pregnant women and their newborn babies.

Materials and Methods

Epidemiological Sample

The epidemiological sample was selected to be representative of the Turin newborn population (Turin, Italy). As we already presented in our previous article [31], subjects were recruited from July 2016 to October 2017 by consulting the register of births of the Sant'Anna Gynecological Hospital and following these selection criteria: (1) Full-term pregnancy (>37 G.A.); (2) Physiological pregnancy conditions; (3) No drugs or pharmacological treatment during pregnancy; (4) Single babies (no twins) with Apgar scores >5; And (5) healthy babies at birth (not admitted to the neonatal intensive care unit or in life-threatening conditions).

Questionnaire

Data on individual, socio-demographic and lifestyle habits (e.g., clinical characteristics, education, smoking, diet, working information, living environment) were obtained from mother during birth-hospitalization through a standardized questionnaire (PRAMS questionnaire), as described in our previous work [31]. Infant data were filled in by neonatologists.

Biological Analysis

A pool of fresh urine (30 mL for mothers/5–8 mL for babies) was collected from each mother before the discharge and before the third day of the newborn's life. Infants' urine samples were collected by means of a specific polypropylene bag (Urinocol® Pediatric, BRAUN, Milano, Italy) placed inside the diaper of each newborn. Urine samples were stored at –80 °C until analysis.

Urinary Biomarkers

- Interleukin 1β (IL-1β): Is a master regulator of inflammation via controlling a variety of innate immune processes [13,23]. The urinary IL-1β concentration was measured by a high-competitive enzyme-linked immunoassay (ELISA) performed with a specific microplate kit (R&D Systems n. DY201, Minneapolis, MI, USA), according to manufacturer's instructions.

- Interleukin 6 (IL-6): Is another well-known inflammatory marker and a target biomarker of inflammatory diseases [7,13]. The urinary IL-6 concentration was measured by a high-competitive enzyme-linked immunoassay (ELISA) performed with a specific microplate kit (R&D Systems n. DY206, Minneapolis, MI, USA), according to manufacturer's instructions.

- 15-F2t-Isoprostane (15-F2t-IsoP): Is a specific indicator of lipid peroxidation, both *in vitro* and *in vivo* [8,13,14]. The urinary 15-F2t-IsoP concentration was measured by a competitive enzyme-linked immunoassay (ELISA) performed with a specific microplate kit (EA85, Oxford Biomedical Research, Inc., Oxford, MI, USA), according to manufacturer's instructions.

- Creatinine: Urinary creatinine was determined to normalize the excretion rate of the all the aforementioned urinary biomarkers as previously described [32].

Statistical Analysis

Due to non-normal distribution, non-parametric correlation tests (Spearman correlation) were performed between maternal and neonatal inflammation marker levels and a group of independent variables: Living environment (i.e., suburban vs high urbanization), life style habits (i.e., active and passive smoking exposure), anthropometric characteristics (i.e., weight and BMI (body mass index) during pregnancy), age and level of education.

Data were expressed as mean ± SD or counts and percentages. BMI was used both as continuous (Spearman cor-

relations) and, in order to perform multinomial logistic regression, as categorized variable (following OMS categorization: Overweight or obese = OwO vs mother not overweight or obese = not OwO) [33]. In addition, 15-F2t-IsoP concentrations were used both as continuous and categorized variable (tertiles), in order to perform multinomial logistic regression.

As the statistical distribution of the quantitative parameters was found to be non-Gaussian (Kolmogorov-Smirnov test), non-parametric tests were used to assess between group differences (Mann-Whitney U-test, Spearman correlations test). Two-sided p value < 0.05 was considered to indicate statistical significance. The Multinomial Logistic Regression model was used to assess the association between neonatal 15-F2t-IsoP concentrations (tertiles), as dependent variable, and OwO and urbanization level, included in the model as categorical variables, controlled for active and passive smoke. To account for intragroup correlation, the Huber-White standard error estimate for cluster sampling was applied. Results were reported as odds ratios (ORs) with 95% confidence intervals (CIs). All analyses were carried out using the software STATA 16.1 (StataCorp LLC: College Station, TX, USA).

Results

Table 1 describes the characteristics of the study population (i.e., newborn and mothers) and the inflammation marker concentrations in the study population, split up for newborn and mother group. This is the same cohort described in a previous our work [31], with 8 subjects removed due to unsuitable urine samples. Newborn and mother samples resulted homogenous in terms of intra and inter-individual characteristics (Levene non-parametric test $p < 0.05$).

