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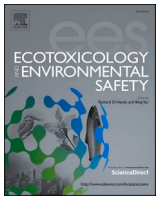

Early genotoxic damage through micronucleus test in exfoliated buccal cells and occupational dust exposure in construction workers: a cross-sectional study in L'Aquila, Italy

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A R T I C L E I N F O

Keywords:
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A B S T R A C T

Aim: The city of L'Aquila (central Italy) was hit by a strong earthquake in 2009 that caused the collapse of several buildings, deaths and injured people. In the following years, a great number of building sites were activated, building workers resulted intensely exposed and represent a relevant target for research on environmental mutagenesis and epidemiological surveillance. Cells of buccal mucosa are considered an appropriate site for early detecting of cytogenetic damage, since it represents the first barrier in inhalation or ingestion and can metabolize carcinogenic agents into reactive chemicals. Our study is aimed 1) at comparing the early genotoxic damage as measured by the buccal mucosa micronucleus test in two subgroups of workers defined by different occupational exposure and 2) at evaluating possible confounding variables such as lifestyle factors.

Methods and results: A cross-sectional study was conducted in L'Aquila, on 24 outdoor workers (OWs) highly exposed on the construction sites and 26 indoor workers (IWs), all subjected to the compulsory occupational surveillance system, in the period 2017–2018. Buccal cells samples were collected and, based on the Micronucleus test, the exfoliated cells were classified in respect of nuclear changes observed. Moreover, a self-report questionnaire composed of 84 items, was administered to the workers.

Results: Significant differences were observed between Exp^+ (OWs) and Exp^- (IWs) in the number of the analyzed cells (expressed as mean value out of 1000 cells): respectively 954.46 *vs* 990.06 normal cells, ($p < 0.001$); 19.79 *vs* 4.95 micronucleated cells, as marker of chromosomal damage ($p < 0.001$); 13.93 vs 8.96 binucleated cells, as marker of failed cytokinesis ($p < 0.001$); 2.09 *vs* 1.18 karyolytic cells, as marker of cell death and damaged DNA ($p < 0.05$). According with a multivariate regression analysis, in addition to the job exposure (OW vs IW, beta = 12.221, $p < 0.001$), the only variable independently associated with an increase in Micronuclei (MNs) is the smoking habit (OWs vs IWs, beta = 6.683, $p < 0.001$) which, even if not associated with dust exposure, worsens cell integrity. Moreover, this worsening effect is weaker in workers not exposed to the site dust (moderation effect). Within social demographic factors, the high educational level only apparently seems to affect MNs number: even if unbalanced in favor of IWs vs OWs, this variable resulted a confounder, since its effect disappears when the interaction between these two factors is considered, because it is a covariate of smoking habit as well as of the job condition.

Conclusion: Despite some limitation, our findings clearly confirm the role of occupational exposure as a marker of cytogenetic damage associated with MNs number in construction workers. Moreover, smoking status appears as the only other investigated factor independently associated to the outcome. The statistical model, in addition, highlights possible moderation and confounding effects, such as interaction between smoking and occupational exposure and the unbalanced school education level in workers. Micronucleus test in exfoliated buccal cells would be considered a suitable method for studying the early genotoxic damage in the construction occupational setting as well as in evaluating the efficacy of preventive practices.

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1. Introduction

Cytogenetics and environmental mutagenesis, today, provides us many tools to study the relationship between exposure to chemicals and their effects on population. The exfoliated buccal epithelial cells (Zeppa, 2014), are currently considered a reliable cell type for Micronuclei (MNs) analysis (Holland et al., 2008; Samanta and Dey, 2012; Arul et al., 2018).

MNs consist of pieces or whole chromosomes which are lost during the cell division (anaphase), forming a secondary nucleus that is smaller than the main one of the cell, so it is called micronucleus. The main mechanisms from which these small nuclei can arise are: chromatide-type or chromosome breakage; anaphasic bridges aberrations; spindle disfunctions or malfunctions of chromosome-division involved organelles (e.g. kinetochore); apoptosis (Hintzsche et al., 2017).

