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Genetic alteration profiling in middle-aged women acutely exposed during the mechanical processing of dental nanocomposites

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ABSTRACT

Nanoparticles (NPs) have become an important part of everyday life, including their application in dentistry. Aside from their undoubted benefits, questions regarding their risk to human health, and/or genome have arisen. However, studies concerning cytogenetic effects are completely absent. A group of women acutely exposed to an aerosol released during dental nanocomposite grinding was sampled before and after the work. Exposure monitoring including nano (PM0.1) and respirable (PM4) fractions was performed. Whole-chromosome painting for autosomes #1, #4, and gonosome X was applied to estimate the pattern of cytogenetic damage including structural and numerical alterations. The results show stable genomic frequency of translocations (F_G/100), in contrast to a significant 37.8% ($p<0.05$) increase of numerical aberrations caused by monosomies ($p<0.05$), but not trisomies. Monosomies were mostly observed for chromosome X. In conclusion, exposure to nanocomposites in stomatology may lead to an increase in numerical aberrations which can be dangerous for dividing cells.

1. Introduction

Nanoparticles (NPs), fractions of particulate matter that have one or more external dimensions up to 100 nm (The European Commission, 2011), have become an important part of everyday life in the last 20 years. Even though many NPs originate in nature, a significant amount have an anthropogenic origin. Some of them are random by-products of material processing, for example milling or grinding; others are intentionally produced with the aim of improving the properties of materials.

Many of the newly composed materials, called nanocomposites, are frequently used in dentistry as a universal aesthetic material for tooth reconstruction. Their acrylic- and methacrylic-based matrix contain inorganic filler particles of different sizes including nano-sized ones, such as silica (SiO_2) or zirconium dioxide (ZrO_2) (Schmalz et al., 2018). The release of NPs during the mechanical processing of nanocomposites raises the risk of chronic and acute exposure of both dental staff, and patients. Published data estimate the inhalation of nano-fraction by dental personnel in the range from 0.00016% to 0.0040% (w/w) of the

Abbreviations: % AB.C., percentage of aberrant cells; AB/1000, aberrations/1000 cells; Ace, acentric fragment; APS, aerodynamic particle sizer; B[*a*]P, benzo[*a*] pyrene; BEAS-2B, normal human bronchial epithelial cells; Bis-EMA, bisphenol A diglycidyl methacrylate ethoxylated; Bis-GMA, bisphenol A diglycidyl methacrylate; BMI, body mass index; BNC, binucleated cell; CEN- MN, micronuclei with the absence of centromere signal; CEN+ MN, micronuclei with the presence of centromere signal; DAPI, 4′,6-diamidino-2-phenylIndole; EOM, extractable organic matter; Dic, dicentric chromosome; F_G/100, genomic frequency of translocations/100 cells; FISH, fluorescence *in situ* hybridization; FITC, fluorescein-5-isothiocianate; Ins, insertion; MN, micronuclei; MWCNT, multi-walled carbon nanotubes; NCJ, number of color junctions/1000 cells; NHEJ, Non-Homologous End Joining; NPs, nanoparticles; PAHs, polycyclic aromatic hydrocarbons; PAINT, Protocol for Aberration Identification and Nomenclature; PBL, peripheral blood lymphocytes; PHA, phytohaemagglutinin; PENS, PErsonal Nanoparticle Sampler; PM, particulate matter; R, ring; Rcp, reciprocal translocations two-way; SD, standard deviation; SMPS, Scanning Mobility Particle Sizer; SWCNT, single-walled carbon nanotubes; T, translocations one-way plus two-way; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate; WCP, Whole Chromosome Paints.

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total dust (Van Landuyt et al., 2012; Bradna et al., 2017). Generally, the extensive use and development of new types of NPs and/or nanomaterials, continually raise questions related to their safety for human health, including the risk of cardiovascular diseases, various cancers, reproductive system disorders and lung injury (Aloisi et al., 2022; Alsaleh et al., 2021; Ding et al.; 2023; Korsiak et al., 2022).

Numerous studies in genetic toxicology use various biomarkers to assess the NPs cytotoxicity, or genotoxicity. Most of the studies are conducted *in vitro*, however, some of these focus on the direct effects on humans. Regarding nanomaterials used in stomatology, the cytotoxic or inflammatory effects of dental composite dust particles including NPs have been observed in human bronchial epithelial cells (16HBE14o-) as well as in human alveolar A549 cells (Cokic et al., 2016; Jiang et al., 2022; Riedelbach et al., 2021). Another study showed that most airborne ultrafine particles released by the grinding of dental resin composites were in the size range of 15–35 nm. Their presence was confirmed within the cell cytoplasm in addition to the proof of their toxicity in human bronchial epithelial cells (HBEC-3KT) (Camassa et al., 2021). Increased attention has also been paid to the potential hazard of exposure to zirconium oxide NPs which have been shown to cross numerous physiological barriers and to cause considerable DNA damage in human T cells, apoptosis, to decrease cell proliferation, to cause cellular oxidative stress, and halt the cell cycle (Asadpour et al., 2016). It is generally accepted that the advantages of their application must balance the possible toxic effects to human health (Hichem et al., 2022). Even though additional *in vitro* studies of NPs used in stomatology have been published, knowledge on the *in vivo* exposure of humans to real-world stomatology material is completely missing, despite the constantly increasing use of nanocomposites in dentistry.

Our present study was inspired by these facts as well as by our previous research focused on an investigation of the effects of NPs exposure of the nanocomposites research workers. During that study, the groups of exposed workers were followed repeatedly and annually sampled for six consecutive years including monitoring of exposure in their workplace. Biomarkers reflecting both chronic and acute exposure were studied and data on oxidative stress, as well as the integrity and function of the genome were published (Novotna et al., 2020; Pelclova et al., 2018a, 2018b, 2020; Rossnerova et al., 2019a, 2019b, 2020, 2021). The results suggested gender-related differences in the frequency of different types of micronuclei (MN). While chromosomal breaks, identified as centromere negative MN (CEN- MN), were comparable for both genders in the NPs exposed group, the frequency of chromosomal losses, identified as centromere positive MN (CEN+ MN), was significantly higher in females (Rossnerova et al., 2019a). An increase of aneuploidies in the subgroup of females was confirmed by the whole chromosome painting method (WCP) (Rossnerova et al., 2019b).

Considering these findings, as well as the fact that frequencies of chromosomal aberrations after acute exposure to NPs from nanocomposites in stomatology have never been studied in females, we conducted the follow-up study presented here. The group of women acutely exposed to particulate matter (PM), including nano-fraction during the grinding of dental nanocomposites, was sampled twice (before and after the work). A broad spectrum of various types of chromosomal aberrations in metaphase cells was assessed in peripheral blood lymphocytes (PBL) by the WCP method. The findings included both types of changes: (i) structural alterations of painted chromosomes reflecting breaks and/or their repair (including translocations, reciprocal translocations, insertions, dicentric or acentric chromosomes), as well as (ii) numerical aberrations indicating mitotic spindle failure subsequently resulting in aneuploidies (including particularly chromosome losses: nullisomies and monosomies; or their gains such as trisomies, tetrasomies etc.). Two autosomes (#1 and #4), and gonosome X were selected as markers of genetic damage (Sigurdson et al., 2008). In this study we verified the hypothesis that acute exposure (without previous exposure) to PM from dental nanocomposites has different impacts on various types of chromosomal aberrations (stable and numerical). Moreover, we expected differences in the sensitivity of autosomes and sex chromosome to numerical aberrations by NPs exposure from dental nanocomposites.