Maternal and neonatal 15-F2t-IsoP levels were found to be significantly associated with each other (Mann-Whitney $p < 0.01$) (Fig. 1). Maternal BMI, living environment and tobacco smoke exposure (both active and passive) showed significant associations with inflammatory markers, both in mothers and in newborns. Maternal BMI at the end of the pregnancy resulted to be significantly associated with maternal IL-1 (Mann-Whitney $p = 0.01$), IL-6 (Mann-Whitney $p = 0.01$), and neonatal Isop (Mann-Whitney $p = 0.04$) levels (Fig. 1).

Maternal IL-1 levels resulted associated with active (cigarette/die, $p < 0.01$ and smoke yes/no $p = 0.01$) and passive (exposition hours, $p = 0.02$ and exposition yes/no $p = 0.03$) smoke. No significant association was found between tobacco smoke exposure and inflammatory levels in newborns.

Moreover, higher urbanization level proved to be significantly associated with higher OS in newborns (Mann-Whitney $p < 0.02$) (Fig. 2).

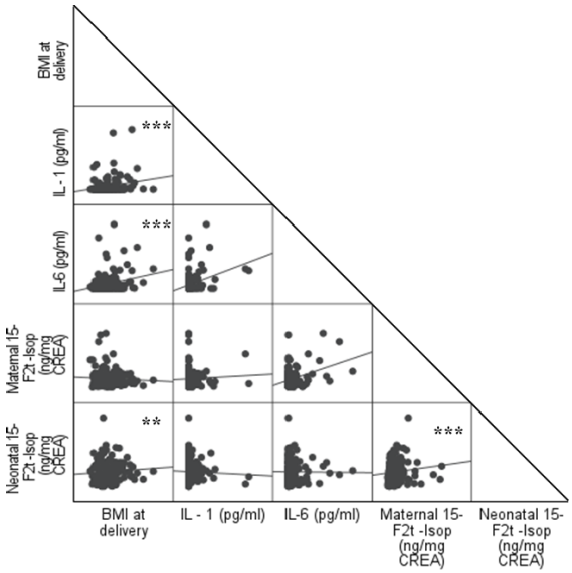

Fig. 1. Multi-panel matrix between BMI at the term of pregnancy and maternal and newborn markers: Neonatal 15-F2t-Isop and maternal 15-F2t-Isop, IL-1, IL-6 ($^{**}p < 0.05$, $^{***}p < 0.01$). The inflammation marker concentrations in the study population, split up for newborn and mother.

Finally, we performed a Multinomial Logistic Regression between neonatal 15-F2t-IsoP (categorized in tertiles) and maternal OwO (Obese/overweight vs Not Obese/overweight), Urbanization (High vs Low), active and passive smoke exposure (Fig. 3). The model proved that newborn with higher maternal OwO showed and increased likelihood of having higher oxidative stress and inflammation level (OR: 3.1, 95% CI: 1.14–6.3, $p = 0.04$) while urbanization, even not statistically associated (OR: –1.02, 95% CI: –1.13–1.01, $p = 0.06$), show a trend of decrease of OS levels in lower urbanized newborns. No other significant associations with active and passive tobacco smoke exposure were found.

Discussion

OS plays a detrimental role in pregnancy and neonatal outcomes. Several human diseases, involving numerous organs and systems, are associated with OS, especially during pregnancy. To investigate the role of OS risk factors on pregnancy and neonatal outcomes, we assessed the levels of some pro-inflammatory markers in pregnant women and their newborns. A comprehensive analysis of maternal clinical characteristics and lifestyle habits allowed identification of three factors strongly associated with OS: Maternal BMI, urbanization levels and tobacco smoke exposure. Our analysis showed a direct and significant association between maternal and neonatal 15-F2t-IsoP levels, proving a direct impact and contribution between mother and newborns, in terms of OS. These results underline how maternal OS is able to strongly influence, not only fetus development, but also directly increase neonatal OS levels. This

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BIOLOGICAL REGULATORS
and Homeostatic Agents

Table 1. Physical characteristics and inflammatory marker levels of the newborn at birth and of the mothers.

