ORIGINAL ARTICLE

The Potential Genotoxic Effects of Antineoplastic Drugs in Occupationally Exposed Nurses

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Abstract

Background: Antineoplastic drugs are commonly used globally. They directly interact with the tumor cell deoxyribonucleic acid and inhibit their growth. These actions are non-selective affecting both tumor cells and healthy cells producing toxic effects. The present study was designed to evaluate oxidative stress and genotoxic effects of antineoplastic drugs in occupationally exposed nurses under routine working conditions.

Methods: The studied groups included 28 nurses working in Hematology and Oncology unit, Benha University Hospitals and 20 non exposed subjects that work in academic jobs in Benha Faculty of Medicine, matched in age, gender & socioeconomic status. Malondoaldehyde (MDA) & reduced glutathione (GSH) were measured for all participants as markers of oxidative stress. Extent of DNA damage in leucocytes was also evaluated by comet assay as a biomarker of genotoxicity.

Results: There were a significant increase in MDA & reduction in GSH in occupationally exposed subjects than control. Also, extent of DNA damage in the lymphocytes of occupationally exposed participants has a high significance than the controls. The work duration and age had a significant impact on degree of DNA damage.

Conclusion: occupationally exposed nurses to antineoplastic agents are at greater risk of oxidative stress with consequent DNA damage and potential genotoxic damage.

Keywords: Antineoplastic, Oxidative stress, Comet assay, DNA damage,.

INTRODUCTION

Antineoplastic drugs are highly cytotoxic agents used in chemotherapy but while their usefulness is undeniable, they have been identified as potentially dangerous to occupational health care professionals (1).

The International Agency for Research on Cancer (IARC) has evaluated several antineoplastic drugs that inhibit tumor growth by disrupting cell division and killing growing cells, some of which were included as human carcinogens in Group1 (2). There are more than 10 million cancer cases around the globe. WHO estimates that the disease will reach more than 13 million by 2030. One of the cancer treatment routes is the use of cytotoxic compounds (3).

Exposure to antineoplastic drugs may occur during production, transportation and delivery and, of course, when used in health care facilities, either in hospitals or in the home, or even when disposed as waste materials. Different routes of exposure to pharmacists, pharmacy technicians and nurses who prepare or administer antineoplastic drugs as through inhalation, dermal absorption or less probably by ingestion (hand-mouth exposure route) (4 , 5).

Occupational exposures to chemicals are well recognized and regulated. However, families of workers may suffer second-hand exposure from contaminated clothes, placing them at greater risk than the general population. Pregnant women and children are especially sensitive subgroups in this scenario (6).

Anticancer drugs can generate reactive oxygen species (ROS) which leads to mutations and DNA damage. Overproduction of ROS can affect lipids, protein, and DNA of the cell and lead to destruction of cell structure and function (7).

Several cytogenetic methods such as the analysis of chromosomal aberrations, sister chromatid exchanges, and micronuclei in peripheral blood lymphocytes had been used frequently to assess the potential genotoxic effect of anticancer agents in occupationally exposed workers (8).

The alkaline single cell gel electrophoresis technique or COMET assay is a relatively new molecular assay which measures strand breaks incomplete excision repair sites, alkali labile sites and cross-linking, in individual cells (9).

The rationale of the present study was to assess possible genotoxicity of occupational exposure to antineoplastic drugs

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and its correlation with oxidative stress in occupationally exposed nurses.

METHODS

Study setting and subjects

This was a prospective comparative cross-sectional study conducted in Hematology and Oncology Unit, Internal Medicine Department, Faculty of Medicine, Benha University Hospitals, Egypt after obtaining the approval from the Research Ethics Committee in Benha Faculty of Medicine with number (RC1-1-2020). Data was collected over a period of 2 months (from 1st March 2020 till 30th April 2020). An informed written consent (in Arabic language) was obtained from the participants. It included their personal data and details about the study as title, objectives, methodology, expected benefits and risks, and confidentiality of data.