2. Materials and methods

2.1. Study group

This study was conducted during two consecutive sampling days in September 2020. The study group was composed of a total of 24 volunteers who had never worked with dental nanocomposites, to satisfy the focus of the study on the effects of acute exposure. All study participants were females (aged 27–50 years), to address their underrepresentation in previous studies (Rossnerova et al., 2019a, 2019b). They were sampled twice a day (first, pre-exposure = pre-shift; second, post-exposure = post-shift). The subjects did not use any respirators or other personal protective equipment. All subjects had physical examinations, both pre-exposure and post-exposure. Detailed questionnaires on basic characteristics, lifestyle and health status including questions focused on: (i) smoking habits, (ii) alcohol consummation, (iii) occupational dust exposure, (iv) vitamin intake, (v) medication use, (vi) occurrence of mold in the household, and (vii) RTG, immunizations, and exposure to chemicals during the last three months were completed during the sampling days. The general characteristics by age and BMI are presented and described in Section 3.1., Table 1.

2.2. Dental nanocomposite processing

The work activities of the study subjects included the grinding of dental light-cured nanocomposite Filtek Ultimate A2 body, 3 M ESPE (Germany), consisting of matrix formed from monomers (bis-GMA, UDMA, TEGMA, bis-EMA6) filled with 78.5 wt% of primary 20 nm SiO_2 and 4–11 nm ZrO_2, particles and their agglomerates (0.6–10 μm). The material was polymerized in 2-mm increments for 20 s each with a Valo polymerization lamp (Ultradent Products, USA) at the radiant exitance 1000 mW/cm^2. (Landuyt et al., 2014; Bradna et al., 2017). The study group was divided into four subgroups of six people each. Each participant was intensively engaged in grinding for ten minutes, followed by a stay in the work room with the rest of the subgroup, while other subjects performed grinding (details in Table 2). The grinding technique consists of the following steps: the specimen surface was ground with a round medium diamond bur (particle size 107–126 μm; Edenta, Switzerland) at 100,000 rpm without water cooling. The burs are fixed to a 1:5 handpiece (Kavo Dental, Germany) attached to an electrical micromotor LA-3 E supplied by a Perfecta 900 (W&H Dentalwerk, Austria). To avoid overheating, the handpiece was changed every two minutes and the used bur was replaced with a new one. The mass of dental material removed during grinding was quantified by its weight for each subject, subgroup, and group (more details in Section 3.2.).

2.3. Exposure monitoring

Two approaches of exposure monitoring to nanoparticles (NPs), as well as to other particulate matter (PM) fractions were used during the grinding of dental nanocomposites in the room with a volume of 135 m^3.

In the first approach, personal exposure was measured by an active personal nanoparticle sampler (PENS) (Tsai et al., 2012), which allows the collection of both respirable particulate mass (RPM) and NP mass simultaneously. The PENS attached app. 30 cm from the mouth to the collar consists of three main components (the first one separates particles with an aerodynamic diameter (D_a) > 4 μm, the second one collects particles with the D_a ranging from 100 nm to 4 μm, and the third component separates NP with D_a < 100 nm). An AirChek XR5000 personal sampling pump was used as the vacuum source, up to a pressure drop of 21 kPa (Ondrackova et al., 2019).

The second approach involved stationary online exposure

monitoring of PM fractions carried out by a Scanning Mobility Particle Sizer (SMPS 3936 L, TSI, USA) and an Aerodynamic Particle Sizer (APS 3321, TSI, USA). Both instruments provided particle number concentrations and were sampled in 5 minute long cycles. The SMPS measured particle number concentration in the size range of 0.01–0.7 μm (mobility diameter, D_m), while the APS monitored particles in the size range 0.5–20 μm (D_a). The SMPS data were subsequently converted to the D_a using an algorithm described previously (Sioutas, 1999), resulting in size-resolved number concentrations with D_a covering an interval from 20 nm to 20 μm. The methodological details were published previously (Talbot et al., 2017; Pelclova et al., 2018a). The measuring devices were placed approximately 1 m from the place where the grinding was performed.

2.4. Venous blood sampling and cultivation

Whole venous blood samples were collected into sodium-heparinized vacutainers (Dialab) and stored at 4°C until processing. The cultures were established within 24 h and harvested following 72 h of incubation at 37°C. Colchicine (Fluka) was added to the final concentration of 0.5 μg/ml 2 h before the end of the incubation process, to obtain a sufficient number of mitoses. The cells were collected by centrifugation, re-suspended in pre-warmed hypotonic solution (0.075 M KCl) and fixed in acetic acid/methanol. The cell suspensions with mitosis were stored at −20°C in fixative and used to prepare microscopic slides for analysis (Rossnerova et al., 2010).

2.5. FISH technique

The fluorescence *in situ* hybridization (FISH) technique was performed according to the manufacturer's instructions (MetaSystems). A set of whole chromosome probes (Customized XCP-Mix #1R-#4 G-# XRG) (MetaSystems,) for autosomes #1 (labeled with TexasRed: a red emitting fluorophore), #4 (labeled with FITC (fluorescein-5-isothiocyanate): a green emitting fluorophore), and gonosome #X (labeled with a combination of the red and green emitting fluorophore visualized as yellow) were used for overnight hybridization with the samples. The unpainted chromosomes were counterstained with 4',6-diamidine-2'-phenylindole dihydrochloride (DAPI) mixed with Vectashield mounting medium (Vector Laboratories) (Rossner et al., 2014).

2.6. Structural and numerical aberrations analysis

Microscopic analysis was performed using a Zeiss Axioscope microscope equipped with a triple filter to simultaneously visualize the DAPI (blue), TexasRed (red), and FITC (green) signals. The final magnification of the studied metaphases was 1000-times. Color images with chromosomal aberrations were collected and analyzed using the ISIS software, version 5.8.14 (MetaSystems). The number of scored metaphases was 1000 per sample (two-times per study subject). Both structural and numerical chromosomal aberrations were recorded in this study.

Structural chromosomal aberrations were classified according to the Protocol for Aberration Identification and Nomenclature (PAINT) (Tucker et al., 1995) including stable and unstable chromosomal aberrations (one-way translocations: t; two-way, reciprocal translocations: rcp; insertions: ins; dicentric chromosomes: dic; acentric fragments: ace; rings: r).

Numerical chromosomal aberrations (aneuploidies) of painted chromosomes were also recorded (including monosomies, and trisomies; as well as other rare cases such as nullisomies, tetrasomies, and additional cases up to +/-6 chromosomes in the final karyotype), as were other rare findings (endoreduplications and complex karyotype rearrangements).

The findings of the studied chromosomes were assigned visually in a total of 480,000 metaphases as follows: (i) unpainted, DAPI counterstained chromosomes: by "A", or "a"; (ii) #1: by "B" or "b"; (iii) #4: by "C" or "c"; (iv) #X: by "D" or "d"; a capital letter refers to the whole chromosomes or a part with centromere; a small letter refers to the part of a chromosome without a centromere. Examples of aberrant metaphases are shown in Fig. 1.