Buccal MNs dosage in exfoliating epithelial cells of the oral mucosa is widely used in biomonitoring studies to assess the human exposure to genotoxic agents. In the genetics, the micronucleus test is one of the cytogenetic tests most frequently used in pre-screening and follow-up activities of precancerous lesions (Fenech and Morley, 1986; Bolognesi and Fenech, 2013; Bolognesi et al., 2015) and it is a minimally invasive tool to assess the genomic damage, the chromosome instability and the aerodigestive tract cell death in humans (Holland et al.,2008; Ribeiro and Angelieri, 2008). Therefore, the micronucleus test is used in several studies to assess occupational (Hutter et al., 2020) and environmental exposure to genotoxins (Bolognesi et al., 2015; Salama et al., 1999; Testa et al., 2002; Alabi et al., 2020); medical procedures (Ribeiro and Angelieri, 2008; Carlin et al., 2010), micronutrient deficiencies (Thomas et al., 2011; Thomas and Fenech, 2015), lifestyle factors (Chandirasekar et al., 2014; Nefic and Handzic, 2013; Kausar et al., 2009), pesticide exposure (de Oliveira et al., 2019), genetic susceptibility (Rosin and Ochs, 1986) and polymorphisms of xenobiotic-metabolizing and DNA repair genes in DNA damage, (de Souza et al., 2020). Moreover, the high presence of MNs in buccal cells seems to be linked to an increased risk of degenerative diseases and cancer (Bolognesi et al., 2015). Cells of buccal mucosa represent the first barrier in inhalation or ingestion, and they can metabolize carcinogenic agents into reactive chemical substances. Chromosomal stability in buccal cells was linked to age but not affected by exercise and nutrients (Franzke et al., 2020). Genomic instability in exfoliated buccal cells has been recently found among cement warehouse workers (Krishna et al. (2020). Since about 90% of human cancers are epithelial, they are considered an appropriate site for early cytogenetic damage detection (Holland et al., 2008).

The city of L'Aquila was hit by a strong earthquake in 2009 that caused the collapse of several buildings, 309 deaths and about 1500 injured people (Del Papa et al., 2019). In the following years, a great number of building sites were activated, particularly in the center of the city, causing an excess of air pollutants (chemicals, particulate matter) as emissions from outdoor/indoor building industry processes and demolition activities (Kirkeskov et al., 2016).

As a consequence, the resident population was permanently exposed to dust and resulted in a higher risk of being affected by respiratory conditions (D'Aloisio et al., 2019).

Moreover, building workers resulted intensely exposed during their working hours on the construction sites but, also, during the rest time spent in the city of L'Aquila, so, they represent a relevant target population subgroup for environmental mutagenesis studies and epidemiological surveillance.

Our study is aimed at comparing the early genotoxic damage as measured by buccal micronucleus test (MNs count) in two subgroups of workers defined by different occupational exposure (outdoor workers highly exposed in the construction sites and other indoor workers) and at evaluating possible confounding variables such as lifestyle factors.

2. Materials and methods

2.1. Study design

The study, with a cross-sectional design, has been carried out on a sample of fifty males voluntary recruited among workers living and working in L'Aquila city (Central Italy) subjected to the compulsory occupational surveillance system, in the period 2017–2018.

Two subgroups have been defined on the basis of occupational exposure to air pollutants from site dust in the post-earthquake reconstruction yard: 24 subjects were outdoor building workers (OWs, exposed to site dust, Exp^{+}) and 26 subjects were in-door workers recruited among academic employees attending biomedical laboratories (IWs, not-exposed to site dust, Exp^{-}).