PART A	NEWBORN GROUP (N = 125)	
Sex (Mean ± SD)		
Male	80 (64%)	
Female	45 (36%)	
Height at birth (Mean ± SD)	50.1 ± 6.7	
Weight at birth (Mean ± SD)	3.3 ± 0.4	
Cranial circumference (Mean ± SD)	33.9 ± 1.16	
Respiratory procedures N (%)		
No	110 (88%)	
Yes	15 (12%)	
General medical check-up N (%)		
No	45 (36%)	
Yes	80 (64%)	
Diseases at birth N (%)		
No	80 (64%)	
Yes	45 (36%)	
PART B	**MOTHER GROUP (N = 125)**	
Age (Mean ± SD)	34.2 ± 4.6	
Height (Mean ± SD)	164.1 ± 7.3	
Weight (Mean ± SD)		
Pre-pregnancy	64.2 ± 13.8	
End of pregnancy	76.8 ± 13.7	
BMI (Mean ± SD)		
Pre-pregnancy	23.8 ± 4.9	
End of pregnancy	28.5 ± 4.8	
Educational level N (%)		
Low level	15 (11.9%)	
Medium level	49 (38.9 %)	
High level	62 (49.2%)	
Occupation N (%)		
Yes	112 (88.9%)	
No	5 (4%)	
Others	9 (7.1%)	
Living place N (%)		
Suburban	69 (54.8%)	
Urban	57 (45.2%)	
Smoking Habits N (%)		
No	101 (80.8%)	
Passive (Average exposure time: 3 hours)	16 (12.8%)	
Yes	8 (6.4%)	
<10 sig/die	4 (50%)	
>10 sig/die	4 (50 %)	
PART C	**INFLAMMATORY MARKERS CONCENTRATIONS**	
Mean ± SD/median [Q1–Q3]	MOTHER	NEWBORN
15-F2t-IsoP	2.6 ± 2.7/1.9 [0.9–3.5]	6.6 ± 4.7/5.9 [3.2–8.9]
IL-1	30.9 ± 75.7/3.8 [3.8–15.5]	/
IL-6	77.4 ± 146/9.3 [9.3–86]	/

(Part A) Physical characteristics of the newborn sample at birth, general information. (Part B) Characteristics of mother population. (Part C) Inflammatory marker levels in newborns and mothers.

finding supports the need for greater attention to preventive strategies and health education campaigns aimed at controlling and reducing maternal OS levels during pregnancy. In this regard, our analysis showed some important factors that could be controlled to reduce maternal OS levels.

Maternal overweight/obesity showed to significantly increase OS levels in newborns. This is an important risk factors for impaired pregnancy and neonatal outcomes [4, 12,34]. First, subclinical metabolic dysfunctions in obese women are proved to be associate with adverse pregnancy

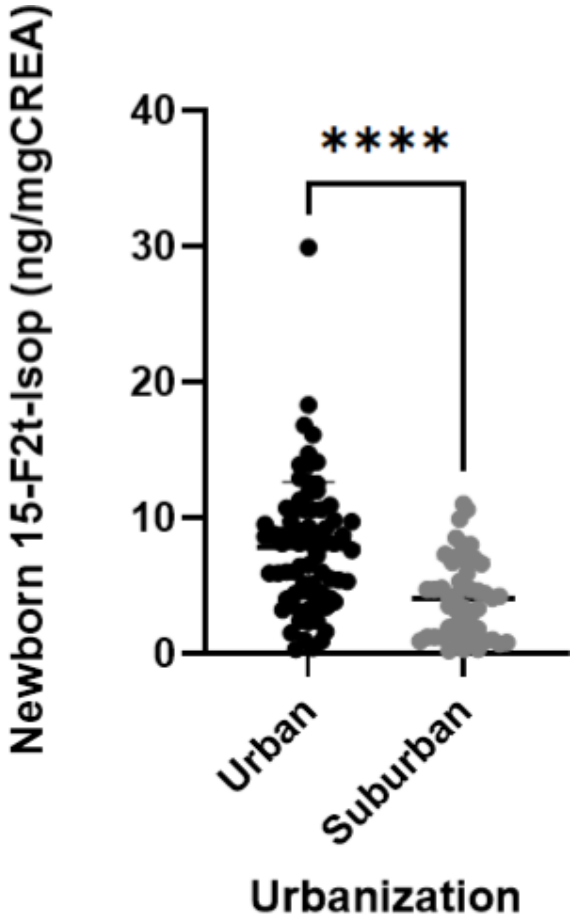

Fig. 2. Non-parametric test (Mann-Whitney) between urbanization and neonatal 15-F2t-IsoP levels ($^{****}p < 0.02$).