2.7. Data analysis

The genomic frequencies of stable translocations (F_G), a result of non-homologous end joining (NHEJ) repair, were calculated as described (Lucas et al., 1992). In detail: Exchange (translocations) frequencies obtained from each chromosome were calculated for the whole genome by dividing the observed frequencies by the factor of 2.05 fp (1 − fp), where fp is the fraction of the painted DNA converted by individual chromosomes. The genomic frequency of translocations in our study obtained from the three chromosomes painted together (including the exchanges between painted chromosomes) was estimated by dividing the observed frequencies by the factor 2.05 {(fr + fg + fy) [1 − (fr + fg + fy)] + frfg+ fgfy + frfy} (Puerto et al., 2000), where fr, fg and fy are the fractions of the genome (Morton, 1991) converted by chromosomes 1, 4 and X. F_G was recalculated per 100 cells and expressed as genomic frequencies of translocations per 100 cells (F_G/100). Additionally, the percentage of aberrant cells (% AB.C.) and aberrations/1000 cells (AB/1000) for structural, numerical, and total aberrations were calculated, as well as the number of color junctions (NCJ) for the painted chromosomes.

Basic descriptive statistics (mean, standard deviation (SD), median, minimum, maximum), including t-test for normally distributed variables, were calculated using Microsoft Excel 2013. Other tests, including the bivariate, and multivariate linear regression analyses were performed using SPSS 20.0 software.

3. Results

3.1. Questionnaire characteristics of the studied subjects

The general characteristics of the study subjects are presented in Table 1. The study group was composed of females with a median age of 35 years. For cytogenetic analyses, the whole group was divided into younger and older females by the median value. BMI profiling revealed the studied group to be slightly overweight (value 25.4). A total of 8.3%/50%/41.7% underweight/normal weight/overweight females, respectively, were involved in the acute exposure experiment. Younger females had lower BMI in comparison with older ones.

Additional questionnaire data revealed other exposure factors for individual participants of the study. In detail: one active smoker (4.2%); no one in risk of occupational exposure to dust; six participants consuming vitamin C supplements (25%); nine participants taking medication for thyroid gland or hypertension or/and hormonal anticonception (37.5%); one participant reported occurrence of mold at home (4.2%); two women had X-ray examination (8.4%), one had been vaccinated (4.2%) and three reported another exposure to chemicals during the last three months (12.5%) in the last three months. Moreover, the majority (62.5%) of the participants had a mild irritation of conjunctivae after exposure; however, no auscultation phenomena on the physical examination of the lungs were detected.

3.2. Exposure evaluations

The exposure risk of particulate matter including nano fraction was evaluated by: (i) a simple measurement of the weight of the total grinded mass; (ii) evaluation of the personal exposure for each participant; (iii) stationary exposure at a single location in the monitored room.

An overview of exposure characteristics in the studied group as well as in the individual subgroups is summarized in Table 2. The presented data shows the total average time of exposure to be 76.83 minutes, a value comparable for individual subgroups. In contrast, the average

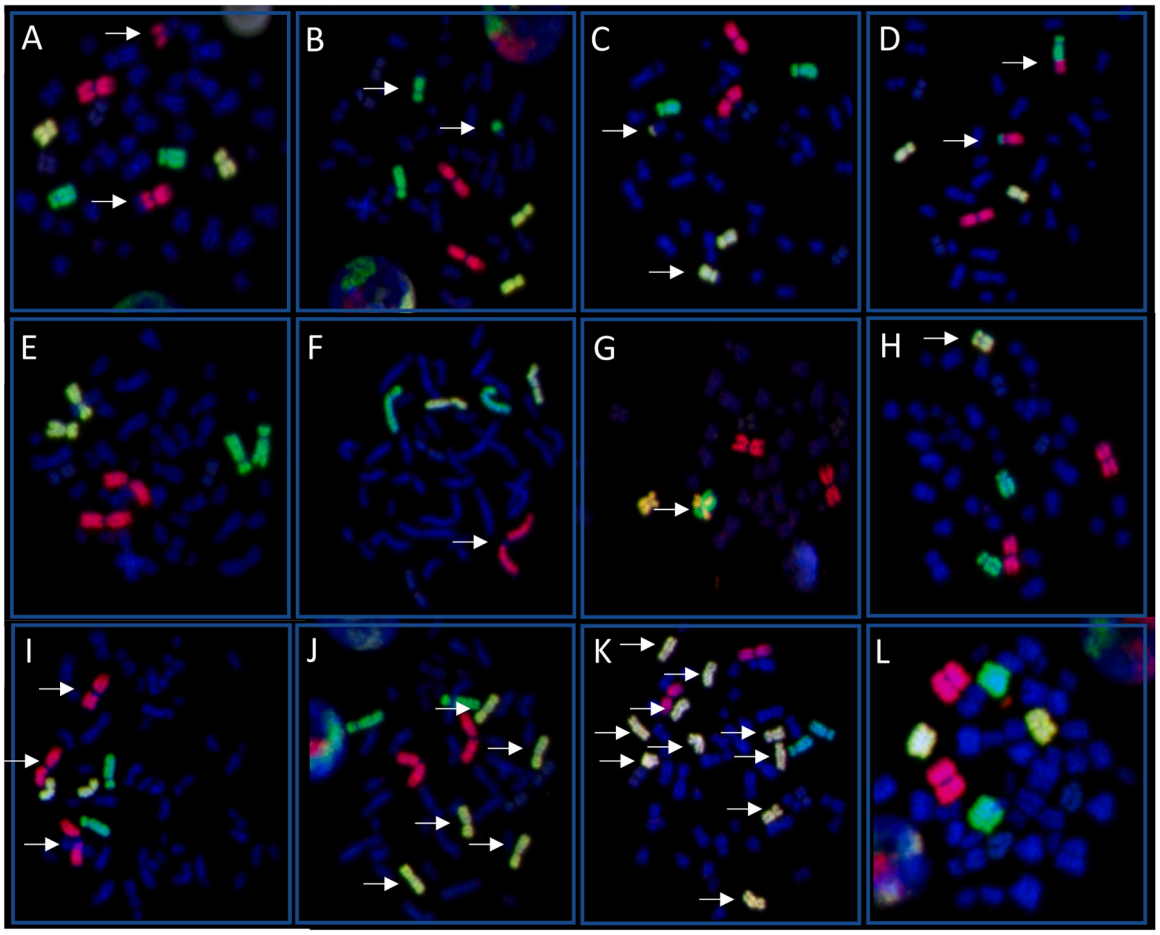

Fig. 1. Examples of metaphases with or without chromosomal aberrations analyzed by fluorescence in situ hybridization using whole chromosome painting (WCP). Findings of structural and numerical aberrations in chromosomes #1 (red, labeled B,b), #4 (green, labeled C,c) and #X (yellow, labeled D,d); other chromosomes stained with DAPI (blue, labeled A,a): A-D stable/structural chromosomal aberrations (A – rcp (Ab)(Ba), B – rcp (Ac)(Ca), C – rcp (Ad)(Da), D – rcp (Bc)(Cb)), E – metaphase without tracked aberrations, F-K numerical aberrations including monosomies, trisomy and others aneuploidies (F – 1x(B), G – 1x(C), H – 1x(D), I – 3x(B), J – 5x(D), K – 10x(D)); L other findings, endoreduplication.

Table 1
Characteristics of the study subjects.

