Applicability of the comet assay in evaluation of DNA damage in healthcare providers' working with antineoplastic drugs: a systematic review and meta-analysis

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Background: Unintended occupational exposure to antineoplastic drugs (ANDs) may occur in medical personnel. Some ANDs are known human carcinogens and exposure can be monitored by genotoxic biomarkers.

Objective: To evaluate the obstacles to obtaining conclusive results from a comet assay test to determine DNA damage among AND exposed healthcare workers.

Methods: We systematically reviewed studies that used alkaline comet assay to determine the magnitude and significance of DNA damage among health care workers with potential AND exposure. Fifteen studies were eligible for review and 14 studies were used in the meta-analysis.

Results: Under random effect assumption, the estimated standardized mean difference (SMD) in the DNA damage of health care workers was 1.93 (95% CI: 1.15–2.71, $p < 0.0001$). The resulting SMD was reduced to 1.756 (95% CI: 0.992–2.52, $p < 0.0001$) when the analysis only included nurses. In subgroup analyses based on gender and smoking, heterogeneity was observed. Only for studies reporting comet moment, I2 test results, as a measure of heterogeneity, dropped to zero. Heterogeneity analysis showed that date of study publication was a possible source of heterogeneity ($B = -0.14$; $p < 0.0001$).

Conclusions: A mixture of personal parameters, comet assay methodological variables, and exposure characteristics may be responsible for heterogenic data from comet assay studies and interfere with obtaining conclusive results. Lack of quantitative environmental exposure measures and variation in comet assay protocols across studies are important obstacles in generalization of results.

Keywords: Antineoplastic drugs, Comet assay, Genotoxicity, DNA damage, Occupational exposure, Health care workers

Introduction

Antineoplastic drugs (ANDs), also known as cytostatic drugs, are a heterogeneous group of chemicals used to control the growth of neoplastic cells. Mustard gas was the first chemical used in the treatment of bone marrow and lymph nodes malignancy in the late 1940s. Subsequently, mustard gas was applied as a therapeutic agent in non-Hodgkin's lymphoma.[1] ANDs are classified into five groups based on the source and mechanisms of action: (a) alkalinating (b) antimitotic (c) antibiotics (d) antimetabolites and (e) miscellaneous agents.[2] Most ANDs interfere with cell proliferation through DNA destruction, RNA synthesis, or duplication process.[3] Unfortunately, ANDs do not distinguish cancer cells from normal ones, leading to unwanted adverse effects in patients receiving chemotherapy. The International Agency for Research on Cancer (IARC) classifies some ANDs as human carcinogens.[4] In addition to patients, health care providers such as nurses, physicians, laboratory personnel, and pharmacists who prepare, handle, and administer ANDs or deal with patients' excreta are also at risk of exposure to ANDs.[5,6]

Biological monitoring can estimate occupational exposure to hazardous agents. Considering the extensive use of

ANDs, it is difficult to select an exposure biomarker that encompasses the total potential exposure to various classes of these compounds. However, because most ANDs interfere with cell genomes, biomarkers of genotoxic effect can serve as a surrogate to determine exposure. Genotoxicity endpoints are preferred due to potential role of ANDs in development of cancer.[7] Falck et al.[8] first investigated occupational exposure to ANDs by measuring the urine mutagenicity in occupationally exposed nurses. Since then, tens of studies have explored the genotoxic risk of working with ANDs using different genotoxicity tests. During the last three decades, numerous studies have used various genotoxic endpoints as the biomarker of effect for exposure to ANDs.[9, 10] Singh et al.[12] developed the comet assay (or single-cell gel electrophoresis) test to assess DNA damage. The simplicity and availability of the comet assay test make it a good choice for screening in occupational and environmental genotoxicity studies. Valverde and Rojas.[13] recently reviewed the applicability of the comet assay in occupational biomonitoring. Ündeğer et al.[14] performed the first study on DNA damage in health care workers by the comet assay. Since that time, several articles using comet assay to examine the extent of DNA damage in various job categories with potential exposure to ANDs have been published.[15] However, results are inconclusive. An important issue in the interpretation of results obtained by comet assay is personal and methodological confounders. In other words, direct comparison of comet assay studies on the similar subjects exposed to the same compound are relatively difficult to perform due to differences in population characteristics, agents analyzed, exposure metrics, and differences in the parameters of comet assay.[13]

There are also concerns about the comparison of comet assay results across studies. Factors such as inter- and intra-laboratory variations and the use of different protocols may explain the observed heterogeneity in comet assay results.[16] Most comet assay studies use pure laboratory settings for optimization of comet assays as a source of their reviews on the effect of various parameters on comet assay results. To the best of our knowledge, there is only one meta-analysis on comet assay and smoking.[17] This manuscript examines the field applicability of the comet assay as a biomonitoring tool in occupational genotoxicology. We found that more than three-quarters of articles related to application of comet assay are for occupational biomonitoring. We identified only one study which narratively reviewed the global application of comet assay in occupational and environmental biomonitoring.[13] However, Valverde and Rojas[13] did not publish a systematic evaluation of studies based on exposure situations. Accordingly, we systematically reviewed and quantitatively analyzed published resources of comet assay to determine the occurrence of DNA damage in health care providers occupationally exposed to ANDs. This review evaluates the magnitude and significance of DNA damage in health care providers working with ANDs compared to non-occupationally exposed control subjects. It also identifies the challenges these studies encountered in obtaining conclusive results.

Data from studies were used to: (a) to calculate the effect size of potential occupational exposure to ANDs in health care providers, (b) to critically evaluate exposure assessment procedures in the case of ANDs, and (c) to evaluate the methodological aspects of comet assay and their effects on drawing conclusions from the data.

Materials and methods
Search strategy
We searched PubMed, Scopus, CINAHL, Google scholar, American society of clinical oncologists (ASCO), Cochrane library, and HealthStar for relevant published studies between 1960 and September 1, 2014. A grey literature search was performed at System for Information on Grey Literature in Europe-SIGLE (http://www.opengrey.eu/) and the health management information consortium (HMIC) databases. References were checked to identify additional publications. Search criteria were prepared by combining a 'exposed population' AND 'intervention' (exposure) AND 'outcome.' Relevant words and headings describing health care providers (e.g. health care personnel, hospital personnel, occupational exposure, oncology service, workplace, and pharmacy service) were used as population proxies. Antineoplastic agents and related synonyms such as chemotherapy, chemotherapeutic, anticancer, cytostatic, ANDs, and the 12 mostly used ANDs were searched as exposures. Examples of outcome terms for DNA damage genotoxicity included: DNA damage, DNA breaks, comet assay, biological monitoring, and single-cell gel electrophoresis. A complete list of search keywords based on PICO (population, intervention, control, and outcome) is shown in Table 1. Three authors (MJZS, MM, and MH) were responsible for the systematic search. Additional details of the review methodology are available on PROSPERO (Registration number: CRD42014009075).

Eligibility criteria and data extraction
All accessible studies examining the role of ANDs potential exposure with primary DNA damage via comet assay in health care providers (ward and cancer day clinic nurses, physicians, pharmacists, pharmacy technicians, and attendants) between 1970 and September 1, 2014 were eligible for systematic review and subsequent meta-analysis. Abstracts, editorials, case reports, and review articles were excluded, as were studies on drug production facility workers, laboratory animals, and *in vitro* studies. Only resources with English full text were reviewed. However, non-English resources were all assessed for their English language availability. For meta-analysis, only studies reporting quantitative results of alkaline comet assay on PBLs were included. All relevant data including author(s) name, publication date, country, sample size (cases and

Table 1   Keywords used for search in databases based on PIO (population, intervention, and outcome) search strategy

