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Sister Chromatid Exchanges and Micronuclei in Lymphocyte of Nurses Handling Antineoplastic Drugs

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ABSTRACT

Individuals handling antineoplastic drugs or their wastes may absorb these potent genotoxic agents. The effects of handling antineoplastic drugs were examined in a group of 24 nurses working in the hematology and oncology departments of two different university hospitals in Shiraz (Iran) and in a group of 18 unexposed nurses as control group. The cytogenetic repercussions of exposure were assessed by examining sister chromatid exchanges (SCEs) and micronuclei (Mn) in circulating lymphocytes. A significant increased frequencies of SCE and Mn is observed in circulating lymphocytes. A significant increased frequencies of SCE and Mn is observed in nurses in daily contact with antineoplastic drugs as compared to control group.

INTRODUCTION

Anticancer drugs target cancers because cell division is rapid in cancerous tissue. These drugs affect other proliferating non-cancerous tissues such as bone marrow, hair follicles, gastrointestinal, nasopharyngeal and genitourinary tract epithelia, and developing embryos. The antineoplastic drugs are known to be carcinogens and teratogens in experimental animals (28). Several anticancer chemotherapeutic agents have cytogenetic effects and induce mutations in bacteria and cultured mammalian cells (28). It is shown that at least some cancer chemotherapeutic drugs, particularly alkylating agents, cause second malignancies, most commonly leukemias, lymphomas, and sarcomas (7).

It has aberrations, the majority of which are balanced rearrangements, persist for many years in children who have survived for extended periods after chemotherapy of cancer (18). Increased frequencies of chromosomal aberrations sister chromatid exchanges (SCE) (6, 11, 12, 17, 20, 22, 25), and micronuclei (Mn) (3,10) have been reported in peripheral lymphocytes of cancer patients been receiving chemotherapy. Scientific articles regarding potential or actual hazards of cytotoxic drug exposure have been appearing in medical, pharmaceutical, and nursing literature for many years (1-4). Direct exposure to cytotoxic agents can occur during admixture, administration, or handling and involve inhalation, ingestion, or absorption.

Setting where many of these drugs are administered or prepared (hospitals, home health agencies, pharmacies, waste handlers, and outpatient settings) need sensitive,selective, non invasive, and in expensive screening tests reflecting absorption of many anticancer drugs.

Analysis of SCE (13, 23, 32) and Mn test (5, 15, 16, 26) are sensitive means of detecting DNA damage in proliferating cells and the tests have also been used for monitoring human populations for exposure to environmental mutagens. The effects of handling antineoplastic drugs on SCEs in lymphocytes in vivo is still being discussed. Some studies report an increase in SCE frequencies (1, 19, 21, 30, 31) while others do not confirm these observations (2, 4, 8, 24,27, 29).

Thus, to detect mutagenic effects of antineoplastic drugs on occupational exposure, SCEs, and Mn were analysed in hospital nurses regularly handling such drugs and in non-exposed controls.

MATERIALS AND METHODS

Subjects

Twenty-four healthy female nurses, in the age range 22 to 43 years, were studies. These nurses had been handling antineoplastic drugs for a range of 1-10 years. Blood samples were obtained from hospital nurses exposed to antineoplastic drugs in oncology and hematology sections at 2 different hospitals of Shiraz, Iran (Nemazi hospital and Ali-Asgar hospital). We have also studied unexposed nurses, as controls from those hospitals. There was no statistically significant age difference between the oncology/hematology nurses (age range = 22 to 43 years; average age = 28.5 years) and the control group (age range = 21 to 41 years; average age = 29.1years).

The most frequently handled drugs included Cylophosphamide, Methotrexate, Vincristine, Adriamycin, Cisplatinum, Etoposide, 5-Fluorouracil and Bleomycin. Eighteen unexposed healthy female nurses ranging in age from 21 to 41 years served as controls.

In order to identify any of the factors that may confound the analysis of SCEs, and micronuclei test, two groups were asked to fill in a questionnaire about their extraoccupational exposure such as smoking, drug consumption, viral diseases, dietary habits and other factors which potentially play a role in the induction or expression and/or alteration of SCE, and Mn.