Group/Subgroup	N	Mean ±SD	Median (Min. – Max.)
GENDER (females)	24		
AGE (years)			
All	24	37.0 ± 7.2	35.0 (27.0 – 50.0)
Younger (≤35)	13	31.5 ± 2.5	31.0 (27.0 – 35.0)
Older (>35)	11	43.6 ± 4.9	44.0 (36.0 – 50.0)
BMI (kg/m^2)			
All	24	25.4 ±6.6	23.74 (18.1 – 42.2)
Underweight (<18.5)	2	18.2 ± 0.2	18.2 (18.1 – 18.3)
Normal (18.5 – 25)	12	21.8 ± 1.8	21.8 (19.2 – 24.5)
Overweight (>25)	10	31.3 ± 6.2	29.5 (25.4 – 42.2)
BMI by AGE			
Younger (≤35)	13	23.5 ± 5.0	22.6 (18.1 – 35.1)
Older (>35)	11	27.7 ± 7.7	25.4 (18.3 – 42.2)

N: number of subjects; BMI: body mass index.

mass weight removed by grinding was 0.88 ± 0.32 g (mean ± SD) for all participants, with a relatively wide range from 0.32 – 1.77 g. A comparison of individual subgroups (S1-S4: 0.97 g, 1.04 g, 0.93 g, and 0.59 g; respectively) revealed significantly lower total grinded mass in the S4 subgroup ($p<0.01$) than in S1 and S2. Personal exposure data obtained from personal nanoparticle samplers (PENS) allowed for the analysis of individual exposures to respirable fraction PM4 (particulate matter up to 4 μm) as well as to nano fraction PM0.1 (particulate matter up to 0.1 μm). Again, a relatively wide range of individual exposure values (similar to the total grinded mass) was observed for both, PM4 and PM0.1 fractions (209 – 927 μg, and 3.3 – 7.6 μg, respectively). Interestingly, there was no correlation between the individual values of grinded mass and personal exposure data (e.g., the highest grinded mass value in subgroup S3, but the highest personal exposure to respirable and nano fraction in subgroup S1).

Additionally, data from stationary monitoring obtained by a combination of results from the scanning mobility particle sizer (SMPS), and the aerodynamic particle sizer (APS) shows the total number concentrations of particles across the spectrum from nano to PM10. The obtained values demonstrate the highest number of particles in the nano fraction with a maximum of 49 300/cm^3. Consistently, the highest values in all fractions were detected in subgroup S2, while the lowest values were found in subgroup S4.

3.3. Structural chromosomal aberrations

The complex evaluation of structural chromosomal aberrations was one of the main goals of this study. The overview of the main results assessed by FISH (Mean ±SD, and Median (Range)) for the individual analyzed parameters (AB/1000 S, % AB.C. S, F_G/100, NCJ, t, rcp, ace, dic, ins) in individual groups and subgroups are shown in Table 3. The data relevant to the acute exposure episode uniformly shows no differences between the pre-shift and post-shift results excluding the frequency of the insertions where a significant increase was observed

Table 2
Exposure characteristics in the studied group and subgroups.

Characteristics	Group/Subgroups (S1 – S4)				
	All	S1	S2	S3	S4
	Value / Mean ± SD Median (Range)				
Basic exposure characteristics					
N	24	6	6	6	6
Exposure time (min)	76.83 ± 1.76	77.33 ± 0.52	74.33 ± 0.82	78.33 ± 0.82	77.33 ± 1.37
	77 (73–79)	77 (77 – 78)	74.50 (73 – 75)	78.50 (77 – 79)	77.50 (75 – 79)
Total grinded mass (g)	0.88 ± 0.32	0.97 ± 0.22	1.04 ± 0.25	0.93 ± 0.44	0.59 ± 0.18*
	0.88 (0.32–1.77)	0.91 (0.80 – 1.40)	0.98 (0.74 – 1.39)	0.88 (0.47 – 1.77)	0.59 (0.32 – 0.80)
Personal PENS exposure data (μg)					
Respirable fraction - PM4	381 ± 146	411 ± 233	352 ± 88	366 ± 105	301 ± 99
	327 (209 – 927)	320 (264 – 927)	372 (294 – 533)	363 (209 – 490)	324 (224–528)
Nano fraction - PM0.1	3.3 ± 1.3	3.4 ± 1.9	3.2 ± 0.7	3.7 ± 1.0	3.2 ± 0.9
	2.9 (2.0 – 7.6)	2.6 (2.2 – 7.6)	3.0 (2.4 – 4.3)	3.6 (2.1 – 4.9)	2.9 (2.0 – 4.7)
Stationary SMPS/APS exp. data (#/cm^3)					
Total number conc. 1 μm - 10 μm	39.2 ± 20.3	44.5 ± 18.5	49.6 ± 24.2	36.0 ± 19.1	28.4 ± 1.1
	38.5 (1.6 – 70.8)	43.4 (2.4 – 67.3)	64.5 (1.72 – 70.8)	41.6 (1.6 – 57.4)	33.6 (2.9 – 40.1)
Total number conc. 100 nm - 1 μm	11700 ± 5310	12200 ± 4420	15800 ± 6780	11500 ± 4190	8040 ± 1860
	10100 (3910–24900)	12800 (3910–18000)	17800 (4200–24900)	12700 (5020–16400)	8920 (4060–9780)
Total number conc. < 100 nm	29500 ± 13200	29800 ± 12000	34000 ± 15000	31200 ± 13900	23400 ± 9180
	29900 (3370–49300)	29500 (5570–45300)	40100 (6930–49300)	37700 (5910–46400)	27400 (3370–33100)

N: number of subjects; PM4: particulate matter of aerodynamic diameter ≤ 4 μm; PM0.1: particulate matter of aerodynamic diameter ≤ 0.1 μm; PENS: personal nanoparticle sampler; SMPS: scanning mobility particle sizer; APS: aerodynamic particle sizer; *, p<0.01 (related to S1 and S2 subgroups).

Table 3
Structural chromosomal aberrations overview in the studied subjects.

Group/Subgroup	Mean ± SD								
	Median (Min. – Max.)								
	AB/1000 S	% AB.C. S	F_G/100	NCJ	t	rcp	ace	dic	ins
All	5.67 ± 3.67	0.35 ± 0.22	1.32 ± 0.95	4.92 ± 3.44	4.50 ± 3.39	1.79 ± 1.88	0.88 ± 1.12	0.13 ± 0.34	0.04 ± 0.20
Pre-exposure	5 (0–14)	0.40 (0–0.80)	1.13 (0–3.96)	4.50 (0–14)	4 (0–14)	2 (0–5)	0.50 (0–4)	0 (0–1)	0 (0–1)
All	5.50 ± 3.53	0.31 ± 0.20	1.25 ± 0.86	5.13 ± 3.66	4.46 ± 2.99	1.58 ± 1.10	0.50 ± 0.88	0.13 ± 0.34	0.25 ± 0.53*
Post-exposure	5 (0–13)	0.30 (0–0.70)	1.13 (0–3.11)	4 (0–13)	4 (0–11)	2 (0–4)	0 (0–3)	0 (0–1)	0 (0–2)
Younger (≤35)	5.38 ± 3.95	0.31 ± 0.21	1.24 ± 0.91	5.15 ± 4.08	4.38 ± 3.20	1.77 ± 1.30	0.46 ± 0.78	0.23 ± 0.44	0.23 ± 0.44
Post-exposure	4 (0–13)	0.20 (0–0.70)	1.13 (0–3.11)	4 (0–13)	4 (0–11)	2 (0–4)	0 (0–2)	0 (0–1)	0 (0–1)
Older (>35)	5.64 ± 3.14	0.32 ± 0.19	1.26 ± 0.84	5.09 ± 3.30	4.55 ± 2.88	1.36 ± 0.81	0.55 ± 1.04	n.d.	0.27 ± 0.65
Post-exposure	6 (0–10)	0.30 (0–0.6)	1.42 (0–2.26)	6 (0–10)	5 (0–8)	2 (0–2)	0 (0–3)	n.d.	0 (0–2)

Abbreviations: AB/1000 S, structural aberrations/1000 cells; % AB.C. S, percentage of aberrant cells with structural aberrations; FG/100, genomic frequency of translocations/100 cells; NCJ, number of color junctions; t, translocations; rcp, reciprocal translocations; ace, acentric fragments; dic, dicentric chromosomes; ins, insertions; n.d., not detected; *, p<0.05.

