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Malondialdehyde-Deoxyguanosine Adduct Formation in Workers of Pathology Wards. The Role of Air Formaldehyde Exposure

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Abstract

Background—Formaldehyde is a ubiquitous pollutant to which humans are exposed. Pathologists can experience high formaldehyde exposure levels. Formaldehyde – among other properties – induce oxidative stress and free radicals, which react with DNA and lipids, leading to oxidative damage and lipid peroxidation, respectively. We measured the levels of air-formaldehyde exposure in a group of Italian pathologists and controls. We analyzed the effect of formaldehyde exposure on leukocyte malondialdehyde-deoxyguanosine adducts (M₁-dG), a biomarker of oxidative stress and lipid peroxidation. We studied the relationship between air-formaldehyde and M₁-dG adducts.

Methods—Air-formaldehyde levels were measured by personal air samplers. M₁-dG adducts were analyzed by ³²P-postlabelling assay.

Results—Reduction rooms pathologists were significantly exposed to air-formaldehyde in respect to controls and to the pathologists working in other laboratory areas ($p < 0.001$). A significant difference for M₁-dG adducts between exposed pathologists and controls was found ($p = 0.045$). The effect becomes stronger when the evaluation of air-formaldehyde exposure was based on personal samplers ($p = 0.018$). Increased M₁dG adduct levels were only found in individuals exposed to air-formaldehyde concentrations higher than 66 $\mu\text{g}/\text{m}^3$. When the exposed workers and controls were subgrouped according to smoking, M₁-dG tended to increase in all the subjects but a significant association between M₁-dG and air-formaldehyde was only found in not smokers ($p = 0.009$). Air formaldehyde played a role positive but not significant ($r = 0.355$, $p = 0.075$, Pearson correlation) in the formation of M₁-dG, only in not smokers.

Conclusions—Working in the reduction rooms and to be exposed to air-formaldehyde concentrations higher than 66 $\mu\text{g}/\text{m}^3$ is associated with increased levels of M₁-dG adducts.

Keywords

Formaldehyde; occupational exposure; oxidative stress; MDA; M₁dG adduct

INTRODUCTION

Formaldehyde (FA) is a ubiquitous pollutant to which humans are chronically exposed via different routes. This chemical is also present in various forms of life, including humans (1). Commercially, it is widely employed in the production of resins containing urea, phenol, melamine and, in a smaller extent, their derivatives. Furthermore, the high chemical reactivity of FA is exploited for preservation and disinfection in pathology laboratories, as well as for antimicrobial agent in consumer and/or cosmetic products. Human exposure to FA in occupational environment has been considered several times by the American Conference Governmental Industrial Hygienists (A.C.G.I.H.), from 1946 until the actual Ceiling limit value (TLV-C) of 0.3 ppm (0,370 mg/m³).

The reactive capacity of FA, shown by direct contact to target tissues, can induce local irritations, acute and chronic toxicity, and genotoxic and carcinogenic activity (2,3,4), as confirmed by an increased incidence of nasopharyngeal cancer in industrial workers, embalmers and pathologist (5,6), by the relationship demonstrated between formaldehyde and leukemia in a recent meta analysis (7) and by a significant positive association between formaldehyde exposure and childhood asthma (8). Thus, the widespread use of FA in many working and life contexts represents a potential risk factor for human health when this pollutant is assumed by contact or breathed. This last type of exposure is probably the principal and more important route of human exposure, both in life and work environments.

FA is mainly genotoxic for chromosome to bacteria (9,10,11) and mammalian cells in culture, including human cells (4,12,13). Some endpoints were analyzed *in vitro* and in epidemiological studies, including the formation of micronucleus (MN) and sister chromatide exchange (SCE) in peripheral blood lymphocytes. To assess the DNA damage in chromosomes of populations occupationally exposed to FA, SCE were used as cytogenetic biomarker in pathology laboratory workers (14,15,16). FA can also induce the formation of DNA adducts. In particular FA-induced DNA adducts can be single-strained type (intrastrand crosslink), double-strained type (interstrand crosslinks) or can involve a reactive group of a protein to form DNA-protein crosslinks (DPCs) (2,17,18,19). DPCs are the primary and principal form of FA-induced DNA damage, in which probably histones are involved (19,20). The DPCs formation due to FA exposure is confirmed by the reduction of electrophoretical migration of DNA damaged observed by means of Comet test and demonstrated also by a *in vitro* study where irradiated cells exposed to increasing FA concentrations show a reduction of DNA migration (4,18).