Data Collection

Convenient sampling was conducted on twenty eight nurses occupationally exposed at Hematology & Oncology Unit. **Inclusion criteria:** they were working at oncology unit for 2 to 7 years, non-alcoholic, non-smokers, had not been subjected to diagnostic X-ray examinations during the year before the beginning of this study. They were not taking oral contraceptive pills during the study period. While, twenty female employees as control group were selected from those working in academic institutions (Benha faculty of medicine). They were not exposed to antineoplastic drugs. They were matched with the study group for age & other inclusion criteria.

The checklist was performed to determine the personal data, occupational and medical history of the participants under this study including age, type and place of work, duration of work, personal protective equipment usage such as masks, gloves and lab-coat, history of chronic diseases and exposure to chemotherapy or radiotherapy and medicine usage.

Laboratory investigations

Peripheral blood samples from exposed and control participants were collected in the morning via venipuncture; the sample was divided into two parts: one kept in a heparinized tube and immediately sent to the laboratory where the comet assay was conducted, at room temperature and protected from light., and the other into a plain tube & left to coagulate at room temperature and centrifuged for 15 minutes at 986 g. The clear non-hemolyzed supernatant serum was rapidly collected, and stored at ($-80°C$) until assessment of oxidative stress parameters. **Malondialdehyde (MDA)** which is a lipid peroxidation marker in lymphocytes was used to determine the thiobarbituric acid reactive substances (TBARS) amount formed during lipid hydroperoxide decomposition by following absorption in a Beckman DU-7 spectrophotometer at 532 nm (10). **Reduced glutathione (GSH)** was detected by measuring in quartz cuvettes by a fluorimeter set at 350 nm excitation and 420 nm emission wavelengths according to the spectrofluorometric method (11).**The comet assay** was done as frosted microscopic slides were cleaned with ethanol and dipped into 1% normal melting point agarose (NMA). Twenty μL of blood cell suspensions is combined with 0.7% low melt agarose(LMA) 80 μL. Vortex and 30 μL of mixed agarose/cell suspension were mixed and put on the microscopic slides. The slides were eventually covered with the cover slip, left on an ice-cooled metal surface for 10 minutes and then deposited 100 μL of LMA on the previous layer of LMA. After 5 minutes, slides were dipped for an hour in an alkaline lysis solution, then removed and gently washed with deionized water. Slides were placed horizontally in an electrophoresis tank filled with an electrolysis buffer (previously set at 4 ° C) and kept in the refrigerator for 30 minutes at 4 ° C. Electrophoresis parameters were done for constant conditions of 0.8V/ and CM300 mA. Slides were set for 5 minutes in neutralization buffer After 30 minutes of electrophoresis, and then washed with deionized water. Slides were stained with Ethidium bromide solution for 5 minutes (12, 13). For Comet capture and analysis, at 400 magnification, a total of 50 randomly collected comets from each slide were analyzed using an epifluorescence microscope (Zeiss, Germany) linked to an image processing device via a black and white camera. (Comet Assay II; Perceptive Instruments Ltd, UK). A computerized image analysis system was used to acquire images, calculate the integrated intensity profile for each cell, estimate the components of the comet cells and assess the range of derived parameters. The following comet parameters were evaluated for the quantification of DNA damage: tail length, percentage of DNA in tail and tail moment. Tail length (i.e. DNA migration length) is directly related to the size of the DNA fragment and shown in micrometers. It was measured from cell core. Calculated as (tail length percentage of DNA in tail)/1.

Statistical analysis

Using SPSS version 20 software (SpssInc, Chicago, ILL Company) the data collected were tabulated and analyzed. Categorical data were presented as numbers and percentages, and they were analyzed using Chi square (l'2). Quantitative data for normality were tested using the Shapiro-Wilks method, assuming normality at P>0.05. Normally distributed variables were expressed as mean ± standard deviation and analyzed by student "t" for 2 independent groups, while non-parametric data were presented as median and inter-quartile range (IQR), and analyzed by Mann Whitney U test. Non parametric correlations were assessed by Spearman's correlation coefficient (rho). P ≤0.05 was considered significant.

