Antineoplastic drug occupational exposure: a new integrated approach to evaluate exposure and early genotoxic and cytotoxic effects by no-invasive Buccal Micronucleus Cytome Assay biomarker

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ABSTRACT

Health-care personnel handling antineoplastic drugs could be at risk for adverse health effects. We aimed to evaluate genotoxic and cytotoxic effects of antineoplastic drug exposure of personnel preparing and administering such drugs in three Oncology Hospitals in Italy enrolling 42 exposed subjects and 53 controls. Furthermore, we aimed to study the possible influence of *XRCC1* and hOGG1 DNA repair genes polymorphisms on genotoxicity induced on buccal cells. We performed workplace and personal monitoring of some drugs and used exposure diary informations to characterize the exposure. Urinary 5-FU metabolite (α-fluoro-β-alanine) was measured. Buccal Micronucleus Cytome (BMCyt) assay was used to evaluate DNA damage and other cellular anomalies. GEM and 5-FU contamination was found in 68% and 42% of wipe/swab samples respectively. GEM deposition was found on workers' pads while no α-fluoro-β-alanine was found. BMCyt-assay showed higher genotoxicity and cytotoxicity on nurses administering antineoplastics than on preparators and controls. Among micronucleus (MN) positive (with MN frequency higher than 1.5‰) exposed subjects, the percentage of those carrying XRCC1 mut/het genotype was higher than in MN positive-controls. Using the sensitive BMCyt assay, we demonstrated that handling antineoplastics still represents a potential occupational health risk for workers that should be better trained/informed regarding such risks.

Keywords: nurses, antineoplastic drug handling, buccal micronucleus cytome (BMCyt) assay, workplace monitoring

1. Introduction

Currently more than 100 different antineoplastic drugs are available for treatment of cancer and many other pathologies such as rheumatoid arthritis, nephritis, multiple sclerosis, and lupus (Vioral & Kennihan, 2012). Several antineoplastic drugs, which inhibit tumor growth by disrupting cell division and killing growing cells, have been evaluated by International Agency for Research on Cancer (IARC) which included some of them as human carcinogens in Group 1 (IARC 2011). Chronic effects linked with exposure to antineoplastic drugs, such as delayed time to conception, spontaneous abortion, miscarriage and congenital anomalies (Connor et al., 2014), as well as genotoxic effects (Rekhadevi et al., 2007; Cornetta et al., 2008, Moretti et al., 2015, Buschini et al., 2013, Ladeira et al., 2014, Mahmoodiet al., 2017) and cancers (Skov et al., 1992) were reported. The studies, which evaluated genotoxic changes, used mainly micronucleus and comet assay, but only some of them are supported by environmental and biological monitoring. Ladeira et al., 2014, detected surface contamination of 5-FU and found both contamination and significant increase of micronucleus in peripheral blood lymphocytes of the exposed subjects. Moretti et al., 2015, who monitored the presence of antineoplastic drugs using Cyclophoshpamide as marker of exposure, found an increase of MN and Chromosome Aberrations frequencies on lymphocytes of workers handling antineoplastic drugs in respect to controls. Our laboratory have previously performed a biomonitoring study on nurses and technicians handling antineoplastic drugs in an Italian Oncology Hospital (Cavallo et al., 2005, Ursini et al., 2006). We used multiple endpoints in both lymphocytes and exfoliated buccal cells and we found that exfoliated buccal cells are more sensitive than lymphocytes to detect cytogenetic damage induced by antineoplastic drug exposure. Therefore, we performed a new study on a larger population, involving also other two Italian big oncology hospitals, where we used the Buccal Micronucleus Cytome (BMCyt) assay, particularly interesting since it represents a noninvasive, although very sensitive, biomarker of exposure to genotoxic substances and mixtures.

The aim of the present study was to evaluate whether the currently used antineoplastic drugs and new procedures of preparation and administration, arising from the increased awareness of the possible health

risk for workers handling such drugs, still induce genotoxic and cytotoxic effects. We also evaluated workplace contamination, personal exposure to antineoplastic drugs and absorption of one specific drug, 5-FU through the measurement of its main metabolite α-fluoro β-alanine in the urine of exposed subjects. We used this specific drug and metabolite pair because 5-FU is among the most widely used antineoplastic drug, and because of a high conversion efficiency of the parent drug into a single metabolite (Rubino et al., 2006).