2.2. Micronucleus test in exfoliated buccal cells

Before starting the collection of cells, the subjects were requested to rinse the mouth two times with 100 mL of water to remove cell debris. The samples were collected by using a small-headed toothbrush against the cheek wall in a circular motion to collect buccal cells. Buccal cell samples were collected into 50 mL Falcon tubes containing 20 mL of Saccomanno's fixative and was stored at 4 °C for one month (Bolognesi and Fenech, 2013). The cell suspension was centrifuged for 10 min at 580 g, the supernatant was aspirated leaving approximately 2 mL of cell suspension; successively 8 mL of buccal buffer (0.013M Tris-HCl, 0.13M EDTA, 0.02M NaCl) were added at room temperature and the cells were resuspended. This step was repeated for three times. To increase the number of clearly separated cells, the cell suspension was passed 5–6 times into a syringe using an 18 G needle, later the cells were passed through a 100 μm nylon filter and collected in 15 ml centrifuge tubes. The cell suspension was centrifugated for 10 min at 580 g. The supernatant was removed, and the cells were resuspended in 1 mL of buccal cell buffer, to further aid in the cellular disaggregation, then 50 μL per mL of DMSO was added. Cells were fixed using the required volume of ethanol: glacial acetic acid 3:5 to give a concentration of 80,000 cells/mL. Using a Pasteur pipette 100–150 μL of cell suspension was dropped onto a pre-cleaned/ready-to-use microscope slide, two slides were prepared for each subject. Finally, the cells were fixed using ethanol: glacial acetic acid 6:1. The slides were drained and dried, then colored with 5% Giemsa for 30 min in the dark and at room temperature. The slides were rinsed in running water for 5 min and then rinsed again in Milli-Q. Immediately the slides were placed face down on Whatman No. 1 filter paper to blot away any residual moisture. Nuclei and MNs are stained blue, while the cytoplasm is pink. The cells (Cytome) were observed at 100 X and 400× magnification and were evaluated to determine micronucleus (MN) frequencies and nuclear changes. A minimum of 1000 cells were evaluated per slide.

For the statistical analysis the nuclear changes have been defined according to the following categories:

- Normal cells: cells presenting a uniformly stained nucleus.
- Micronucleated cells (MNs count): proportion of cells presenting at least one MN out of 1000 normal cells. MNs are round or oval, not linked with the main nucleus, they show the same texture and stain intensity as the main nucleus but with a size that is 1/3–1/16 of the nucleus.
- Binucleated cells: proportion of cells presenting two main nuclei of similar size and stain out of 1000 normal cells
- Karyolytic cells: proportion of cells with nuclei with complete DNA depletion out of 1000 normal cells.

2.3. Genotoxicity factor calculation

The Genotoxicity Factor (GF) was introduced to evaluate the level of genotoxicity expressed by the samples, based on the criteria outlined by

the Regional Agency Environmental Protection, Emilia Romagna, Italy, ARPA ER,1997–2001. It was calculated using the mean values displayed in results section and it corresponds to the ratio between the frequency of micronucleated cells of OW samples (Exp^+, exposed to site dust) and frequency of micronucleated cells of IW samples (Exp^-); Table 1 reports the outcome classification based on the ranges of GF ratios.

2.4. Questionnaire

Selected workers filled in a structured questionnaire drafted by the Occupational Medicine Service of the University of L'Aquila. The questionnaire was anonymous, self-administered and voluntary based. It covered different areas: the occupational exposure to environmental pollutants, lifestyle habits, medical history and any other significant unhealthy exposures (such as drugs, ionizing radiations) to assess other confounding factors. It is composed by 84 items divided in the following subsections: general information, familiar history, personal history, smoking habits, work history, exposure to mutagen factors, alcohol consumption, comorbidities, respiratory history and symptoms, safety, perceptions and attitudes towards occupational risks. All data collected was recorded on a computerized database in an anonymous way; the file was protected by password, known only to the researchers. Informed consent was obtained from all participants through the electronic form.

2.5. Statistical analysis

The statistical significance level chosen for the entire analysis was 0.05. For the qualitative variables, absolute and relative frequencies were calculated, and associations analyzed by means of Pearson's Chi-square test (χ^2). For quantitative variables arithmetic mean and standard deviation were calculated, and differences analyzed by means of Student's t-test. Multivariate stepwise regression was performed to identify the independent contribution of each variable with the unique outcome dependent variable, i.e. the average MNs count. The analyses were performed using the Stata Statistical software/IC version 15 for Windows/Microsoft (Stata Statistical Software: College Station, TX: StataCorp, 2015).