RESULTS

The main characteristics of the study and control groups are presented in Table 1. All subjects were nonsmokers, with no history of cancer or radiation exposure. The nurses were working in well ventilated rooms with fans according to the information obtained from the questionnaire. There was no statistical significant difference between study and control groups regarding age and residence. All the subjects exposed had been in touch with antineoplastic drugs 5.0±2.0 years. During preparation and administration of antineoplastic drugs, 50% of studied nurses wore gloves and face masks. Regarding risky behavior (eat and drink during work), 50% of studied nurses were accustoming to eat and drink during work.

Table 1. Comparison between the study and control groups regarding demographic data.

Variable		Study group (n=28)		Control group (n=20)		P
Age (ys)	Mean±SD	39.8±9.9		38.7±9.8		0.71
	Range	25-53		25-52		
		No.	%	No.	%	P
Residence	Urban	12	42.9	10	50.0	0.62
	Rural	16	57.1	10	50.0	

Student "t" test was used

Table 2. Comparison between the study and control groups regarding Tail length (µm), Tail moment, MDA, Glutathione reductase.

Variable	Study group (n=28)			Control group (n=20)			P
	Mean	± SD	Range	Mean	± SD	Range	
Tail length (µm)	8.69	1.84	5.14-11.95	2.06	0.65	0.91-3.68	<0.001
Tail moment	1.62	0.49	0.81-2.2	0.38	0.20	0.1-0.66	<0.001*
MDA	12.56	0.65	11.57-13.57	10.08	0.43	9.56-10.85	<0.001*
Glutathione reductase	5.95	0.34	5-6.53	7.19	0.29	6.85-7.65	<0.001

**ZMWU= Z value of Mann Whitney U test was used

Compared with occupationally exposed subjects, the mean values of tail length and moment were significantly higher than the controls. Regarding the oxidative stress assessment, there was a highly significant increase in mean values of MDA. While, there was a highly significant decrease in mean values of Glutathione reductase in the study group compared with control group (Table 2). According to age of the control group, there was non-significant correlation between it and studied parameters (Table 3).

The study illustrated the influence of the duration of occupational exposure to antineoplastic drugs among the exposed nurses on the level of chromosome damage, as there was a significant positive correlation with DNA aberrations which include tail length and tail moment. Also, there was a significant positive correlation with MDA. Likewise, the duration of exposure in work places negatively correlated with glutathione reductase level (Figure 1).

Table (4) revealed that there was a statistical high significant increase of the level of chromosome damage among the occupationally exposed nurses with risky behavior. Also, there was high significant increase of MDA level. Moreover, there was statistical significant decrease regarding glutathione reductase level.

Since age and work duration is highly correlated, analysis of correlation indicated the influence of age on the level of chromosome damage among exposed nurses which revealed a statistical high significant increase in tail length and tail moment.

Moreover, there was a high significant increase of MDA level and statistical significant decrease regarding glutathione reductase level (Figure 2, 3).

Examination of peripheral blood lymphocytes by comet assay revealed normal intact nuclei in control group while there was degree of damage depended on duration of work of exposed nurses (Figure 4).

DISCUSSION

Antineoplastic dugs consist of a clustered group of chemicals popularly used for cancer treatment and other non-neoplastic diseases. These drugs have proven to be carcinogenic, mutagenic, and teratogenic (14, 15). The risks of side effects from antineoplastic drugs in treating patients with cancer are often outweighed by the benefits of the drugs and are often avoided, reduced or decreased by different treatment steps. Nevertheless, these medications are exposed to nurses and pharmacists in a wide-spectrum subtherapeutic concentration with long cumulative duration, of uncertain biological consequences (16).

Table 3. Relation between age of control group and Tail length (µm), Tail moment, MDA, Glutathione reductase.

