American Journal of Cancer Biology

American Journals of Cancer Biology
http://www.ivyunion.org/index.php/ajcb
Vol. 1, Article ID 201300042, 7 pages

Research Article

Anticancer Drugs Induced Chromosomal Rearrangements in Lymphocytes of Breast Cancer Patients

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Abstract

Breast cancer is one of the most commonly diagnosed malignancies in women around the world. Chromosomal rearrangements are known to play important role in the pathogenesis of many diseases including cancer. In case of breast cancer, chromosomal changes are detectable at all stages of tumour development providing excellent opportunity for prognosis and therapy. Present work aimed to study the frequency of chromosomal aberrations and satellite associations in human peripheral blood lymphocyte culture of freshly diagnosed breast cancer patients after in vitro exposure to combination of anticancer drug treatment. The present study reveals that, combination of anticancer drugs significantly increases chromosomal aberrations without altering the frequency of satellite associations.

Keywords: Chromosomal aberrations; Satellite associations; Breast cancer; Peripheral blood lymphocyte culture (PBLC)

Peer Reviewers: Jongdae Lee, PhD, Department of Medicine, University of California, San Diego, United States. Sarita Saraswati, PhD, Department of Pharmacology, King Saud University, Saudi Arabia

Received: November 24, 2012; **Accepted**: February 23, 2013; **Published:** March 5, 2013

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Breast cancer is one of the most common malignancies in women. It continues to be a major burden and cause of death worldwide. In cases of early detection, it is treatable by surgery, radiation and chemotherapy, but the prognosis is influenced by many factors. The majority of cancer cells represent dynamic karyotypic changes, including

chromosomal rearrangements [1]. A positive association between the frequency of chromosomal aberrations in peripheral blood lymphocytes (PBLs) and the risk of cancer at different sites has been supported by numerous clinical observations [2]. Different case-control studies have also reported a significant increase in the frequency of aberrant cells in PBLs of cancer patients [3-11]. However in case of breast cancer; chromosomal aberrations are found to be a dominant genetic event and may play an important role in cancer progression.

In the present study, chromosomal rearrangements were observed in freshly diagnosed breast patients who had not undergone any chemotherapeutic and/or radiation treatment. Further, peripheral blood lymphocytes of patients were exposed to combination of anticancer drugs namely 5-Fluorouracil {5-FU}, Cyclophosphamide and Adriamycin commonly used for breast cancer treatment.

5-FU causes DNA damage, specifically double strand (and single-strand) breaks, during S phase of cell cycle [12-13]. On the other hand, cyclophosphamide is a potent alkylating agent being cytotoxic to tumour cells via crosslinking of DNA strands and inhibition of protein synthesis [14]. Mechanism of action of adriamycin is still not fully understood but suggested mechanisms includes formation of DNA adducts, production of free radicals and inhibition of protein synthesis [15].

Therefore, it is essential to assign, role of cytogenetic endpoints such as chromosomal aberrations and satellite associations for the diagnosis and treatment of breast cancer.

Materials and Methods

Lymphocyte Culture

Lymphocyte cultures were set up by Hungerford [16] with slight modifications [17]. Heparinized whole blood (0.5 ml) was added to a mixture containing 5 ml of culture medium RPMI 1640 and 0.1 ml phytohemagglutinin (Lectin). Then the culture vials were kept in *HERA cell* ^{150} CO_2 incubator for 71 hrs, at 37 °C with 5 % CO_2. Then 0.1 ml demecolcine solution was added at last 2 hours of incubation period to arrest cells at metaphase. The cells were collected by centrifugation, resuspended in a prewarmed hypotonic solution (KCL, 0.075 M) for 20-25 minutes and fixed in chilled methanol/acetic acid (3:1 v/v) solution (Carnoy's fixative). Then drops of cell suspension were allowed to fall from at least 2.5 feet height on pre chilled and chemically cleaned slides. These slides were air dried on a hot plate at 50-60 °C. All slides were blind coded and labelled soon after assuring about well spread chromosome.

