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Original Article

Monitoring of Oxidative Stress in Nurses Occupationally Exposed to Antineoplastic Drugs

M. Mahboob, M. F. Rahman, P. V. Rekhadevi, N. Sailaja, A. Balasubramanyam, P. V. Prabhakar, Shailendra Pratap Singh, Utkarsh A. Reddy, G. Sankara Rao, Paramjit Grover

Toxicology Unit, Biology Division, Indian Institute of Chemical Technology, Tarnaka, Habsiguda, Hyderabad - 500 607, India

ABSTRACT

Antineoplastic drugs (ANDs) have been in clinical usage for more than five decades. The nonselective mechanism of action of ANDs between cancerous and noncancerous cells had well documented side effects such as acute symptoms, reproductive health issues, and potential cancer development in healthcare workers as a result of occupational exposure. The anticancer mechanism of ANDs is the generation of reactive oxygen species (ROS) which are responsible for various side effects in patients undergoing chemotherapy and the healthcare personnel occupationally exposed to them. ROS have potential to damage lipids, DNA, proteins, and so on leading to oxidative stress condition. The aim of this study was to evaluate the possible oxidative stress effect of antineoplastic drugs in nurses who routinely handle ANDs in an oncology hospital in south India. Malondialdehyde levels, reduced glutathione content, and glutathione *S*-transferase activity were analyzed in serum collected from 60 female nurses handling ANDs and compared with equal number of healthy volunteers matched by age and sex except AND exposure. The results showed statistically significant ($P < 0.05$) increase in malondialdehyde levels in the serum of exposed nurses. However, glutathione content and glutathione *S*-transferase activity was significantly decreased in these nurses. Our study suggests that the nurses occupationally exposed to ANDs were susceptible to the oxidative stress and emphasizes the need for a harmonized safe handling approach that assures minimal risk to the working nurses.

Key words: Antineoplastic drugs, antioxidant, nurses, oxidative stress

INTRODUCTION

Antineoplastic drugs are used worldwide during chemotherapy in curing the cancer. Presently, more than 50 different ANDs are in use, most of them have been classified as mutagens, carcinogens, and teratogens to humans.[1] The nurses may be occupationally exposed to ANDs while preparing and administering antineoplastic agents to patients, cleaning up chemotherapy spills, and handling patient's sweat, emesis, feces, urine, and contaminated linen of AND-treated patients.[2,3] Although nurses handling ANDs are well instructed about risks of exposure, detectable levels of these drugs are still reported in their urine which indicates the occupational exposure.[4,5] Many studies have reported that the workers who occupationally handle ANDs showed side effects such as nausea, vomiting, diarrhea, eye and throat irritation, menstrual irregularities, skin reactions, hair loss, headache, and dizziness[2,6]; cancer, miscarriage, and offspring malformation[7]; and genotoxicity.[5,7,8] Accumulating evidence indicates that exposure to ANDs generate free radicals which in turn leads to oxidative stress condition. Indeed, oxidative stress is being increasingly

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Address for correspondence: Dr. M. Mahboob, Toxicology Unit, Biology Division, Indian Institute of Chemical Technology, Tarnaka, Hyderabad - 500 607, India. E-mail: mahboobm1983@gmail.com

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recognized as a possible mechanism in the toxicity and carcinogenesis of most of the ANDs.[9] Till date, very limited studies on occupational risk for hospital nurses handling ANDs and oxidative stress evaluation[8] have been reported in the literature, and in India no research has been done. Hence, it was considered important to evaluate end points of oxidative stress such as lipid peroxidation (LPO), reduced glutathione (GSH), and glutathione *S*-transferase (GST) in occupational exposed nurses in order to monitor occupational hazards. In this article, we present occupational exposure and biomonitoring of oxidative stress in hospital nurses employed in one of a south Indian oncology hospital.

MATERIALS AND METHODS

Chemicals

Thiobarbituric acid (TBA), trichloroacetic acid (TCA), HCl, potassium phosphate mono- and dibasic, 5,5-dithiobis (3-nitrobenzoic acid), EDTA, 5-sulfosalicyclic acid, sodium hydroxide, sodium carbonate, potassium sodium tartarate, 1,chloro,2-4, dinitrobenzene, and bovine serum albumin were obtained from Sigma Chemical Company (St. Louis, Mo, USA). All the reagents used were analytical grade.