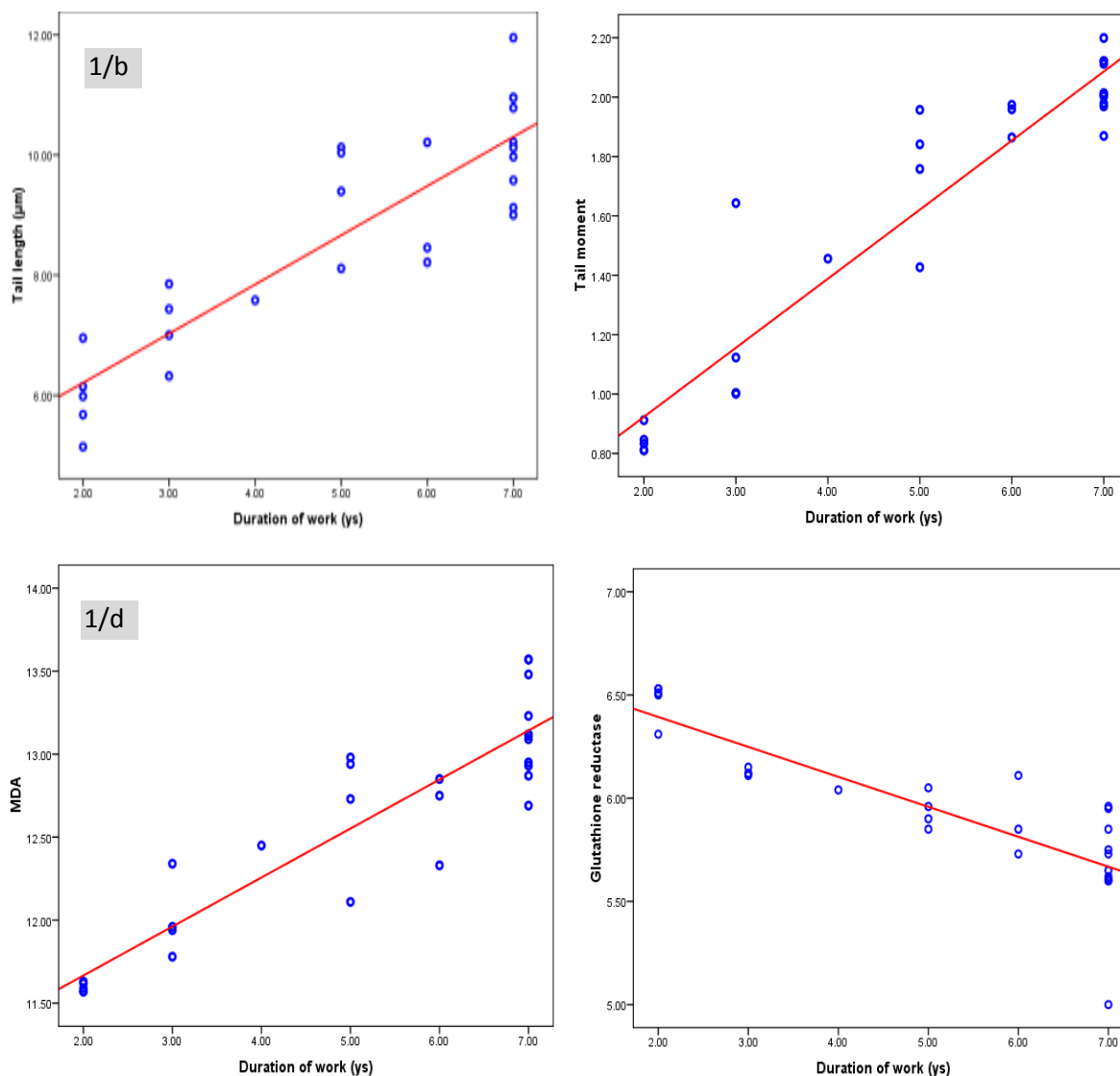| With	Age of control group (N=20)	
	rho	P
Tail length (µm)	0.062	0.79
Tail moment	0.096	0.68
MDA	0.244	0.30
Glutathione reductase	-0.081	0.91

rho: Spearman's correlation coefficient

Fig. 1. Scatter graph showing significant positive correlation between duration of work and tail length, tail moment, MDA and negative correlation with glutathione reductase in the study group nurses.
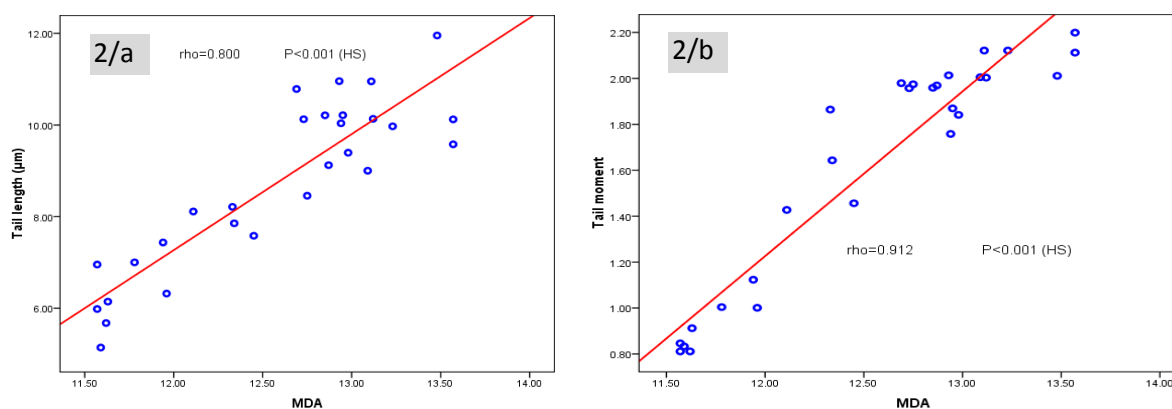

Fig. 2. Scatter graph showing correlation between MDA level and tail length and tail moment in the study group nurses.

Table 4. Relation between the studied markers according to risky behavior (eat and drink during work) of the study group nurses.

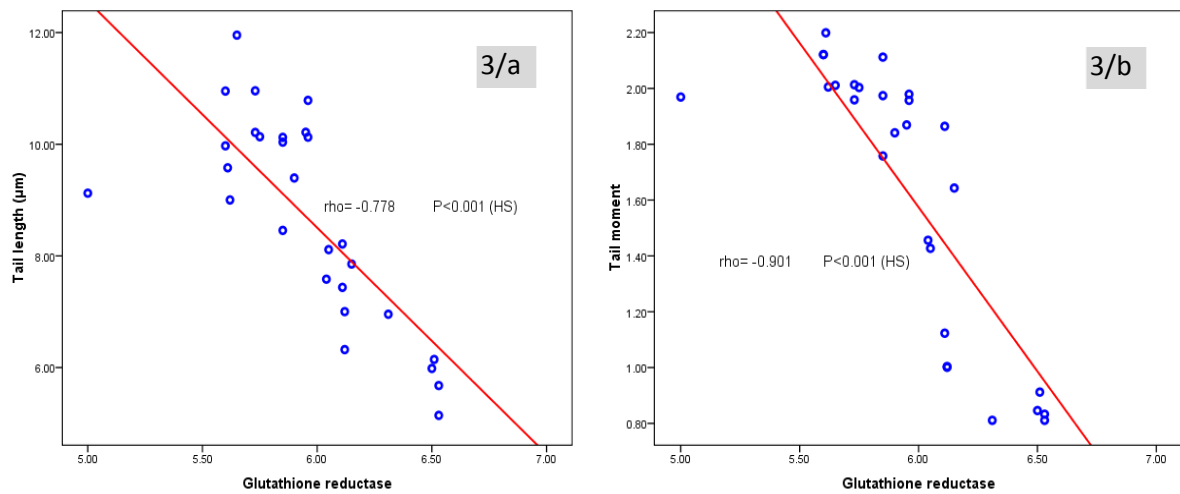| Variable	Risky behavior						P
	No (n=14)			Yes (n=14)			
	Mean	± SD	Range	Mean	± SD	Range	
Tail length (µm)	7.30	1.49	5.14-10.78	10.07	0.86	8.46-11.95	<0.001
Tail moment	1.26	0.45	0.81-1.98	1.99	0.11	1.76-2.2	<0.001*
MDA	12.03	0.44	11.57-12.87	13.09	0.27	12.73-13.57	<0.001*
Glutathione reductase	6.14	0.38	5-6.53	5.76	0.13	5.6-5.96	0.002

* ZMWU= Z value of Mann Whitney U test was used

Fig. 3. Scatter graph showing correlation between glutathione reductase level and tail length and tail moment in the study group nurses.
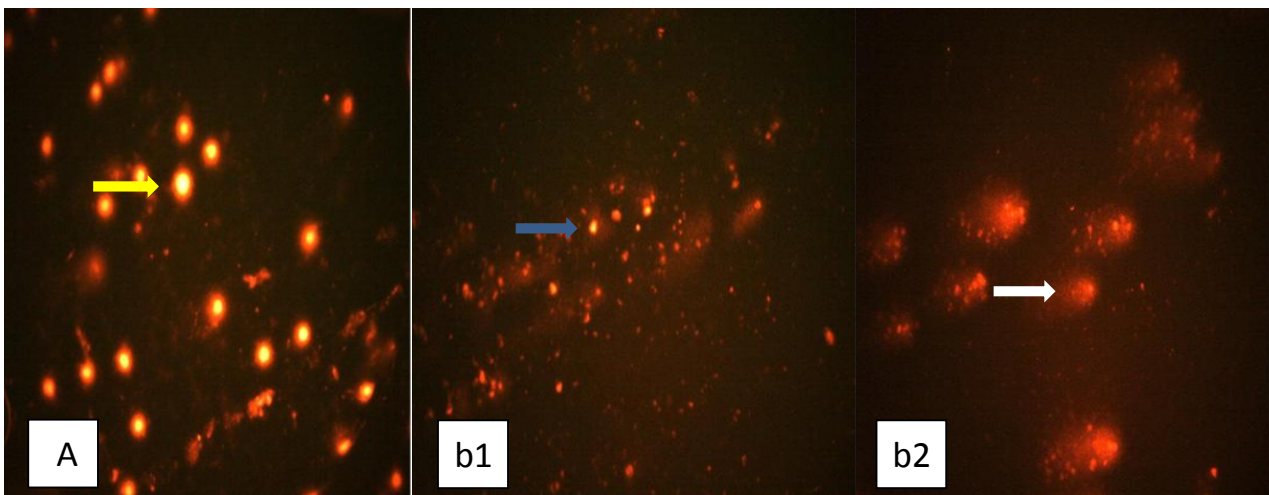

Fig. 4. (A) Photomicrograph of peripheral blood lymphocytes of the control group with intact nuclei (yellow arrow), (b1) photomicrograph of peripheral blood lymphocytes of occupationally exposed nurses [2 years work duration] show low degree of damage (blue arrow), (b2) photomicrograph of peripheral blood lymphocytes of occupationally exposed nurses [7 years work duration] show high degree of damage (white arrow) (stained with 20ug/ml ethidium bromide with 400X).

As regard oxidative stress parameters, the current study revealed a significant increase in MDA, the chief marker of lipid peroxidation. While, there was a significant reduction in glutathione reductase (GSH), as a marker of antioxidants, in occupationally exposed nurses as compared to non-exposed control group.

These results were in line with the study of *Eghbalk et al. (2018)* (17) who found significant formation of MDA in isolated lymphocytes from occupationally exposed nurses to antineoplastic drugs as compared lymphocytes isolated from healthy unexposed nurses. Also, they found a significant decrease in reduced glutathione (GSH) level followed by a large increase in the levels of oxidized glutathione in the isolated lymphocytes of nurses who are occupationally exposed to anticancer drugs compared to those of healthy non-exposed workers in similar circumstances.

Studies of neoplastic disease illustrated an increase in reactive oxygen species (ROS) and decrease in plasma levels of vitamins C and E as well as glutathione peroxidase in treatment with anti-neoplastic drugs (18). Also, the current study agreed with many studies that evaluated oxidative stress induced by anti-neoplastics both in vitro and in vivo (19, 20, 21).

Anti-neoplastic drugs can generate reactive oxygen species (ROS) via the xanthine-xanthine oxidase system, mitochondria, and NADPH oxidase in cells (22). Excess generated ROS will bind to membrane lipids, inducing lipid peroxidation. Also, increased ROS formation may directly oxidize the prosthetic protein group or reacts with the peptide chain, leading to functional changes in cellular organelles such as mitochondria and lysosomes (23).

The present study showed correlation between chromosome damage and oxidative stress among the occupationally exposed nurses with risky behavior as eat and drink during work and wear protective measures as gloves and face masks, these results were in accordance with *El-Ebiaryet al. (2013)* and *Aristizabal-Pachon & Castillo (2020)* (24, 8) who established in different practitioners that protective measures applied to the handling of antineoplastics can substantially reduce the genotoxic effect of these agents.

A rapid, inexpensive, simple, and sensitive technique is used to detect early DNA damage that occurs at the single cell level, this is called comet assay test. Using these test different forms of DNA damage can be found (25, 26). In the current study; participants were investigated for genotoxicity using comet assay. The mean comet tail length & moment were significantly higher in antineoplastic occupationally exposed nurses than the level of the control group.

These results agreed with the study of *Yoshida et al. (2006)* (27) who reported lymphocyte DNA damage (increased tail length) measured by comet assay in nurses occupationally exposed to chemotherapeutics. Several studies that had assessed the genotoxic potential of individuals handling anti-neoplastic agents using comet assay had found DNA damage to be significantly higher than the controls (28, 29, 30). Contrary to these findings, researchers examining DNA damage in health care staff who handle anti-neoplastic drugs found no statistically significant increase in DNA damage in nurses with regard to controls detected by comet assay (31).

The present study revealed a significant increase in MDA, comet tail length & moment, and reduction in GSH in correlation with increased age & duration of work and exposure among occupationally exposed nurses. In line with these results, other studies on genotoxic effect of exposure to anti-neoplastic drugs had concluded a similar increase in DNA damage with the years of exposure (29). Also, a study done by Hessel et al. (2001) and Kopjar et al. (2009) (32, 33) had demonstrated increase of micronuclei frequency in peripheral blood of exposed workers tended to rise with age Cytogenetically, aging is linked with a range of gross cellular changes, which include changed size and morphology, genomic instability, and expression and proliferation changes (34).

This genotoxic effect is mediated by initiation of the phenotype of genomic instability, which allows the initiated cell to transform into a cancer cell by promoting higher proliferative capacity. It is well recognized that cancer results from an accumulation of several genetic changes that can be controlled by chromosomal changes that can be cytogenetically discovered (35).

LIMITATIONS

The current study is limited by its small sample size and the use of a convenience sample. A larger, representative sample size would offer some generalizability. Because of the limited sample, these findings can only be attributed to the participants who were willing to participate in a study of this type.

CONCLUSION

The current study indicated that occupational exposure to antineoplastic drugs led to oxidative stress & genotoxic DNA damage in nurses working in hematology and oncology unit, internal medicine department, Benha University Hospital, in comparison to non-exposed participants.

RECOMMENDATIONS

1. Determination of biomarkers of oxidative stress and DNA damage should be used as early detectors of genotoxic effects of antineoplastic drugs in order to prevent health hazards in occupationally exposed nurses.
2. Further researches should be done to explain the exact molecular mechanism of chemotherapeutics that might be used as a cornerstone for determination of exposure limits and work environment standards.
3. Frequent change of position of work of subjects handling these chemotherapeutics.

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