Nucleolar Organizing Regions staining by AgNO3

Nucleolar Organizing Regions (NOR) staining was performed according to the silver nitrate ($AgNO_3$) method of Verma and Babu [18]. $AgNO_3$ solution was prepared by mixing 4 g $AgNO_3$ in 8 ml distilled water and stored light protected at 4 °C. Few drops of silver nitrate solution were applied on slide along with 2 % gelatine solution mixed with formic acid. Heat was applied till brownish colour appeared. Prepared slides were blind coded and scored for observations of NORs.

Preparation of drug

All the drugs (5-FU, adriamycin and cyclophosphamide) were prepared in sterile distilled water and 1 M concentration of stock solutions was prepared. After optimization of various dilutions in the present study, the sublethal concentrations of 5-FU (30 ng/30 µl), adriamycin (15 ng/15 µl) and cyclophosphamide (15 ng/15 µl) were used.

Experimental Protocol

Total of 22 breast cancer patients (blood was collected from Lions Cancer Detection Centre, Surat) were studied along with 22 age and sex matched female controls. Written consent of patients was obtained.

All blood samples collected from breast cancer patients were divided in two parts.

Part A- Total 11 PBL cultures were set up without chemotherapeutic exposure.

Part B- Total 11 PBL cultures of breast cancer patient were exposed to a combination of 5-FU {30 ng/30 µl}, adriamycin {15 ng/15 µl} and cyclophosphamide {15 ng/15 µl} added after 24 hours of initiation of lymphocyte culture. Cells were exposed to drugs combination for 48 hours.

Similarly 22 PBL cultures were prepared from healthy

females' which serve as a control.

Results were analysed using student t-test with aid of SPSS software.

Results

Results indicated frequency of chromosomal aberrations observed with and without chemotherapeutic exposure to peripheral blood lymphocytes of breast cancer patients in comparison to controls (Table 1). We found significant increase in chromosomal aberrations in breast cancer patients in comparison to that of control ($P < 0.05$). PBLs of patients not exposed to any chemotherapeutic agents showed significant increase in chromatid gaps and endoreduplication as compared to controls. In addition, the frequency of hyperdiploid configuration was also found to be significantly higher in breast cancer patients ($P < 0.05$).

Table 1 Frequency of chromosomal aberrations in breast cancer

Groups	Chromosome type aberrations				Chromatid type aberrations				Others		
	B	G	D	R	B	G	Int.	ER	TA	Hypo	Hyper
Before Chemotherapy											
Control (Mean)	0.818	0.272	1.181	0.454	0.727	0.545	0.363	0.454	0.454	3.545	0.636
Breast cancer (Mean)	0.363	0.272	0.818	0.909	1.090	1.818	0.363	2.636	1.000	5.818	1.636
P value	0.075	0.500	0.251	0.134	0.208	**0.016***	0.500	**0.005***	0.211	0.146	**0.037***
After chemotherapy											
Control (Mean)	0.181	0.363	0.363	0.636	0.454	0.272	0.181	0.727	0.636	4.272	0.363
Breast cancer (Mean)	0.636	0.363	0.636	0.454	1.454	0.272	0.545	1.090	1.000	4.818	1.000
P value	**0.034***	0.500	0.147	0.341	0.164	0.500	0.101	0.152	0.147	0.295	**0.008***

(* - P value < 0.05 significantly different from control)

(B- Break, G- Gap, D- Dicentric, R-Ring, Int- Chromatid Interchange, ER- Endoreduplication, TA- Telomeric association, Hypo- Hypodiploid, Hyper- Hyperdiploid)

Lymphocytes of patients and controls were exposed to a combination of 5-FU (30 ng/30 µl), adriamycin (15 ng/15 µl) and cyclophosphamide (15 ng/15 µl) drugs added at 24th hours after initiation of culture, exhibited significant increase in frequency of chromosome breaks and hyperdiploid configuration (P value < 0.005) as compared to controls (Figure 1).

