Micronuclei in families exposed to air pollution

A pilot study in the Czech Republic

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Introduction

Biomarkers of effect may relate health outcomes to individual personal exposures and take the various individual factors like uptake, metabolism and excretion into consideration. Differences in genetic background and age are considered. Also, biomarkers often reflect aggregated exposures and confounders. In family biomonitoring studies, including more than one child and parents, useful information; may be provided about how children differ from each other, and from the parents in response to genotoxic exposure.

Micronucleus (MN) is a biomarker of genotoxic exposure and early biological effect. A high frequency of MN indicates an early step in carcinogenesis, as DNA damage at the chromosome level is an important event in carcinogenesis [1] (Figure 1).

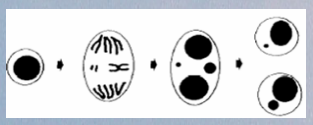
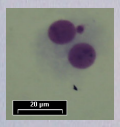


Figure 1. Micronuclei expression in a dividing nucleated cell
MN are expressed in dividing cells that either contain chromosome breaks lacking centromeres and/or whole chromosomes that are unable to travel to the spindle poles during mitosis or meiosis. At telophase, a nuclear envelope forms around the lagging chromosomes and fragments, which then uncoil and gradually assume the morphology of an interphase nucleus with the exception that it is smaller than the main nuclei in the cell, hence the term micronucleus [2].



BN lymphocyte with a micronucleus (MN).
Photomicrograph (× 400) of PBL stained in Giemsa.

Children differ from adults in exposure to the environment due to differences in behaviour, e.g. inability to remove themselves from a noxious environment, hand-to-mouth behaviour and playing on the floor. Compared with adults, children have a higher daily intake of food, water and air per kg body weight, and therefore they may have a proportionally higher intake of toxic agents than adults.

The susceptibility of children are different from adults. Increased susceptibility is related to altered rates of distribution of toxins, detoxification, DNA repair processes, and cell proliferation. Children have immature organs, immune system, metabolic and excretory pathways, and alterations in target tissue, organs and CNS susceptibility dependent on age. Moreover children have a longer life span in which to express illness [3]. Hence biomonitering studies in children are necessary for risk assessment of children.

Children from Teplice area in Northern Czech Republic

Epidemiological studies have been reviewed and the association between exposure to ambient particulate matter (PM) and mortality and morbidity of urban populations has been stated [4]. Especially ultrafine particles (UFP) and fine particles (FP) are of concern. Clinical studies indicate that UFP are retained in the alveolar regions of the lungs and penetrate to the bloodstream. UFP might carry PAHs, VOCs, and they may contain trace metals, e.g., Pb, Cd, V, Ni, Cu, Zn. Concentrations can reach 100.000 UFP/ml in urban air.

Exposures to **air pollution** in the Teplice district situated in the northwest of the Czech Republic (Figure 2) revealed serious adverse respiratory health problems for children and affecting reproduction in adults. Elevated levels of air pollutants, even during short winter inversions, resulted in measurable uptake, metabolism, and excretion of genotoxic organic compounds as well as increased blood concentrations of toxic metals and DNA damage [5].

Objectives

The objective of the family pilot project was to assess the relationship between genotoxic exposure from ambient air and a range of biomarkers measured in children and mothers [6].

The exposure assessment included air sampling of ultrafine and fine particles at the front doors of 24 families living in the Czech Republic. Measurements and analyses of other particle fractions and gaseous air pollutants from stationary monitoring stations along with meteorological conditions on the sampling days were obtained. Relevant information on exposure to indoor sources, including ETS, was compiled from questionnaires and biomarker results.

The biological material (blood and urine) was collected in 48 children (two siblings) and their mothers. Families selected for the study were drawn from the Teplice area, a former mining region, and compared with families from the rural area of Prachatice (Figure 2).

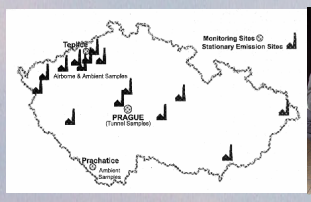


Figure 2. Study areas
Teplice is located in a valley surrounded by mountains and during winter periods of cold temperature and absence of wind, air pollution may be trapped and elevated concentrations may be formed. The combustion of brown coal (for home heating and power plants) combined with heavy local industrialization (e.g., glass factories and lignite mines) and motor vehicles over the last decades has provided some of the worst levels of pollution in all of Europe [7].

Air sampling of PM at the front doors of the families
Repeated 20-minute sampling was carried out in February and March 2004. A handheld condensation particle counter (TSI, model 3007) and a photometer (TSI, Dust-track model 8520) equipped with a 2.5 µm impactor was used.

Methods

The cytokinesis-block micronucleus (CBMN) test was performed in peripheral blood lymphocytes (Figure 3).

The principle of the CBMN test is to stimulate a culture of nondividing (G_0) cells to divide with the mitogenic compound PHA (phyto-haemagglutinin). Cytochalasin-B (Cyt-B), a cytokinesis-blocking agent, is added at the last part of the culture period, and the frequency of MN is counted only in binucleated cells (BN) to ensure that a single nuclear division that is essential for MN development [8].

The CBMN test is used in biomonitoring of human populations, screening of chemicals and other specific purposes [9].

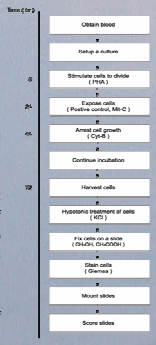


Figure 3. CBMN test

Scoring criteria:

- Only viable cells with an intact cytoplasmic membrane are scored
- The two main nuclei of the BN cells should be equal in size, staining pattern and staining intensity. They may touch but not overlap. The two nuclei within a BN cell may be attached by a fine nucleoplasmic bridge
- MN to be scored are morphologically identical to, but smaller than the main nuclei
- The BN cells should not contain more than 6 MN
- 1000 BN lymphocytes are scored for MN
- Cells and MN are not scored if uncertain and unclear [10]

Results

The air sampling at the front doors resulted in low levels of UFP and FP in both areas. The difference between Teplice and Prachatice appeared to be small during this particular time of sampling. Air monitoring data and the results of the chemical analyses of the PM indicated a significant exposure difference between the areas, especially during winter.

Children and mothers living in the Teplice area have higher frequencies of MN cells as compared with children and mothers from the Prachatice area (Table 1) and (Figure 4).

Table 1. Characteristic of the study population and MN frequencies

Subpopulation	no. of subjects	Gender M/F	no. of smokers	Age Mean (S.D)	Age Range	no. of MN per 1000 BN cells Mean	no. of MN per 1000 BN cells Range
Prachatice							
Younger children	12	7 / 5	none	6.24 (0.5)	5 – 7	5.2	1 – 12
Older children	12	6 / 6	none	9.43 (1.2)	7 – 11	7.5	0 - 27
Mothers	9		3	34.97 (4.5)	29 – 44	10.0	1 - 21
Teplice							
Younger children	12	6 / 6	none	6.48 (0.6)	6 – 7	7.0	2 - 13
Older children	11	5 / 6	none	9.31 (1.2)	7 – 11	9.6	2 – 23
Mothers	12		1	32.86 (4.1)	28 – 40	16.7	6 - 43

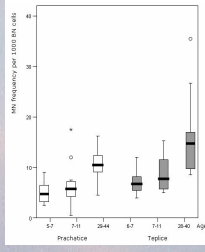


Table 2. Effects on MN frequencies

Variable	OR	95 % C.I.	P - value
Area [1]	1.50	1.19; 1.88	0.002
Age of children	1.09	1.05; 1.14	0.0004
Age of mothers	0.98	0.96; 1.00	0.110
Gender [2]	0.79	0.67; 0.94	0.014
Family	0.24		
Traffic [3]	1.74	1.36; 2.12	0.009
Cooking [4]	1.44	1.11; 1.77	0.039
Heating [5]	1.54	1.25; 1.83	0.008
Cotinine [6]	1.03	1.09; 0.98	0.285

[1] Teplice vs. Prachatice, [2] boys vs. girls, [3] high vs. low UFP/ml air, [4] gas vs. electric, [5] furnace inside vs. outside, [6] cotinine (ng/ml urine) (n = 68)

Figure 4. MN frequencies
Box-plots illustrating the median MN frequency, S.D., quartiles and extremes according to the subgroups (clustered by age) for each area.

In order to analyze the effect of several variables binomial mixed regression models were applied. As shown in table 2, children and mothers from the Teplice area had significantly increased MN frequencies of 50 % compared with the study population from Prachatice.
A significant effect of age on the MN frequency was revealed in children. An age difference of one year increases the formation of MN by 9 %. Moreover increased MN frequencies of 21 % was found in girls as compared to boys. A low inter family variation was found as expressed by the S.D.

MN frequencies were analyzed in order to test whether the effect of area could be explained by exposure to emissions from traffic or indoor sources. Higher MN frequencies were found in the two families living in proximity to high level of traffic. Exposure to indoor sources showed an adverse effect, however these findings are not conclusive.

Conclusions

The family pilot study showed that MN is a valuable and sensitive biomarker for early effect, which can be used to reveal MN frequency differences in the blood of families exposed to different levels of pollution.

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Acknowledgements

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