Since it is very difficult to analyse all the handled drugs, to better characterize individual exposure, we used a diary of exposure which each worker filled in reporting daily (in the last three weeks before the sampling of buccal cells) all the handled drugs and the relative amounts. We aimed to obtain such informations also to evaluate the possible correlation between antineoplastic drug exposure and their genotoxic and cytotoxic effects.

Moreover, we aimed to study the influence of single nucleotide polymorphisms of XRCC1 and hOGG1 DNA repair genes on genetic damage of exfoliated buccal cells of workers exposed to antineoplastic drugs. We choose the Arg^{399}Gln polymorphism of XRCC1 gene, which affects DNA repair efficiency, and in addition the Ser^{326}Cys hOGG1 polymorphism, a DNA glycosylase that plays a vital role in preventing carcinogenesis by repairing oxidative damage to DNA. The role of the two polymorphic gene variants, have been analyzed to verify a potential association between increased cancer risk and reduced DNA repair ability.

2. Materials and methods

2.1 Subjects

Ninety-five workers employed in three Italian big Oncology Hospitals (A, B and C) were enrolled. In particular, we studied 42 exposed subjects, of which 17 were technicians or nurses preparing antineoplastic drugs and 25 were nurses who administrated them. Table 1 shows the main characteristics of studied subjects. Fifty-three healthy subjects non-occupationally exposed to antineoplasticc drugs, working in the administrative offices, were selected as control group. All

subjects gave their consent for participating in this study. Data collection was by questionnaire, which included information on age, gender, smoking habits and job seniority. An exposure diary filled in by each worker in the last 3 weeks before the sampling was used to obtain information on the amounts of gemcitabine and total drugs handled by the preparators and administrators of the three hospitals. The study was approved by the Ethic Committees of the involved hospitals.

2.2 Workplace monitoring

Workplace monitoring was performed by collecting 350 samples in areas of pharmacy and administering wards. Sampling for workplace and personal monitoring at the pharmacy units of all hospitals took place on Monday. In hospital A, also workplace and personal monitoring of Day Hospital was performed on Monday. In hospitals B and C the monitoring at the day hospital and wards were performed in successive days within a week. Urine collection took place within 24h (2 samples shift hours, out-shift hours) simultaneously to pads collection. Buccal cells were collected on the third day of the week in all the hospitals.

To allow comparison of the results obtained in the hospitals, sampling positions were standardized. In principle, the sampling protocol described by Rubino et al., 1999 is used to select positions in the preparation rooms of the oncologic pharmacies, and further wipe samples are collected in specific positions that are suggested by the hospital staff, because of their workplace experience. In the out-patient treatment facilities, wipe samples were collected from the body of the infusion pump, the mechanism of the peristaltic organ, the holding pole, the wheel-tray and a section of the floor below the pump holding pole and close to the side of the armchair corresponding to the position of the patient's infusion site. Workplace samples were taken by wipe-sampling surfaces with a normalized equipment used since 1999 (Floridia et al., 1999), with minimal modifications. Detailed sampling and sample preparation protocols are summarized in **Supplementary S1**. Personal exposure was monitored by pads placed on worker protective clothes. Personal pads are manufactured in the

laboratory as 100 to 200-piece batches that are subject to internal quality control to ensure uniformity. Three pads were pasted on the operator's forearms and on the outer layer of professional garment, on the upper chest, at the beginning of the considered work period at the end of the working time. Detailed sampling and sample preparation protocols are summarized in **Supplementary S1**. Measurement of traces of antineoplastic drugs in wipes, swabs and pads was accomplished by HPLC with UV-Vis detection, by using established methods developed by the laboratory, or modifications that account for the availability of improved equipment. Two methods were used, one for nucleoside analogs (5-Fluorouracil, Cytarabine, Gemcitabine, Azacytidine), one for anthracyclines (Daunorubicin, Doxorubicin and Epirubicin). The sample preparation protocol was the same for the two analyses. Briefly, a Thermo Instruments P2000 integrated LC system with autosampler and UV-Vis detector (Thermo Instruments, Rodano, Italy) with 3-mm i.d. chromatographic columns was used. Typical minimum detected amounts of nucleoside analogs were approx. 0.02 micrograms, of anthracyclines of 0.50 micrograms in the sample. A full description of the methods is summarized in **Supplementary S2, S3**. Drug concentration in the examined water extracts was converted to drug mass in the sample and to drug mass *per* sample area.