However, FA can induce also oxidative stress. Indeed, FA can increase the formation of reactive oxygen species (ROS) in many tissues, which can interact with DNA and lipids, leading to oxidative damage and lipid peroxidation (LPO), respectively (21,22,23). Malondialdehyde (MDA) is a natural product of lipid peroxidation that can react with DNA to form exocyclic adducts, including the 3-(2-deoxy- β -D-erythro-pentafulanosyl)pyrimido[1,2- α]purin-10(3H)-one dG (M₁dG), that, if not repaired, can induce base pair mutations and cause frame-shift mutations in reiterated sequences (24,25). Previous studies have also shown that the formation of M₁dG adducts could be associated to increased cancer risk and tumor progression (26,27,28). M₁dG adduct measurement is considered to be also a biomarker that reflect exposures to air pollutants capable to induce oxidative stress and ROS (29). In addition, dose-related induction of MDA production in

plasma and liver were demonstrated by some experiments carried out *in vivo* on rats FA exposed. (21,30).

Therefore, in the present study we measured the levels of personal air-FA exposure in a group of pathologists working in three Italian pathology wards and in a group of controls. Then, we analyzed the effect of air-FA exposure on the levels of leukocyte M₁dG adducts, a biomarker of oxidative stress and lipid peroxidation. The relationship between air-FA exposure levels and M₁dG adducts was also evaluated. Our results could provide first indications to evaluate the doses of FA to which the workers are exposed and a consequent biological response planning future preventive actions.

MATERIALS AND METHOD

Epidemiological sample

44 workers, recruited in three pathology wards of Piedmont region in Italy, were recruited as subjects potentially exposed to FA referred as cases in this paper. Contemporaneously, 32 students and workers in scientific laboratories of University of Torino not professionally exposed to FA were recruited as controls. For each of 76 subjects an air-FA sample was collected for an entire working shift (8 hours); data on personal medical history, smoking habits, drug intake, were also collected through a questionnaire administered at the end of the working shift when a sample of venous blood was collected too. The description of smoking status of all the subjects was *a priori* established, classifying as “not smokers” the never smokers and the former smokers who had ceased smoking for at least 1 month and as “smokers” who smoked at least one cigarette *per* day. All subjects were informed about the objective of the study and they gave written informed consent.

Personal air-FA

FA air samples were collected for a whole working shift (8 hours) on Wednesday using passive personal air samplers working with radial symmetry (Radiello®), clipped near the breathing zone of the subject. Samplers were equipped with a specific sorbent tube containing florisis® 35–50 mesh coated with 2,4-dinitrophenylhydrazine (DNPH). DNPH reacting with FA, changes by derivatization to the specific 2,4-dinitrophenylhydrazone-FA which was quantified with a HPLC Perkin Elmer equipped with an UV detector regulated at 360 nm: NIOSH Method n. 2016 (31). The instrument was set according the following specifications: pump Perkin Elmer series 200, detector UV-VIS Perkin Elmer LC 295, dilutor model 401 Gilson, automatic sampler Gilson model 231, HPLC column, cartridge 10 m LiChro CART 250-4. The instrumental conditions were: mobile phase: 45% acetonitrile, 55% water; flux in column 1 mL/min; injection volume 20 µL. Estimate Limit Of Detection (LOD) was 0.05 µg/mL. The chemical desorption was done as follows: elution with 10 mL of acetonitrile, sonication for 20 min. 200 µL of DNPH solution and a drop of concentrated perchloric acid were added to 1 mL of bubbled sample to promote derivatization to dinitrophenylhydrazone. The reaction proceeded for at least 30 minutes at room temperature and then the samples were transferred in micro vials (300µL) for HPLC analysis. Calibration curve was prepared using a calibration standard (the specific 2,4 dinitrophenylhydrazone formed as above) provided by the sampler manufacturer of Radiello® having a certificated concentration of 3.83 µg/mL, expressed as FA. The calibration curve was prepared with a range of concentration between 0.05 and 3 µg/mL. The standard solutions and the blank were treated as samples.

