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MALES-FEMALES DIFFERENCES IN THE SPECTRUM OF CHROMOSOMAL ABERRATIONS IN THE GROUP OF NANOCOMPOSITES PRODUCTION WORKERS

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Abstract

An increase in the use of nanomaterials (NM) has been witnessed in many areas of human life. Therefore, assessment of genotoxicity of NM and nanoparticles (NP) is one of the main objectives of genetic toxicology. Despite this fact, human cytogenetic studies following the exposure to NP are still rare. Moreover, no relevant information on possible differences in sensitivity to NP related to gender is available.

In this study we periodically (in September 2016, 2017 and 2018; pre-shift and post-shift each year) analyzed a group of workers (both genders), working long time in nanocomposites research, and matched controls. Aerosol exposure monitoring of particulate matter including nano-sized fractions was carried out during working shift. Micronucleus assay using Human Pan Centromeric probes, was applied to distinguish, besides the frequency of total MN in binucleated cells (BNC), also other types of chromosomal damage (losses and breaks). Moreover, whole-chromosome painting (WCP) for autosome #1 and both gonosomes (X and Y) were applied in third sampling period (2018) with the aim to identify the particular structural and numerical chromosomal aberrations.

Obtained results showed: (i) differences in the risk of exposure to NP related to individual working processes (welding, smelting and machining); (ii) differences in chemical composition of nano-fraction; (iii) no effect of chronic exposure of NP (total MN) opposite to significant effect of acute exposure; (iv) gender-related DNA damage differences (females seem to be more sensitive to chromosomal losses). Additional data from WCP suggested increased frequency of numerical aberrations in gonosomes.

Keywords: DNA damage, gender, chromosomal aberrations, micronuclei, nanoparticles, occupational exposure

1. INTRODUCTION

A use of nanomaterials (NM) in numerous areas of human life is logically associated with the risk of exposure to nanoparticles (NP). Regardless, the cytogenetic studies in real human population are still rare. Even though 2 papers focusing on the frequency of micronuclei in peripheral blood lymphocytes of nanocomposites

production workers [1,2] were published recently and disrupted the zero knowledge in this area of research [3], more studies are still needed especially due to high diversity of properties of NP. Moreover, detailed information about particular chromosomal rearrangements in individual chromosomes are still completely missing.

Even though various methodological approaches were used frequently during the years in numerous human biomonitoring studies (e.g. conventional cytogenetic analysis (CCA), analysis of total micronuclei (MN) and sister chromatid exchanges (SCE)), none of them is able to give detailed information related to stable chromosomal rearrangements, such as translocations or insertions in particular chromosomes. Analysis of chromosomal aberrations in combination with whole chromosome painting (WCP) by fluorescence in situ hybridization method (FISH) is a promising method developed already in 1986 by Pinkel et al. [4] especially for clinical and tumor cytogenetics. Nevertheless, it is occasionally also used in human biomonitoring [5]. The aberrant cells are classified according to the Protocol for Aberration Identification and Nomenclature (PAINT) describing their various individual types [6]. Moreover, besides the structural rearrangements, this method has also potential to assess numerical aberrations of painted chromosomes. No type of aberrations by WCP of selected chromosomes have ever been analyzed in human subjects exposed to NP.

In this study, we continued to study a group of nanocomposite researchers (sampled in September 2016, 2017 and 2018; pre-shift and post-shift) and besides summary of obtained data related to frequency of total, centromere positive (CEN+) and centromere negative (CEN-) MN in binucleated cells (BNC), we also focused on the differences in the level of DNA damage related to gender. Because the data from micronucleus assay showed high differences in CEN+ MN and CEN- MN between males and females, we decided to continue in this research by involving of WCP into our cytogenetic part of research. We painted two (in females: autosome #1 and gonosome X) or three (in males: autosome #1 and gonosomes X, Y) chromosomes with the aim to obtain most detailed information about the frequency of stable as well as numerical chromosomal aberrations related to gender in this group of nanocomposite research workers. Selection of both groups of chromosomes (autosomes and gonosomes) was based by the previously published knowledge on preferential content of X chromosome in micronuclei of females [7,8].