3. Results

3.1. Micronucleus test in exfoliated buccal cells

The various distinct cell populations used in the buccal cytome assay were determined based on criteria outlined by Tolbert et al. (1992) which are intended to classify the buccal cells based on nuclear morphology, in 'normal' cells (Fig. 1 A, B) and cells that are considered 'abnormal'. These abnormal nuclear morphologies are thought to be indicative of DNA damage or cell death. Cells with MNs are characterized by the presence of a main nucleus and one or more smaller nuclei called MNs (Fig. 1 C, D).

Description: A: differentiated cells. B: binucleated cell. C, D: Cells with MNs (micronuclei) are characterized by the presence of a main nucleus and one or more smaller nuclei called MNs. Magnification 40 X.

The MNs are usually round in shape and their diameter may range between 1/3 and 1/16 of the diameter of the main nucleus and must be located within the cytoplasm of the cells. The presence of MNs is indicative of chromosome loss or fragmentation occurring during previous nuclear division (Fenech and Morley, 1986).

Table 1
Genotoxicity factor classification.

Genotoxicity Factor range	Outcome
$GF \leq 1.4$	Negative
$1.5 \leq GF \leq 2.9$	Slightly positive
$3.0 \leq GF \leq 14.9$	Positive
$GF \geq 15.0$	Strongly positive

All workers were males, overall aged 36.6 ± 10.3 years. As described in Table 2, the two subgroups resulted significantly different in their general characteristics: the OWs are older than IWs ($p < 0.01$), less frequently smoker ($p < 0.05$), more frequently foreign citizens (n.s.) and with a lower education level ($p < 0.001$).

Normal, micronucleated, binucleated and karyolytic cells are reported in Table 3, as proportion on 1000 cells examined. Significant differences were observed between Exp^+ (OWs) and Exp^- (IWs) in the number of the analyzed cells: respectively 954.46 *vs* 990.06 normal cells, ($p < 0.001$); 19.79 *vs* 4.95 micronucleated cells, as marker of chromosomal damage ($p < 0.001$); 13.93 vs 8.96 binucleated cells, as marker of failed cytokinesis ($p < 0.001$); 2.09 *vs* 1.18 karyolytic cells, as marker of cell death and damaged DNA ($p < 0.05$). The Genotoxity Factor value related to micronucleated cells resulted is 4.01 and, on the basis of classification proposed in MM section (Table 1), it falls in the 'positive' rank ($3.0 \leq GF \leq 14.9$) being coherent to the statistically significant differences found in MNs count between the two subgroups (OWs-Exp^+ vs IWs-Exp^-).

3.2. Micronuclei, lifestyle habits and potential protective factors in Exp^+ and Exp^-

Table 4 reports the arithmetic mean values of MNs counts stratified by socio-demographic variables (age, citizenship), lifestyle habits (smoking, alcohol consumption) and potential protective factors (training on job security and educational level) in OW-Exp^+ and IW-Exp^-. Considering the subgroups of workers separately, for all factor the MNs counts didn't show significant differences, except for smoking habits and educational level. By comparing smokers and no-smokers, the values increased significantly both in OWs (23.56 vs 17.09, $p < 0.001$) and in IWs (5.47 vs 4.67, $p < 0.01$), while by considering the educational level, the values increased in OWs with low level of education vs OWs with high level (respectively, 21.41 vs 17.65, $p < 0.05$). This last comparison wasn't possible in the IWs since none had low educational level.

A multivariate regression was performed to estimate the strength of the independent associations between the factors considered in the above univariate analysis (independent variables) and the MNs count values as outcome of interest (dependent variable). This approach is needed to better understand the role of job exposure in relation to the other potential predictors, i.e. to exclude such confounding or interaction effects, also considering the differences between the two groups (OWs vs IWs) in respect to the same variables as already shown (Table 2). In Table 5 three models are compared: the first one includes all the factors, the second one includes only the independent variables with a significant regression coefficient after a stepwise procedure ($p < 0.05$) and the third one includes an interaction analysis.

In Model 2 only three explicative variables maintained statistical significance in the regression coefficients: occupational exposure (OW vs IW, beta = 13.378, $p < 0.001$), smoking (smoker vs no-smoker, beta = 2.799, $p < 0.001$) and educational level (high vs low educational level, beta = −2.111, $p < 0.01$) and they explain circa 95% of the MNs variance in the sample.