Questionnaire

The same day of the air and blood sampling, at the end of shift, to each subject a questionnaire was administered to acquire information concerning the individual and clinical

features (age, sex, place of residence, hobbies, therapies), smoking habits, profession (qualifications, seniority and job-specific work) and the presence and use of environmental and personal devices to prevent air exposure and health risks.

Blood sampling, DNA extraction and purification

For each subject 10 mL of venous blood were collected at the end of the working shift in Vacutainer tubes containing Na-EDTA as an anticoagulant. Blood tubes were then kept in the dark at -20°C and processed within few days. DNA was extracted from whole blood using Salting Out method (32) to measure M_1dG adducts. Briefly, 1mL of whole blood was washed with RCLB buffer (Red Cells Lysis Buffer: TRIS 10 mM, MgCl_2 5 mM, NaCl 10 mM) to remove erythrocytes and then washed again with WCLB buffer (White Cells Lysis Buffer: TRIS 10 mM, EDTA 10 mM, NaCl 50 mM) to lyse leukocytes; furthermore, sodium dodecylsulfate (SDS) for breaking cytoplasmic membrane and proteinase-K for histonic proteins digestion were added.

After incubation at 55°C for at least 60 min, sodium acetate was added to the samples, allowing protein precipitation to the bottom by centrifugation. The supernatant containing DNA was transferred to another tube and isopropyl alcohol was added to facilitate the precipitation of DNA. After some washes with ethanol (70%) DNA was collected and dissolved in low-TE and finally stored at -20°C .

Preparation of the reference M_1dG adduct standards

Two reference adduct standards using MDA or H_2O_2 were prepared. Calf-thymus (CT)-DNA or leukocyte DNA from a blood-donor were treated with 10 mM MDA according to published conditions (33,34). Epithelial lung carcinoma cell line A549 were exposed to 100 μM H_2O_2 . MDA-treated CT-DNA was then diluted with untreated CT-DNA to obtain decreasing levels of the reference adduct standard to generate a calibration curve.

MALDI-TOF-MS analysis

DNA adducts also were detected in MDA-treated calf thymus DNA by mass spectrometry (Voyager DE STR from Applied Biosystems, Framingham, MA, USA) through the following sequence of steps: (1) react DNA with NaBH_4 (35) followed by precipitation with isopropanol; (2) digest with snake venom phosphodiesterase and nuclease P1; (3) extract DNA adducts that are less polar than normal nucleotides on an OASIS cartridge (Waters Corporation); (4) tag with an isotopologue pair of benzoylhistamines (d_0 and d_4) in a phosphate-specific labeling reaction in the presence of carbodiimide (36); (5) remove residual reagents by ion exchange solid-phase extraction; (6) resolve tagged adducts by capillary reversed-phase HPLC with collection of drops onto a MALDI plate; (7) add matrix (α -cyano-4-hydroxycinnamic acid); and (8) analyze by MALDI-TOF-MS.

M_1dG adduct analysis

As previously mentioned, the production of MDA have been demonstrated *in vivo* on rats exposed to FA (21,30). Thus, we measured the levels of M_1dG adducts in FA exposed workers and controls using our previously described method (34). DNA (1–2 μg) was digested by micrococcal nuclease and spleen phosphodiesterase. Hydrolysed samples were treated with nuclease P1 (2.5 μg) for 30 min at 37°C . The nuclease P1 treated samples were incubated with 15–25 μCi of $[\gamma\text{-}^{32}\text{P}]\text{-ATP}$ and T4-polynucleotide kinase (0.75 U/ μl) to generate labelled M_1dG adducts. Samples were applied to the origin of chromatograms and developed with 0.35 MgCl_2 up to 2.0 cm on a filter paper wick. Plates were developed in the opposite direction with 2.1 M lithium formate, 3.75 M urea pH 3.75, and then run at the right angle to the previous development with 0.24 M sodium phosphate, 2.4 M urea pH 6.4.