2.3 Urine sampling and analysis

The collaborating hospital workers yielded urine samples over the 24 hours that start at the beginning of the considered work period. Standard 2-litre plastic hospital bottles were used. One sample was taken from the beginning of the work period to the end of the daily working shift, the other from that moment to the beginning of the next-day work shift. Appropriate representative sub-samples were obtained at the facility for refrigerated storage, delivery and analysis by the laboratory. Urine supplied by the participating hospital workers was screened for the presence of the main metabolite of 5FU, the un-natural amino acid alpha-fluoro-beta-alanine (AFBA),

employing chemical derivatization with dabsyl chloride (Rubino et al., 2006) and liquid chromatography-tandem mass spectrometry (LC-MS-MS) in the MRM mode. A commercially available, structurally close amino acid (beta-fluoro-valine), added before sample derivatization, was used as the internal standard for quantification. The typical minimum detectable amount of AFBA is approx. 0.02 micrograms in the sample. A full description of the methods is summarized in **Supplementary S4**.

2.4 Buccal Micronucleus Cytome (BMCyt) assay

The exfoliated buccal cells were collected at the start-shift of the Wednesday. The cells were obtained by gently scraping the right and left cheeks with a wet toothbrush (previously immersed in Phosphate Buffer Solution), after washing out the mouth with water. The obtained cells were suspended in 25 ml of buffer solution containing 0.01 M Tris-HCl, 0.1 M EDTA and 0.02 M NaCl (pH 7), and transferred to the laboratory where BMCyt assay was performed. The collected cells were washed twice in the same buffer solution. Then 50 µl of the final cell suspension ($1.5x10^6$-$2x10^6$ /ml) were dropped on pre-warmed slides (37°C). Cells were air dried and fixed in 80% methanol for 48 h, then they were stained with orange acridine (0.005%, Sigma) and observed by fluorescence microscope at 400 X magnification (Leica, Germany). At least 2000 differentiated cells were analysed for each subject, by two expert readers according to the criterion established by Titenko-Holland et al., 1998. The presence of cells with MN, nuclear buds and broken eggs (indicative of DNA damage), of binucleated (indicative of cytokinesis defect or arrest) and of karyolytic cells (advanced stage of necrosis and apoptosis) and of condensed chromatin (indicative of early stages of apoptosis) were recorded separately. For each subject the frequency of each abnormality was estimated on total differentiated exfoliated cells and expresses as ‰. Moreover, subjects with micronucleated cells frequency exceeding a fixed cut-off value (1.5‰) were considered positive to MN assay. We chose 1.5 MN‰ threshold on the basis of the results of HUMNXL (Human MicroNucleus project on eXfoLiated buccal cells) published by Bonassi et al.

2011 that report the estimated spontaneous MN frequency of 0.74‰ (95% CI 0.52-1.05). HUMNXL project involved 5424 subjects obtained from 30 laboratories worldwide included our laboratory (EU7) using different staining methods. In particular for our staining method (Acridine Orange) a mean value of 0.98‰ (95% CI 0.39-1.14) was reported, therefore we established a cut off value of 1.5 above both the upper limits of confidence intervals.

