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Buccal micronucleus cytome assay in primary school children: A descriptive analysis of the MAPEC_LIFE multicenter cohort study

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ABSTRACT

Background: Recent data support the hypothesis that genetic damage occurring early in life during childhood can play an important role in the development of chronic diseases in adulthood, including cancer.
Objectives: The objective of this paper, part of the MAPEC_LIFE project, is to describe the frequency of micronuclei and meta-nuclear alterations in exfoliated buccal cells of 6–8year-old Italian children recruited in five Italian towns (i.e., Brescia, Torino, Pisa, Perugia and Lecce) with different air pollution levels.
Methods: About 200 children per town were recruited from primary schools. Biological samples were collected twice from the same children, in two different seasons (winter 2014-15 and late spring 2015). Cytogenetic damage was evaluated by the buccal micronucleus cytome assay.
Results: Overall,n = 1046 children represent the final cohort of the MAPEC_LIFE study. On the whole, the results showed a higher mean MN frequency in winter (0.42 ± 0.54‰) than late-spring (0.22 ± 0.34‰). MN frequency observed among the five Italian towns showed a trend that follows broadly the levels of air pollution in Italy: the highest MN frequency was observed in Brescia during both seasons, the lowest in Lecce (winter) and Perugia (late-spring).
Conclusions: To the best of our knowledge, the number of recruited children included in the analysis (n = 1046) is the highest compared to previous studies evaluating the frequency of MN in exfoliated buccal cells so far. MN

Abbreviations: MN, Micronuclei; NBUD, nuclear buds; BMCyt, buccal micronucleus cytome assay; PBS, phosphate-buffered saline, pH 7.4; BC, basal cells; BNC, binucleated cells; CCC, condensed chromatin cells; KHC, karyorrhectic cells; PYK, pyknotic cells; KYL, karyolitic cells; RI, repair index; SD, standard deviations; BMI, body mass index; IMI, Italian Mediterranean Index; $PM_{2.5}$, particulate matter with aerodynamic diameter ≤ 2.5 μm; NO_2, nitrogen dioxide

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frequency was associated with winter season and living in towns at various levels of air pollution, suggesting an important role of this exposure in determining early cytogenetic effects.

1. Introduction

Several studies have shown an increased susceptibility of children population to the effects of genotoxic agents from both environment and lifestyle (Neri et al., 2006a, b; Merlo et al., 2007). Children are considered a high-risk group in terms of the health effects because of their different and unique pathways of exposure, their dynamic developmental physiology and their longer life expectancy (WHO, 2008). Moreover, recent data support the hypothesis that genetic damage occurring early in life during childhood can play an important role in the development of chronic diseases in adulthood, including cancer (Wild and Kleinjans, 2003; Landrigan, 2004; WHO, 2005; Bateson and Schwartz, 2008; Grigg, 2009). The higher susceptibility of children, with respect to adults, to the noxious effects of environmental pollutants might depend on smaller airways, immature detoxification and metabolic systems, as well as frequent exposure to outdoor air of children (Kurt et al., 2016).

In the last decades, numerous epidemiological studies have used a molecular approach to study health and disease conditions and related risk factors, for improving measurement of exposure and for early detection of health effects (Bennett and Waters, 2000). Biomonitoring of genotoxic hazards has been reported in several studies by the use of different genotoxicity endpoints, such as analysis of primary DNA damage (by the comet assay), or cytogenetic effects, such as micronuclei (MN), chromosomal aberrations and sister chromatid exchanges. Among genotoxicity endpoints, MN is one of the most commonly used biomarker in molecular epidemiology studies to assess the presence and extent of chromosomal damage in human population exposed to genotoxic agents and for the identification of genetic and lifestyle factors able to affect genome stability (Fenech et al., 1999; Knudsen and Hansen, 2007). MN appear in the cytoplasm of interphasic cells as small additional nuclei, smaller than the main nucleus. MN typically generate during the anaphase from acentric chromosome fragments (chromosome breakage produced by clastogen agents) or whole chromosomes (chromosome malsegregation caused by aneugen agents). Acentric or whole chromosomes are left behind during mitotic cellular division and, consequently, are excluded from both daughter nuclei (Fenech et al., 2011b). Because of the ability to detect both clastogenic (e.g., chromosome breakage) and aneugenic (e.g., spindle disruption) effects, MN are considered biomarkers of early biological effect (NRC, 2006; Kirsch-Volders et al., 2011).