Detection and quantification of M₁dG adducts and total nucleotides was obtained by storage phosphor imaging technique employing intensifying screens from Molecular Dynamics (Sunnyvale, California, USA) for 0.20–48 hr. The screens were scanned using a Typhoon 9210 (Amersham, Buckinghamshire, U.K.). Software used to process the data was ImageQuant (Molecular Dynamics). After background subtraction, the levels of M₁dG adducts were expressed such as relative adduct labelling (RAL) = screen pixel in adducted nucleotides/screen pixel in total normal nucleotides (nn). To calculate the levels of total nn, aliquots of hydrolysed DNA were appropriately diluted and reacted in the mixtures used for M₁dG adduct labelling. The obtained ³²P-labelled total nucleotides were separated on Merck PEI-cellulose TLC plates using 280 mM (NH₄)₂SO₄, 50 mM NaH₂PO₄. The values measured for the M₁dG adducts were corrected across experiments based on the recovery of the internal standard. Cochromatography of leukocyte DNA samples with MDA treated CT standard were performed to identify the M₁dG adducts detected in the chromatograms of human samples using 2.1 M lithium formate, 3.75 M urea, pH= 3.75 and 0.24 M sodium phosphate, 2.4 M urea, pH= 6.4 or 0.24 M sodium phosphate, 2.7 M urea, pH= 6.4. Furthermore, since previous studies have shown the hydrolytic ring-opening of M₁dG at pH = 11.7 (37;38), we have also decided to evaluate whether the alkaline hydrolysis can induce the autoradiography disappearance of leukocyte M₁dG adduct spots. Thus, M₁dG adduct spots, that have been previously identified in the chromatograms of volunteers, were excised and incubated with - without increased concentrations of ammonium hydroxide 0.1 M, 0.5 M, 1.0 M and 2.0 M, pH = 11.7 for 10' to induce the following ring-opening reaction:

After alkaline hydrolysis, the samples were transferred to the same chromatogram and run side by side with 0.24 M sodium phosphate, 2.4 M urea, pH = 6.4. Our results indicate that the alkaline hydrolysis of M₁dG induces the autoradiography disappearance of M₁dG adduct spots, supporting the identity of leukocyte adduct spots such as M₁dG.

Statistical analysis

Statistical analyses were performed on log transformed M₁dG data to stabilize the variance and normalize the distribution of adducts. FA exposed workers and controls were *a priori* grouped according to tertiles for levels of personal FA exposure. Mean concentrations of FA and M₁dG adduct levels across variable levels were compared by analysis of covariance, introducing into each model terms for sex, age (continuous), smoking, exposure status, and air-FA measurements, as appropriate. *Post hoc* Dunnett tests were performed for multiple comparisons among variable levels. A *p*-value of < 0.05 (two-tailed) was considered significant for all of the tests. The data were analyzed using SPSS 13.0 (SPSS, USA).

RESULTS

The 76 subjects were subgrouped (a) in pathologists working in reduction rooms, (b) in pathologists working in other laboratory areas of the three Italian pathology wards and (c) in controls. Frequency of epidemiological characteristics, personal air-FA concentrations and M₁dG adduct levels are reported in tables I and II, respectively.

FA in personal air

Table 1 shows a general description of air-FA concentrations (μg/m³) measured with the personal samplers. Our results show that the subjects working in the reduction rooms

showed personal air-FA values 7.7 times higher than those of controls ($p < 0.001$) and 6.5 fold higher values than those of pathologists working in other laboratory areas ($p < 0.001$).

Among the reduction room workers, four subjects showed values higher than the limit set by ACGIH at $370 \mu\text{g}/\text{m}^3$. The levels of air-FA measurements tended to be increased in smokers. A positive and statistically significant correlation between minutes spent in the reduction room and the level of air-FA was also found ($r = 0.599$, $p = 0.014$) (Figure 1).