(p<0.05). Nevertheless, due to the generally low frequency of insertions, this finding could be random. Overall, no effects of short-term acute exposure were observed. This observation is supported mainly by the frequency of total translocations (t) including: (i) one-way and two-way reciprocal translocations (rcp) which are almost identical before and after exposure episodes; (ii) the genomic frequency of translocations/100 cells (F_G/100) whose median values are identical. Additional analysis by age showed a gradual increase of F_G/100: median values 1.13 and 1.42, in younger (≤35 years) and older women (>35 years), respectively.

3.4. Numerical chromosomal aberrations

An evaluation of numerical chromosomal aberrations was another goal of this study. An overview of the main results assessed by the WCP method of the three selected chromosomes: Mean ± SD, and Median (Range) for the individual analyzed parameters (AB/1000 N, % AB.C. N, Monosomies, Trisomies, and other aneuploidies) in individual groups and subgroups is shown in Table 4. The data for the acute exposure episodes shows significant differences in the number of aberrations (AB/1000 N) and frequency of monosomies (both, p<0.05). Moreover, the

Table 4
Numerical chromosomal aberrations in the studied subjects.

Group/Subgroup	Mean ± SD				
	Median (Min. – Max.)				
	AB/1000 N	% AB.C. N	Monosomies	Trisomies	Other aneuploidies
All	15.21 ± 8.89	1.51 ± 0.88	8.17 ± 6.02	6.42 ± 3.45	1.50 ± 0.52
Pre-exposure	14.50 (3–48)	1.45 (0.30–4.70)	7 (0–30)	6.5 (1–16)	1.50 (1–2)
All	18.67 ± 7.23*	1.80 ± 0.67**	11.27 ± 5.46*	5.86 ± 2.73	2.12 ± 1.32
Post-exposure	18.50 (8–30)	1.85 (0.80–3)	11 (2–21)	5 (1–10)	2 (1–5)
Younger (≤35)	18.31 ± 6.22	1.77 ± 0.56	11.04 ± 4.99	6.05 ± 2.39	1.93 ± 1.08
Post-exposure	18 (10–30)	1.80 (1–2.80)	10 (5–21)	5 (3–10)	1.65 (1–4)
Older (>35)	19.09 ± 8.56	1.85 ± 0.81	11.55 ± 6.20	4.73 ± 3.26	2.33 ± 1.58
Post-exposure	23 (8–30)	2 (0.80–3)	14 (2–21)	5 (0–9)	2 (1–5)

Abbreviations: AB/1000 N, numerical aberrations/1000 cells; % AB.C. N, percentage of aberrant cells with numerical aberrations; Other aneuploidies, including nullisomies, tetrasomies, and others; *, p<0.05; ** p=0.05.

post-exposure value of % AB.C. N was on the border of significance (p=0.05). With respect to maximum values higher in the pre-exposure period than post-exposure (Table 4), we compared individual values of AB/1000 N in pairs and revealed the following: (i) increased number of chromosomal aberrations as a results of NPs exposure was found in 62.5% of participants; (ii) identical, or almost identical values in both periods was observed in 25% of participants; and (iii) three outlier values were detected with an opposite trend associated with NPs exposure. In contrast, the frequency of trisomies was not different after the acute exposure period, while the median values of the monosomies increased. In addition, the number of monosomies increased with age: a 40% increase was observed in older (age 36–50 years) women when compared with younger women (age 27–35 years).

3.5. Overall pattern of chromosomal aberrations

Proportions of individual types of chromosomal aberrations for the two sampling times: pre-shift (pre-exposure) and post-shift (post-exposure), as well as proportions of rearrangements for the individual painted chromosomes are presented in Fig. 2, for the structural aberrations, and in Fig. 3 for the numerical aberrations, respectively. Data presented in Fig. 2, part A and B, support the data presented in Table 3 and show the overall stability of the structural alterations with dominancy of translocations (one-way and two-way), excluding a significant increase of insertions which could be a random result due to the generally low frequency of these findings. Additional data in Fig. 2, parts C and D, show the ratio of the structural alterations for the individual painted chromosomes. Both pie charts show the majority of the absolute numbers of structural aberrations in chromosome #1 followed by the #4 and #X. Considering the estimated frequencies of these types of aberrations in relation to the size of the individual painted chromosomes (41.7%, 31.6%, 26.7%, respectively), the obtained data show slightly higher values for both autosomes in contrast to the slightly lower values for the painted gonosome for the pre-shift sampling, as well as for the post-shift sampling excluding chromosome #4.

While the total number of structural aberrations was stable when the pre-shift (pre-exposure) and post-shift (post-exposure) results were compared, the total number of numerical aberrations (including monosomies, trisomies, and other alterations as nullisomies, tetrasomies etc.) increased by about 22.5% from 368 to 451 cases as shown in Fig. 3. Data presented in Fig. 3, parts A and B, supported the results presented in Table 4 and show the overall increase of the monosomies after the acute exposure episode. While the increase of monosomies in the overall pattern of all aneuploidies is +6%, the absolute increase for monosomies alone reached the value +37.8%. Additional data in Fig. 3, parts C and D, show the ratio of numerical alterations for the individual painted chromosomes. For each of the painted chromosomes, the results are divided into chromosomal losses (monosomies) and chromosomal gains (trisomies). Both pie charts show most of the numbers of the numerical aberrations in gonosome X, when compared with both autosomes. In addition, an increase of monosomies is visible in all painted chromosomes: an increase in absolute numbers of cells [11, 19 and 50 cells, for chromosome #1 (red), #4 (green) and X (yellow), respectively], corresponding to a relative increase of cell numbers of 1.2%, 3.1% and 5.9% for the given chromosomes (Fig. 3, parts C and D). All data indicate the highest increase of monosomies in gonosome X.

3.6. The overall effects of acute exposure from dental nanocomposites on the cytogenetic markers

The frequencies of structural (AB/1000 S), and numerical (AB/1000 N) aberrations in the studied subjects before and after the exposure episode are shown in Fig. 4. Here, the dominancy of the numerical alterations over the structural rearrangements for both sampling periods as well as their post-exposure increase is clearly visible. Interestingly, the total number of mitoses containing both numerical and stable aberrations increased after the exposure 3.14-times (from 7 to 22 cases).

