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Sex Chromatin Frequency Variation among Breast and Ovarian Cancer Patients

Ismaiel NA, Rassul FE and Ahmad BJ*

Department of Biology, Faculty of Science, University of Soran, Iraq

Abstract

Background: An epigenetics paradigm and an effective model system for the generation of facultative heterochromatin throughout development are represented by the inactivation of the X chromosome. The expression of the XIST gene from the X chromosome that will be inactivated in females is the first step in the process of X Chromosomal Inactivation (XCI) (Xi). The idea of a direct role for BRCA1 in the localization of XIST was highlighted by the apparent cytological overlap between BRCA1 and XIST RNA across the Xi.

Objectives: The goal of the current study was to compare the prevalence of sex chromatin in breast and ovarian cancer patients with that of healthy, normal females.

Material and Methods: The blood smear samples from 30 healthy normal girls (25 with breast cancer and 25 with ovarian cancer) served as the study's main source of data. The modal occurrence rate of sex chromatin was determined by analyzing 100 polymorphonuclear neutrophils from each person.

Results: Our findings showed that there was a significant variation in the mean prevalence of sex chromatin among cancer patients.

Conclusion: This finding suggests that cancer patients' latent X chromosomes have been reactivated. With the help of this research, it may be possible to use the inactive X chromosome as an epigenetic biomarker in cancer at the molecular and cytological levels.

Keywords: Sex Chromatin; Polymorphonuclear neutrophils; Breast cancer; Ovarian cancer

Introduction

In mammalian females, dosage compensation of X-linked genes is attained by random inactivation of one of the two X chromosomes in somatic cells [1]. Sex chromatin is convergent 1 micron of condensed heterochromatin seen commonly at the periphery of female nuclei in certain tissues like corneal epithelium, vaginal mucosa, oral, fibroblasts and vaginal mucosa, etc. and in blood smears as a drumstick [2-5]. In neutrophils inactive X chromosome appears in one of the five forms. They are drumsticks, racquet forms, small club, minor lobe and sessile nodules. Davidson and Smith are the first to recognize and report the presence of neutrophil drumsticks and nonspecific appendages and their differences in sexes. A drumstick consists of a small nuclear mass of about 1.5 µ in diameter, attached to the body of the nucleus by means of a thin stalk and the incidence of drumsticks varied between 1% to 17% with an average of 2.9% [2].

X Chromosome Inactivation (XCI) takes place early in embryogenesis and can be randomly divided into a series of events: Counting and choice, initiation, propagation, and maintenance of inactive state [1]. Initiation begins with the XIST expression and stabilization of its noncoding RNA transcript in cis, along the X chromosome that is designed for inactivation [1,6]. Recently, Ganesan et al. [7] indicated that XIST RNA concentration on the inactive X chromosome is dependent on the product of the BRCA1 tumor suppressor gene. This is a nuclear protein playing a role in several important cellular processes, including DNA damage repair, transcription regulation, and cell cycle checkpoints [8,9]. Heterozygous carriers of BRCA1 mutations are particularly liable to breast and ovarian cancer development [10-12]. Several evidences show the occurrence of XCI changes in breast cancer. In early studies, several authors noted that a few destructive breast and ovarian tumors did not show a detectable Barr body [13-16]. More recently, cytogenetic studies of breast carcinomas evidenced the earning of an additional active X chromosome (Xa), together with the lack of the inactive one [17-19]. Women carrying a germline mutation in BRCA1 experience a

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Correspondence:
Narmeen Abdulsamad Ismaiel, Department of Biology, Faculty of Science, University of Soran, Erbil, Iraq, E-mail: shamsabd10@yahoo.com

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significantly increased lifetime risk of breast and ovarian cancer. The tumors that arise in these individuals all disclose loss of the wild-type and holding of the mutant allele of BRCA1, implying that this gene functions as a tumor suppressor. Interestingly, men who harbor a BRCA1 mutation do not have a clear increase in cancer predisposition [20,21]. BRCA1 is a nuclear protein that has been identified in all examined proliferating cells and whose expression seems to be cell-cycle regulated. Numerous studies have revealed that BRCA1 likely contributes to a number of essential biological functions, such as the regulation of cell-cycle checkpoints, transcriptional control, and the preservation of genomic integrity [8-10].

The present study is an attempt to compare the prevalence of sex chromatin changes, if any, in breast and ovarian cancer patients with normal healthy female's Peripheral blood smear.

Materials and Methods

A total of 80 females were included in the study, out of which 30 of normal healthy females (age range 20-45), 25 were diagnosed as breast carcinoma, and 25 diagnosed as ovarian cancer (age range 18-60) in Nanakaly Hospital. The diagnosis of these breast and ovarian cancer patients was confirmed by histopathologic biopsy. A structured schedule was used to collect the data on the demography, life style pattern, reproductive history and clinical details from cancer patients.