Sister Chromatid Exchange (SCE) Analysis

For the SCE analysis, standard cultures with 0.4 ml whole blood, 8 ml RPMI-1640 medium, 15% heat-inactivated fetal calf serum 0.2 ml, PHA-M and 3mg/ml 5-bromodeoxy uridine (BrdU) were used. The Cultures were incubated in complete darkness at 37°C for 72 h, and Colchicine (0.9 mg/ml) was present in the cultures for the final 1.5 h. The cells were harvested by exposure to

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hypotonic solution with 0.075 M KCl for 20 min at 37°C, and fixed in methanol and acetic acid (3:1). Slides were prepared and stained using the Giemsa technique (9). SCEs were analyzed in 30 cells containing 46 chromosomes in each preparation, and the mean SCE frequency was calculated as SCEs, per cell of each subject.

Micronuclei Test

In order to study the Mn, the blood smear were prepared and the slides were stained using 5% Giemsa solution as described (14).

Statistical Analysis

The significance of differences was assessed using unpaired Student's t-test and proportional Z-test. A probability of $P<0.05$ considered statistically significant.

RESULTS

Observations

Direct observation revealed the following potential exposure situations:

a) During preparation: powder particles and liquid droplets aerosolize. Also, spills, leaks, and container/syringe breakage occure during transport from or to the pharmacy or during shaking.

b) During adminstration: Syringes leak during transport, priming of intravenous sets, expelling of air, and connection to or removal from the patient. Aerosols form during priming, expelling of air, and connection to or removal from the patient.

c) Miscellaneous exposures: Discarded containers contaminate housekeeping workers. Also, improperly cleaned equipment/containers and patient excreta are sources of contamination.

SCE Analysis

A statistically significant difference in the number of SCE was observed between the exposed and control goups (Table 1). The mean frequency of SCE/cells was 7.12 ± 0.80 and 5.81 ± 1.20 in the oncology/hematology and control group nurses, respectively. Which shows significant difference between the studied groups ($t = 4.32$; $df = 40$; $p<0.05$).

Micronucleus Test

The results of micronuclei determination are indicated in Table 2. The frequency of micronuclei amoung oncology/hematology nurses, was significantly higher (Z-value =3.65) as compared to those of the control group.

Table1. **Frequency of SCE in blood lymphocytes among oncology/hematology nurses and control nurses**

Groups	N	SCE/cell*	$t_{df}=42$
Oncology/hematology	24	7.12±0.80	4.32 **
Control	18	5.81±1.20	

*Values are mean± SD
** P<0.01

Table 2. **Micronuclei in peripheral blood lymphocytes (micronuclei/1000 cells) in oncology/hematology nurses and control nurses**

Groups	N	Mn/1000 cells	Z-value
Oncology/hematology	12	0.75	3.65
Control	10	0.25	

DISCUSSION

This study is the first to report the effect of handling anticancer drugs on oncology nurses in Iran. The results of the present study shows that among nurses working in hematology and oncology departments, those handling antineoplastic drugs exhibited a significant increases in the number of SCEs and micronuclei in circulating lymphocytes.

Biological monitoring with SCE, chromosomal aberration, and forward mutation assays has also produced positive results in nurses (1, 19, 21, 27, 28, 30, 31); however, several studies could not demonstrate any relationship between occupational exposure to cytostatic drugs and increased SCEs or other system assays (2, 4,8, 24, 29). We assume that different results may occur from the low levels and average duration of exposure and that some of the nurses may have been using protective measures while handling these drugs; i.e.wearing

surgical masks, gloves, and using vertical laminar flow hoods for drug mixing.

Biomonitoring of occupationally exposed people appears to be a sensitive way to evaluate the genotoxic effects of cytostatic drugs exposures (and radiation exposures). This type of monitoring may be used as an indicator to detect early damage and to demand more controls.

The purpose of this work was to provide data on the genetic hazards due to the occupational exposure to antineoplastic drugs. Since the potential risks and biological consequences of anticancer drugs have been attained through the extrapolation from acute exposures.

Our results are also particularly interesting for a developing country such as ours, where biological security controls are not so strict and extended work days are common. For health surveillance, the detection of early genotoxic effects may permit the adoption of preventive biological controls such as hygienic improvements in the workplace or the reduction of hours of occupational exposure.

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