Additional data presented in Table 5 summarizes the results of the bivariate and multivariate linear regression of the studied parameters and their impact on the level of structural and numerical aberrations,

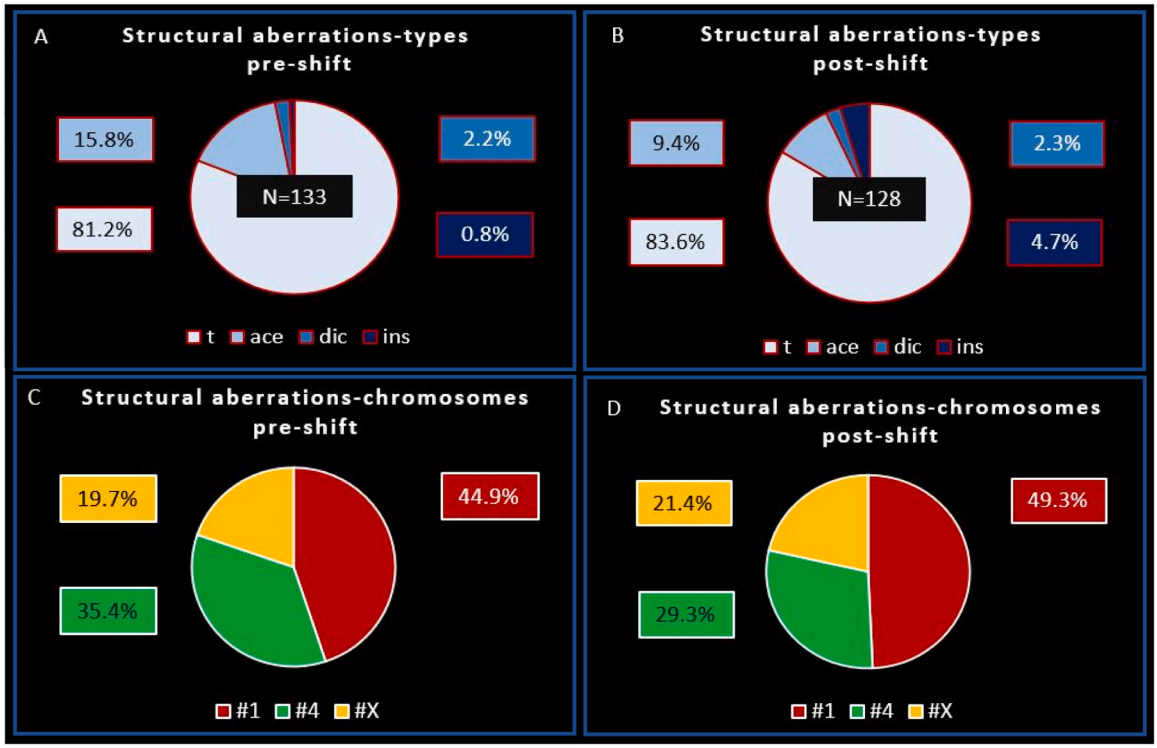

Fig. 2. Pattern of structural chromosomal aberrations in the studied group (A and C pre-shift, B and D post-shift): A-B by the types of aberrations; C-D by the individual painted chromosomes.

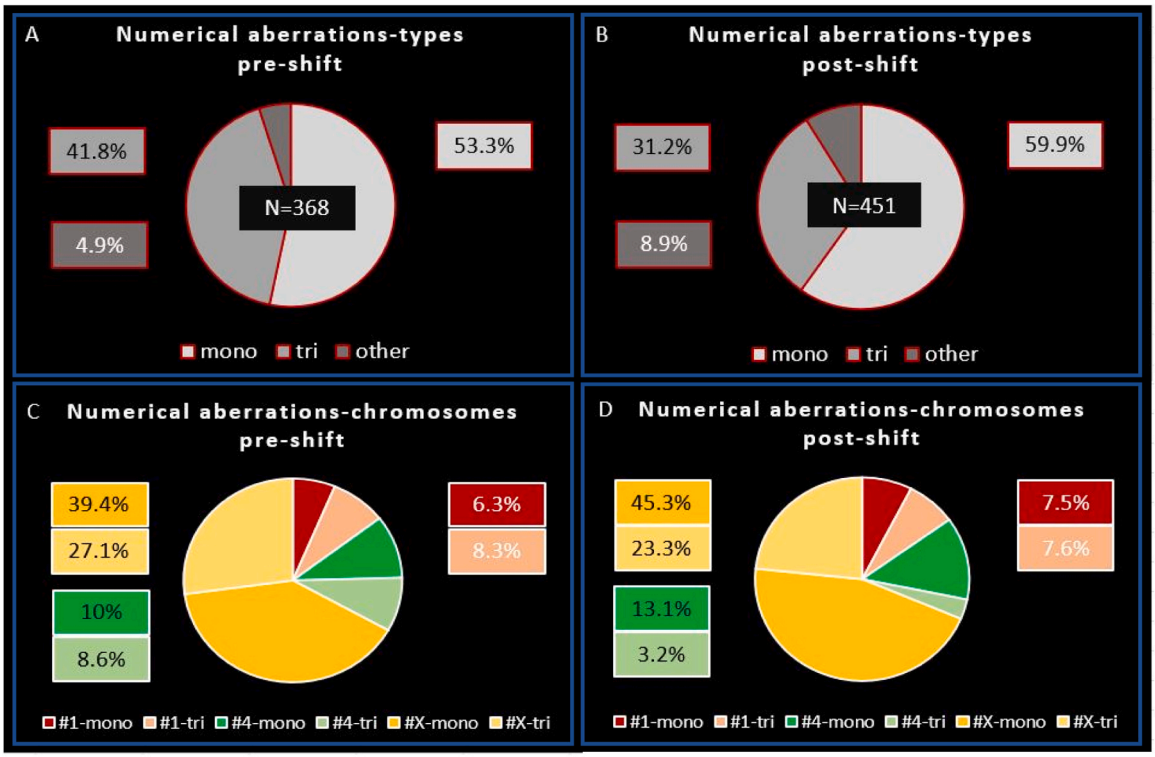

Fig. 3. Pattern of numerical chromosomal aberrations in the studied group (A and C pre-shift, B and D post-shift): A-B by the types of aneuploidies; C-D by the individual painted chromosomes and type of aneuploidies.

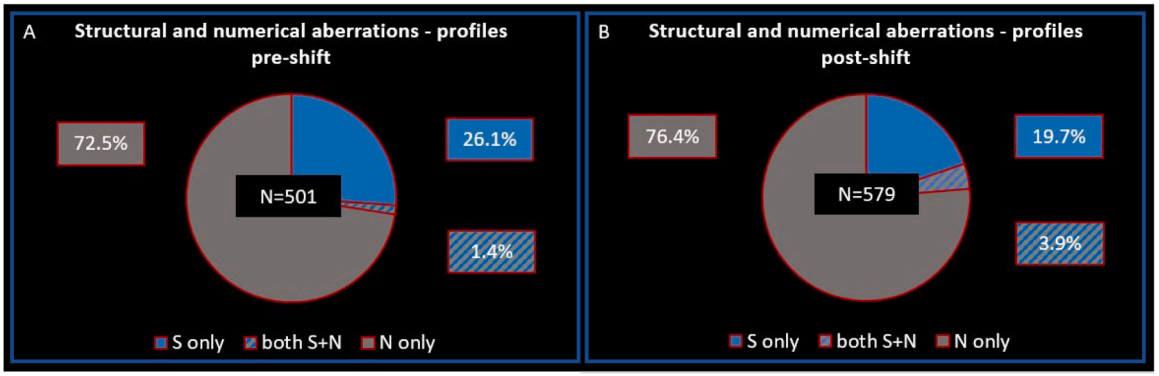

Fig. 4. Overall structural and numerical chromosomal aberration profiles in the studied group (A pre-shift, B post-shift). Three categories represent: S only, cells with exclusively structural aberrations; N only, cells with exclusively numerical aberrations; both S+N, cells with both types of aberrations.

calculated as the difference between frequencies after and before and after exposure to respirable PM4 and nano PM0.1 fractions during the processing of the tested nanocomposites. Even though no significant results were observed for these personal exposure data, the obtained results indicate the effect of age on the level of stable translocations (F_G/100) in the bivariate model (p=0.080), as well as in the multivariate model when analyzed together with BMI and personal exposure data to PM4 (p=0.088) or nano fraction PM0.1 (p=0.095).