2. METHODS

2.1. Study groups

A group of researchers (both genders: 75% males/25% females), working long time in nanocomposites research, and matched controls was periodically analyzed in September 2016, 2017 and 2018 (pre-shift and post-shift each year). A total of 211 blood samples was collected during this period. The sample set included a group of workers 20-63 years old, involved in the processes such as welding mild steel S355J2 and smelting in workshop 1; and machining including the milling and grinding of epoxide resin with nanoSiO_2, and geopolymer nanocomposites in workshop 2 and control volunteers matched by age and place of residence. The exposed subjects did not use personal respiratory protection. More details related to basic characteristics were presented previously [1,2].

2.2. Exposure

Questionnaire: The detailed times of exposure to NP were recorded for participants of the study (particularly: (i) long-time exposure (years); (ii) common daily exposure (min); (iii) monitoring day exposure (min)). Concrete data are mentioned in section Results and Discussion.

Monitoring: The online aerosol monitoring during the shift for individual processes included application of two standard aerosol spectrometers [Scanning Mobility Particle Sizer (SMPS) (TSI SMPS 3936L, USA) and Aerodynamic Particle Sizer (APS) (TSI APS 3321, USA)] used to obtain more details on exposure in the

nanoscale range from 6 nm up. The offline Berner Low Pressure Impactor (BLPI) (HAUKE Gmbh., Austria) was used to sample aerosol particles onto 10 stages up to 13.6 µm including two nanoscales (25-56 nm and 56-100 nm) during each process. These samples were consecutively analyzed by gravimetry; ion chromatography [9] and Scanning Electron Microscopy (SEM) (Tescan Indusem, Czech Republic) equipped with Energy-Dispersive X-Ray Spectroscopy (EDS) (XFlash detector 5010, Bruker, Germany) were then used to analyze ionic and elemental compositions of size-resolved aerosol fractions. More details on aerosol exposure monitoring were published previously [10].

2.3. Cytogenetic analysis

***Blood samples*:** Blood cultures were incubated at 37 °C for 72 h. Cytochalasin B (Sigma, USA) was added to a final concentration of 5 µg/ml after 44 h [11] in case of micronucleus test, or colchicine (Sigma, USA) was added to a final concentration of 0.5 µg/ml 2 h before the end of the incubation in case of metaphases evaluation. Cultures were harvested by centrifugation, treated with hypotonic solution of KCl and fixed with methanol/acetic acid.

***Micronucleus test with Pan-centromeric FISH*:** The protocol performed with fluorescein-5-isothiocyanate (FITC) labelled Human Pan Centromeric probes was adapted from the manufacturer's protocol (Cambio, UK). Briefly: slides were dehydrated, denatured, hybridized with probes and washed. Stained slides were counterstained with 4, 6-diamidino-2-phenylindole (DAPI) mixed with Vectashield (Vector Laboratories, USA) mounting medium.

Whole-chromosome FISH: The protocol to perform whole-chromosome FISH painting (WCP) for selected chromosomes (customized XCP-Mix: autosome #1 (red (B,b)) and both gomosomes X (green (C,c)), Y (yellow (D,d))) was adapted from the manufacturer's protocol (MetaSystems, Germany). Briefly: fresh slides were denatured simultaneously with probe, hybridized and washed. Stained slides (unpainted chromosomes) were counterstained with DAPI (blue (A,a)) as already described.