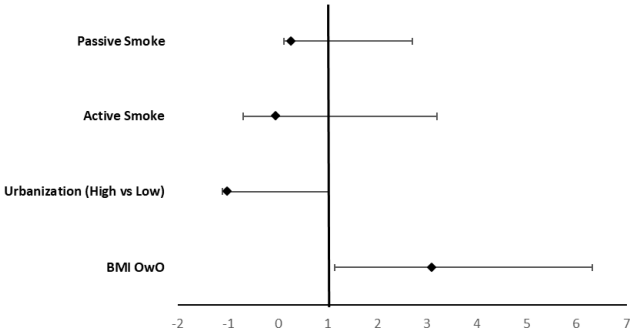

Fig. 3. Multinomial Logistic Regression between neonatal 15-F2t-IsoP (categorized in tertiles) and maternal OwO and urbanization, controlled for active and passive smoke exposure.

outcomes, such as GDM and pre-eclampsia [2,11,35]. Furthermore, obese women have an increased risk of spontaneous preterm births and perinatal mortality [36]. Neonates born to obese women have an increased risk of overgrowth, greater insulin resistance and they are more susceptible to develop chronic diseases during adolescence and lifespan [37,38]. In particular, newborns of obese mothers have higher concentrations pro-oxidant and pro-inflammatory species (e.g., IL-6) than infants of lean mothers [34]. In these perspectives, our results are in line with the previous literature and showed a link between maternal and neonatal OS levels. Moreover, this direct link may help explaining underlying mechanisms of different neonatal chronic diseases associated with maternal overweight/obesity, such as bronchopulmonary dysplasia, chronic lung disease, retinopathy, necrotizing enterocolitis, periventricular leukomalacia or patent ductus arteriosus.

In addition, we could observe a trend, although not statistically significant, between urban air pollution and the increase in neonatal OS levels. Thus, this may suggest that air pollution can represent a risk factor for pregnancy and neonatal outcomes, in particular, for its role in inducing an increase in inflammatory processes and OS. In general, the urban environment has a number of features that could have adverse effects, mainly on children's respiratory health, especially during the first few years of life, when the immune system is rapidly developing [39]. Prenatal and maternal exposures to urban pollution can influence immune development and therefore the variability of the newborn's immune responses could depend on this condition: In effect, the development of the fetal immune system during the perinatal and postnatal periods appears to be responsive to maternal experiences and characteristics [39,40]. Comparing mother and newborn, this positive trend with OS may underline the conceivable association between the urban environmental exposure of the mother and the inflammatory immune response of the newborn, further demonstrating the importance of environmental quality with the health of the populations. This could be an important evidence on which develop preventive measures aimed to reduce risks associated with air pollution exposure in this delicate life phase. Finally, smoking during pregnancy constitutes the largest modifiable risk factor for maternal and child health, in order to reduce risks of obstetric complications, higher rates of spontaneous abortions, ectopic pregnancies, placental abruption, premature labor, and preterm birth [41,42]. Furthermore, maternal tobacco smoking is a well-known source of active oxidizing agents that release free radicals harmful for health [43]. Confirming the literature, our results showed that tobacco smoke, breathed in active and passive form, testified a strong influence on increased maternal OS and inflammation levels, as proved by its significant association with IL-1/IL-6.

Limitations

The cross-sectional design of the present study represents the main limitation, as it does not allow us to assess the causal direction of the associations, with consequences on data/results interpretation. We must consider this sample size population as a sample too small to perform strong and parametric statistical analyses, which are more predictive and prognostic. Furthermore, maternal individual data

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BIOLOGICAL REGULATORS
and Homeostatic Agents

were self-reported and this can mean a possible bias in the data interpretation. Finally, we measured OS and inflammatory biomarkers in mothers and only OS biomarkers in newborns. This is a limitations in the description and analyses of the bio-molecular mechanisms.

Conclusions

Overall, our study confirmed literature evidence of maternal overweight/obesity, air pollution exposure and tobacco smoking influence on OS levels. Moreover, the direct association identified between neonatal and maternal OS levels further advocate for public health preventive measures that, reducing risks connected to lifestyle habits, can improve pregnancy and neonatal outcomes.

Further studies are needed to deeper investigate characteristics, weight and underlying molecular mechanisms of such relationships, especially regarding air pollution exposure.

Availability of Data and Materials

The data presented in this study are available on request from the corresponding author.

Author Contributions

EC, VB and RB—conceived of the manuscript and planning the writing; VB, GS, FG—performed sampling and analyses; EC and VB—wrote the draft; FG, GS, CP, TM, LM, and RB—reviewed the draft and offered substantial revisions. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

The study was conducted according to the Declaration of Helsinki. Informed consent was obtained from all subjects involved in the study. Each mother was enrolled and informed about the aim of this study and signed a written informed consent. The local Ethics Committee of "A.O.U. Città della Salute e della Scienza" of Turin (22 October 2015, file No. CS/709) approved the study protocol and, subsequently, sensitive data were replaced by an anonymous identification code to ensure full privacy of data.

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Conflict of Interest

The authors declare no conflict of interest.

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