Frequency of satellite associations of acrocentric chromosomes in patients and controls was studied after exposure to chemotherapeutic agents (Figure 1). A significant increase ($P < 0.05$) was observed in DD (between D group chromosomes) and DG (between D & G group chromosomes) associations of freshly diagnosed cancer patient. Whereas, SAs were not significant after exposure to chemotherapeutic drugs (Table 2).

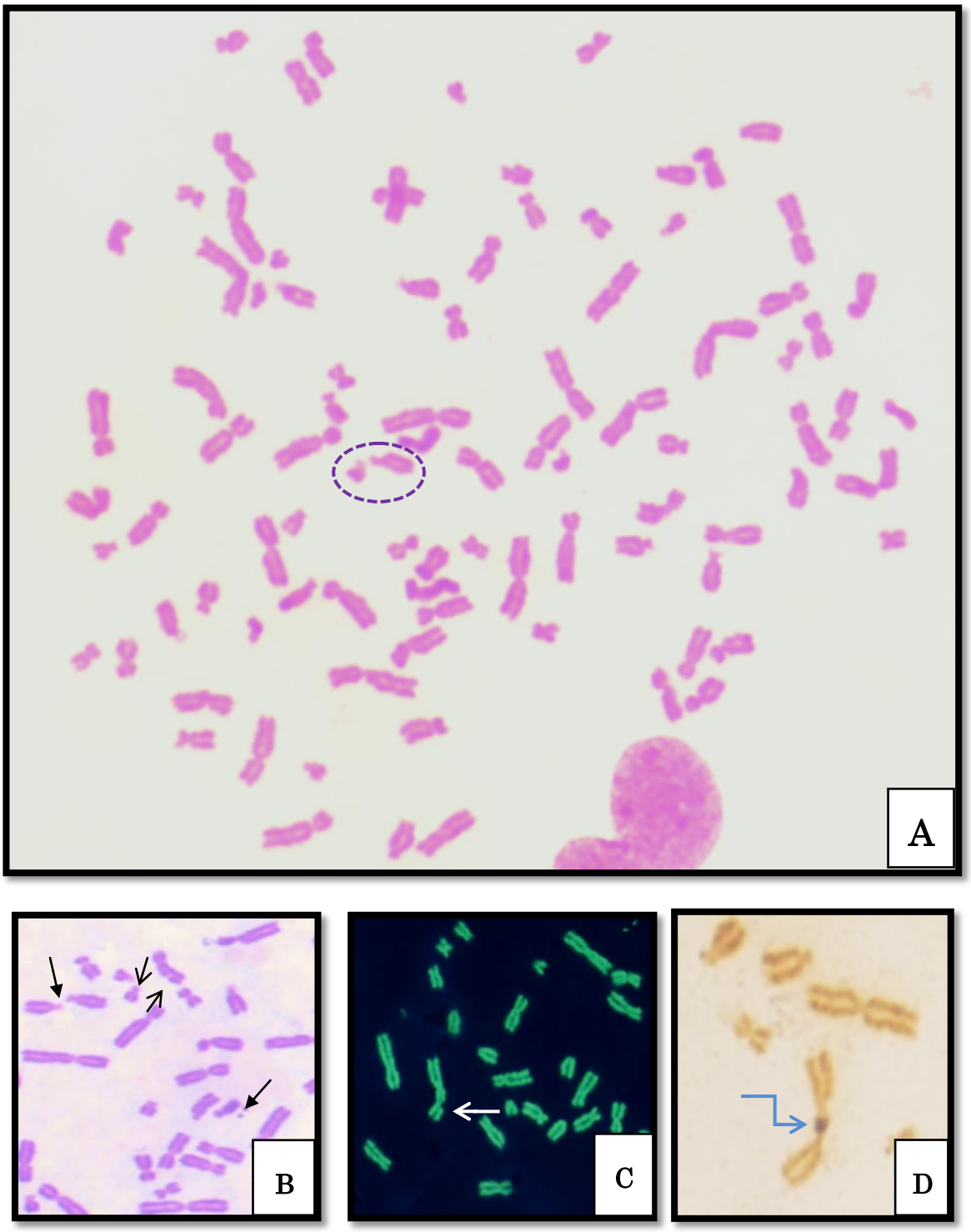

Figure 1 Chromosomal rearrangements in breast cancer patients. (**A**) atellite association (DG type) in hyperdiploid metaphase. (**B**) Chromatid gap, double minutes DD and GG acrocentric associations (partial plate). (**C**) Chromosome break (partial plate). (**D**) NOR stained in DD type acrocentric association (partial plate).