2.5 Polymerase chain reaction and Restriction Fragment Length Polymorphism

Genomic DNA was isolated from whole blood of the recruited participants by using the QiAmp DNA blood mini kit cat. N. 51306 (Qiagen, Germany) following the manufacturer's instructions. Polymerase Chain Reaction (PCR) of XRCC1 $Arg^{399}Gln$ (rs25487) and hOGG1$Ser^{326}Cys$ (rs1052133) has been performed in the thermocycler (Multigene optimax thermal cycler, Aurogene SRL, Italy). The reaction mixture contained 1X PCR buffer, 100 ng of DNA and 2.5 Unit of AmpliTaq Gold polymerase per DNA sample, (Applied Biosystems Cat. N8080161, ThermoFisher Scientific MA, US), 0.5µM of Forward and Reverse primers, 0.2 mM of dNTP and 2 mM of $MgCl_2$. The oligonucleotides were purchased from Metabion GmbH (Germany-Dasit Carlo Erba-Italy); XRCC1 $Arg^{399}Gln$: Forward primer 5'-TTGTGCTTTCTCTGTGTCCA-3'; XRCC1 Reverse primer 5'-TCCTCCAGCCTTTTCTGATA-3'; hOGG1 $Ser^{326}Cys$: Forward primer 5′-ACTGTCACTAGTCTCACCAG-3′ and hOGG1Reverse Primer 5′-GGAAGGTGCTTGGGGAAT -3′. Amplification conditions of the XRCC1$Arg^{399}Gln$ gene were 95°C 5 min, 95°C 30 sec, 56°C 30 sec, 72°C 1 min, 72°C, 7 min and 4°C ∞ (35 cycles). Amplification conditions of the hOGG1 $Ser^{326}Cys$ gene were 94°C 5 min, 94°C 30 sec, 60 °C 30 sec, 72°C 30 sec, 72°C 7 min and 4°C ∞ (35 cycles). After amplification the PCR products of XRCC1 (615 bp) and of hOGG1 (207 bp) were separated on 1% agarose gel (Cat. BMR 918100 Euroclone MI, Italy) with TBE (Tris, Boric acid, EDTA) buffer and stained with gel red staining solution (Biotium CA, US). Gel images were visualized by ultraviolet transillumination and acquired using a Microsoft Lumia 950 mobile phone with a 20 MP photocamera. Twenty µL of each amplicon obtained from PCR of XRCC1 $Arg^{399}Gln$ gene were digested overnight at 37°C in a total volume of 50 µl with 10U of MspI restriction enzyme in the presence of 1X buffer IV (New England Biolabs, MA, US). The digested products were: wt (Arg/Arg): 221, 374 bp; het (Arg/Gln): 615, 374, 221bp; mut (Gln/Gln) 615 bp.

Twenty µL of amplicon obtained from PCR of hOGG1 $Ser^{326}Cys$ gene were digested for 2h at 37°C in a total volume of 50 µl with 15U of Fnu4HI restriction enzyme in the presence of 1X buffer IV

(New England Biolabs, MA, US), followed by enzyme inactivation at 65°C for 20 min. Samples were run on 2% agarose and the gel and images were acquired as described above. The digested products were: wt (Arg/Arg): 207 bp; het (Arg/Gln): 207, 107, 100 bp; mut (Gln/Gln) 100, 107bp.

2.7 Statistical methods

Statistical analysis was performed with IBM SPSS software version 22. The chi square test was performed to test the significance of the association between categorical variables and groups analysed. One-way ANOVA and non parametric tests (Mann-Whitney U-test and Kruskal-Wallis) were also used to test the significance of median values differences between exposed and controls subgroups. Pairwise comparisons were performed using Dunn's procedure with a Bonferroni correction for multiple comparisons. Multiple regression analysis was also performed, using as dependent variables the studied biomarkers of effects and as independent variables the exposure, confounding factors and polymorphisms. Values of $p < 0.05$ were considered significant. The correlation between two variables were determined by Pearson correlation coefficients. A p value <0.05 was considered significant.

3. Results

Table 1 shows the characteristics of the studied population, where it is possible to observe that there are no statistically significant differences for gender, smoking habits, age, job seniority and polymorphism of both the genes hOGG1 and XRCC1 among control subjects, administrators and preparators.

3.1 *Workplace and biological monitoring*

Tables 2-3 summarize the analytical results of pharmacies and administration wards of the three hospitals (A, B and C), and report the only two nucleoside analog drugs, 5FU and GEM, that

yielded positive results in at least one tested sample. In two hospitals (A and C) there was also a limited use of anthracycline therapies with doxorubicin, and the 38 wipe and swab samples taken in the booths where patients were treated with the drug were also analyzed with the anthracycline method. No anthracyclines were measured in any of the samples. Table 3 summarizes the drug deposition results of the pads that were positioned on the personnel working in the pharmacy and in patient treatment facilities. In all three hospitals, only GEM, but no 5-FU (just one positive out of 123 pads) could be detected. In hospital A GEM deposition was much higher, with a 20-fold variability between the workers, but no statistical difference between pharmacy preparators and administrators was found. In the other two hospitals, levels were consistently lower by an order of magnitude, and deposition measured on administrators was distinctly higher than in pharmacy preparators. Distribution of the deposition of GEM between chest, right and left forearm was very homogeneous, with no difference between the three hospitals and the different tasks. We found total amounts of prepared GEM of 141, 103 and 113 g in hospital A, B and C respectively. The amounts of GEM handled by administrators were 99.21, 59.81 and 9.52 g in hospital A, B and C respectively. Biological monitoring of exposure to 5FU by measurement of AFBA in urine did not find any sample with AFBA above its detection limit of 0.02 μg/mL.