Reference M₁dG adduct standards

The capability of MDA treatment to induce in vitro the formation of M₁dG adducts in CT-DNA was evaluated (34). A significant increment in the formation of M₁dG adducts was found in MDA treated DNA relative to control DNA ($p < 0.001$). The average levels of M₁dG adducts per 10^6 nn $\pm$ SE were 0.3 ± 0.1 , 1.6 ± 0.2 and 5.0 ± 0.4 in 1 mM, 4 mM and 10 mM MDA treated DNA, respectively, while a mean level of 0.1 M₁dG adducts per 10^6 nn (± 0.01) was detected in untreated DNA. A calibration curve was generate ($R^2 = 0.99$).

We then analyzed whether MDA treatment was capable of inducing M₁dG in human leukocyte DNA (Figure 2, A). A significant increase in M₁dG adducts was found relative to control DNA ($p < 0.001$). The average level of M₁dG adducts per 10^6 nn $\pm$ SE was 2.2 ± 0.6 and 0.02 ± 0.01 in MDA treated and untreated DNA, respectively. This adduct was previously identified as M₁dG using different techniques (33, 39, 40). The presence of MDA adducts in the MDA-treated CT-DNA (10 mM MDA), was also confirmed by us subjecting it to analysis by mass spectrometry, giving the results shown in Figure 3. As seen, 6 MDA DNA adducts apparently were identified, including one (M3mdC) that has not been reported before. The presence of a background adduct-spot in the untreated samples is in keeping with previous studies reporting endogenous levels of M₁dG adducts in control DNA (33, 39).

Finally, the induction from free radicals of the same DNA lesion in an in vitro system by incubating epithelial lung carcinoma cell line A549 with H_2O_2 was also analyzed. Our findings showed that treatment with $100 \mu\text{M}$ H_2O_2 induced a significant increase in M₁dG adducts in the lung carcinoma cells relative to the unexposed cells ($p < 0.001$). The mean level of M₁dG adducts per 10^6 nn was 0.25 ± 0.09 and 0.07 ± 0.01 in H_2O_2 treated and untreated cells, respectively.

M₁dG adducts in the leukocytes of pathologists

M₁dG adduct analysis was focused in the pathologists that were exposed to FA and in controls. Figure 2 reports the pattern of M₁dG adducts detected in the chromatograms of the study volunteers. The intensity of M₁dG adducts was generally stronger in the chromatograms of FA exposed pathologists compared with those of controls (Figure 2, Bvs C).

The co-chromatography confirmed the presence of M₁dG in leukocyte DNA. Then, alkaline hydrolysis experiments showed that the autoradiography disappearance of leukocyte M₁dG adducts was associated to increased OH^- concentrations, supporting the identity of leukocyte adduct spots such as M₁dG adducts.

Any differences were found taking into account gender and age in the 40 subjects considered for M₁dG analysis (table 2). Table 2 shows also mean levels of M₁dG adducts in the subgroups B and C of figure 2. The difference for M₁dG adduct levels between the pathologists working in the reduction rooms ($5.7 \text{ M}_1\text{dG adducts per } 10^8 \text{ nn} \pm 1.3$) and controls ($2.4 \text{ M}_1\text{dG adducts per } 10^8 \pm 0.3$) was statistically significant ($p = 0.021$) and persisted after adjusting by sex, age, and smoking ($p = 0.045$). The association with M₁dG

adducts becomes stronger when the evaluation of air-FA exposure was based on personal samplers ($p = 0.003$) and persisted after adjusting by sex, age, and smoking ($p = 0.018$).

When the study population was subgrouped according to smoking, a general direct relationship between M₁dG adduct levels and personal air-FA exposure was observed. Nevertheless, a statistically significant higher level of M₁dG adducts ($p = 0.009$) was recorded in exposed to higher concentrations of air-FA only in non-smokers (table 3), probably due to the small number of analyzed subjects. These findings were confirmed sub grouping smokers and non smokers in only two levels of air-FA exposure: a larger group (10 + 9 non smokers and 4 + 3 smokers) of less exposed to formaldehyde ($\leq 66 \mu\text{g}/\text{m}^3$) and the same groups (7 non smokers and 6 smokers) more heavily exposed to air-FA ($> 66 \mu\text{g}/\text{m}^3$).