Table 2 Frequency of satellite associations in breast cancer

Groups	Type of Satellite Association								
	DD	DG	GG	DDG	DGG	DDD	GGG	3DG/3GD	DDGG
Before chemotherapy									
Control (Mean)	1.727	3.363	1.363	0.272	1.090	0.0	0.0	0.0	0.0
Breast cancer (Mean)	3.727	6.363	1.909	0.545	0.818	0.0	0.0	0.0	0.0
P value	0.040*	0.029*	0.250	0.105	0.332	-	-	-	-
After chemotherapy									
Control (Mean)	1.272	0.727	0.818	0.090	0.090	0.090	0.0	0.0	0.0
Breast cancer (Mean)	1.000	0.727	0.727	0.090	0.090	0.090	0.0	0.0	0.0
P value	0.265	0.500	0.392	0.500	0.500	0.500	-	-	-

(* - Significant at p value < 0.05)

Discussion

It has been hypothesized that the frequency of chromosomal aberrations in peripheral blood lymphocytes of healthy individuals represents a marker of susceptibility to cancer [19-20]. Chromosomal aberrations are usually considered to derive from unrepaired or misrepaired DNA lesions induced by exogenous or endogenous exposure to DNA-damaging agents. A comprehensive review of genetic rearrangements consequent to chromosome aberrations and their role in the pathogenesis of solid and hematologic cancers was reported [21].

A Significant higher frequency of aberrant metaphases in PBLs of breast cancer patients has been well documented [22-23]. In addition, chromosome breaks have also been reported by Ochi et al. [24], in peripheral blood leucocytes of untreated breast cancer patients. Our results are in good agreement with that of Ochi et al. We report a high frequency of chromosome breaks in breast cancer patients. Mirfakhraie et al. [25] have indicated the loss of chromosomes 1, 3 and r (11) in PBLs of breast cancer patients. However we didn't find any such change. High frequency of aberrations in PBLs of cancer patients may throw light on the defective genetic mechanisms.

It is interesting to note that significantly higher frequency of hyperdiploid configuration was found in both drug treated as well as untreated lymphocytes of patients as compared to controls. The similar results have been shown by Sophia et al. [26], in Non-Hodgkin Lymphoma.

There have been few reports available on frequency of satellite associations in healthy individuals [27-31]; however the frequency of satellite associations was mainly studied in various age groups [32-35]. There is a paucity of information on the study of satellite association with special reference to female cancers [1]. Therefore, an attempt was made to study freshly diagnosed breast cancer patients who had not undergone chemotherapeutic treatment. The comparison was made with age and sex matched female controls. Results revealed significant higher frequency ($P < 0.05$) of SA between DD and DG group of acrocentric chromosomes as compared to control (Table 2). This shows significant involvement of D and G group chromosomes in the pathogenesis of breast cancer. However, exposure to combination of anticancer drugs used in present study did not reveal any significant change in frequency of SA.

From the foregoing discussion, it is concluded that higher frequency of hyperdiploid configuration remained unchanged in both treated as well as untreated breast cancer patients. In case of untreated lymphocytes of breast cancer patients, the possible cause of hyperdiploidy could be due to

genomic instability and/ or an exposure to environmental factors which includes carcinogens that may alter chromosome copy. The exact mechanism of action of chemotherapeutic treatment in peripheral blood lymphocytes of breast cancer patients is not fully understood with special reference to chromosomal rearrangements.

Acknowledgement

Authors are highly thankful to Dr. Shrivastava, Director, Dr. Roshni Jariwala of Lions cancer detection centre for providing blood samples. Special thanks to Dr. M. G. Saralai, Principal, C.K.Pithawalla Institute of Pharmaceutical Science & Research for his kind support.

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