3.2 Buccal micronucleus cytome (BMCyt) assay

Table 4 shows the results of BMCyt assay with the statistical analysis performed between exposed subjects and controls and among the three groups according the different task (preparators, administrators and controls). The non parametric Mann-Withney test showed in the exposed group higher median values of MN, CC and MN+NB+BE than those found in control group. The comparison of median values of the studied anomalies, among the different tasks, shows that on the preparators MN, NB and MN+NB+BE median values resulted significantly higher than controls. In the administrators the median values of MN, CC and MN+NB+BE were higher as compared to controls.

Multiple regression analysis performed on confounding factors (age, gender and smoking habit), exposure and gene polymorphisms on biomarker frequencies found in total population a significant rice for MN, MN+NB+BE and CC, for the exposure variable (Table 5). Also hOGG1 polymorphism exerted its influence on CC, with a decrease of frequency of such biomarker in the hOGG1mut/het. In the exposed group, gender variable influenced only NB frequency, while in control group smoking habit influenced only CC frequency. Only the statistically significant results are reported in such Table.

Relatively to the positivity to MN we found higher percentages of MN positive subjects in both exposed groups (64.7% in the preparators and 68.0% in the administrators) vs 30.2% of controls (χ^2 p value 0.002).

We also correlated the amounts of GEM on pads and those reported on the diary of exposure (GEM, and total drugs) with MN, NB, CC and MN+NB+BE frequencies found by BMCyt assay. Taking into account all the exposed subjects, we found only a negative correlation between total amount of handled drugs (reported on the personal diary) and CC frequency (Pearson's r = - 0.352, p=0.026). When we analysed the group of preparators, we found positive correlations between GEM amounts, found on pads, and frequencies of MN (Pearson's r = 0.621, p=0.024), NB (Pearson's r = 0.723, p=0.005) and MN+NB+BE (Pearson's r = 0.753, p=0.003). We also found on preparators a negative correlation between GEM amounts reported on the diary with NB frequency (Pearson's r = -.561, P=0.024). We did not find any correlation in the administrator group.

3.3 Gene Polymorphisms

We did not find any association between the tested polymorphisms and MN frequency in exfoliated buccal cells, but we found higher CC frequency in hOGG1 wild type subjects of the administrator group (Mann Whitney p=0.040).

We also compared the positivity to MN with the above cited polymorphisms but we did not find differences among MN positivity and presence of mutant/heterozygous (mut/het) neither for hOGG1 nor for XRCC1 considering all the studied subjects independently from the exposure and job task assignment. When we focused on the difference in the type of job task, for the administrators, we found that 83% of MN positive subjects were mut/het for hOGG1 and that 75% of MN positive subjects were mut/het for XRCC1 polymorphisms, although χ^2 p values (hOGG1 p=0.114; XRCC1 p=0.317) were not statistically significant. Taking into account only MN positive subjects, we analysed the association between hOGG1 and XRCC1 workers' genotype and job task and found that the percentage of administrators with hOGG1 mutant/heterozygous genotype was higher than in the other two groups, although not statistically significant (χ^2 p =0.368). In the case of XRCC1 the percentage of administrators and preparators with XRCC1 mutant/heterozygous genotype was similar (70.6% and 72.7% respectively) and higher than in the control group (50%) (χ^2 p=0.362), suggesting a possible influence of presence of mut/het polymorphism on MN induction due to antineoplastic drug exposure. When we considered all the exposed subjects as compared to controls, we found that the percentage of total exposed subjects with XRCC1 mut/het genotype was 71.4% with χ^2 p=0.155.

4. Discussion

This study represents one of the few available ones that, simultaneously, evaluates in oncology hospitals, the presence of antineoplastic drugs on surfaces and on workers, the genotoxicity and cytotoxicity on exfoliated buccal cells of exposed workers and that studies the potential susceptibility in subjects with variant hOGG1 and XRCC1 gene polymorphisms on the induction of MN and other cellular anomalies on exfoliated buccal cells. It confirms the results of our previous studies (Cavallo et al., 2005 and Ursini et al., 2006) performed in one of the three hospitals currently analyzed and demonstrates that, after several years from such studies, 5-FU surface contamination is still present although at very lower extent particularly in drug administration areas.