A final statistical analysis was performed by evaluating the correlation between M₁dG adduct and air-FA exposure levels. Pearson correlation showed a role positive but not significant of air-FA in M₁dG levels in non smokers ($r = 0.355$, $p = 0.075$, Figure 4), but not in smokers.

DISCUSSION

Oxidative stress has been related to the etiopathogenesis of several chronic diseases (aging, hepatic and renal diseases, cancer) mainly caused by the presence of aldehydes formed by lipids oxidation. LPO is one of processes induced by oxidative stress. (41,42). MDA is the principal and most studied aldehyde produced by polyunsaturated fatty acid peroxidation. In the physiological state, at neutral pH, MDA is present as an enolate anion and shows low chemical reactivity. Nevertheless, this molecule is able to interact with nucleic acid bases to form several different adducts (43). FA breathed can induce oxidative stress through different ways, between which is an example the production increase of enzymes utilized in FA detoxification after FA exposure (21,30). For this reason, we decided to study the relationship between occupational exposure to air-FA and the levels of M₁dG adducts.

The results of the present survey show that about the fifty *percent* of the pathologists recruited in the three Italian pathology wards were truly professionally exposed to air-FA. The “real cases” were those working in the “reduction rooms”, where FA is directly used to fix the biological tissues (see figure 1). Statistical analysis in table 1 confirms the “real cases” as the sole subgroup with higher personal air-FA levels if compared to all the other subjects enrolled both in the pathologists and in control subgroups. This evidence emphasizes how the laboratories where tissue reduction is performed are the most risky in pathology departments where the best preventive measures are desirable.

Four subjects working in the “reduction rooms” have shown personal air-FA levels superior to the ACGIH limit ($0.370 \text{ mg}/\text{m}^3$). Given the good environmental work conditions of these workers and the relatively low FA levels shown by the other workers operating in the “reduction room”, we can assume that these high values could depend by improper work behavior rather than by poor environmental working conditions. However, some authors described workers exposed to similar or higher FA pollution levels if compared to the ACGIH limit (15,16,44). Thus, air-FA does not represent an indistinct risk factor for all the workers in the pathology wards (doctors, students, technicians, etc) but “only” a preventive item connected to a specific workstation.

Then, we focused the measurements of M₁dG adducts in the group of pathologists really exposed to FA and in controls. Our results show that working in reduction room was associated with increased leukocyte M₁dG adduct levels; indeed, the levels of M₁dG adducts of air-FA professionally exposed pathologists were significantly higher than those of controls (table 2). This association persisted also when personal air samplers were used to

measure the extent of air-FA exposure. Interestingly, the analysis of the dose-response relationship shows an increased adduct formation only in workers exposed to external air-FA levels higher than $66 \mu\text{g}/\text{m}^3$, but not in subjects exposed to lower air-FA values. In addition, the effect of air-FA exposure was more evident in the group of non smokers, suggesting that the role of FA exposure in the M₁dG adducts may have been masked by smoking (table 3).

FA is the main product used in the pathology laboratories where tissue reduction is performed, thus it can be involved in the induction of increased levels of M₁dG adducts of the group of laboratory workers exposed to FA. FA can induce increased levels oxidative stress and enhanced formation of ROS by different ways, including by the activation of oxidases and the inhibition of scavenger systems. For instance, FA is a substrate for the action of cytochrome P-450 monooxygenase system II E1 isozyme and can be oxidized by peroxidase, aldehyde oxidase and xanthine oxidase with the subsequent ROS formation (22). A pathway independent from MDA production can be also involved in the formation of M₁dG adducts. Indeed Dedon (45) proposed a mechanism in which M₁dG can also be formed directly upon ROS exposure through the formation of base propenal. In addition, impaired antioxidant activities of enzymes, in metabolic detoxification of oxidative by-products have been also reported in experimental animals treated with FA (22,46). Finally, FA exposure can lead to inflammation and to a consequent excess of oxidative stress (47). Activated macrophages and neutrophils can release ROS, such as hydrogen peroxide and hypochlorite acid, that can induce LPO and MDA formation. Indeed, we have recently shown that ROS generated by activated inflammatory neutrophils are involved in M₁dG adduct formation (48).