The introduction of an interaction factor between the two variables 'occupational exposure' and 'smoking' in the regression analysis (Model 3) highlights a negative moderation effect (−5.900, $p < 0.001$), i.e. the effect of smoking in the IWs (not exposed to dust) is lower than in the OWs (exposed). Moreover, in Model 3 the coefficient of the variable 'educational level' losses statistical significance (0.177, n.s.), i.e. this variable is a confounding factor. In Model 3 the explained variance is circa 98%, so higher than in Model 1 and 2.

4. Discussion

Although there are several techniques and procedures available to detect MNs frequencies, the human buccal micronucleus assay is one of

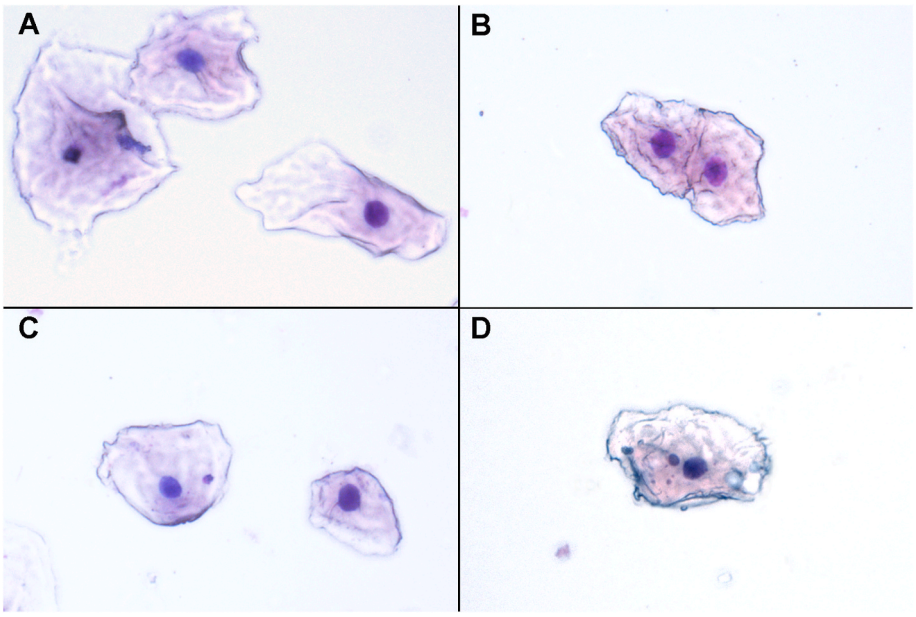

Fig. 1. Micronucleus test in exfoliated buccal cells.

Table 2
Sample description.

	Outdoor workers (24)	Indoor workers (26)	p value [c]
Age (years)	41.4 ± 11.55	32.3 ± 6.7	$p < 0.01$
No. Smoker (%)	10 (41.7%)	19 (79.3%)	$p < 0.05$
No. Italian citizenship (%)	19 (79.2%)	25 (96.2%)	$p = 0.065$
No. with high education [a b] (%)	9 (47.4%)	25 (100.0%)	$p < 0.001$

[a] 6 missing data.
[b] High education level corresponds to 13 years of school education or above (i.e. secondary school upper level and university graduation level).
[c] Chi-square test.

Table 3
Normal, micronucleated, binucleated and karyolytic cells in the population study (ratio every 1000 cells): arithmetic mean ± standard deviation and p-value at Student t-test.

	Outdoor workers (24)	Indoor workers (26)	p value
Normal cells	954.46 ± 18.77	990.06 ± 1.36	$p < 0.001$
Micronucleated cells	19.79 ± 3.54	4.95 ± 7.11	$p < 0.001$
Binucleated cells	13.93 ± 2.10	8.96 ± 1.29	$p < 0.001$
Karyolytic cells	2.09 ± 1.71	1.18 ± 0.38	$p < 0.05$

Table 4
MNs counts stratified by lifestyle habits and potential protective factors (arithmetic mean values ± standard deviation and significance at Student t-test).

Outdoor workers (24)	*Yes*	*No*	Sign.
Age ≥40 years	19.62 ± 3.80	19.98 ± 3.38	n.s.
Italian citizenship	19.84 ± 3.60	19.60 ± 3.68	n.s.
Smoke	23.56 ± 0.48	17.09 ± 0.36	$p < 0.001$
Spirits	18.54 ± 5.34	19.96 ± 3.36	n.s.
Wine	19.71 ± 4.23	19.82 ± 3.36	n.s.
Beer	18.39 ± 3.57	20.97 ± 3.18	n.s.
Training on job security [a]	19.85 ± 3.46	19.86 ± 3.76	n.s.
High education level [b]	17.65 ± 2.52	21.41 ± 3.56	$p < 0.05$
Indoor workers (26)	*Yes*	*No*	Sign.
Age ≥40 years	4.81 ± 0.79	4.98 ± 0.71	n.s.
Italian citizenship	5.00 ± 0.69	3.8[c]	[d]
Smoke	5.47 ± 0.55	4.67 ± 0.33	$p < 0.01$
Spirits	4.98 ± 0.76	4.90 ± 0.67	n.s.
Wine	4.93 ± 0.82	4.99 ± 0.41	n.s.
Beer	4.91 ± 0.73	5.09 ± 0.69	n.s.
Training on job security [a]	4.99 ± 0.85	4.86 ± 0.00	n.s.
High education level [b]	4.97 ± 0.72	–	–

[a] 9 missing data.
[b] 6 missing data.
[c] Only one observation.
[d] Not calculable.

the most widely used techniques to measure genetic damage in human population studies (Bolognesi and Fenech, 2013; Bolognesi et al., 2015). In particular, the MN test was performed: it is used as a biomarker for the evaluation of the early effects from exposure to mutagenic and carcinogenic compounds. Our results show that the group exposed to site dust (OWs-Exp$^+$) has mean MNs values significantly higher than the values reported in the not exposed group (IWs-Exp$^-$), overall, 19.8 vs 5.0, that means almost four-fold. We found similar results, also when the mean MNs values are stratified for each factor considered (age, citizenship, education level, smoking, alcohol consumption, training on job security, Table 4). Since MNs are considered indicators of genotoxic-related chromosomic damage, chromosome breakage or spindle disfunction, they are used as carcinogenic potential predictors of environmental agents and as efficacy indicators of cancer prevention. Moreover, an approach that seems to be in accord with our findings comes from the assessment of synergic actions among several harmful sources and kinds of exposure, such as type of job, tasks, work practices and organization. Control measures were explored using distinct exposure models for respirable dust and quartz (Van Deurssen et al., 2014). The level and quality of exposure to airborne dust and free crystalline silica was assessed in a study conducted on another sample of construction workers in the same city of L'Aquila. Four local authorities (the Joint Local Committee, the University of L'Aquila, the Local Health Authority of Abruzzo and the National Institute for Insurance against Accidents at Work) promoted a project aimed at studying air pollutants assessment and detection (SiO_2, fibers, respirable and inhalable dusts and VOCs) by personal air sampling on the construction sites (Pettinaro et al., 2016). Comparing different tasks, manual demolition workers are the most exposed to considerably high levels of 'inhalable' dusts, exceeding the precautionary thresholds defined by the Industrial Hygienist organizations (10 mg/m^3), while for 'respirable' dust and crystalline silica dust

Table 5
Multiple regression models (dependent variable MNs count).

	Model 1			Model 2			Model 3		
	β-coeff	95% I.C.	p-value	β -coeff	95% I.C.	p-value	β -coeff	95% I.C.	p-value
Indipendent variables									
Age (≥40 years)	0.012	−1.392–1.416	p = 0.986						
Citizenship (Italian vs foreign)	1.391	−0.571–3.353	p = 0.159						
High educational level (yes vs no)	−2.305	−4.343–−0.267	*p < 0.05*	−2.111	−3.780–−0.442	*p < 0.05*	0.177	−1.000–1.352	p = 0.763
Training on Job security (yes vs no)	−0.314	−1.005–0.378	p = 0.363						
Beer (yes vs no)	−0.816	−2.357–0.726	p = 0.290						
Wine (yes vs no)	−0.460	−1.740–0.820	p = 0.470						
Spirits (yes vs no)	−0.327	−1.990–1.336	p = 0.692						
Smoking (yes vs no)	2.715	1.377–4.052	*p < 0.001*	2.799	1.672–3.926	*p < 0.001*	6.683	5.494–7.872	*p < 0.001*
Occupational exposure (OW vs IW)	12.754	11.398–14.109	*p < 0.001*	13.378	12.052–14.703	*p < 0.001*	12.221	11.354–13.087	*p < 0.001*
Occupational exposure * Smoking (interaction)							−5.900	−7.365–−4.43473	*p < 0.001*
Model parameters									
No. observations	44			44			44		
p-value (F test)	p < 0.001			p < 0.001			p < 0.001		
Adj R-squared	0.9578			0.9544			0.9827		