This particular result is explained by the partial replacement of 5-FU with the orally administrated capecitabine to treat colon and breast tumors. Moreover, this new study differently from previous ones, highlights gemcitabine contamination on both surfaces and pads of workers handling antineoplastic drugs, due to the enhanced use of such drug to treat the increased pancreatic and lung tumors. The amounts of GEM found on pads from preparators seem to reflect those that they handled, in fact total GEM amount handled in hospital A, was higher than that handled in the other two hospitals. The findings also show higher levels of 5-FU and GEM surface contamination in patient treatment facilities in respect to the more controlled pharmacy. BMCyt assay results obtained in exfoliated buccal cells confirm the induction of genotoxicity particularly on nurses administrating antineoplastic drugs and show, for such task, also cytotoxic effects in terms of higher frequency of CC, indicative of apoptosis. This result is consistent with the higher amounts of GEM and 5-FU in the areas of patient treatment in respect to those found in the pharmacies. However, also the preparation task seems to induce genotoxic effects particularly in terms of NB induction indicative of gene amplification. The evaluation of the possible influence of confounding factors and exposure on biomarkers of cytotoxicity and genotoxicity, confirmed that the exposure is the main factor causing genotoxic effects. Such findings confirm the suitability of BMCyt assay as sensitive and no invasive biomarker of early genotoxic and cytotoxic effect for occupational antineoplastic exposure. In the last years, several Italian biomonitoring studies are available that found genotoxic effects of occupational antineoplastic drug exposure by comet assay, chromosomal aberrations analysis and MN assay in peripheral blood lymphocytes (Cornetta et al. 2008, Buschini et al 2013, Moretti et al. 2015). Also Ladeira et al., 2014 evaluated genetic damage by MN assay on lymphocytes of oncology nurses in two Portuguese hospitals and found higher MN frequency in exposed workers compared with controls. A more recent study demonstrated that the mean frequency of cytogenetic damages in terms of CAs, MN formation, and SCE in lymphocytes of personnel handling antineoplastic drugs were significantly higher than those found in control unexposed group (Mahmoodi et al., 2017). However, there are few studies, besides those performed

by our laboratory, that performed Buccal Micronucleus Cytome assay on workers handling antineoplastic drugs. Machado-Santelli et al. 1994 found increase of micronucleated buccal cells in exposed subjects as compared with the control group (Machado-Santelli et al 1994). Burgaz et al., 1999, who performed MN assay both on lymphocytes and buccal cells of oncology nurses, found increase of MN only on lymphocytes whereas they found an increasing trend on buccal cells. Another study performed by MN assay on lymphocytes and exfoliated buccal cells, on nurses handling antineoplastics found higher MN frequency on both cell types in exposed subjects correlating with higher DNA damage evaluated by comet assay on lymphocytes (Rekhadevi et al., 2007).

Our present study represents the only one that correlated the amounts of GEM detected by personal monitoring and those obtained by a diary of exposure with MN frequency and other cellular anomalies of buccal cells. The positive correlation found on preparators between GEM amounts (found on pads) and genotoxicity on buccal cells, encourages us to suggest to use such approach to evaluate genotoxicity induced on workers handling antiblastic drugs. The unexpected negative correlation found, in the group of all the exposed workers, between total amounts of handled drugs (reported on the diary) and CC, could be explained by the lower response, in terms of apoptosis, induced by chronically total drug exposure in those subjects who handled the highest amounts of drugs. However, this is only an hypotesis to be confirmed by further studies.

All together the results of the correlation between amount of drugs and biological effects suggests the usefulness of both the tools (personal monitoring and diary of exposure) to better characterize the occupational exposure to anticancer drugs. Therefore, since it is difficult to analyse on pads all the handled drugs, the diary of exposure could furnish further informations on the possible occupational exposure to such drugs, but only if all the workers fill in it with much attention and if they handle anticancer drugs exactly with the same procedures.

In this study we also demonstrated that the presence of both *het* and *mut* genotype of XRCC1 gene, could represent a susceptibility factor for MN induction in exfoliated buccal cells confirming the

results of Cornetta et al., 2008 on lymphocytes. Our findings also show that the hOGG1 polymorphism influences the induction of CC that could be explained with the impairment of cell capacity to repair the damage undergoing to apoptosis in presence of reduced or modified hOGG1 enzyme activity. These findings encourage performing further studies on larger populations to confirm the suitability of MN assay on exfoliated buccal cells, also in consideration of the non-invasiveness of such assay compared to other tests. The higher percentages of MN positive subjects in both exposed groups in respect to controls confirms the suitability of the used criterion to identify the positive subjects to MN assay and its usefulness to demonstrate the induced genotoxic effect in the biomonitoring of populations exposed to potential genotoxic mixtures.