Our findings can be useful to characterize the risk, at least in term of DNA damage, experienced from the subjects working in the "reduction rooms" of the pathology wards. However, predictive value of biomarkers, especially those of internal dose and early biological effects, is quite limited to assess individual risk. This is because the processes that lead from exposure to disease are affected by many factors, many of which are unknown or their real impact is not estimable (e.g. individual genetic profile, age, diet, lifestyle and health status, mode of exposure, etc.). Conversely, the predictive values of biomarkers of DNA damage in a given population remain of relevant importance at the level of public health concern (49,50).

Finally, the role of tobacco smoke habits was investigated. Cigarette smoke contains a broad range of carcinogens and ROS derived from tobacco pyrolysis products that can lead to M₁dG adduct formation. The levels of M₁dG adducts tended to increased in smokers in respect to not smokers, but without reaching a statistical significance (table 2). Moreover, the association of M₁dG adducts with smoking tended to be more present in subjects exposed at air-FA exposure levels lower than $66 \mu\text{g}/\text{m}^3$ than in individuals exposed to higher air-FA concentrations (table 3).

The relationship between smoking and M₁dG adducts was previously examined by us in two hospital based studies and higher amount of DNA lesions were found in bronchi and larynx of smokers (27,28). The levels of endogenous DNA damage were also increased in lung cancer cases with respect to controls, but only in smokers and lung cancer cases with levels of MDA-DNA adducts above the population median had also reduced survival (28). Similar relationship were observed in the leukocytes and oral mucosa of smokers (29,51), but other authors reported no differences for tobacco smoking in breast and colon mucosa (26,52). Previous experimental studies have also indicated that cigarette smoking cause the elevations of urinary F₂-isoprostanes, an indicator of oxidative stress (53,54).

In summary, our study shows that working in the reduction rooms of pathology wards and to be exposed to air-formaldehyde concentrations higher than $66 \mu\text{g}/\text{m}^3$ is associated with increased levels of malondialdehyde-dG adducts. Taking into account that the TLV-TWA level for FA is almost 6 times higher than $66 \mu\text{g}/\text{m}^3$ ($370 \mu\text{g}/\text{m}^3$), we must consider this last biological finding as an important knowledge for the future preventive actions aimed to this kind of workers.

The present work lays the basis for further investigations with increased statistical power and based on less invasive and more reliable surrogate target sampling, e.g. nasal epithelial cells instead of venous blood, to assess the biological effects on tissues more affected by exposure to FA.

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LIST OF ABBREVIATIONS

ACGIH	American Conference Governmental Industrial Hygienists
CT	calf-thymus
DNPH	2,4-dinitrophenylhydrazine
DPCs	DNA-protein crosslinks
LOD	limit of detection
M₁dG	3-(2-deoxy-β-D-erythro-pentafuranosyl)pyrimido[1,2-α]purin-10(3H)-one dG adduct
FA	formaldehyde
LPO	lipid peroxidation
MDA	malondialdehyde
MN	micronucleus
NN	normal nucleotides
RCLB buffer	red cells lysis buffer
ROS	reactive oxygen species
RAL	relative adduct labelling
SDS	sodium dodecylsulfate
SCE	sister chromatide exchange
WCL buffer	white cells lysis buffer

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Fig. 1.
Correlation between air-FA and minutes spent in reduction rooms in subjects “true exposed”.

Fig. 2. M₁dG adduct pattern in 10 mM MDA treated human leukocyte DNA (A), in pathologists working in the reduction rooms (B), and in controls (C).

Fig. 3. Detection of DNA adducts after mass tagging by MALDI-TOF-MS in MDA-treated, NaBH₄-reduced calf thymus DNA. Relative to the accurate masses that are seen in the spectrum from one spot, the exact masses are as follows using the M nomenclature of Goda and Marnett (1991): M1dG (581.166); M1dA (583.182); M2dG (653.187); M3dC (667.192); M3dA (693.219); and, in the inset (from another MALDI spot), M3mdC (681.207).