all the workers have shown concentration below the thresholds (respectively 10 mg/m^3 and 0,025 mg/m^3) (Mastrantonio et al., 2019).

Nevertheless, the genotoxicity assessment with the MNs test needs to consider the possible confounding factors such as age (Ramsey et al., 1995; Ganguly, 1993; Richard et al., 1994), female gender (Richard et al., 1994), smoking behaviour (Fontham et al., 1986), alcohol consumption (Schmidt et al., 1981; Stich and Rosin, 1983). In our results, the multivariate regression analysis revealed that, in addition to the job exposure, when all the factors are considered together and in their potential interaction, the only variable independently associated with an increase in MNs is the smoking habit which, even if not associated with dust exposition, worsens cell integrity. Moreover, this worsening effect is weaker in workers not exposed to the site dust (moderation effect). Within social demographic factors, the high educational level only apparently seems to affect MNs number: even if unbalanced in favor of IWs-Exp$^-$ vs OWs-Exp$^+$ (Table 2), this variable resulted a confounder (Table 5), since its effect disappears when the interaction between these two factors is considered, because it is a covariate of smoking habit as well as of the job condition. The 'job training' factor resulted not to be associated with MNs number in both workers group as did the 'alcoholic drinks' factor. The role of smoke seems to be confirmed according to Bonassi et al. (2011), that reported a significant result for age (p < 0.001) and significantly increased MNs frequencies in heavy smokers (≥40 cig/day). The use of tobacco is associated with cytotoxic and genotoxic effects as demonstrated by a higher frequency of MNs in exfoliated cells of smokers; in addition, the increase of MNs frequency in buccal cells is strongly dependent on the type and number of cigarettes daily consumed (Nersesyan et al., 2011; De Geus et al., 2018; Benvindo-Souza et al., 2017). A study conducted by Palaskar and Jindal (2010), reported MNs frequencies of 17.57 for smokers and 3.53 for no-smokers, even if the sample size is quite small.

Our study has some limitations such as cross-sectional design, small sample dimension, lacking data about exposure (chemical qualitative and quantitative analysis of dusts) and no information about the specific tasks of construction workers.

5. Conclusion

Occupational exposure is one of the main factors that may play a key role in explaining an increase of MNs number in construction workers. Moreover, smoking status could influence the effect of dust exposure and can be considered an important factor in worsening early genotoxic effects. Our study confirmed findings of previous ones on occupational risks and, in addition, clarifies possible moderation and confounding effects, such as interaction between smoking and occupational exposure and the unbalanced school education level in workers. Further research is needed on wider samples, with more accurate recruiting procedures and more complete data collection, such as chemical and physical characteristics of dust, its association with biomarkers and a dose-response effect. Moreover, the compliance and efficacy of preventive practices such as PPE (Personal Protective Equipment) and of construction site safety planning should be evaluated.

Author statement file

L F, AMG P, S L, LT: Conceptualization. AMG P, M S, S C, S L: Data curation. M S , R M, F DA: Formal analysis; L F: Funding acquisition; S L, S C, AMG P, L T, L F: Investigation; S L, F DA, R M, M S, SC: Methodology; L F, L T: Project administration; L F, AMG P: Resources; M S, O Z, F DA: Software; L F, AMG P, M S: Supervision; M S, F DA, Validation; AMG P, M S, S L, S C: Visualization; AMG P, L F, S L, M S:Roles/Writing - original draft; AMG P, L F, S L, M S:Writing - review & editing.

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.

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