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Outdoor air pollution exposures and micronuclei frequencies in lymphocytes from pregnant women and newborns in Crete, Greece (Rhea cohort)

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ABSTRACT

Background: Micronuclei (MN) are biomarkers of early genetic effects that have been used to investigate the association between environmental exposures and cancer. However, few studies have examined the association between environmental exposures during pregnancy and MN in mothers and newborns.
Objectives: We examined MN frequency in maternal blood and in cord blood, in relation to maternal air pollution exposure, and the potential interaction with maternal vitamin C intake and maternal smoking.
Methods: We used the cytokinesis-block micronucleus assay to assess MN frequency per 1000 bi-nucleated T-lymphocytes from 181 mothers and 183 newborns born in 2007–2008 in Heraklion (Crete, Greece). The ESCAPE land-use regression methods were used to estimate annual mean exposure to outdoor air pollution [particulate matter (PM), black carbon, nitrogen dioxide (NO_2) and nitrogen oxides (NO_x)] at maternal home addresses. Food frequency questionnaires were used to estimate maternal dietary vitamin C intake during pregnancy. Smoking habits were self-reported using questionnaires which were checked by measuring maternal urinary cotinine levels.
Results: Exposure to $PM_{2.5}$ was associated with increased MN frequencies in pregnant women [rate ratio [RR (95%CI)] per 5 $\mu g/m^3$ = 1.53 (1.02, 2.29)]. This increase was considerably higher among women who did not fulfill the recommended vitamin C dietary allowances [RR = 9.35 (2.77, 31.61); n = 20]. Exposure to $PM_{2.5-10}$, PM_{10}, NO_2 and NO_x were also associated with a higher incidence of MN frequencies in smoker women (n = 56). No associations were found for newborns.
Conclusions: We found an association between air pollution, particularly $PM_{2.5}$, and MN frequency in mothers but not in newborns. This association was more pronounced among women with a lower dietary intake of vitamin C during pregnancy and among women who smoked during pregnancy. While

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results are clear in mothers, the association between maternal carcinogenic exposures during pregnancy and biomarkers of early biologic effect in the newborn remains poorly understood.

1. Introduction

Outdoor air pollution has been evaluated by the International Agency for Research on Cancer (IARC) Monographs Programme as carcinogenic to humans (Group 1). The IARC evaluation showed an increasing risk of lung cancer with increasing levels of exposure to particulate matter and air pollution (Loomis et al., 2013). Whether air pollution may affect other cancers or cancer in susceptible populations is unknown. Cancer incidence among European children has been steadily increasing in the last decades (Kaatsch, 2010) but causes of this increase are unknown. Maternal exposures before and during pregnancy may modulate gene expression in the fetus, which might lead to long-lasting changes that influence health outcomes during childhood and adulthood (Sly and Carpenter, 2012).

The NewGeneris research project (Newborns and Genotoxic exposure Risk; https://www.newgeneris.org/) was conducted to test the hypothesis that maternal intake of dietary carcinogens results in in utero exposure and early biological effects in the unborn child, possibly leading to increased risk of cancer in later childhood (Merlo et al., 2009). Results from the NewGeneris analysis showed that newborns from Crete, Greece (Rhea Mother–Child cohort), presented the highest mean frequencies of micronuclei (MN) in cord blood compared with newborns from other European countries (Merlo et al., 2014). MN are small extra-nuclear bodies that result from acentric chromosome/chromatid fragments or whole chromosomes/chromatids that lag behind in anaphase stage and are not included in the daughter nuclei in telophase stage (Kirsch-Volders et al., 2014). MN indicate genome damage and MN frequency in peripheral blood lymphocytes had been associated with increased risk of cancer in adults (Bonassi et al., 2007). We have previously reported association of water disinfection by-products and reproductive factors in relation to MN frequency in the Rhea Mother–Child cohort (Stayner et al., 2014; Vande Loock et al., 2011). Here we analyze the association between maternal outdoor air pollution exposure during pregnancy and MN frequency, considering potential interactions with maternal vitamin C intake and smoking.

2. Methods

2.1. Study population

The present study included mothers and newborns from the Rhea Mother–Child cohort (Heraklion, Crete, Greece) described elsewhere (Chatzi et al., 2011). The first contact with potential participants was made at around 10th–13th weeks of pregnancy, at the time of the first ultrasound examination. Briefly, women who became pregnant during February 2007–February 2008 in the prefecture of Heraklion were asked to participate in the study. Women were eligible to participate in the study if they were residents of the study area, other than 16 years of age, visiting a participating hospital or private clinic during the 10th–13th week of gestation, and did not have communication limitations. Participating women were contacted again during the 14th–18th, 28th–32nd weeks of pregnancy and at birth. During recruitment, 1610 eligible women agreed to participate, and 1459 (91%) were followed through delivery. A random subset of 408 participants, among pregnancies with no complications such as preterm births, donated maternal and/or cord blood for a wide range of biomarkers measurement as part of the NewGeneris study. 232 samples were randomly selected for MN measurement (Merlo et al., 2009). Mothers and newborns with MN analysis in maternal and cord blood lymphocytes, from singleton pregnancies and data on outdoor air pollution at maternal home addresses were considered for this study. Overall, 181 mothers and 183 newborns were included. Of these, 136 were mother–child pairs.

The Ethics Committee of the University Hospital in Heraklion, Greece, approved the study and all participants provided written informed consent.

2.2. Exposures assessment

We used face-to-face structured questionnaires, self-administered questionnaires and medical records to obtain demographical information on mothers and newborns (Chatzi et al., 2011). Maternal home address was recorded around 10th–13th weeks of gestation (during first visit). Around 14th–18th weeks of gestation (during second visit), validated food frequency questionnaires (FFQ) were administered to collect information on diet during pregnancy (Chatzi et al., 2011). Vitamin C intake was derived from information recorded in FFQ using food composition tables (McCance and Widdowson's, 1991). During the same visit, self-reported information on active smoking (nonsmoker/ex-smoker/active smoker) and passive smoking (at home and work place) was collected. We also collected urine samples to measure cotinine levels and we used the 100 ng/ml cut-off value, previously defined for this study population (Vardavas et al., 2011), to differentiate smokers from passive-smokers/non-smokers. Exposure to second hand smoke in Crete is much higher than in other populations and therefore a specific cut-off value had to be defined for the Rhea cohort. Distribution of cotinine in the Rhea cohort led to two very distinct groups, with a large gap between them. The 100 ng/ml cut-off differentiated well these two groups.

Outdoor air concentrations of particulate matter (PM) with an aerodynamic diameter below 2.5 μm ($PM_{2.5}$), between 2.5 μm and 10 μm ($PM_{2.5-10}$, coarse PM), below 10 μm (PM_{10}), $PM_{2.5}$ absorbance (a measure of black carbon), nitrogen dioxide (NO_2) and nitrogen oxides (NO_x) were measured between February 2009 and 2010 in Heraklion. Measures were collected in 40 different sites during two weeks period in three different seasons (cold, warm and intermediate)(Cyrys et al., 2012). Measured average concentrations were combined with geographic predictors to develop land use regression (LUR) models following the protocols developed as part of the European Study of Cohorts for Air Pollution Effects (ESCAPE: http://www.escapeproject.eu) (Beelen et al., 2013; Eeftens et al., 2012). Annual mean concentrations between February 2009 and 2010 were estimated at maternal home address assuming that annual mean estimations are stable from year to year (Cesaroni et al., 2012; Eeftens et al., 2011; Madsen et al., 2011).