MN in peripheral blood lymphocytes have been extensively used in human biomonitoring studies to identify potential genotoxic exposures as well as chromosomal instability (Fenech, 2002a; Fenech, 2002b) and the frequency of MN in circulating lymphocytes is recognized to be a predictor of cancer risk in human populations (Bonassi et al., 2007; Murgia et al., 2008; Bonassi et al., 2011b). Moreover, a significant increase in MN frequency in lymphocytes was found in patients with cancer or preneoplatic lesions (El-Zein et al., 2006; El-Zein et al., 2011; Maffei et al., 2014), neurodegenerative diseases (Migliore et al., 2011), cardiovascular diseases and diabetes (Andreassi et al., 2011).

In recent years, exfoliated cells from epithelial tissues have been increasingly used in the MN assay. Buccal mucosa (BM) is an easily accessible tissue for sampling cells in a minimally invasive manner and does not cause undue stress to study subjects (Thomas et al., 2009). Moreover, evaluation of genotoxic end-points in rapidly dividing cells (such as BM cells) allows the assessment of cytogenetic damage (i.e., MN) without establishing the *ex vivo* cell replication step typically required by classical metaphase or interphase analyses (e.g., the cytokinesis-block MN assay in binucleated lymphocytes) (Bonassi et al., 2011a). The assessment of MN in exfoliated epithelial cells from oral mucosa has thus provided a complementary method for cytogenetic analyses in an easily accessible tissue without cell culture requirement (Fenech et al., 2011a). Nowadays, the human buccal micronucleus cytome (BMCyt) assay is one of the most widely used techniques to measure genetic damage in human population studies (Bonassi et al., 2011a; Fenech et al., 2011a; Bolognesi et al., 2013). Moreover, MN frequency measured in peripheral blood lymphocytes and in buccal cells, even if occurring at different frequency, showed to be highly correlated, and hence to have a similar ability to detect effects of exposure to genotoxic agents (Ceppi et al., 2010).

Through the micronucleus cytome assay in buccal exfoliated cells it is possible to evaluate, aside to chromosomal and DNA damage markers (MN, and nuclear buds), cell proliferation markers (basal and binucleated cells), cell death/apoptosis markers (cells with condensed chromatin, or karyorrhectic, pyknotic and karyolytic cells), and repair index (Thomas et al., 2009; Thomas and Fenech, 2011). Moreover, the micronucleus cytome assay on exfoliated cells is particularly useful in biomonitoring studies involving children to avoid traumatic and painful sampling procedures causing children any discomforts.

The objective of this paper, part of the MAPEC_LIFE project ("Monitoring Air Pollution Effects on Children for Supporting Public Health Policy"), is to describe the frequency of MN and meta-nuclear alterations in exfoliated buccal cells of 6–8year-old Italian children recruited in five Italian towns (i.e., Brescia, Torino, Pisa, Perugia and Lecce). Cytogenetic data are presented in relation to children's characteristic, such as socio-demographic and anthropometric features, lifestyle, parent's characteristic and outdoor/indoor exposure to genotoxic agents.

2. Material and methods

2.1. Study design

The MAPEC_LIFE project ("Monitoring Air Pollution Effects on Children for Supporting Public Health Policy"), is a prospective epidemiological cohort study funded by the European Life + Programme (LIFE12 ENV/IT/000614), which aimed to investigate the association between air pollution exposure and early biological effects in children. Details of the study design have been described elsewhere (Feretti et al., 2014). Briefly, the study was conducted in five Italian towns (Fig. 1) characterized by different levels and features of air pollution. Brescia and Torino are located in the Po Valley in Northern Italy, a highly industrialized area with unfavorable climate conditions, at the highest levels of air pollution in Europe; Pisa and Perugia are located in a medium-low polluted area in Central Italy, where air pollutants only occasionally exceed law limit values; Lecce is located in a very low polluted area, in Southern Italy, where air pollutants never exceed limit values. The five towns have also different demographic and socio-economic characteristics (Bagordo et al., 2017).

About 200 children per town were recruited from primary schools to evaluate, in their BM cells, biomarkers indicative of DNA damage (*i.e.*, micronuclei and/or nuclear buds), cellular proliferation potential (*i.e.*, basal and/or binucleated cells), and/or cell death (*i.e.*, condensed chromatin, karyorrhectic, pyknotic, and karyolytic cells). Biological samples were collected twice from the same children, in two different seasons (winter 2014-15 and late spring 2015).

The children's parents were interviewed to gather information on exposure to air pollutants from both indoor and outdoor sources and children's lifestyle (Zani et al., 2015). Children with severe diseases and