Element	Keywords
Population	'Health personnel'[Mesh] OR 'occupational exposure'[Mesh] OR 'Oncology Service, Hospital'[Mesh] OR 'Medical Staff'[Mesh] OR 'Oncology Nursing'[Mesh] OR 'Professional Role'[Mesh] OR 'Occupational Groups'[Mesh] OR 'Pharmacy Service, Hospital'[Mesh] OR 'Workplace'[Mesh] OR 'Nurses'[Mesh] OR 'professional Practice'[Mesh] OR 'Pharmacy technicians'[tiab] OR 'occupational setting'[tiab] OR 'Hospital personnel'[tiab] OR 'Personnel'[tiab] OR 'Occupationally'[tiab] OR 'Occupational'[tiab] OR 'Oncology personnel'[tiab] OR 'Oncology department'[tiab] OR 'Health care staff'[tiab] OR 'Health care staff'[tiab] OR 'health care personnel'[tiab] OR 'Health care personnel'[tiab] OR 'Medical staff'[tiab] OR 'Worker'[tiab] OR 'Health care workers'[tiab] OR 'Hospital staff'[tiab] OR 'Professionally'[tiab] OR 'Health care workers'[tiab] OR 'work environment'[tiab] OR 'oncologic nurses'[tiab] OR 'oncology worker'[tiab] OR 'medical worker' [tiab] OR 'oncology'[tiab] OR 'pharmacists' [tiab] OR 'workstation' [tiab]
Intervention Outcome	'Antineoplastic Agents'[Mesh] OR 'drug Therapy'[Mesh] OR 'Cytostatic Agents' [Mesh] OR 'Electrochemotherapy'[Mesh] OR 'Mutagenicity Tests'[Mesh] OR 'Drug Compounding'[Mesh] 'Cytotoxic'[tiab] OR 'Chemotherapy'[tiab] OR 'Antineoplastic drug'[tiab] OR 'Antineoplastic'[tiab] OR 'anti cancer'[tiab] OR 'Chemotherapeutic'[tiab] OR 'chemotherapy'[tiab] OR 'anti-cancer'[tiab] OR 'cytostatic'[tiab] OR 'anticancer'[tiab] OR 'genotoxic agent' [tiab] OR 'antitumor' [tiab] OR 'ANPD'[tiab] OR 'Cyclophosphamide'[Mesh] OR 'Doxorubicin'[Mesh] OR 'Chlorambucil'[Mesh] OR 'tamoxien'[Mesh] OR 'cisplatin'[Mesh] OR 'bleomycin'[Mesh] OR 'merphalan'[Mesh] OR 'mitomycin'[Mesh] OR 'etoposide'[Mesh] OR 'cytarabine'[Mesh] OR 'fluorouracil'[Mesh] OR 'nitrosourea'[tiab] DNA Damage'[Mesh] OR 'DNA Breaks'[Mesh] OR 'toxicity'[Mesh] OR 'Mutation'[Mesh] OR 'DNA damage'[tiab] OR 'mutagenic'[tiab] OR 'mutagens'[tiab] OR 'mutagenicity'[tiab] OR 'genotoxic'[tiab] OR 'Comet Assay' [Mesh] OR 'Mutation'[tiab] OR 'adverse effects'[Mesh] OR 'comet assay' [tiab] OR 'micronucleus'[tiab] OR 'Cytogenetics'[Mesh] OR 'cytogenetics'[tiab] OR 'carcinogenic'[tiab] OR 'Carcinogenesis'[Mesh] OR 'genome damage'[tiab] OR 'biological monitoring'[tiab] OR 'toxicological'[tiab] OR 'teratogens'[tiab]

controls), matching, age, sex, the use of personal protective equipment (PPEs), safety measures, drug types, comet assay results, statistical tests, and any significance in results were organized into a spreadsheet. Comet assay test results can be presented in different forms. Therefore, we used hierarchal selection process of the best comet parameter to include into meta-analysis similar to the meta-analysis performed by Hoffman et al.[17] Briefly, percent of DNA in the comet tail was selected as a priori measure.[18] In the case of absence of percent of DNA in tail, comet moment and comet length were selected, respectively.

Statistical analysis

Relevant data from selected studies were imported into comprehensive meta-analysis (CMA) software version 2 (CMA, Biostat, Englewood, NJ).[19] For each study, the sample size, mean value, and standard deviations (SD) for both cases and controls were entered into the software. In cases where standard error (SE) was reported in lieu of SD, SD was calculated using the formula: $SD = SE \times (N)^{1/2}$.[20] In studies where findings were reported as mean and a range, SD was calculated for both case and control groups by dividing the range by four according to the procedure described by Hozo et al.[21] Because there is no way to compare different comet parameters to each other, all results were computed for standardized mean difference (SMD). Pooled data were first analyzed according to fixed effect model, however due to heterogeneity, analysis was subsequently performed using a random effect model (results of fixed effect analysis are accessible upon request). Funnel plot was used to demonstrate publication bias. Funnel plot represents SMD in the selected model against standard error of results.[22] Studies with wider confidence intervals and smaller sample sizes fill the bottom of funnel.[23] To investigate the source of heterogeneity, data were extracted and analyzed separately based on smoking status, comet parameter, and gender.

Results

Titles and abstracts of 813 retrieved studies were reviewed and 19 articles were selected for full-text assessment (Fig. 1). One of these 19 articles[24] was based on alkaline elution method, which is different from comet assay, and was subsequently not considered. The population of two studies was drug production workers and both were excluded.[25, 26] Another study[27] did not report any new data, and only reanalyzed previous published studies and was excluded. Table 2 summarizes the general characteristics of 15 studies included in the systematic review. All 15 studies included cases from hospitals' nurses, pharmacists, and medical doctors who were potentially occupationally exposed to ANDs. Three studies combined results for nurses, medical doctors, and pharmacists.[10, 28, 29] Controls were selected from different job classes including hospital personnel,[14, 28–34] office workers, students,[35, 36] and the general population.[10, 38, 39] All studies matched cases and controls by age and gender, and some studies matched on additional parameters including coffee consumption, lifestyle, medical history, alcohol consumption, and radiation exposure history.[38] In one study, the smoking rate in controls was higher than cases, and a significant increase in tail length but not in tail moment was observed in cases with the history of working with ANDs.[38]

Exposure assessment

Nine of studies collected exposure data via participant questionnaires and relied on employment in an oncology unit as a surrogate for AND exposure. Table 3 shows exposure information including participants' task, workload, urine, or air sampling and work experience. Two studies performed air sampling as an exposure assessment method.[28] Four studies also measured surface contamination via wipe or pad sampling.[28, 33, 34, 39] Duration of exposure to ANDs varied by study. In the

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│ duplicates were removed              │
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                 │                  │ 1#Used data from 3 other │
                 │                  │ studies [3]              │
                 │                  │ 1# used alkaline elution │
                 │                  │ method [4]               │
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│ 15 of studies were       │──────▶│ 1# study excluded because│
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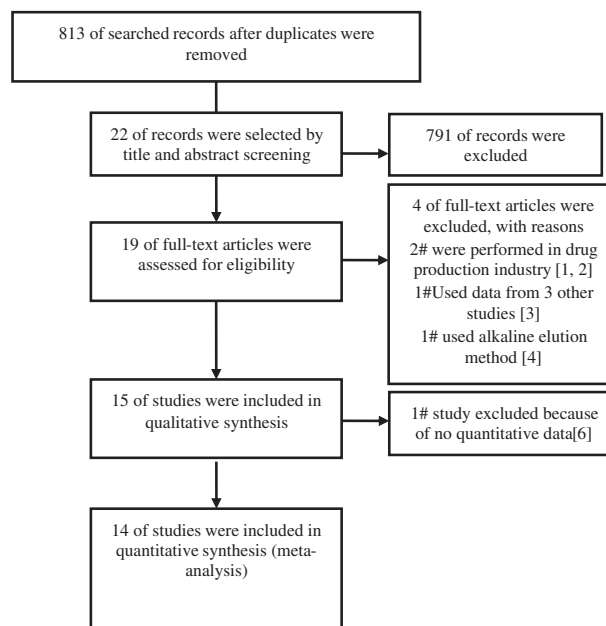
Figure 1 The flow diagram of search strategy and study selection process

study of Undeger et al.,[14] cases were employed ranging from 6 months to 13 years, whereas in the study by Rekhadevi et al.[38] cases were employed between 6 and 23 years. Buschine et al.[30] used duration of employment as the surrogate of exposure duration and calculated the exposure of subjects as a function of the number of preparations plus the number of administration multiple by maximum number of daily drug preparation. However, they found no significant correlation between calculated frequency and observed damage. In the selected studies, subjects were heterogenic in accordance with their job. For example, cases in Villarini et al.[39] performed transportation, preparation, administration, and handling body fluids, whereas in the Laffon et al.[32] and Undeger et al.[14] cases only performed preparation and administration of ANDs.

Assessment of DNA damage

All studies selected for systematic review included examinations of PBLs. However, two also incorporated the results of the comet assay of buccal cells.[27, 33] Four studies sampled blood sampling in the morning, [10, 33, 38] three sampled blood at the end of shift[14, 30, 39], one at mid-day[29], and the rest of the studies did not mention the time of blood sampling. There was no consistency in reporting comet assay parameters across studies. Most of the studies reported tail length, however different units for reporting were common.[10, 32, 34, 36, 38] Mader et al.[33] reported the tail factor while five studies reported percent of DNA in tail.[28, 30, 31, 36, 39] One study reported DNA damage index[29] and another study reported total comet score.[35] Comet parameter values varied widely across studies.

We also evaluated the methodological variables (Table 4). The composition of lysis buffer was the same in the

eight studies that reported it. Among selected studies, lysis time varied from 45 min to overnight. However, four studies did not mention their lysis time. In 12 studies, the preferred lysis time was one hour, although 3 studies used 45 min[34], 90 min[33], and overnight.[30] Unwinding time in six studies was 20 min[14, 30, 31, 33, 36, 39], was 40 min in two[34], and was 30 min in one study.[29] Remaining studies did not report unwinding time. Electrophoresis time varied between 20 and 40 min, however most studies, adjusted this time to 20 min. Electrophoresis in Yoshida et al.[34] lasted 40 and 60 min in Sasaki et al.[38] Eight studies set their electrophoresis current at 300 mA and current was not reported in other papers. Different methodologies were used for visual scoring of DNA damage in the comet assay tests. Three studies used subjective visual scoring system for their evaluations (Table 2). However, there was also inconsistency in the studies according to their procedure for conducting microscopic evaluations. Microscopic evaluations were performed in the range of 200–1000 magnifications (Table 3).

Findings from meta-analysis

Among 15 selected studies for systematic review, 14 studies with 620 health care providers and 558 controls were included in the meta-analysis. One study did not have quantitative results.[33] Genetic damage index (GDI)[41] was used to prepare results for inclusion.[14] into the meta-analysis. Accordingly, GDI was calculated by merging results of DNA damage in different levels as follows:

$$GDI = (\text{Type I} + 2 \times \text{Type II} + 3 \times \text{Type III})/(\text{Type 0} + \text{I} + \text{II} + \text{III})$$

The smallest sample size for health care providers and controls was 12.[29] Maximum sample sizes for health care providers and controls were 83[31] and 74[30], respectively.

Table 2 Overview of studies eligible to include in the systematic review

Author	Country	Sex/N Case	Sex/N Control	Job Case	Job Control	Smoking	Preventive Measures	Drug type	Exclusion Criteria	Work History	Blood Sampling Time	Reported parameter	Statistics
Undeger et al. (1999)	Turkey	M:29 F:1	M:29 F:1	Nurses	Nurses, Secretaries, Technicians	Y/N	PPE	AL,AM,MI,AB	?	0.5 – 13 YR	End shift	GDI	P
Maluf et al. (2000)	Brazil	M:1 F:11	M:1 :11	Nurse& pharmacist	Hospital workers	Y/N	?	?	?	3.42 ± 2.02	Mid-day	DDI	NP
Kopjar et al. (2001)	Croatia	F:50	F:20	Nurse, pharmacist	Students & Office workers	Y/N	G,M,SC	AL,AM,MI,AB	Radiation, Birth control pill	12.9 ± 9.4	?	TL,TD,TM	NP
Laffon et al. (2005)	Portugal			Nurse	Nurses from School		GW,M,SC,-Coat,H	AL,AM,AB	?	6.4 ± 6.22	?	TL	
Ursini et al. (2006)	Italy	F:23 M:7	F:25 M:25	Nurse, pharmacy technician	Hospital workers	Y/N	G,GW,HC,GOG	?	?	NI	Morning	TM	P
Yoshida et al. (2006)	Japan	F:19	F:18	Hematology Nurse	Nurses	Y/N	?	AL, AB,O	?	4.2(0.2 – 25.2)	?	TL	NP
Rekhadevi et al. (2007)	India	F:60	F:60	Oncology Nurse	General populations	N	No	AL,AB	Tenure>5, no smoker, no alcoholic, no radiation, no medicine	13.61(6 – 23)	Morning	TL	P
Cornetta et al. (2008)	Italy	F:67 M:16	F:53 M:20	Oncology Nurse	Hospital workers	Y/N	G,G-W,M,GOG,SC	AL,AM,MI,AB,O	?	12.2 ± 7.3	?	TD	NP
Mader et al. (2008)	Austria	F:15	F:15	Nurses oncology care unit	Nurses	Y/N	NI	?	History of chemotherapy or radiotherapy	?	?	TF	?
Sasaki et al. (2008)	Japan	F:57	F:46	Nurse	Clerks from hospital	Y/N	?	?	Radiation exposed, tenure< 0.5 year	?	Morning	TM,TL	P
Izdes et al. (2009)	Japan	M,f:19	M,f:19	Nurse	Office Workers	Y/N	G,M,SC	AL,AM,MI,AB	?	?	?	TCS	NP
Kopjar et al. (2009)	Croatia	F:50	F:50	Nurse& Doctor	General populations	Y/N	G, M, SC	?	Alcohol consumption, X-ray exposure, medical use, non-ionizing radiation exposure	?	Morning	TI, LTN	NP
Connor et al. (2010)	USA	M,f:66	M,f: 52	Nurse& Pharmacist	Nurses & Pharmacists	N	SC II(NIOSH Prot.), CST-D(SOME)	AL,MI,AM, O	History of chemotherapy, radiotherapy, smoker, genotoxic drug therapy, hormone therapy	?	?	TD,TM	?

(Continued)

Table 2   (Continued)

Author	Country	Sex/N		Job		Smoking	Preventive Measures	Drug type	Exclusion Criteria	Work History	Blood Sampling Time	Reported parameter	Statistics
		Case	Control	Case	Control								
Villarini et al. (2011)	Italy	M:7 F:45	M:12 F:40	Nurse, pharmacy technician, attendant	Public Structures	Y/N	?	?	Radiation exposure	?	End Shift	TD,TL	NP
Buschini et al. (2013)	Italy	F:63	F:74	Oncology Nurse	Nurses	N	?	AL,AM,MI,AB	Radiation exposure radiotherapy, smoking	110 ± 86	End shift	TI	NP

Sex: M: male, F: female.
Smoking: Y: yes, N: non smoker.
Protective measure: PPE: personal protective equipment, G: gown, GOG: goggle, SC: safety cabinet, GW: glove.
Drug type: AL: alkalinating agents, AM: antimetabolite, MI: mitotic inhibitor, AB: antibiotics, O: other miscellaneous drugs.
Reported parameter: TL: tail length, TM: tail momentum, TI: tail intensity, TF: tail factor, TCS: total comet score, DDI: DNA damage index, GDI: Genetic damage index.
Statistics: p: parametric statistics, NP: non- parametric statistics.

Among the studies selected for meta-analysis, three reported mean and standard error of mean (SE).[10, 30, 32] In one study, results were presented as mean and range.[34] Only one study reported a P-value and sample size[34], therefore, the effect size was calculated using P-value and sample size. The rest of studies reported their results as mean and SD.

Under the random effect assumption, the SMD of all studies was 1.93 (95% CI: 1.15–2.71, $p < 0.0001$) (Fig. 2). Table 5 provides calculated SMDs of all studies and the corresponding weight of each study under the random effect model. Studies by Cornetta et al.[31] and Buschini et al.[30] showed the highest weight (7.52 and 7.51%, respectively). These studies had larger sample sizes compared to the other studies. However, their findings were not consistent with each other. Cornetta et al.[31] reported a significant effect of potential exposure on the increase of DNA damage, while Buschini et al.[30] reported a non-significant effect. To explore the source of heterogeneity, subgroup analysis was performed based on smoking status and gender of participants. Even under subgroup analysis based on smoking status, large heterogeneity was observed ($I^2 = 96.65$, $p < 0.001$) (Fig. 3) and there was no significant association between smoking status and increased DNA damage (Q test: $Q = 0.007$, df = 1, $p = 0.931$). However, the observed mean effect size for studies with smokers was higher than the studies with non-smokers ($m = 2.45$, 95% CI = 1.19–3.71 vs. $m = 2.42$, 95% CI = 1.44–3.41). Subgroup analysis of gender showed no significant association between gender and increased DNA damage (Q test: $Q = 0.353$, df = 1, $p = 0.553$) (Fig. 4). Meta-analysis was performed on 11 studies that provided explicit data for nurses (Fig. 5). Under random effect assumption, the estimated SMD of these studies was 1.756 (95% CI: 0.992–2.52, $p < 0.001$). To investigate the role of comet assay reporting parameters as a possible methodological variable on observed heterogeneity, selected studies were evaluated separately based on reported comet parameter (Table 6). Separating findings based on comet parameters reduced the observed heterogeneity.

Publication bias and sensitivity analysis

Under the random effects model, sensitivity analysis ($n = 14$) showed that removing the Buschini et al.[30] study increased the effect size from 1.93 to 2.11. On the other hand, removing the Kopjar and Garaj-Vrhovac[36] and Laffon et al.[32] studies, led to a significant decrease of overall effect size to 1.50 and 1.56, respectively. Sensitivity analysis on studies with explicit data for nurses ($n = 11$) under the random effects model showed that removing Buschini et al.[30] study resulted in an increase of the effect size from 1.756 to 1.965. On the other hand, the removal of the Laffon et al.[32] and Rekhadevi et al.[38] studies significantly decreased the overall effect size to 1.296 and 1.426 respectively. An asymmetry was seen in Begg's funnel plot

Table 3   Evaluation of studies according to their methodological variables

Study name	Sampling Time	Sampling Type	PBL* treatment	Dilution	LMPA%#	Lysis Time	Lysis Buffer	Alkaline unwinding pH	Alkaline unwinding Time	Alkaline unwinding Solution	Electrophoresis pH	Electrophoresis Time (Min)	Electrophoresis Current (ma)	Electrophoresis Field	Neutralization	Stain	Microscopic analysis Magnification	Microscopic analysis N	Special point	Protocol
Undeger et al. (1999)	End of week	–	Ficol	2×10^5	0.5	60 min	2.5 M NaCl, 100 mM Na EDTA, 10 mM Tris, 1% sodium sarcosinate, 1% Triton-X 100 and 10% DMSO, ph10	13	20 min	1 mM Na EDTA and 300 mM NaOH, pH 13	13	20	24 v, 300ma	–	0.4 M Tris, pH 7.5 (3 time, 5 min)	65 ll ethidium bromide (20 lg/mL)	1000	200 (100 for each)	Dim light	Singh
Maluf et al. (2000)	Tuesday lunch time	–	–	–	0.5	60 min	2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Na Sarcosinate to which 1% Triton X-100 and 10% DMSO	13	30 min	300 mM NaOH, 1 mM EDTA	–	20	25v	0.9v/cm	0.4 M Tris, pH 7.5 (3tim, 5 min)	70 ll ethidium bromide (20 lg/mL)	200	50/subj	under red light	
Kopjar et al. (2001)		Within 24 h	–	–	0.5	60 min	2.5 M NaCl, 100 mM disodium EDTA, 10 mM Tris–HCl, 1% sodium sarcosinate pH 10] with 1% Triton X-100 (Sigma) and 10% dimethyl sulfoxide (Kemika) added fresh	13	20 min	300 mM NaOH, 1 mM disodium EDTA,	–	20	25 v300 ma	–	0.4 M Tris–HCl, pH 7.5 (3 times)	20 µg/ml ebr	400	50	dim light	Singh
Laffon et al. (2005)	–	Freeze-till 2 weeks via>80%	–	10^7cells/ml	??	–	–	–	–	–	–	–	–	–	–		400	50 (2 per subject)	–	Singh
Ursini et al. (2006)	–	–	Ficoll-	–	0.7%	1 h	2.5 M NaCl, 100 mM Na2EDTA, 10 mM Tris with 1% Triton X-100, and 10% DMSO added fresh	13	20 min	1 mM Na2EDTA and 300 mM NaOH	–	20	300, 20 v	–	0.4 M Tris HCl(5 min)	50 ll ethidium bromide (10 lg/mL)	200	50	under dim light	Singh
Yoshida et al. (2006)	Morning	Immediately to lab	Polymor-phprep	2 × 10^5	??	45 min	10% dimethylsulfoxide	–	40 min	300 mM NaOH, 1 mM EDTA	–	40	35 v	1v/cm	water	SYBR Green	200	100		Comet assay kit [2]
Rekhadevi et al. (2007)	–	Within 2 h	–	–	??	–	–	–	–	–	–	–	–	–	–		400	Each 50(duplicate / subj)	–	Singh
Cornetta et al. (2008)	Beginning of the work shift, of the third day of the week	–	Ficoll	20000000	0.7%	1 h	2.5 M NaCl, 100 mM Na2EDTA, 10 mM Tris with 1% Triton X-100, and 10% DMSO added fresh	13	20 min	1 mM Na2EDTA and 300 mM NaOH,	13	20	300	–	0.4 M Tris HCl (3 times)	50 ll ethidium bromide (10 lg/mL)	200	1000 examin, 50 analyzed	under dim light	Singh

(Continued)

Table 3 (Continued)

Study name	Sampling Time	Sampling Type	PBL* treatment Dilution	LMPA%#	Lysis Time	Lysis Buffer	Alkaline pH	Alkaline Time	Alkaline Solution	Electro pH	Electro Time (Min)	Electro Current (ma)	Electro Field	Neutralization	Stain	Magnification	Microscopic N	Special point	Protocol
Mader et al. (2008)	–	–	–	0.6	90	2Æ5 MNaCl, 100 mMNa2EDTA, 10 mM Tris, 1% sodium sarcosinate, 1% Triton X-100, 10% DMSO, pH 10	>13	40 min	1 mM Na2EDTA, 300 mM NaOH	–	20	25v,300ma	–	–	–	400	1000	under dimmed	–
Sasaki et al. (2008)	Morning of the day or day-off shift	24 h	–	??	–	–	–	–	–	–	60	300	–	–	–	400	100	–	Comet assay kit [2]
Izdes et al. (2009)	–	–	–	??	–	–	–	–	–	–	–	–	–	–	–	400	100(duplicate slide)	–	Singh
Kopjar et al. (2009)	Morning	Within 2 h	–	0.5	–	–	–	–	–	–	–	–	–	–	–	250	50–100(2 replicate/sub)	–	Singh
Connor et al. (2010)	–	–	–	??	–	–	–	–	–	–	–	–	–	–	–	–	100	–	Toraason [3]
Villarini et al. (2011)	–	Simultaneously). Case control. 4 h	–	0.7	1 h	2.5 MNaCl, 100mMNa2EDTA, 10mMTris–HCl, NaOH to pH 10; 1% Triton X-100 and 10% DMSO	>13	20 min	10 mM Na4EDTA, 300 mM NaOH	>13	20	300	1v/cm	0.4 MTris HCl pH 7.5	50 Il ethidium bromide (20 lg/mL	500	150(3 REPETITION, 50)	end of the work-shift after at least four consecutive days	–
Buschini et al. (2013)	–	–	Fresh leukocyte	??	Overnight	2.5 M NaCl, 0.1 M EDTA, 10 mM Tris, 10% DMSO, 1% (v/v) Triton X-100	10	20 min	0.3 M NaOH, 1 mM EDTA	13	20	300 mA (	0.78 V/cm	0.4 MTris pH 7.5	0.75 mLof 10 mg/mL EB	–	100/slide (2 slide/participant)	–	Singh[1]

*PBL.: Peripheral blood lymphocytes.

#low melting point agarose concentration.

[1]according to procedure described by Singh et al. (1988).

[2]By the Trevigen Inc. kit for comet assay: 'Comet Assay: reagent kit for single-cell gel electrophoresis assay'.

[3]Toraason et al. (2006). M, Lynch DW, DeBord DG, et al. DNA damage in leukocytes of workers occupationally exposed to 1-bromopropane. Mutat Res. 2006; 603:1–14.

Table 4   Exposure-related characteristics of cases in the selected studies

Author	Duty	Drugs	Protection	Exposure parameters	Remarks
Buschini et al. (2013)	Preparation, handling	50 different drugs, from 1,2A,2B,3, and not classified IARC group	—	Questionnaire, Duration of employment as a surrogate for duration of exposure. Calculated frequency score	No relation between calculated exposure frequency and DNA damage
Villarini et al. (2011)	Transportation, Preparation, administration, handling body fluids	16 different drug from 1,2A,2B,3,and not classified IARC group	Glove, mask	Wipe sample (surface contaminant) Pad sample (dermal exposure) Urine CP (exposure biomarker)	Higher but not significant DNA damage in those without personal protection. Urinary CP in 17.5% of nurses. 29.3% of wipe samples were positive.
Connor et al. (2010)	Preparation, Handling, administration	Different classes of ANDs include: Paclitaxel, 5-fluorouracil, cyclophosphamide, cytarabine, ifosfamide.	NIOSH safety recommendation, class II BSC, CSTD	Surface Wipe Sampling, Area and Personal Air Sampling, Urine Sample (cyclophosphamide and paclitaxel) work load = 106.72/week	No significant associations were observed considering different exposure variables. Positive contamination in 60% of wipe samples. Nearly no air contamination.
Izdes et al. (2009)	Preparation, Handling,	Mostly cyclophosphamide, cisplatin, etoposide, 5 Fluorouracil, vinblastine, Bleomycin, Doxorubicin	Gloves and masks for preparation, vertical flow safety cabinet	No exposure assessment. Cases had at last two year exposure with ANDs.	No significant difference observed between cases and controls.
Kopjar et al. (2009)	Preparation, administration, handling of body fluids	Different drugs from 1, 2A, 2B, 3 IARC group. Mostly 2A and 3 groups.	Varied across cases (glove, mask, and hood).	1 to 6 h a day work with ANDs. Exposure history ranged from 1 to 30 years(mean = 12.9 years)	The use of appropriate protective equipment significantly reduce the level of DNA damage.
Sasaki et al. (2008)	Preparation, Handling	No relevant data	No relevant data	Self-rated questionnaire	Cases had significantly higher tail length but no tail moment.
Cornetta et al. (2008)	Handling	More than twelve drugs	Gloves, overalls, goggles, masks, vertical air-flow cabinet	Weekly working time = 36 h/week, 12.2 ± 7.3 years of work history	—
Rekhadevi et al. (2007)	Preparation, Administration, handling body fluids	Mostly cisplatin, carboplatin, adriamycin, bleomycin and endoxane	No protective measures	Questionnaire. 8 h/day for 6 days a week (ANDs handling = 4.06 ± 0.73 h/d). Work history >5 years. Urinary cyclophosphamide	Positive but not significant association between duration handling anti-neoplastic drugs per day and urinary CP concentrations.
Yoshida et al. (2006)	Preparation, administration	19 drugs	Only latex glove	Questionnaire Umu assay of wipe samples	Level of DNA damage increased with increase of contamination of surface
Ursini et al. (2006)	Preparation, administration	35 drugs	Personal protective Equipment, including gloves, caps, overalls, and goggles.	Urine and wipe sample Workload = 300 preparation/week,	Relationship between the amount of ANDs handled, Surface contamination, and DNA damage. Surface pollution = 18 μg/m²
Laffon et al. (2005)	Preparation, administration	6 drug (from 1,2A,2B,3 IARC group)	Laboratory coat, mask, gloves, use of laminar airflow hoods while Preparing the drugs.	Questionnaire	—
Kopjar et al. (2001)	Preparation, handling	More than 5 drug, from 1,,2B,3, and not classified IARC group	–	Self-report of occupational exposure to ANDs, use of safety precaution during handling, daily exposure time (1–6hr/day)	1–30(M = 12.9)
Maluf et al. (2000)	—	—	—	No relevant exposure information Work history = 3.42 ± 2.02	—
Undeger et al. (1999)	Preparation, administration	10 drugs from 1,2A,2B,3,and not classified IARC group	Use of protective equipment, ventilation hoods, existence of policies governing antineoplastic exposure	Questionnaire Exposure history = 0.5–13y(m = 3.73)	Nurses who had taken the necessary individual safety precautions have less DNA damage.
Mader et al. (2008)	—	—	—	Questionnaire, Air sampling (5- fluorouracil or anthracyclines)regular contact with cytostatics and patients, classification and frequency of handling cytostatic drugs	No traces of airborne 5- fluorouracil or anthracyclines were detected.

List of abbreviations: BSC: biosafety cabinet, IARC: International Agency for Research on Cancer,.
CSTD: closed-system drug transfer device, CP: Cyclo Phosphamide.

when analysis was carried out for health care providers data ($n = 14$). Using Egger's test, we found significant evidence of publication bias (P for bias = 0.0003). Trim and fill tests on studies showed that after adjustment for symmetry using five studies, the effect size was reduced to 0.464 (95% CI = −0.439 to 1.367) (Fig. 6). Studies by Laffon et al.[32] and Kopjar et al.[10] considerably modified the effect size. In studies with nurses, asymmetry was also

Study name		Statistics for each study					Std diff in means and 95% CI
	Std diff in means	Standard error	Variance	Lower limit	Upper limit	p-Value	
Undeger (1999)	1.046	0.275	0.076	0.507	1.586	0.000	
Maluf (2000)	1.579	0.468	0.219	0.662	2.495	0.001	
Kopjar (2001)	1.321	0.287	0.082	0.758	1.884	0.000	
Laffon (2005)	7.907	0.832	0.693	6.276	9.539	0.000	
Yoshida (2006)	1.391	0.367	0.134	0.672	2.109	0.000	
Ursini (2006)	-0.008	0.258	0.067	-0.514	0.498	0.974	
Rekhadevi (2007)	4.144	0.324	0.105	3.509	4.779	0.000	
Sasaki (2008)	0.147	0.198	0.039	-0.242	0.536	0.458	
Cornetta (2008)	0.574	0.164	0.027	0.253	0.895	0.000	
Izdes (2009)	3.193	0.489	0.239	2.234	4.152	0.000	
Kopjar (2009)	7.350	0.557	0.310	6.259	8.442	0.000	
Connor (2010)	-0.008	0.185	0.034	-0.372	0.355	0.965	
Villarini (2011)	0.664	0.201	0.041	0.269	1.059	0.001	
Buschini (2013)	-0.161	0.172	0.029	-0.497	0.176	0.350	
	1.931	0.399	0.159	1.149	2.713	0.000	

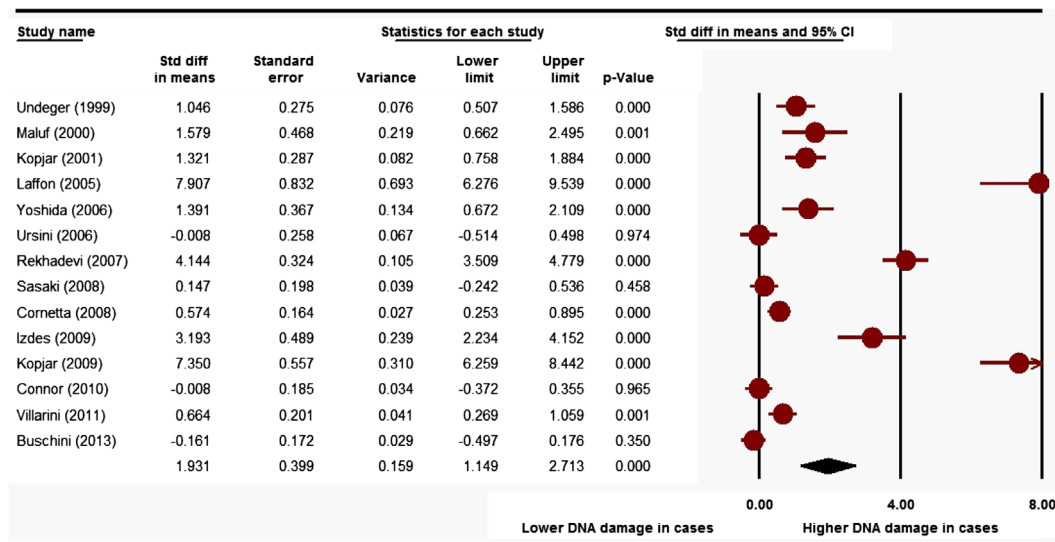Lower DNA damage in cases   Higher DNA damage in cases

Figure 2   **Forest plot of studies that assessed the difference between DNA damage (according to comet assay test) in all health care workers working with antineoplastic drugs**

Table 5   **Reported effect size of studies included in the meta-analysis (n = 14)**

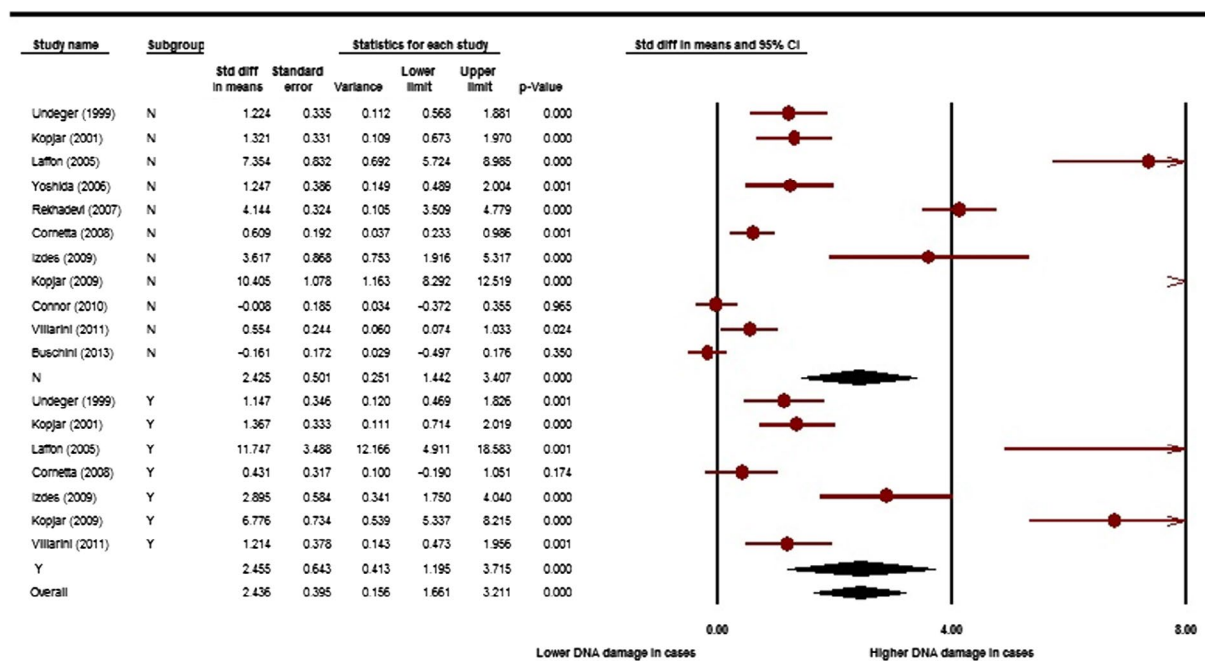| Study name	Exposed			Control			SMD (95%CI)			Random (%)
	N	Mean	SD	N	Mean	SD	SMD	Lower limit	Upper limit	
Undeger (1999)	30	0.49	0.38	30	0.19	0.15	1.05	0.51	1.59	7.35
Maluf (2000)	12	20.83	10.19	12	8.08	5.16	1.58	0.66	2.49	6.89
Kopjar (2001)	50	81.49	4.31	20	76.01	3.70	1.32	0.76	1.88	7.33
Laffon (2005)	29	46.46	0.48	22	42.68	0.47	7.91	6.28	9.54	5.72
Yoshida (2006)*	19	8.80	2.27	18	6.00	1.70	1.39	0.67	2.11	7.16
Ursini (2006)	30	16.03	8.71	30	16.10	8.10	−0.01	−0.51	0.50	7.38
Rekhadevi (2007)	60	13.66	2.37	60	6.21	0.92	4.14	3.51	4.78	7.25
Sasaki (2008)	57	2.05	1.79	46	1.79	1.73	0.15	−0.24	0.54	7.47
Cornetta (2008)	83	1.16	0.82	73	0.77	0.47	0.57	0.25	0.90	7.52
Izdes (2009)	19	19.89	4.84	19	6.84	3.16	3.19	2.23	4.15	6.83
Kopjar (2009)	50	17.46	0.65	50	14.00	0.14	7.35	6.26	8.44	6.63
Connor (2010)	66	53.06	7.32	52	53.12	7.50	−0.01	−0.37	0.36	7.49
Villarini (2011)	52	2.73	2.02	52	1.67	1.01	0.66	0.27	1.06	7.47
Buschini (2013)	63	0.95	0.24	74	0.99	0.26	−0.16	−0.50	0.18	7.51
Total	620			558						

Results reported as mean and range. SD was calculated for both case and control groups through dividing the reported range by 4;
*$P < 0.05$.

observed in Begg's funnel plot (Egger's test $p = 0.0013$). We also examined publication bias for subgroups, but even under this situation, asymmetry was observed in all conditions. To examine whether the publication date, as a proxy for date of exposure measurement, contributed to the between-study heterogeneity, we used meta-regression with the time of publication as a covariate. This analysis showed that date of publication was a possible source of heterogeneity ($B = −0.14$; $p < 0.0001$).
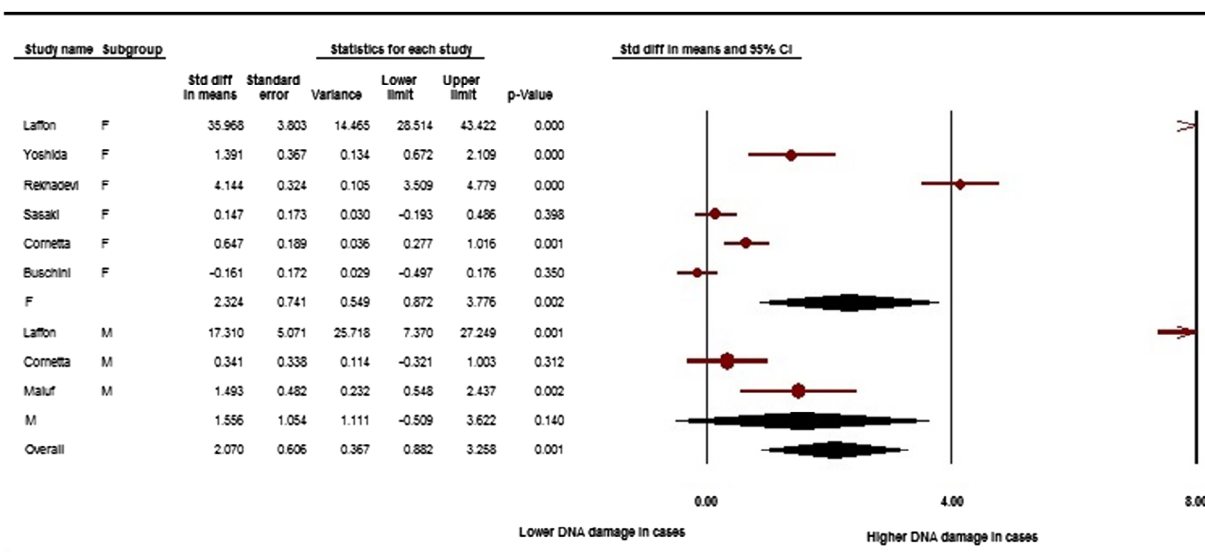
Discussion

In this review, 15 studies reporting comet assay results from health care providers occupationally exposed to ANDs were identified and evaluated. Fourteen studies were eligible for inclusion in the meta-analysis. Pooled data indicated that DNA damage in cases working with ANDs was significantly higher than control groups. The observed effect size in this study, 1.93, is threefold larger than the effect size in the meta-analysis on smokers and non-smokers using comet assay.[17] However, in a study on PBLs treated with therapeutic doses of ANDs (Bleomycin 0.39 µg/mL; Mitomycin C 0.31 µg/mL), the calculated SMD for combined treatment of cells with both drugs was 1.971, similar to our finding. The calculated SMD for observed tail length in control PBLs and the ones treated with Bleomycin was 1.378, however the observed SMD for Mitomycin C was 1.083.[42] In a study of patients exposed to a therapeutic dose of cyclophosphamide (50–200 mg/day), the observed SMD was 2.28.[43] Results lead to the conclusion that DNA damage in people occupationally exposed to ANDs is higher than smokers, however the effect is lower than therapeutic doses. There was considerable heterogeneity under the random effects analysis, with no reduction in heterogeneity after subgroup

Study name	Subgroup	Std diff in means	Standard error	Variance	Lower limit	Upper limit	p-Value
Undeger (1999)	N	1.224	0.335	0.112	0.568	1.881	0.000
Kopjar (2001)	N	1.321	0.331	0.109	0.673	1.970	0.000
Laffon (2005)	N	7.354	0.832	0.692	5.724	8.985	0.000
Yoshida (2006)	N	1.247	0.386	0.149	0.489	2.004	0.001
Rekhadevi (2007)	N	4.144	0.324	0.105	3.509	4.779	0.000
Cornetta (2008)	N	0.609	0.192	0.037	0.233	0.986	0.001
Izdes (2009)	N	3.617	0.868	0.753	1.916	5.317	0.000
Kopjar (2009)	N	10.405	1.078	1.163	8.292	12.519	0.000
Connor (2010)	N	-0.008	0.185	0.034	-0.372	0.355	0.965
Villarini (2011)	N	0.554	0.244	0.060	0.074	1.033	0.024
Buschini (2013)	N	-0.161	0.172	0.029	-0.497	0.176	0.350
N		2.425	0.501	0.251	1.442	3.407	0.000
Undeger (1999)	Y	1.147	0.346	0.120	0.469	1.826	0.001
Kopjar (2001)	Y	1.367	0.333	0.111	0.714	2.019	0.000
Laffon (2005)	Y	11.747	3.488	12.166	4.911	18.583	0.001
Cornetta (2008)	Y	0.431	0.317	0.100	-0.190	1.051	0.174
Izdes (2009)	Y	2.895	0.584	0.341	1.750	4.040	0.000
Kopjar (2009)	Y	6.776	0.734	0.539	5.337	8.215	0.000
Villarini (2011)	Y	1.214	0.378	0.143	0.473	1.956	0.001
Y		2.455	0.643	0.413	1.195	3.715	0.000
Overall		2.436	0.395	0.156	1.661	3.211	0.000

Y: smokers, N: non-smokers

Figure 3   Forest plot of studies that assessed the difference between DNA damage (according to comet assay test) in health care workers working with antineoplastic drugs based on subgroup analysis for smoking status of participants

Study name	Subgroup	Std diff in means	Standard error	Variance	Lower limit	Upper limit	p-Value
Laffon	F	35.968	3.803	14.465	28.514	43.422	0.000
Yoshida	F	1.391	0.367	0.134	0.672	2.109	0.000
Rekhadevi	F	4.144	0.324	0.105	3.509	4.779	0.000
Sasaki	F	0.147	0.173	0.030	-0.193	0.486	0.398
Cornetta	F	0.647	0.189	0.036	0.277	1.016	0.001
Buschini	F	-0.161	0.172	0.029	-0.497	0.176	0.350
F		2.324	0.741	0.549	0.872	3.776	0.002
Laffon	M	17.310	5.071	25.718	7.370	27.249	0.001
Cornetta	M	0.341	0.338	0.114	-0.321	1.003	0.312
Maluf	M	1.493	0.482	0.232	0.548	2.437	0.002
M		1.556	1.054	1.111	-0.509	3.622	0.140
Overall		2.070	0.606	0.367	0.882	3.258	0.001

F: Female; M: Male

Figure 4   Forest plot of studies that assessed the difference between DNA damage (according to comet assay test) and in health care workers working with antineoplastic drugs based on subgroup analysis for sex of participants

analysis based on gender and smoking status. The observed effect size and coefficient of variation of studies by Laffon et al.[32] and Kopjar et al.[10] are distinct from the rest of the studies (approximately 1%; the mean coefficient of variation for the other studies is 45%). It is difficult in the comet assay to have little variation and large effect between groups. However, sensitivity analysis showed that removing these studies from the meta-analysis did not change the significance and direction of total effect size.

Lack of quantitative inhalational exposure assessment (air sampling), dermal exposure (pad or wipe sampling), or biomarkers of exposure is a weakness of selected studies from an occupational health point of view. Variation in comet assay protocols and lack of reporting important parameters of the comet assay, such as electrophoresis field strength, was another weakness of the studies. In the following sections, we discussed the possible confounders and sources of heterogeneity in four groups: (a) work environment
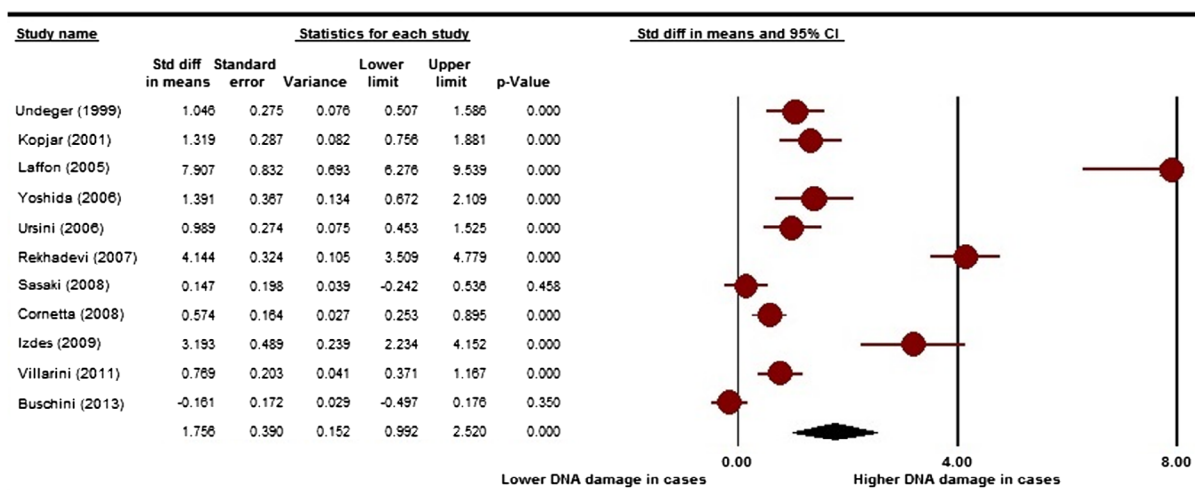
Study name		Statistics for each study						Std diff in means and 95% CI
	Std diff in means	Standard error	Variance	Lower limit	Upper limit	p-Value		
Undeger (1999)	1.046	0.275	0.076	0.507	1.586	0.000		
Kopjar (2001)	1.319	0.287	0.082	0.756	1.881	0.000		
Laffon (2005)	7.907	0.832	0.693	6.276	9.539	0.000		
Yoshida (2006)	1.391	0.367	0.134	0.672	2.109	0.000		
Ursini (2006)	0.989	0.274	0.075	0.453	1.525	0.000		
Rekhadevi (2007)	4.144	0.324	0.105	3.509	4.779	0.000		
Sasaki (2008)	0.147	0.198	0.039	-0.242	0.536	0.458		
Cornetta (2008)	0.574	0.164	0.027	0.253	0.895	0.000		
Izdes (2009)	3.193	0.489	0.239	2.234	4.152	0.000		
Villarini (2011)	0.769	0.203	0.041	0.371	1.167	0.000		
Buschini (2013)	-0.161	0.172	0.029	-0.497	0.176	0.350		
	1.756	0.390	0.152	0.992	2.520	0.000		

Figure 5   Forest plot of studies that assessed the difference between DNA damage (according to comet assay test) in nurses working with antineoplastic drugs

Table 6   Calculated effect size and observed heterogeneity after meta-analysis based on reported comet parameter

Reported Factor	N studies	N cases	N controls	SMD (95% CI)	I^2
Tail length	6	261	216	3.94 (1.91–5.97)	97.96
Tail momentum	3	153	128	0.066 (-0.17–0.30)	0.00
Tail density	5	314	271	0.448 (-0.01–0.90)	85.87
GDI	1	30	30	1.05 (0.51–1.59)	–
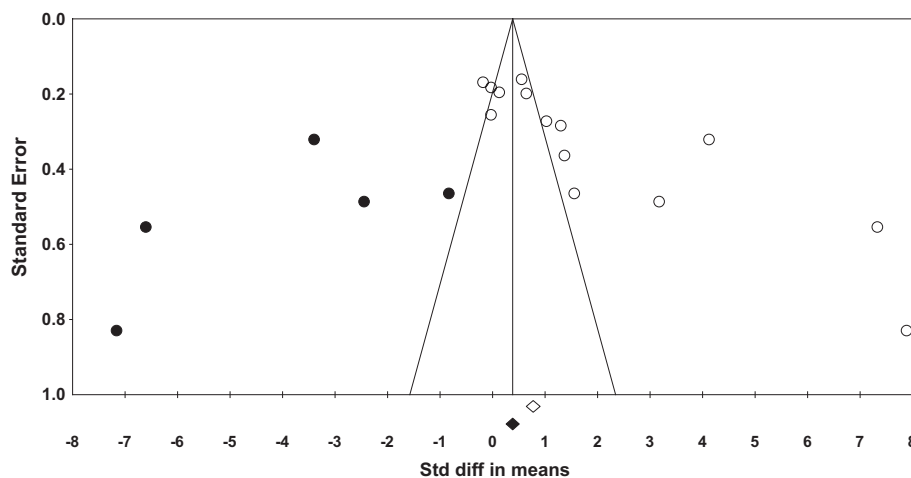
Figure 6   Begg's funnel plots (with pseudo 95% CIs) of the difference in means (DMs) vs. the standard error of the SMDs (standardized mean differences) for studies that assessed the effect of working with antineoplastic drugs in health care workers and DNA damage by comet assay; open circles represent retrieved studies; solid circles represent trim and filled studies; open diamond represent observed SMD for retrieved studies; dark diamond represents the SMD of studies after trim and fill test.

and exposure characteristics, (b) personal variability, (c) study design and methodological confounders, and (d) comet assay methodological variability.

Exposure allocation in most studies was based on indirect methods of exposure assessment (self-report questionnaires with determinants such as job title, use of PPE, and daily workload in work with ANDs). Different levels of contamination with ANDs are found in occupational environments and subsequent exposure to these compounds reported in oncology personnel.[44, 45] Therefore, working in oncology departments (or similar units) does not

necessarily indicate ANDs exposure. It can be concluded that exposure allocation based on job title could be used as a proxy of exposure occurrence to ANDs, but it is not an ideal measure for examination of exposure intensity and dose response studies. The actual magnitude of such exposures largely depends on tasks, workload, use of PPE, type of drug administration system, use of specific safety protocols, and workplace safety culture.[46] Type and level of protection was different among studies. A high level of personal protection (such as application of NIOSH recommendations or simultaneous use of various PPEs) was

associated with no significant difference in DNA damage between cases and controls.[14, 28, 33] The use of inappropriate or single PPE by itself should not be regarded as measure of prevention against ANDs. Most ANDs can permeate through latex and polyethylene gloves.[47] Villarini et al.[39] found no significant difference in DNA damage between hospital personnel wearing masks or gloves compared to those without.

Use of ANDs for chemotherapy requires preparation, transportation, administration, and handling of patient's body fluids. Nurses or other health care personnel, completely or in part, typically perform these activities. For example, Cornetta et al.[31] reported exposed subjects duties as handling of ANDs. In contrast, workers in Rekhadevi et al.[38] study, performed various duties such as preparation, administration, and handling of patient body fluids. Accordingly, the lower workload may explain the lower effect size in two of the studies.[31, 33] Villariani et al.[39] found that variation in magnitude of DNA damage in cases who handle ANDs could be due to job title and tasks. Use of different classes of ANDs is also an important factor. Milic and Kopjar[42] applied therapeutic doses of Bleomycin and Mitomycin C *in vitro* alone and in combination in human lymphocytes. They found significant differences in increased primary DNA damage between alone and combined administration of drugs. Exposure to ANDs can occur via several routes including inhalation, injection, dermal, and ingestion. However, there is no consistency between results and most studies showed no AND contamination in breathing air, but several showed varying levels of contamination of work surfaces and outside of ANDs vials and storage containers which could result in dermal exposure.[28, 48, 49] Surface contamination differs by location and is positively correlated with the number of handling events.[28] ANDs have low vapor pressure resulting in a higher chance of exposure through surface contamination compared to airborne fraction. Contamination of food and beverages from these drugs is also a source of ingestion. Widespread contamination of workrooms with ANDs, even when personnel are not involved in the preparation and handling of these drugs, they result in additional exposures.[5]

History of medication use, hormone therapy, smoking, age, employment in radiology wards, alcohol consumption, gene polymorphism, area of residency, and job history are potential individual-level confounders. DNA repair ability differs among individuals and some of the observed variations in studies maybe due to this inter-personal variation. Gene polymorphism as found in Laffon et al.[32] also has a significant effect on comet assay results. Most studies in this review adjusted for personal confounders such as gender, age and medication usage. Subgroup analysis based on smoking status did not reduce the heterogeneity. There are no conclusive results on the effect of smoking on DNA damage in comet assay test.[17] Smoking status did not indicate the same level of DNA damage in

group members and hence the level of DNA damage may be related to severity and frequency of smoking, tobacco type, and smoking style. Another consideration is the selection of controls. Control group members likely had varying levels of baseline DNA damage, a problem also relevant to the exposed groups, influenced by factors such as geographical area and proximity to industrial sources of outdoor pollutants.[50] The use of older controls may also mask differences in the results of the exposed group. This condition may be responsible for the similarity between results of cases and controls in Sasaki et al.[38] However, duration of exposure to ANDs and age did not significantly influence levels of DNA damage in the Kopjar et al. study.[10] The effect of age as an influential parameter in comet assay results is questionable. Two studies found that age had no effect on endogenous single-strand break levels and repair capacity,[51, 52] whereas other studies reported an increase in DNA damage in comet assay with an increase in age.[53–55] The study by Tsilimigaki et al. found baseline DNA damage to be relatively similar (with low coefficient of variance) in a younger population. However, in subjects older than 40, the observed mean and coefficient of variation of baseline DNA damage determined by the comet assay was significantly higher.[56] Buschine et al.[30] used duration of employment as a surrogate for exposure duration. Therefore, considering cases as a homogenous exposure group (HEG) is not feasible due to the large variation in employment durations (e.g. one to 30 years of employment in Kopjar et al.[36]). However, other studies found no significant association between duration of employment or age with ANDs and the observed DNA damage by comet assay.[14, 39] Buschine et al. calculated the exposure of subjects as a function of the number of preparations plus the number of administrations multiplied by the maximum number of daily drug preparations. They found no significant correlation between calculated frequency and observed damage.[30]

Comet assay methodological variation across studies is also an important issue in interpretation and generalization of results. The use of different comet assay protocols, different visual scoring procedures, various systems for reporting of DNA damage, and different types of statistical analysis for interpretation of data are methodological confounders and possible causes of inter-laboratory variability observed across studies. The ranges of observed comet parameters across studies were large. Even in the case of the same comet formation procedure, the use of different visual scoring systems prohibits direct comparison of reported values across studies. Other meta-analyses on comet assay studies (e.g. comet assay and smoking[17] or sperm DNA fragmentation[57] had the same problem. Inter-laboratory variation is the strongest contributor (56.7%) to observed variation in comet assay tests.[16] Concentration of low melting agarose, alkaline treatment time, and electrophoresis time are among the most important methodological variables that may be responsible for

the observed heterogeneity in the results.[58, 59] However, lysis time in Triton and high salt solution in the studies of this review was in the range of 45 min to 24 h. However, it is believed that variation in lysis time has no significant effect on the result of comet assay test.[60, 61] Green et al.[62] examined the effect of lysis at one and five hours and found no significant difference in the results. Conversely, unwinding time has a large effect on tail parameter formation in the comet assay test even for untreated cells.[60] In another study, tail parameter in cells exposed for 20 was the same as control cells with 8 hour unwinding time.[63] In this review, Yoshida et al.[34] used the longest unwinding time (40 min) and the calculated SMD in their study was considerably higher than 10 of 14 studies evaluated in our meta-analysis. Yoshid et al.[34] also used larger duration of electrophoresis compared to other studies, possibly explaining their findings. In general, the electrophoresis condition is the major technical variable affecting the sensitivity of the comet assay depending on the type of cell and the damage being investigated.[64] Yoshida et al.[34] and Sasaki et al.[38] employed the largest electrophoresis time and had some of the largest observed SMDs. Unfortunately, most studies did not reported electrophoresis parameters in detail. Of the 15 studies, only four reported voltage gradient. Increases in electrophoresis time and voltage could lead to increases in comet parameters.[61] The use of electrophoresis chambers with different configurations could results in significant difference in observed results across laboratories.[59, 65] The use of different parameters in reporting DNA damage in the comet assay may be another source of observed heterogeneity in selected studies. Different comet assay parameters such as comet length and comet tail should not be considered endpoints with the same sensitivity and different scales. Sasaki et al.[38], found a significant increase in tail length but not in tail moment in cases with a history of working with ANDs. The use of tail length for assessment of DNA damage is not recommended in studies examining the effect of prolonged past exposures since it is only useful for narrow exposure times and low levels of damage..[60] Although there are no clear guidelines for the selection of the best comet parameter, international expert groups recommend the use of percent DNA in the tail for practical and theoretical reasons (Collins 2004; Møller 2006). The percent DNA in the tail is also highly correlated with human visual scoring systems.[66, 67] We found that when studies were analyzed separately based on reported parameters, heterogeneity was reduced, and in the case of tail moment, heterogeneity reached zero. However, there is evidence of a strong correlation between comet assay results and comet parameters.[18] Time of sampling is important in macro and micro scale. Several studies found seasonal variation in baseline DNA damage.[68, 69] In the case of acute exposures, even the time window between the end of exposure and DNA damage measurement is important, as an increase in the time window increases the observed DNA damage.[13]

In conclusion, we found that health care personnel occupationally exposed to ANDs showed significant increases in DNA damage according to comet assay results compared to their non-occupationally exposed counterparts. There is a need for additional research to quantify the exposure intensity and effect of these drugs in developing countries. The perceived risk toward ANDs and behavior of nurses also may influence results. If nurses working with ANDs are knowledgeable about the effects of ANDs, they may be more likely to practice preventative measures, result in less DNA damage as detected by comet assay. However, the results of meta-regression suggest that in recent years the observed SMD in studies decreased, implying increased safety precautions or exposure prevention measures. The use of comet assay as a universal DNA damage biomarker and biomarker of effect is a good choice for risk assessment purposes in the case of occupational biomonitoring activities. Ease of use and consistency between comet assay and tests such as the micronuclei (MN) test or sister chromatid exchanges (SCEs) are strengths. Due to the use of different drugs in each setting, it is impossible to ascertain the observed effects of specific drugs. Since some ANDs are human carcinogens (*Group 1 carcinogens by IARC*) and also the cytogenetic biomarkers were positively correlated with risk of cancer, it is advisable to increase the current level of safety in health care personnel who handle these drugs.[70, 71] Use of uniform protocols across studies, such as those recommended by The International Programme on Chemical Safety (IPCS), will help reduce the heterogeneity of results.[72] The role of environmental variability in work practice, daily fluctuations of work load is likely responsible for a considerable portion of variation across studies. Precise description of exposure and control groups and thorough documentation and measurement of exposure characteristic and magnitude are also recommended.

Statement of author contributions

Zare and Mostaghaci designed the study and applied for Research Ethics Board approval. Zare, Mostaghaci, and Hajaghazadeh collected the data. Zare, Mostaghaci, Hajaghazadeh, Mehrparvar, and Mrs. Zare analyzed the data and prepared draft figures and tables. Zare, Mostaghaci, and Hajaghazadeh prepared the manuscript draft with important intellectual input from Mehrparvar and Mrs. Zare. All authors approved the final manuscript. All authors had complete access to the study data.

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Disclosure statement

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