5. Conclusion

This study shows drug contamination in both drug preparation and administration facilities, although in low amounts. We also found a positive correlation between GEM amounts found on pads of preparators and genotoxicity in terms of MN, NB and MN+NB+BE frequencies demonstrating the suitability of BMCyt assay, a sensitive and no invasive biomarker of early effect, as useful tool for the biomonitoring of occupational antineoplastic exposure. Such assay allowed us to highlight that the handling of antineoplastic drugs, particularly the administration task still represents a potential occupational health risk and that workers should be better informed and formed on such risks. So all together, these findings suggest for health care workers handling antineoplastic drugs, the need to be fully aware of the hazards and precautionary measures to minimize exposure to these toxic drugs.

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Table 1. Characteristics of the studied populations and statistical analysis of the differences

Variables		**Controls** n=53	**Exposed subjects** n=42		Statistical significance of differences among groups
			Administrators n=25	Preparators n=17	*p* value
Gender	Males	15 (28.3%)	5 (20.0%)	9 (52.9%)	0.065[a]
	Females	38 (71.7%)	20 (80.0%)	8 (47.1%)	
Smoking	Smokers	12 (22.64%)	5 (20.0%)	6 (35.3%)	0.833[a]
	No smokers	24 (45.28%)	12 (48.0%)	7 (41.2%)	
	Ex smokers	10 (18.87%)	7 (28.0%)	4 (23.5%)	
	Missing	7 (13.21%)	1 (4.0%)	0	
Age	Mean value ±SD (years)	40.9±11.0	40.0±9.2	42.8±9.3	0.698[b]
Job Seniority	Mean value ±SD (years)	12.9±9.4	15.6±7.3	18.4±10.2	0.087[b]
OGG1	wt	28 (59.6%)	13 (52.0%)	10 (58.8%)	0.818[a]
	mut/het	19 (40.4%)	12 (48.0%)	7 (41.2%)	
XRCC1	wt	21 (44.7%)	9 (36.0%)	5 (29.4%)	0.501[a]
	mut/het	26 (55.3%)	16 (64.0%)	12 (70.6%)	

[a]χ^2 test; [b]Analysis of variance (ANOVA)

Table 2. Deposition of 5FU and GEM on several surfaces of the examined drug preparation and administration facilities of the three studied Hospital.

Facility	Total samples N.	Drug	Drug absent samples N.	<LoD [a] samples N.	>LoD samples N. (median values in µg; min-max range)	not analyzable samples N.
A -Pharmacy	27	5FU GEM	15 12	1 2	*7 (1.08; 0.30 – 3.00)*[b] *9 (1.80; 0.54-18.00)*[c]	4 4
A -Patient treatment	47	5FU GEM	35 8	0 0	*3 (0.90; 0.60-1.20)*[b] *30 (7.17; 1.44-93.90)*[c]	9 9
B -Pharmacy	16	5FU GEM	4 4	6 4	*5 (0.67; 0.59 – 6.68)*[b] *7 (10.64; 1.12-19.38)*[c]	1 1
B -Patient treatment	57	5FU GEM	35 13	6 2	*8 (0.84; 0.62-18.19)*[b] *34 (6.25; 0.27-47.82)*[c]	8 8
C -Pharmacy	32	5FU GEM	25 20	2 2	*4 (0.80; 0.40 – 141.83)*[b] *9 (0.76; 0.18-5.97)*[c]	1 1
C -Patient treatment	58	5FU GEM	22 18	0 6	*36 (3.31; 0.26 -196.83)*[b] *34 (2.93; 0.64 - 46.83)*[c]	0 0
Total Pharmacy	75	5FU GEM	44 36	9 8	*16 (0.70; 0.30 –141.83)* *25 (1.58; 0.18 – 19.38)*	6 6
Total Patient treatment	162	5FU GEM	92 39	6 8	*47 (2.49; 0.26 -196.83)* *98 (5.07; 0.27-93.90)*	17 17

[a] LoD: Limit-of-Detection 0.5 µg; [b] $p < 0.01$ (Kruskal-Wallis test); [c] $p < 0.01$ (Kruskal-Wallis test)