Peripheral blood smears are made by placing a drop of blood 1 mm to 2 mm in diameter 1/4 inch from the frosted edge of the slide. The drop was placed in the center line approximately. The purpose is to get a region where the cells are spaced far enough apart to be counted and differentiated. The blood smear is then fixed with methanol for proper microscopic examination. The smear is stained by Giemsa's stain then wash in running tap water, air dried, and field stain to counting of drumstick, and non-specific appendages was done in "Z" pattern. A total number of 100 neutrophils were counted in each slide and observed under oil immersion. polynuclear neutrophils were classified into drumsticks, and nonspecific appendages like sessile nodule and small club (Figure 1).

Statistical analysis

The mean and standard error of the mean are used to express all of the results. To compare the frequency of sex chromatin types in polymorphonuclear leucocytes cells and other nuclear abnormalities in the studied classes, the data were analyzed statistically using student's t-tests for independent samples. Statistical significance was described as a P-value of less than 0.05. All statistical studies were carried out with the GraphPad prism statistical software program (9.3.1).

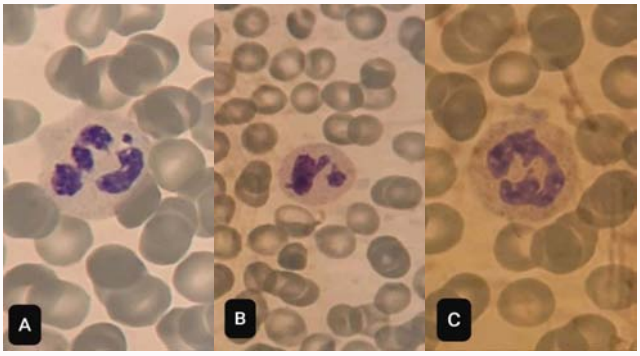

Figure 1: (a) Drum stick cell, (b) sessile nodule and (c) small club cell. Giemsa-stained photomicrographs displayed at 1000x magnification (objective: 100x). Olympus compound binocular microscope magnification.

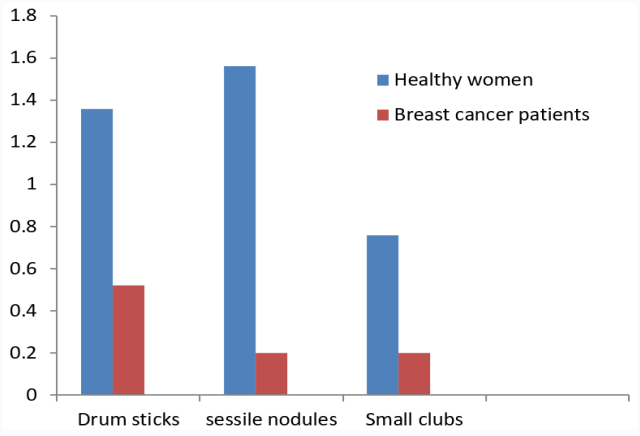

Figure 2: Frequency distribution of sex chromatin in breast cancer.

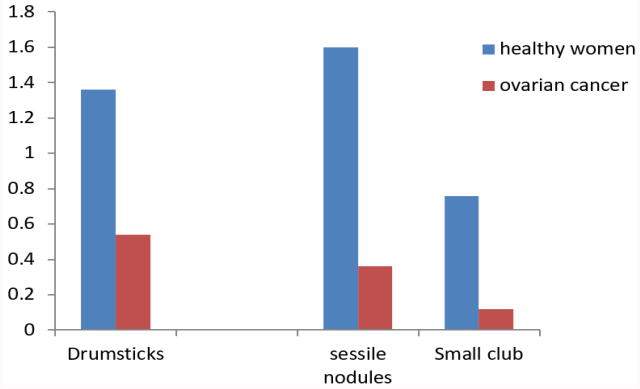

Figure 3: Frequency distribution of sex chromatin in ovarian cancer.

Results

Results for frequency of sex chromatin in cancer patients and healthy female are shown in Table 1, 2 and Figures 1-3. A total of 8,000 neutrophils were observed in blood smear collected from 30 healthy women (18-45 years age) and 25 breast and 25 ovarian cancer patients of (17-55 years age) for evaluation of frequency of sex chromatin. Table analysis revealed a significant association between frequency of sex chromatin distribution and Groups (breast and ovarian cancer patients and normal females) ($p<0.05$). In other words, the presence of sex chromatin in breast and ovarian cancer patients is comparatively lower than the normal healthy females (Table 1, 2).

Frequencies of Small clubs and Drum sticks were significantly lower in breast cancer patients (0.20 ± 0.08, 0.52 ± 0.14) respectively compared to the healthy female (0.76 ± 0.16, 1.36 ± 0.27) respectively ($p<0.05$). Sessile nodules frequency was found to be highly significant ($P=0.0001$) in breast cancer patient (0.20 ± 0.08) than healthy female (1.56 ± 0.24).