4. Discussion

In this study we focused on genetic alteration profiling in middle–aged women acutely exposed during the processing of dental nanocomposites. The overall pattern of chromosomal aberrations including structural and numerical alterations in two autosomes (chromosomes #1 and #4) and gonosome X was analyzed simultaneously using the FISH method applied for WCP. This topic was inspired by our previous cytogenetic results obtained in a group of research workers chronically exposed to NPs (Rossnerova et al., 2019a, 2019b). An additional reason was the absence of human *vivo* studies evaluating the risks of exposure to SiO_2 and ZrO_2 NPs in stomatology. As the application of nanomaterials in stomatology represents a health risk not only to professional workers, but to the entire population during dental treatment, we designed our study to mimic exposure relevant to patients. In addition to the cytogenetic endpoints, we also focused on the monitoring of NPs exposure using both personal (PENS) and stationary monitors including an analysis of the broad spectrum of PM from < 100 nm to PM10.

Compared to previous studies (Novotna et al., 2020; Pelclova et al., 2018a, 2018b, 2020; Rossnerova et al., 2019a, 2019b, 2020, 2021), in this present report personal monitors (PENS) were used to assess PM exposure. The total duration of acute exposure (77 min on average) was selected to simulate a dental treatment of average duration. Comparison of all available data: ((i) for total grinded mass, (ii) stationary SMPS/APS

Table 5
Overview of bivariate and multivariate linear regression results related to personal acute exposure and studied cytogenetic parameters.

A. BIVARIATE linear regression results & cytogenetic parameters								
	% AB.C. S B	*p*	F_G/100 B	*p*	% AB.C. N B	*p*	Monosomies B	*p*
PM4 exposure	-0.074	0.733	-0.049	0.820	0.125	0.560	0.058	0.787
PM0.1 exposure	-0.099	0.644	-0.102	0.636	0.157	0.463	0.102	0.634
Age	0.202	0.345	0.365	0.080	0.066	0.760	0.127	0.554
BMI	-0.016	0.939	0.001	0.995	0.045	0.834	0.032	0.884
B. MULTIVARIATE linear regression results & cytogenetic parameters								
	% AB.C. S B	*p*	F_G/100 B	*p*	% AB.C. N B	*p*	Monosomies B	*p*
PM4 exposure	-0.112	0.631	-0.170	0.435	0.067	0.771	0.064	0.788
Age	-0.202	0.414	-0.401	0.088	-0.015	0.951	0.017	0.947
BMI	0.104	0.680	0.384	0.112	0.230	0.362	-0.019	0.940
PM0.1 exposure	-0.119	0.605	-0.186	0.384	0.103	0.648	0.109	0.640
Age	-0.191	0.433	0.386	0.095	-0.019	0.937	0.014	0.954
BMI	0.101	0.685	0.381	0.109	0.222	0.370	-0.029	0.909

Abbreviations: % AB.C. S, percentage of aberrant cells with structural aberrations; FG/100, genomic frequency of translocations/100 cells; % AB.C. N, percentage of aberrant cells with numerical aberrations; Monosomies, cells missing one of the painted chromosomes.

exposure data for the total number concentrations/cm^3 measured across all PM fraction, at a distance of approximately 1 m from the source of exposure, and (iii) personal exposure data providing information about real individual exposure to nano and PM4 respirable fraction), indicated additional impacts affecting the total real exposure. Since there is no clear relationship between the amount of ground material and the exposure profiles: ((i) the highest maximum level of ground material in subgroup S3, (ii) the highest measured personal exposures for both PM4 and the nano fraction in subgroup S1, and (iii) the highest values measured by a stationary monitor in the subgroup S2), we can assume that various factors including the style of work when grinding the material plays a significant role in the final personal exposure level (e.g. the pressure on the material, the different kinetic energy of the particles, the angle of holding the tool, and also the height of the person).

An analysis of structural chromosomal aberrations was one of the aims in the assessment of a broad spectrum of alterations associated with exposure risks to PM, including nano fraction, in stomatology. In this study, the traditional methodological approach of the WCP of selected chromosomes (Tucker et al., 1995) was used as in many previous reports (Sigurdson et al., 2008). Our results did not show the effects of a short acute exposure on the level of structural aberrations (excluding a random finding, a significant increase of low frequent aberrations - insertions), most likely because induction of chromosomal breaks and their subsequent repair by NHEJ requires an extended time period. However, we observed an increase of stable translocation with age in agreement with the published data (Sigurdson et al., 2008; Rossnerova et al., 2010) and additionally, also data on the overall spectrum of both, structural and numerical chromosomal aberrations which are not commonly evaluated together by this technique (details discussed below).

For the calculation of genomic frequency of translocation per 100 cells (F_G/100) a formula used in many other studies was applied (Sigurdson et al., 2008). It considers equal induction and persistence of chromosome aberrations for individual chromosomes as shown for chromosomes #1, #4, and X in thyroid cancer patients treated with radioactive iodine (Puerto et al., 2000). Similarly, in our study the frequencies of detected aberrations relevant to the length of chromosomes were comparable or slightly higher than expected in both autosomes, while in the sex chromosome the level of detected structural aberrations was lower, about 6.1%. Interestingly, to the best of our knowledge, the sensitivity of the gonosomes was not tested in any human toxicological studies, but it has been reported that aberrations of chromosomes #1–6 assessed in lymphocytes of human subjects exposed to polycyclic aromatic hydrocarbons PAHs were nonrandomly distributed, and chromosome #6 was the most frequently damaged (Bocskay et al., 2007). Similarly, another study that concentrated on the identification of chromosomes damage after benzo[*a*]pyrene (B[*a*]P) and extractable organic matter (EOM) treatment showed chromosome #7 as the most affected regardless of the tested compound (Rossner et al., 2014). Based on the published data, we can hypothesize that there are additional factors that can increase/decrease the probability of the stable aberrations of individual chromosomes. The territoriality of chromosomes and their proximity to the nucleus periphery may be one of these (Cremer and Cremer, 2010), as well as a specific chemical compound or nano sized PM.

Simultaneously, an analysis of the numerical aberrations was performed in this study. In contrast to the structural alteration results, the frequency of overall aneuploidies (including monosomies, trisomies and other numerical abnormalities detected in the metaphase chromosomes) significantly differed and increased after acute exposure. Moreover, a comparison of the quantity of structural and numerical alterations showed a 3.77 times higher number of aneuploidies (losses or gains of chromosomes) before an exposure episode in comparison with structural aberrations accompanied by *ds* DNA breaks. These differences were 4.52 times higher after the acute exposure episode after stomatology nanocomposite grinding. A survey of the relevant literature shows that involving both types of rearrangement into the chromosomal abnormality assessment is relatively rare in studies, which have focused on structural aberrations involving stable translocations and/or unstable acentric fragments (Balajee et al., 2021; Zedan et al., 2022). However, one study evaluated the spectrum of both types in a methyl isocyanate (MIS) gas-exposed population by combination of G-banded metaphases and karyotyping across all chromosomes with the help of IKAROS software (Ganguly et al., 2019).