Fig. 4. Pearson's correlation between M₁dG adduct level and personal air-FA ($r = 0.355$, $p = 0.075$) in the all 27 non smokers among the 40 subject selected for M₁dG.

Table 1

Distributions and percentage of epidemiological characteristics and the average levels of formaldehyde ($\mu\text{g}/\text{m}^3$) according to study variables.

	<i>n</i> ^a	Formaldehyde		<i>p</i>
		mean $\pm$ SE	median	
Gender				
male	22	97.3 $\pm$ 40.9	25.6	0.769
female	53	67.2 $\pm$ 13.0	29.7	
Age				
< 30 years	24	66.1 $\pm$ 24.2	27.0	0.010
30 – 39 years	23	94.8 $\pm$ 34.4	29.0	
> 39 years	28	60.0 $\pm$ 20.5	27.4	
Smoking				
not smokers	57	67.2 $\pm$ 13.1	29.7	0.065
smokers	18	136.9 $\pm$ 51.1	26.2	
Exposure status				
Controls ^b pathologists	32	27.7 $\pm$ 2.5	25.6	< 0.001
working in reduction room	19	212.4 $\pm$ 47.0	128.6	
working in other areas	24	32.4 $\pm$ 6.1	23.3	

^a Some figures do not add up to the total because of missing values

^b Reference level

Table 2

Distributions and percentage of epidemiological characteristics and the mean levels of M₁dG adducts *per* 10⁸ normal nucleotides according to study variables.

	M ₁ dG adducts			
	<i>n</i> ^a	mean ± SE	median	<i>p</i> ^c
Status				
Controls	20	2.4 ± 0.3	1.8	0.045
Reduction room pathologists	20	5.7 ± 1.3	3.3	
Gender				
Male	12	3.8 ± 1.3	2.4	0.345
Female	28	4.1 ± 0.9	2.6	
Age				
< 30 years	14	3.2 ± 1.1	1.5	0.990
30 – 39 years	13	4.8 ± 1.5	2.8	
> 39 years	13	4.2 ± 1.2	3.0	
Smoking				
Not smokers	27	3.8 ± 0.9	2.1	0.494
smokers	13	4.5 ± 1.3	3.0	
Air-FA				
< 22 µg/m ³ ^b	13	2.3 ± 0.44	1.8	0.775
23–66 µg/m ³	13	2.7 ± 0.55	2.0	
> 66 µg/m ³	13	7.3 ± 1.90	4.2	

^a some figures do not add up to the total because of missing values,

^b reference level,

^c adjusted by sex, age, and smoking

Distributions and percentage of epidemiological characteristics mean levels of M₁dG per 10⁸ normal nucleotides according to smoking.

Table 3

	M ₁ dG adducts NOT SMOKERS		n ^a	M ₁ dG adducts SMOKERS		n ^a	p ^c
	mean ± SE	median		mean ± SE	median		
Gender							
Male	3.1 ± 1.0	2.3	6	4.5 ± 2.5	2.4		
Female	4.0 ± 1.1	2.1	21	4.5 ± 1.3	3.9		0.696
Age							
< 30 years	2.0 ± 0.5	1.65	8	4.8 ± 2.5	3.2		
30 – 39 years	4.9 ± 1.8	2.8	11	4.5 ± 1.6	4.5		
> 39 years	4.2 ± 1.7	2.6	8	4.1 ± 1.8	2.0		0.701
Air-FA							
< 22 µg/m ³ ^b	2.1 ± 0.4	1.8	9	2.7 ± 1.2	1.9		
23–66 µg/m ³	2.6 ± 0.7	1.7	10	3.0 ± 0.5	2.0		0.883
> 66 µg/m ³	8.0 ± 2.9	4.2	7	7.3 ± 1.9	4.2		0.647

^a some figures do not add up to the total because of missing values

^b reference level

^c adjusted by sex and age