Frequencies of Small clubs and Drum sticks were significantly lower in breast cancer patients (0.12 ± 0.06, 0.54 ± 0.15) respectively compared to the healthy female (0.76 ± 0.16, 1.36 ± 0.27) respectively ($p<0.05$). Sessile nodules frequency was found to be highly significant ($P=0.0001$) in breast cancer patient (0.36 ± 0.12) than healthy female (1.60 ± 0.24).

Table 1: Mean number of various types of drum sticks of breast cancer patients and healthy women.

Types of sex chromatin	Healthy women (mean ± S.E)	Breast cancer patients (mean ± S.E)	P value and significance
Drum sticks	1.36 ± 0.27	0.52 ± 0.14	0.013*
Sessile nodules	1.56 ± 0.24	0.20 ± 0.08	0.0001****
Small clubs	0.76 ± 0.16	0.20 ± 0.08	0.005**

p>0.05 patients compared with healthy individual (control), unpaired-test

Table 2: Mean number of various types of drum sticks of ovarian cancer patients and healthy women.

Types of sex chromatin	Healthy women (mean ± S.E)	ovarian cancer patients (mean ± S.E)	P value and significance
Drumsticks	**1.36 ± 0.27**	**0.54 ± 0.15**	**0.019***
Sessile nodules	**1.60 ± 0.24**	**0.36 ± 0.12**	**0.0001*****
Small club	**0.76 ± 0.16**	**0.12 ± 0.06**	**0.0013****

p>0.05 patients compared with healthy female (control), unpaired-test

Discussion

Despite reports of a higher prevalence of sex chromatin in breast cancer patients [12], another study found no appreciable change in the frequency of X chromatin in case of breast cancer patients [22]. In addition to these, earlier research [18,23-28] showed a much-reduced prevalence of sex chromatin in several malignancies. Studies on carcinoma, such as esophageal cancer and breast cancer, have indicated comparable findings demonstrating reduced incidence of sex chromatin [29-31]. In our present study the prevalence of sex chromatin in breast and ovarian cancer patients have been shown significantly lower value as compared to the normal healthy females. The reactivation of the inactive X chromosome may be the cause of the considerably reduced prevalence of sex chromatin in breast cancer patients.

It's unclear whether this feature of BRCA1 activity is connected to its tumor suppression feature. However, it is tempting to hypothesize that loss of BRCA1 may also result in increased expression of specific X chromosome genes that may contribute to the onset of breast and ovarian cancer in particular types of female cells. In this regard, it was recently discovered that a group of X-chromosomal genes were overexpressed in BRCA1-deficient ovarian tumors [32]. A subset of malignant breast and ovarian tumors lack a detectable Barr body [14,15,33,34].

The most current proof that the X chromosome is inactivated as a result of the BRCA1 breast cancer susceptibility gene [7]. Although XIST is essential for inactivation, nothing is known about how the non-coding RNA molecule that this gene produces mediates the silencing of a whole chromosome [1]. Particularly, supporting proteins crucial to this process have not been discovered. Now, in an unexpected new discovery, it has been established that the BRCA1 protein is essential for silence the X chromosome [6].

The inactive X chromosome (Xi) varies from its active homologue (Xa) in a variety of ways, such as enhanced methylation of specific CpGs, replication occurring later in the S-phase, expression of the Xist gene with Xist RNA binding, and under acetylation of core histones [17]. The stability and maintenance of gene silence in the inactive X chromosome is significantly influenced by DNA methylation and histone acetylation [1,35]. Numerous studies have demonstrated a substantial association between DNA hypermethylation, transcriptional silencing, and tightly packed chromatin [36-38]. The under acetylation of histone plays a crucial role in the stability of a gene's inactive status, and numerous studies have demonstrated that inactive X in females contains under acetylated H4 [17,39]. However, changes in DNA methylation and acetylation are frequent in a number of different tumor types as well as during development [39]. It has been proposed that hypomethylation of DNAs, which results in transcriptional activation, contributes to oncogenesis by activating oncogenes, which are present in a variety of cancers including breast cancer, cervical cancer, and brain cancer [40,41]. Histone H3 and H4 deacetylation as well as changes in the pattern of histone methylation are associated with DNA methylation [42,43]. In this manner, the pattern of histone modification modifies a landscape that establishes and permits the expression pattern of particular genes inside chromatin areas while organizing and preserving the integrity of the nuclear architecture [44].

Conclusion

Our work has shown that sex chromatin prevalence in breast and ovarian cancer patients is significantly lower than that of an apparently healthy female individual. The results of the current study suggest that the altered DNA methylation pattern, Histon acetylation in cancer cells, and the important role played by the BRCA1 breast cancer susceptibility gene in this epigenetic phenomenon may be the reason for the lower prevalence of sex chromatin in females with breast and ovarian cancer than in normal females.

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