Notably, even though the frequency of aneuploidies was relatively high in both sampling times, these results were driven by the increase of monosomies, and not trisomies whose level remained lower and comparable before and after the exposure. This trend was also clearly visible with age. These observations can be explained by the effects of exposure on the M phase, especially mitotic spindle geometry leading to chromosome segregation errors (Silkworth et al., 2012). We can hypothesize that there are several possibilities of sources of non-balanced division of chromosomes including: (i) kinetochores of chromosomes could establish different types of attachment with microtubules during the early stages of mitosis (merotelic, monotelic or syntelic); (ii) fragmented centrosomes followed by a multipolarity of spindle; or (iii) an incomplete spindle pole. Another question is, why are there more monosomies than trisomies. This can be explained either by the multipolarity of the mitotic spindle leading to the formation of a small nucleus with only a few chromosomes, or by the escaping individual chromosomes which

were not drawn to any pole of the dividing spindle. Similarly, as in our study, other authors have reported a risk of NPs exposure associated with an increase of aneuploidies. Specifically, mitotic spindle disruption was observed after exposure to single-walled carbon nanotubes (SWCNT) in immortalized normal human bronchial epithelial cells (BEAS-2B), similarly as for multi-walled carbon nanotubes (MWCNT) in the same cell line, where monopolar spindle morphologies and fragmented centrosomes were detected (Sargent et al., 2012; Siegrist et al., 2019). Another study showed that specifically modified NPs may act as aneugen-like spindle poisons via interference with tubulin polymerization, leading to an increase of aneuploidies (Buliakova et al., 2017). The question arises, of how can nondisjunctions, that are relevant to dividing cells, be formed with an exposure time of only approximately 77 minutes. This can be explained by the fact that we worked with cells cultivated for 72 h during which time 1–2 cell cycles were completed. This approach allowed us to show the late effects of NPs exposure due to e.g., changes in transcriptome followed by possible changes in proteome induced during an exposure episode.

An evaluation of the frequencies of individual types of aberrations in selected chromosomes was another task of this study. A slightly lower representation of structural aberrations was observed for gonosome X, while aneuploidies, specifically monosomies at both time points, particularly after exposure, were observed in this chromosome. These results correspond with previously published data that also pointed to X chromosome aneuploidies analyzed in interphase or metaphase chromosomes (Norppa and Falck, 2003; Rossnerova et al., 2019b; Ganguly et al., 2023).

Generally, this type of error has its origin mostly in mitotic spindle assembly fail including centrosome polarization or kinetochore connection to microtubules during cell division (Silkworth et al., 2012). Hence, the increased risk of this type of aberrations is specifically relevant for dividing cells, including oocytes, which are more sensitive than male cells when exposed to chemicals that affect meiotic spindle (Pacchierotti et al., 2007). So, this type of exposure can be dangerous for the progeny of women just before or in the early stages of pregnancy. Even though chromosomal aberrations are a frequent reason of abortions in the first trimester (Hassold et al., 2007), many syndromes caused by aneuploidies of the gonosomes are compatible with life and despite the accessibility of excellent prenatal diagnostics in many countries, increased risk of the birth of children with numerical chromosomal aberrations is still significant. Considering our results, we can hypothesize, that this type of exposure might be accompanied by an increased risk of Turner syndrome characterized by the loss of one of the X chromosomes with the final karyotype 45,X0, or by mosaicisms in a combination of the normal karyotype with the aberrant one.

Considering these risks, acute exposure to NPs from nanocomposites in stomatology including ZrO_2 (Asadpour et al., 2016; Hichem et al., 2022; Umaparthy et al., 2022), may cause harmful effects, especially before and in the very early stages of pregnancy, when the first cells start dividing, and is the basis for the karyotype of cells, for additional innumerous divisions. Additionally, errors in mitosis caused by exposure to NPs, or other compounds, could have important implication in tumorigenesis (Buliakova et al., 2017; Kirsch-Volders et al., 2019; Klaseen, et al., 2022).

Even though our study suggests a risk of numerical aberrations, especially monosomies of chromosome X in females after acute exposure to PM, including NPs produced from grinding nanocomposites used in stomatology, a direct link with personal data was not observed. As in all research, this study also implicates innumerous questions, gaps and challenges which could be addressed in the future. These include: (i) the increased number of participants of the study as a major, most important challenge, including a comparison of the effects between males and females; (ii) exposure from grinding of other nanocomposite materials is an interesting topic for follow-up research; chemical analysis of the released material will be needed; (iii) the influence of specific genetic polymorphisms should also be taken into account in a larger study; (iv) an analysis of the effects on additional chromosomes which were not painted in this study is an additional task for the future; (v) additional biomarkers including expression profiles of genes involved in the mitotic spindle, centriole, and/or kinetochore formation should be studied; (vi) long term effects of chronic exposure to NPs from nanocomposites used in stomatology including epigenetic changes could also provide valuable information for dental staff regarding the potential risks of inhalation.

5. Conclusion

An analysis of the frequencies of the broad type of structural and numerical aberrations in women acutely exposed to PM, including NPs, during the grinding of nanocomposite materials in stomatology was the main task of this study. Exposure conditions during dental treatment of the general population were simulated and innovative personal monitoring of exposure, including nano fraction, was applied. An observed increase of numerical aberrations, particularly monosomies of gonosome X, was the main outcome of this study, although a direct correlation with personal PM4 and PM0.1 data was not confirmed. Thus, individual susceptibility and/or other factors and interactions must also be considered. An investigation of a larger cohort accompanied by a detailed chemical analysis of the released PM with the aim of identifying major risk factors is the main recommendation for future studies. In summary, despite clear advantages of the use of nanocomposites in stomatology (Sreenivasalu et al., 2022), the obtained data suggest, that primarily women who plan pregnancy or are in its earliest stages should carefully consider a visit to the dentist, or if necessary, use the maximum available protective equipment and limit inhalation of particles emitted during the treatment to minimize the risk of an increased frequency of monosomies. Finally, the new results may also be related to an adaptation mechanism to adverse environmental conditions leading to a temporary reduction in reproductive capacity.

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Human study statement

The study was conducted according to the guidelines of the Declaration of Helsinki II and approved by the two Ethics Committees: (i) General University Hospital in Prague and First Medical Faculty; Charles University approved the sampling in the study (registration number of permission 426/20 S-IV); (ii) Institute of Experimental Medicine approved the analysis of the samples in the grant of the Czech Science Foundation (registration number of permission 2021/04). All participants signed an informed consent form and had the opportunity to withdraw from participation at any time during the study.

Author contributions

AnR, DP, VZ, and PB designed the study. AnR, IC, and FE conducted the cytogenetic part of the study (blood cultivation, FISH staining, and microscopic analysis). VZ, LO, PB, and AdR conducted personal and stationary monitoring of the exposure to dental nanocomposites. AnR and PR conducted statistical analysis. DP, PK, VZ, LO, PB, AdR, AnR, IC, and FE, organized the sampling and investigation of volunteers. AnR wrote the manuscript and prepared the figures. AnR, IC, FE, ZS, DP, PK, VZ, LO, PB, AdR, and PR revised the manuscript. All authors read and approved the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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