Subjects

Sixty female nurses working in an oncology hospital in Hyderabad, India, who had daily occupational exposure with ANDs while preparation and administration and body fluids of patients undergoing chemotherapy were selected for this study. All the study subjects were 22–54 years of age, weighing 40–80 kg, and height 135–186 cm. The study subjects handled various ANDs such as cisplatin, carboplatin, adriamycin, bleomycin, and endoxane, and the handling time varied from 4 to 8 h per day and total experience period ranges 6–23 years. Sixty unexposed controls matched by age and sex were selected among students, administration staff, or their families. All subjects gave their consent for participating before the beginning of this study. Data were collected with a questionnaire, which included information regarding age, gender, life habits (dietary, smoking, alcohol assumption, etc.), type of antineoplastic drugs handled and the number of mixtures prepared and administered, working hours per day, years of exposure, use of protective equipment (gloves, masks, goggles, protective clothes, and vertical laminar flow safety hoods), and so on. All the study subjects including controls were reported free from alcohol consumption and smoking. The subjects having minimum 5 years working experience in an oncology department were selected for this study. The study protocol was approved by Ethical Committee of our Institution.

Sampling

The blood was collected from six controls and six exposed nurses every week over a period of 3 months. The test samples were coded to avoid possible bias. The blood (2 ml) was collected from each individual on day 4 of the week (Thursday) between 12 and 12.30 pm. Immediately after the collection, the blood was transferred into sterilized tubes, allowed to clot, and the serum was separated by centrifugation at 3000 rpm for 5 min in the hospital. The collected serum was transferred to research laboratory on dry ice and stored at -80°C until use.

Lipid peroxidation

Malondialdehyde (MDA), a LPO end product in serum, was measured according to the method described by Wills[10] with some modifications; 200 μl of serum was mixed with 2 ml of TBA–TCA reagent (0.375% and 15%, respectively). The volume was made up to 3 ml with distilled water and heated on a water bath at 95°C for 20 min. The solution was then cooled under the tap. The reaction product (TBA–MDA complex) was extracted with 3 ml of *n*-butanol. The absorbance of the pink colored extract was measured at 532 nm (Spectramax Plus, Molecular Devices). The amount of MDA was calculated using a molar extinction coefficient of 1.56×10^5/M/cm and expressed as μmol MDA formed/ml serum.

Reduced glutathione

The GSH content was measured using by the method of Ellman.[11] An aliquot of 0.5 ml of each serum sample was incubated with 0.5 ml of sulphosalicylic acid (4% w/v) for 1 h on ice and centrifuged at 10,000 rpm for 10 min. The supernatant (0.4 ml) collected after centrifugation was mixed with 0.4 ml of DTNB (4 mg/ml in 5% sodium citrate) and 2.2 ml KPB (0.1 M, pH 7.4). The yellow color developed in the mixture was read at 412 nm. The amount of GSH present was expressed as μmol GSH/ml serum.

Glutathione *S*-transferase

The GST activity was determined according to the method of Habig *et al*.[12] The reaction mixture consisted of 2.75 ml KPB (0.1 M, pH 6.5), 0.1 ml GSH (75 mM), 0.1 ml 1-chloro 2,4 dinitro benzene (CDNB) (30 mM in 95% ethanol) and 0.05 ml serum in a total volume of 3 ml was taken in cuvette. The change in absorbance was recorded at 340 nm for 2 min. The enzyme activity was expressed as ρmol CDNB conjugate formed per min per mg protein using a molar extinction coefficient of 9.6×10^3/M/cm. The serum protein level was quantified by the method described by Lowry *et al*.[13]

Statistical analysis

The data were analyzed using one-way analysis of variance

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for the comparison of data between exposed and control groups. A *P*-value of 0.05 or less was taken as criteria for a statistically significant difference.

RESULTS

The demographic characteristics of the study subjects considered in this study are indicated in Table 1. According to the information obtained in the questionnaire answered by the nurses, the two study groups, controls, and exposed nurses were age- and sex matched, age ranged between 22–54 and 25–51, respectively. All the exposed nurses were involved in daily preparation and in the administration of the ANDs for an average period of 13.6 ± 4.8 years and daily average of 6.3 ± 1.1 h/day. The study subjects did not use proper and regular defensive measures, such as wearing protective clothes, goggles, gloves, masks, and worked without safety hood. The control and exposed groups subjects selected were nonsmokers and did not consume alcohol and medicines. Oxidative stress induced by antineoplastic drugs in 60 occupationally exposed nurses and 60 matched controls was studied by assaying LPO, GSH content, and GST activity in serum [Table 2]. The serum MDA level was significantly ($P < 0.05$) increased in occupational exposed nurses. However, the GSH content was significantly depleted in the serum of exposed group. Similarly, the GST activity was significantly inhibited in exposed nurses when compared with controls.

Table 1: Characterization of hospital nurses exposed to antineoplastic drugs and healthy volunteers

	Parameters	Exposed	Control
Professionals	Nurses (infusion)	60	60
Sex: female	Unmarried	24	24
	Married	36	36
Age (years)	Mean ± SD	38.21 ± 5.64	37.95 ± 5.64
	Range	22–54	25–51
Weight (kg)	Mean ± SD	56.55 ± 11.63	59.22 ± 10.47
	Range	40–80	39–79
Height (cm)	Mean ± SD	155.29 ± 10.20	160 ± 7.94
	Range	135–186	153–180
Daily exposure time (h)	Mean ± SD	6.30 ± 1.10	Nil
	Range	4–8	
Total exposure period (years)	Mean ± SD	13.61 ± 4.81	Nil
	Range	6–23	
Diet	Vegetarian	25	25
	Nonvegetarian	35	35

Data are presented as mean ± SD (n = 60).

Table 2: Malondialdehyde, glutathione, glutathione *S*-transferase levels in hospital nurses exposed to antineoplastic drugs

Parameter	Control	Exposed
MDA (μM/ml)	19.06 ± 0.73	25.07 ± 1.33* (31.53% ↑)
GSH (μmoles/ml)	4.74 ± 0.14	3.23 ± 0.15* (31.85% ↓)
GST (pM/min/mg protein)	6.18 ± 0.41	4.35 ± 0.25* (29.61% ↓)

Data are presented as mean ± SD. *$P < 0.05$ (n = 60)., ↓, decrease in the content/activity; ↑, increase in the content/activity.

DISCUSSION

For the past decade, concern has been growing regarding the safety of nurses who handle chemotherapy drugs. In general, nurses may be exposed to ANDs by inhalation through vapors when they create aerosols, generate as dust while crushing tablets, dermal exposure through touching contaminated surfaces during the preparation, administration, or disposal of drugs, and oral exposure through hand to mouth contact. Although very uncommon, accidental injection of antineoplastic drugs has been documented.[14] Many studies have shown oxidative stress condition, genotoxic risk such as DNA damage, micronuclei frequency in peripheral lymphocytes, exfoliated buccal epithelial cells, cytogenetic effects, and so on in occupational workers handling ANDs.[5,6,8,15-18]

ANDs were shown to induce reactive oxygen species (ROS) via the xanthine-xanthine oxidase system, mitochondria, and NADPH oxidase in cells.[19] Excess generated ROS interact with cellular macromolecules, including DNA, protein, and lipids and interfere with vital cellular functions resulting oxidative stress.[20-23] Cells contain antioxidant defense mechanism which includes enzymatic and nonenzymatic antioxidant systems. Increased rate of ROS production commonly brings a response, an increase in activities of antioxidant enzymes. But under high rate of ROS production, the enzyme inactivation prevails, leading to reduced antioxidant enzyme activities and to autocatalysis of oxidative damage process.[24,25] *In vitro*[26,27] studies showed that ANDs generate ROS. Indeed, oxidative stress is being increasingly recognized as a possible mechanism in the toxicity and carcinogenesis.[24] Hence, the aim of this study was to monitor oxidative stress induced in occupationally exposed nurses. MDA and GSH levels have been recognized as relevant oxidative stress markers and found to be significantly altered in pathological conditions.[28] GST catalyzes the glutathione-dependent detoxification of several ANDs or their metabolites, thereby protecting cells against free radical attack.[29] In this study, a significant induction of LPO was observed along with the depletion in GSH content and GST activity in serum of occupationally exposed nurses irrespective of their age, marital status, working period, diet, and length of exposure. These observations suggest that the ANDs increased the oxidative stress by lowering the protective mechanism in exposed nurses. ANDs were reported to generate free radicals such as superoxide and hydrogen peroxide which were reported to cause LPO. GSH directly reacts with ROS

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and neutralize them; many enzymes such as glutathione reductase, GST, and glutathione peroxidase utilize GSH to deactivate ROS. This might be the reason for enhanced LPO and depleted GSH content observed in the present study. Oxidative stress studies of ANDs in occupationally exposed workers are scanty but similar increase in serum MDA levels in occupationally exposed workers was reported by Rombaldi *et al.*[8] Previous studies in rat model were also reported significant depletions in glutathione levels with subsequent increase in LPO in liver after cisplatin treatment.[30-32] Jelena Kasapovic *et al*[33] showed that ANDs were responsible for the various side effects including oxidative stress by inducing LPO, depleting GSH content, and inactivating GST in patients undergoing chemotherapy. Our previous study using the same occupationally exposed nurses which have been used in present study demonstrated significant genetic damage in blood using comet assay and micronucleus test. We also reported significant urinary levels of ANDs (cyclophosphamide) in these nurses.[5] This adds to the evidence that the nurses are prone to occupational hazards to ANDs in oncology hospitals.

In conclusion, our results indicate the possible oxidative stress condition due to ANDs exposure in occupationally exposed nurses which contributes to the effects of such drugs. Even though nurses use individual protective equipment while handling ANDs, which certainly reduces the risks, they are not adequate to prevent exposure. Special attention must be given for elimination of the occupational exposure to these drugs by introducing the special protective measures, by automating some of the high-risk activities and safety guidelines are needed.

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