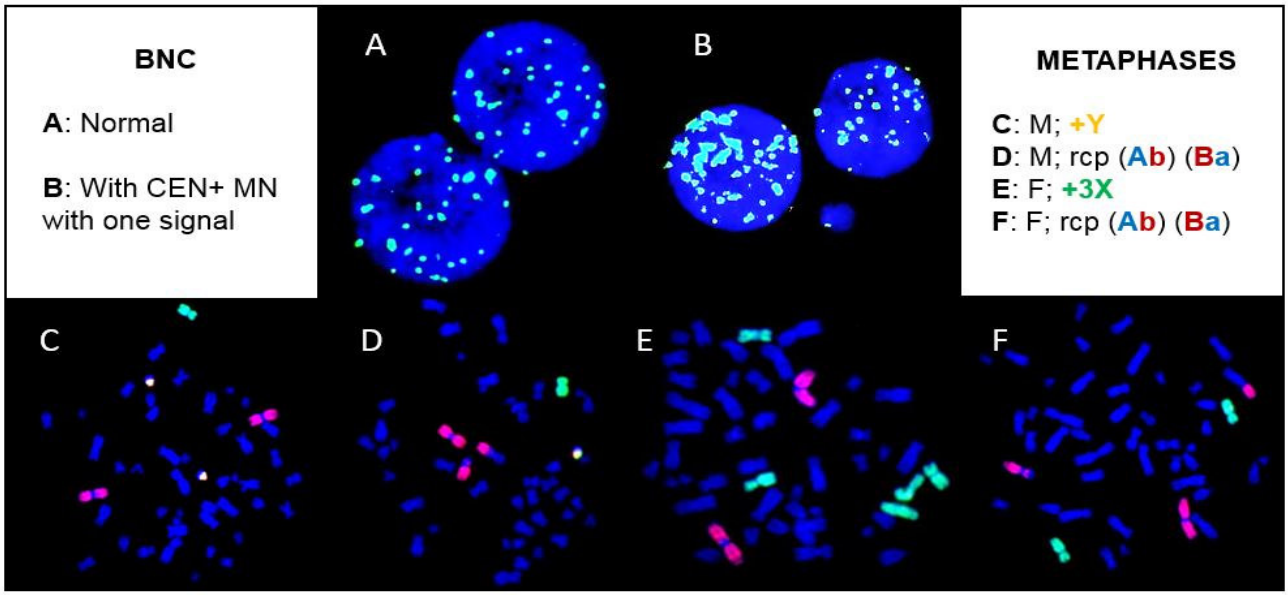

Figure 1 Examples of cytogenetics findings analyzed by Micronucleus test with Pan-centromeric FISH (A-B) and Whole-chromosome FISH (C-F)

***Microscopic analysis*:** One thousand binucleated cells (BNC), or 1000 metaphases were analyzed in case of micronucleus test or structural/numerical chromosomal aberrations (CA) analysis for each subject,

respectively, with an immersion oil objective lens for a final magnification of 1000x. Fluorescence microscope (Axioskop - Zeiss) equipped with filters for DAPI (blue signal), FITC (green signal) and Texas Red (red signal) was used. BNC with micronuclei (MN), centromere positive (CEN+) and/or centromere negative (CEN-), were analyzed in case of micronucleus test. Metaphases with structural CA including e.g. translocations: t, reciprocal translocations: rcp, or insertions: ins [6] and numerical CA were analyzed in case of chromosomal analysis. ISIS software version 5.0 (MetaSystems, Germany) was used. See examples of observations for both methods (micronuclei analysis and WCP) and genders (M: males, F: females) in **Figure 1**.

2.4. Statistical analysis

Basic descriptive statistics were calculated using Microsoft Excel 2013. T-test, Mann-Whitney *U* test, paired sample t-test or the Wilcoxon signed-rank test were used for the comparison of the studied parameters between analyzed groups. Results were expressed as a total MN/1000 BNC, CEN+ MN/1000 BNC and CEN- MN/1000 BNC or number of metaphases with structural or numerical aberration/1000 metaphases (SAB/1000 met. or NAB/1000 met., respectively).

3. RESULTS AND DISCUSSION

Main exposure characteristics of the exposed groups followed in September 2016 and 2017 obtained from questionnaires are summarized in **Table 1**. These data showed longer long-time exposure of subjects analyzed in 2016 when compared with common daily exposure and exposure in monitoring day which were longer in 2017.

Table 1 NP exposure characteristics of the study subjects

Exposure record N=20	2016		2017	
	Mean ± SD	Median (range)	Mean ± SD	Median (range)
Long-time (years)	17.8±10.0	15 (5-40)	12.15±9.27	8 (2-31)
Common daily (min)	101.4±60.0	105 (30-240)	128.25±87.26	120 (30-360)
Monitoring day (min)	156.3±61.9	150 (60-330)	203.5±54.7	180 (150-360)

Moreover, aerosol exposure monitoring from individual working processes were accompanied by differences in nano-fraction exposure and their chemical composition [1,10]. Obtained data (not shown) indicated high differences in the risk of exposure to nanoparticles including differences in chemical composition of nano-fractions related to individual working processes. Particularly, for welding, a substantially higher absolute elemental mass concentration of the nano-sized fraction was observed (9.55x and 107x, respectively, higher total level of nano-sized fractions (25-56 nm + 56-100 nm)) when compared with smelting and machining. Generally, individual processes differed in the chemical composition of the nano-sized fraction with a dominant content of Fe common to all processes. Other common elements included Si, S and Cl. The production of Mn and Na was specific for welding and smelting; Al for smelting and machining; and K was detected in the smelting process only.

Previously published data [1,2] concentrated in details on the clastogenic and aneuogenic effect of chronic (long-term) and acute (short-term) exposure to nano-fraction in the group of nanocomposite researchers involved in three various processes (welding, smelting and machining). These results confirmed repeatedly a lack of effect of chronic exposure of NP on total frequency of MN. This observation can be interpreted as a possible adaptation. DNA damage pattern can change depending on the type of exposure (break increase in welders in comparison with controls). In contrast, significant effects of acute exposure (chromosomal losses related to process of machining) were observed.

The new data presented here that focus on the spectrum of chromosomal aberrations in these groups showed high differences related to gender. This was firstly indicated by micronucleus assay using Human Pan Centromeric probes, where the females have repeatedly significantly higher frequency of CEN+ MN/1000 BNC in comparison with males (p=0.002 and p=0.018 for the groups followed in years 2016 and 2017, respectively). This observation suggests significantly higher tendency to aneuploidy (see **Table 2**).

Table 2 CEN+ and CEN- MN/1000 BNC in males and females

Gender	2016			2017		
	N	CEN+ MN Mean ± SD	CEN- MN Mean ± SD	N	CEN+ MN Mean ± SD	CEN- MN Mean ± SD
Males	30	5.03 ± 2.64	4.66 ± 2.25	26	6.23 ± 2.08	3.92 ± 1.74
Females	11	11.00 ± 5.45*	5.09 ± 3.45	14	8.00 ± 3.33*	5.57 ± 2.79

Follow-up experiments concerning the frequency of numerical and structural aberrations confirmed data from analysis of micronuclei and further showed dramatically higher frequency of numerical aberrations especially in X gonosome of females. Detailed data on both types of aberrations in individual chromosomes (autosome 1 and both gonosomes X and Y) related to gender and evaluated in 1000 metaphases per participant of the study are shown in **Figure 2**.

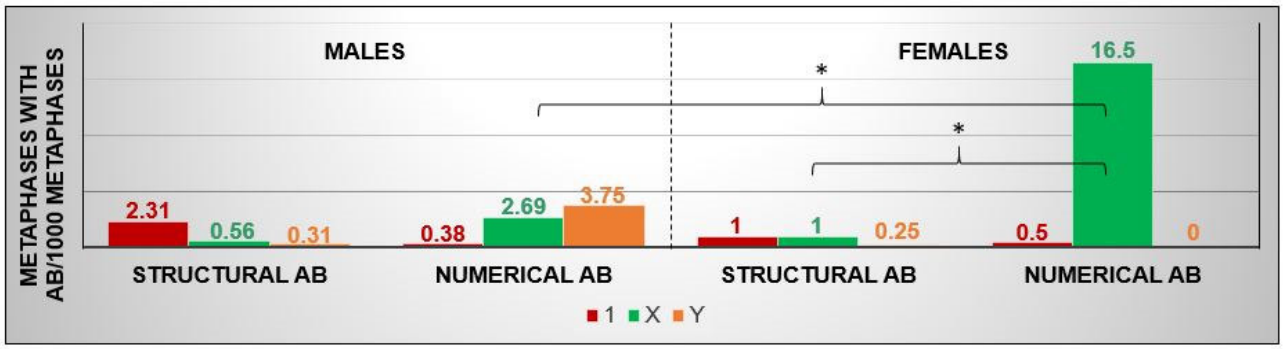

Figure 2 Structural and numerical chromosomal aberrations in chromosomes # 1, X and Y in males and females

4. CONCLUSION

Summary of exposure and cytogenetic results obtained from the repeated collection of samples in September 2016, 2017 and 2018 during the examination of occupationally exposed subjects working long time in nanocomposites research, showed relatively consistent results. Concerning exposure data, besides the high differences in chemical composition of nano-fractions, differences in the risk of exposure to NP associated with individual working processes were shown. Data obtained from analysis of micronuclei confirmed repeatedly a lack of effect of chronic exposure to NP. This result can be interpreted as a possible adaptation. This observation is in contrast with significant effect of acute exposure. Moreover, significant differences related to gender, analyzed by two cytogenetic methods showed females to be more sensitive to chromosomal losses. WCP of autosome 1 and both gonosomes X and Y revealed, besides increased frequency of numerical aberrations in gonosomes, also higher frequency of structural aberrations in autosomes of males in comparison with females.

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