Table 3. Deposition of 5FU and GEM on the external body pads placed on protective clothes of the personnel working in the pharmacy and patient treatment facilities

Facility	Total samples N.	Drug	Drug absent samples N.	<LoD[a] samples N.	>LoD samples N. (median values in µg; min-max range)
A - Pharmacy	15	5FU	15	0	0
		GEM	0	0	15 (*6.47; 3.51 – 14.85*)
A – patient treatment	24	5FU	23	0	1 (*2.49*)
		GEM	0	0	24 (*5.06; 0.51 – 15.21*)
B - Pharmacy	24	5FU	24	0	0
		GEM	2	21	1 (*0.40*)
B – patient treatment	24	5FU	24	0	0
		GEM	0	4	20 (*0.75; 0.29 – 5.38*)
C - Pharmacy	9	5FU	9	0	0
		GEM	6	3	0
C - patient treatment	27	5FU	27	0	0
		GEM	0	7	20 *(0.30; 0.18 – 2.11*)
Total – Pharmacy	48	5FU	48	0	0
		GEM	8	24	16 (*6.44; 0.40 – 14.85*)
Total -patient treatment	75	5FU	74	0	1 (*2.49*)
		GEM	0	11	64 (*0.72; 0.18 – 15.21*)
Total	123	5FU	122	0	1 (*2.49*)
		GEM	8	35	80 (*1.02; 0.18 – 15.21*)

[a] LoD: Limit-of-Detection 0.5 µg

Table 4. BMCyt assay results by exposure and specific task

Group	MN ‰ Median (IQR)	NB ‰ Median (IQR)	CC ‰ Median (IQR)	MN+NB+BE ‰ Median (IQR)
Exposed	2.25 (2.33)[a]	0.98 (1.71)	2.38 (4.73)[e]	4.16 (3.21)[g]
Preparators	1.90 (1.40)[b]	1.47 (2.78)[d]	1.87 (1.23)	4.37 (3.41)[h]
Administrators	2.90 (3.23)[c]	0.84 (1.60)	5.45 (6.40)[f]	3.96 (3.12)[i]
Controls	0.92 (1.44)	0.48 (1.45)	1.41 (2.04)	2.08 (1.83)

MN: Micronucleus; NB: Nuclear Buds; CC: Condensed Chromatin; BE: Broken Egg; IQR: Interquartile Range.

[a] Mann Whitney test p<0.001 vs controls
[b] Kruskal Wallis test. Multiple comparisons: Dunn's procedure with Bonferroni correction (adjusted p-value=0.009)
[c] Kruskal Wallis test. Multiple comparisons: Dunn's procedure with Bonferroni correction (adjusted p-value<0.001)
[d] Kruskal Wallis test. Multiple comparisons: Dunn's procedure with Bonferroni correction (adjusted p-value=0.034)
[e] Mann Whitney test p=0.001
[f] Kruskal Wallis test. Multiple comparisons: Dunn's procedure with Bonferroni correction (adjusted p-value=0.001)
[g] Mann Whitney test p<0.001
[h] Kruskal Wallis test. Multiple comparisons: Dunn's procedure with Bonferroni correction (adjusted p-value=0.002)
[i] Kruskal Wallis test. Multiple comparisons: Dunn's procedure with Bonferroni correction (adjusted p-value= 0.002)

Table 5

Table 5. Multiple regression analysis of confounding factors (age, gender and smoking habits), exposure and gene polymorphisms (XRCC1 and hOGG1) on biomarkers frequencies in exposed workers and controls

Population	Biomarker	Indipendent variables	Regression coefficients				*P-Value*
			Unstandardised β	95% CI		Standardised β	
All (n=95)	MN	Exposure[a]	1.441	0.483	- 2.400	0.319	**0.004**
	CC	Exposure	2.067	0.439	- 3.694	0.272	**0.013**
		hOGG1[b]	-1.882	-3.521	- -0.242	-0.246	**0.025**
	MN+NB+BE	Exposure	2.447	0.880	- 4.015	0.325	**0.003**
Exposed (n=42)	NB	Gender[c]	-1.686	-3.134	- -0.238	-0.383	**0.024**
Controls (n=53)	CC	Smoking habit[d]	1.208	0.040	- 2.376	0.316	**0.043**

[a]Baseline: unexposed; [b]Baseline: wt; [c]Baseline: male; [d]Baseline: smoker

Supplementary

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***Declaration of Interest Statement**

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: