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Fetal microchimerism and X chromosome aneuploidies in women with breast cancer

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Abstract
Breast cancer (BC) which is accepted as the most common invasive cancer among women, can be caused by many factors. Fetal microchimerism (FMc) known as the long-term persistence of small numbers of fetus-derived allogeneic cells in mother is a potential contributor or a protective factor in some diseases, including breast cancer. The presence of Y chromosome and X-chromosome aneuploidies have been associated with BC. We investigated the possible roles of microchimeric cells (McCs) in BC development by detecting the presence of Y chromosome and X-chromosome aneuploidies in a selected group of patients. For this purpose, the fluorescent in situ hybridization (FISH) technique was applied to malignant tumor tissues of 49 BC patients and blood samples of 32 healthy controls. While fetal McCs (FMcCs) were found in malignant tumor tissues of 10 (20.41%) BC patients, all of the healthy controls showed no detectable Y chromosome signals in their blood (p=0.000). Besides, it was found that X-chromosome polysomies increased in BC patients with raised age (p=0.020) and who breastfed their children (0.039), and X-chromosome monosomies were increased in patients who delivered a son at their first pregnancy (p=0.016). On the other hand, X-chromosome monosomies was found significantly higher in early-stage tumors (I and II) than in advanced-stage tumors (III and IV) (p=0.020). FMcCs was present in breast tumor of women who have a male child and, probably allogeneic maternal immune reaction may have led to cancer development in those cases. The presence of FMcCs in breast tissue may be protecting women from cancer for a period of time after delivery, but have the potential to cause cancer in the later period. Detection of X-chromosome aneuploidies in BC patients with raised age and X-chromosome monosomies in the early stages of BC can be used as early markers in the diagnosis of BC.

Keywords: breast cancer, fetal microchimerism, aneuploidy, fluorescent *in situ* hybridization

Introduction

Breast cancer (BC) which is the most common cancer and a leading cause of death in women affects 2.1 million women each year. In 2018, it has been reported that almost 2 million (11, 6%) women were diagnosed with BC and 627,000 (6,6%) women died from this disease [1]. Many environmental, genetic, and epigenetic factors affect the pathogenesis of BC [2]. The epidemiological factors such as age, female reproductive status, delayed first full-term pregnancy, decreased parity or null parity, short duration of lactation or no breastfeeding, usage of oral contraceptive, hormone replacement therapy, unhealthy lifestyles, previous benign breast tumors, and positive family history also influence the development of BC [2, 3]. There is evidence that microchimerism may also have an inverse relationship with BC [4]. Microchimerism (Mc) is defined as the presence of a small amount of genetically different cells or DNA within the tissues or circulation of an individual. Natural Mc which is most commonly caused by pregnancy occurs as a result of bilateral exchange of hematopoietic cells through the placenta. The presence of fetal cells in the mother's circulating blood has been known for over a century. Fetal microchimeric cells (FMcCs) remain in the mother's bone marrow for many years after birth and migrate from bone marrow to blood and tissues. How these cells migrate and adapt to the new environment, and how their differentiation ability is affected by this new environment is not yet known. Although the role of fetal Mc (FMc) is partially known, there is evidence that the presence of McCs, even within physiological limits, may be harmful. The evidence has suggested that FMcCs could be effective in rejuvenating the progenitor cell source, repairing maternal tissues, controlling malignant cells [5]. Due to their above-mentioned functions, many researchers have been focused on the possible roles of FMcCs in tissue repair and cancer pathogenesis in recent years [6-8]. Similarly, the outcome of FMc on long-term maternal health has attracted much attention in recent years and has been increasingly investigated [9]. Many studies also showed the presence of fetal cells in tumors of breast, brain, lung, thyroid, skin, cervix, and colon [5, 10-15].

Having chromosomal anomalies (CA) is one of the most striking features of neoplastic cells, and the presence of chromosome instability and aneuploidy has been demonstrated in various types of human cancers [16-19]. Sex chromosome aneuploidies (SCA) include an extensive group of chromosome disorders characterized by the loss or gain of one or more sex chromosomes. It has been reported that raised cancer risk is associated with SCAs in breast and gonadal cancers [17, 18, 20-22]. Aneuploidies were also reported in a variety of other cancers, including lung, pancreatic, colorectal, and hematological cancers [18, 23-25].

In line with these studies, we also aimed to investigate the roles of FMcCs and SCA in BC development in this study.

Materials and Methods

Study Population

A total of 49 women who have at least one son and were diagnosed with BC were included in this study. Biopsy specimens were obtained from women who underwent a mastectomy in the Department of General Surgery, Faculty of Medicine, Çukurova University. After surgery, malignant breast tissues were taken and sent to the genetic laboratory of the Department of Medical Biology, Faculty of Medicine, Çukurova University. Samples were stored at -20 ˚C until processing. Patients' data regarding age, age at first pregnancy, number of pregnancies, number of children, number of sons, number of boy pregnancies, number of abortions, menopausal status, breastfeeding status, cancer histology/stage, and family history of cancer were recorded. The control group included 32 healthy women who have at least one son. Three ml-heparinized blood was taken from each control subject and the number of children and sons they have were recorded. Ethical approval was obtained from the ethics committee of Çukurova University (ÇÜ2014/36-12), and the participants were informed about the study and their permission was obtained before inclusion.

Breast Tumor Touch Preparation

The surface of freshly excised BC tissues obtained from mastectomy materials was pressed on microscope slides and after drying, placed in cold methanol and fixative solution (methanol: acetic acid; 3:1) for 20 min, respectively. Slides were air-dried and stored at -20 ˚C for after processing.

Blood Preparation

Standard techniques (excluding incubation period) were used for harvesting and slide preparation. After incubation, slides were kept at 37 ˚C overnight and then stored at -20 ˚C for after processing.

Fluorescence In Situ Hybridization (FISH)

FISH procedure was applied according to the method modified by Taştemir *et al.* [26], using CEP X SpectrumOrange/Y SpectrumGreen DNA Probe Kit (Abbott). Firstly, slides were pretreated with 2XSSC for 5 min at room temperature and then immersed in the solution containing HCl (1N), distilled water, and pepsin A (2:200:2 v/v/v) for 30 min at 37˚C. After 30 min, slides were passed through a series of distilled water, PBS, PBS/$MgCl_2.6H_2O$ for 2 min each, and PBS/$MgCl_2.6H_2O$ with paraformaldehyde for 10 min, respectively. After passing through a dehydration series of 70, 85, and 100% ethanol for 3 min each, slides were left to dry. Ten µl of each probe mixtures were dropped on slides immediately, and a coverslip was sealed onto the slides with rubber cement in a dark room. The slides were put in ThermoBrite Denaturation/ Hybridization System (Leica) and denatured for 5 min at 95˚C and hybridized overnight at 37˚C. Following hybridization, slides were washed with 0.4XSSC/0.3% Tween20 for 2 min at 73˚C and 2XSSC/0.1% Tween20 for 1 min at room temperature, respectively. After drying, 10 µl of DAPI was applied to each slide and after coverslipping, slides were kept in a -20 ˚C freezer for 30 min. An average of 500 interphase cells was analyzed using a BX51 Olympus fluorescence microscope equipped with Cytovision Probe Software (Applied Imaging, Santa Clara, CA). Monosomic and polysomic cells for the X chromosome and Y-positive cells were recorded.

Statistical Analysis

SPSS 17.0 statistics software package was used for data analysis. For comparing X chromosome copy number (gain/lost) with clinicopathological parameters between groups, one-way analysis of the variance (ANOVA), the LSD post hoc test, and independent-samples T-test were used. Chi-square ($\chi 2$) test was used to compare the numbers of fetal microchimeric cells in patient and control groups. Results were presented as mean ± standard deviation (SD). The significance level was determined as $p \leq 0.05$ in all tests.

Results

The present study included 49 BC women having at least one male child and 32 healthy women. Demographic and pathological findings of women with BC were provided in Table 1. The mean ages of BC and healthy women were 57, 18 ± 10,515 (ranged between 32-85) and 29,44 ± 5,003 (ranged between 21-38), respectively. Overall, 10 out of 49 women with BC (20,41%) were positive and 39 women (79,59%) were negative for FMcCs. Similarly, 10 women with BC showed Y-chromosome presence and the other 39 did not. All of the healthy women had no detectable Y chromosome in their blood. The prevalence of FMcCs was significantly greater in BC women than healthy controls (20,41%, 0%, respectively and p=0,000). Five (50%) of 10 BC women with FMcCs had one son, 5 (50%) had two or more sons; and 24 (75%) of 32 controls had one son and 8 (25%) had two or more sons. Women with BC delivered more boys than healthy controls, however, the difference was not statistically significant (p=0,238). A 50% (5/10) of Y-positive BC women have 3 or more children, 71,9% (23/32) of the healthy controls have 1-2 children. But the difference was not statistically significant (p=0,149) (Table 2). BC patients (including Y-negative and Y-positive patients) have more children than Y-negative healthy ones.

FISH analysis revealed a loss/gain of X-chromosome copies in tumor cells. At least one X chromosome loss (monosomy) / gain (polysomy) was recorded in 8244 (35.68%) out of 23106 cells examined. X chromosome polysomies were categorized as 3X, 4X, 5X, 6X, 7X, 8X, and above. The ratios of X-chromosome monosomies and polysomies were calculated according to the number of cells examined and compared with the demographic and pathologic findings of patients as mean ± SD. Age affects the frequency of total X polysomy and there was a statistical difference between age groups in this respect (p = 0.020). The frequency of X polysomy in 71-years-and-older age group was compared with the frequencies of X polysomy in age groups of under-50, 51-60, 61-70 years, and a significant difference was found in the comparisons with under-50 and 51-60-years age groups (p = 0.048 and p = 0.006, respectively). Also, a statistically significant difference was found between the age groups of 51-60-years and 61-70 years (p=0.041).

The frequency of X polysomy increased with raised age and the highest frequency was recorded in the 71-years-and-older age group. The significant difference between age groups was mainly due to increased frequency of 4X polysomy (p = 0.012), however, there was a borderline significant difference between age groups for cells including 7 (7X polysomy), 8 (8X polysomy), and more copies X chromosome (p = 0.082 and p = 0.066, respectively) (Table

3 and Table 4). Concerning the number of sons, there was no significant difference between women who have monosomy X and polysomy X conditions (p>0.05). However, there was a significant increase of X monosomies in women who gave birth to a boy at their first pregnancies as compared with other women (p = 0.016). On the other hand, there was no significant difference between the women who gave birth to a boy at their first pregnancies and the other women for polysomy X condition (p>0.05) and there was a borderline significant difference for the presence of 5X, 6X and 7X polysomies between those two groups (p=0,067, p=0,083, p=0,087, respectively) (Table 3 and Table 4).

In the present study, the data provided by patients showed that a large proportion (94.9%) of BC women were breastfeeding their children, and the rate of X polysomy was higher in these patients as compared with those who did not breastfeed (p = 0.039) (Table 3 and Table 4). There was no statistically significant relationship between parameters such as first gestational age, the number of pregnancies, age of menopause, familial cancer history and cancer histology and monosomy/polysomy X condition and BC development (p>0,05). The rate of X monosomies was significantly higher in the early stages of BC (I and II) than in the advanced stages (III and IV) (p=0,020). On contrary, X polysomy rate was found higher in the advanced stages than in the early stages, but the difference was not statistically significant (p> 0.05) (Table 3 and Table 4).

Table 1: Clinicopathological Characteristics of the breast cancer patients

Characteristic	**Patients (n=49)**
Age (years)	
Average	57,18 ± 10,515
Median (Range)	57 (32-85)
First gestational age	
≤ 20	18 (36,7%)
21-30	18 (36,7%)
31-40	4 (8,2%)
No data available	9 (18,4%)
Number of pregnancy	
1	1 (2%)
2-3	19 (38,8%)
4-5	10 (20,4%)
6-7	6 (12,2%)
8 ≥	4 (8,2%)
No data available	9 (18,4%)
Number of children	
Average	3,15 ± 1,388
Median (Range)	3 (1-7)
Number of sons	
1	28 (57,1%)
2 ≥	21 (42,9%)
Rank of a son birth	
First	25 (51%)
2nd ≥	15 (30,6%)
No data available	9 (18,4%)
Breastfeeding status	
No	2 (4,1%)
Yes	37 (75,5%)
No data available	10 (20,4%)
Number of miscarriage	
No	20 (40,8%)
1	9 (18,4%)
2 ≥	11 (22,4%)
No data available	9 (18,4%)
Menapausal status	
Pre	7 (14,3%)
Post	33 (67,3%)
No data available	9 (18,4%)
Mean of menapause age	10,78 ± 8,55 (4 month-34 years)
Family history of cancer	
No	19 (38,8%)
Yes	22 (44,9%)
No data available	8 (16,3%)
Histological type of cancer	
Invasive Ductal Carcinoma	31 (63,3%)
Invasive Lobuler Carcinoma	3 (6,1%)
Mixed	15 (30,6%)
TNM Classification	
I	6 (12,2%)
II	21 (42,9%)
III	20 (40,8%)
IV	2 (4,1%)

Table 2: Comparision of the number of all children and boys between Y-positive and Y-negative groups

	Groups				
	Y-positive Breast CA Patients(n=10)	**Y-negative Breast CA Patients (n=39)**	**p-value**	**Y-negative Healthy Control (n=32)**	**p-value**
Number of Children					
1-2	5 (50%)	11 (28,2%)	0,281	23 (71,9%)	0,149
3-4	4 (40%)	16 (41%)		7 (21,9%)	
5-6	0	3 (7,7%)		2 (6,3%)	
7≥	1 (10%)	1 (2,6%)		0	
No data available	0	8 (20,5%)		0	
Number of Boys					
1	5 (50%)	23 (59%)	0,726	24 (75%)	0,238
2≥	5 (50%)	16 (41%)		8 (25%)	

Table 3. Comparison of X chromosome losses and total X chromosome gains with clinicopathological features (Number of cells carrying X chromosome loss and gain /Number of cells analyzed, Mean ± SD)

Patients characterics	**n**	**X Monosomy (Mean ± SD)**	**p-value**	**X Polysomy (Mean ± SD)**	**p-value**
Age					
≤ 50	9	8,787 ± 14,176		22,727 ± 28,619	0,020[b]
51-60	27	15,156 ± 24,405		15,259 ± 21,744	
61-70	8	2,125 ± 1,995		38,457 ± 38,591	
71 ≥	5	2,945 ± 2,276		53,771 ± 33,576	
Number of children					
1-2	16	11,631±20,309		14,580±22,171	
3-4	19	10,966±19,931		23,561±29,888	
5-6	3	4,682±3,290		27,420±39,989	
7 ≥	2	2,632±1,866		56,072±2,158	0,064[b]
Rank of a son birth					
First	25	14,776 ± 22,568	0,016[a]	21,048 ± 28,374	
2nd ≥	15	2,958 ± 2,374		23,276 ± 27,546	
Breastfeeding status					
No	2	3,827 ± 0,735		6,145 ± 5,616	0,039[a]
Yes	37	9,683 ± 18,337		22,744 ± 28,596	
Menapausal status					
Pre	7	10,726±19,374		23,519±26,938	
Post	33	8,542±16,192		21,537±28,293	
TNM Classification					
Early (I and II)	27	16,057 ± 25,214	0,020[a]	19,437 ± 24,078	
Advanced (III and IV)	22	3,930 ± 3,695		30,376 ± 34,799	

[a] Comparision with independent samples-T test; [b] Comparision with one-way analysis of the variance (ANOVA) followed by the LSD post hoc test

Table 4: Comparision of X chromosome copies with clinicopathological parameters (Number of cells carrying X chromosome copies/Number of cells analyzed, Mean ± SD)

Patients characterics	**n**	**3 X**	**p-value**	**4 X**	**p-value**	**5 X**	**p-value**	**6X**	**p-value**	**7X**	**p-value**	**8X≥**	**p-value**
Age													
≤ 50	9	10,211 ± 21,053		9,520 ± 16,004	0,012[b]	2,652 ± 7,205		0,343 ± 0,611		0	0,082[b]	0	0,066[b]
51-60	27	10,077 ± 17,107		3,437 ± 6,693		0,720 ± 1,888		0,650 ± 2,006		0,243 ± 0,792		0,131 ± 0,522	
61-70	8	10,253 ± 8,837		24,916 ± 27,599		2,020 ± 5,480		0,478 ± 1,205		0,695 ± 1,428		0,092 ± 0,194	
71 ≥	5	23,578 ± 22,933		19,587 ± 31,597		2,880 ± 5,998		3,807 ± 8,293		2,407 ± 5,273		1,511 ± 3,379	
Number of children													
1-2	16	7,141 ±13,251		4,558 ±6,405		1,969 ±5,621		0,677 ±2,184	0,034[b]	0,074 ± 0,248	0,014[b]	0,160 ±0,642	0,007[b]
3-4	19	11,405 ±17,023		10,647 ±20,507		0,483 ±1,517		0,473 ±1,421		0,489 ±1,243		0,061 ±0,226	
5-6	3	4,326 ±3,203		5,881 ±7,787		4,534 ±7,853		6,213 ±10,761		3,946 ± 6,835		2,518 ±4,362	
7 ≥	2	31,271 ±30,957		24,217 ±33,410		0,286 ±0,125		0,197 ±0,279		0,098 ± 0,139		0	
Rank of a son birth													
First	25	10,286 ± 17,122		5,761 ± 11,438		2,124 ±5,216	0,067[a]	1,531 ±4,124	0,083[a]	0,901 ±2,527	0,087[a]	0,444 ±1,577	
2nd ≥	15	9,955 ± 14,542		13,153 ± 21,747		0,116 ±0,264		0,039 ±0,109		0		0,012 ±0,050	

Breastfeeding status													
No	2	1,275 ± 0,152		4,772 ± 5,631		0,097 ± 0,137		0		0		0	
Yes	37	10,532 ± 16,515		8,817 ± 16,834		1,429 ± 4,372		1,050 ± 3,441		0,609 ± 2,107		0,305 ± 1,304	
Menapausal status													
Pre	7	9,353 ± 16,289		10,631 ± 18,320		3,171 ± 8,226		0,363 ± 0,660		0		0	
Post	33	10,333 ± 16,202		8,088 ± 16,030		0,989 ± 2,840		1,101 ± 3,638		0,682 ±2,223		0,342 ± 1,378	
TNM Classification													
Early (I and II)	27	10,028 ± 15,033		8,104 ± 14,622		1,004 ± 4,183		0,139 ± 0,368		0,160 ± 0,756		0	
Advanced (III and IV)	22	13,325 ± 20,248		11,678 ± 22,067		2,126 ± 4,535		1,806 ± 4,385	0,09[a]	0,901 ± 2,608		0,528 ± 1,670	

[a] Comparision with independent samples-T test; [b] Comparision with one-way analysis of the variance (ANOVA) followed by the LSD post hoc test

Discussion

Mc is accepted as an important phenomenon that has the potential to expand new horizons in human health, but fetal Mc can also be seen as a normal physiological event arising during pregnancy. We are just beginning to understand the implications of McCs in the host body, which may be beneficial or detrimental to host health. Some hypotheses regarding Mc have been formulated over the past decade and much of the controversy has focused around the role of McCs in the pathogenesis of various diseases and tissue repair. In all situations, feto-maternal Mc should be considered as a physiological event that its beneficial/detrimental effects and their underlying mechanisms are unknown. In this study, we investigated the potential role of FMc in BC development.

The present study results showed that BC patients (including Y-negative and Y-positive patients) have more children than Y-negative healthy ones. It can be thought that the increased number of pregnancies will increase the number of fetal cells passing to the mother's circulation and tissues and consequently the increased amount of fetal cells in the mother's body will elevate the risk of BC. A previous study reported that FMc was detected in 100% of samples from tumors and their surrounding tissues and in 64% of samples from normal breast tissue. The same study also reported that the relative expression of the SRY gene had a median 5.5 times larger in BC tumor than in its periphery [11], a result which may be interpreted that FMc condition caused overexpression of the SRY locus on the Y chromosome in FMcCs which migrated to tumor tissues in BC women having a son(s). In the present study, tumoral breast tissues were positive for FMcCs in 20, 4% of cases. But, no FMcC was found in the blood of any control woman. The prevalence of FMcCs was significantly greater in women with BC than healthy controls (20, 4%, 0%, respectively and p=0,000). Indeed, in a previous study, the positive FMcCs rate was also found to be 21% [27], and this rate was consistent with the rate we found.

In our previous study, the FMcCs were found in 26, 9% of lung cancer and in 0.9% of bladder cancer women who gave birth to boys. The FMcCs were not seen in the healthy control group in this study [18]. It has also been shown that FMcCs cluster in lung tumors in women decades after pregnancy, so they have the ability to migrate toward injury sites and function as stem cells in the woman's body [14].

A normal physiological phenomenon can turn into physiopathology under inappropriate conditions. In this context, the presence of FMcCs can emerge as a potential contributing factor in the development of certain diseases, including cancer, in women. These cells sometimes help to suppress tumor development by taking on an immunosurveillance role, and other times they may behave like cancer stem cells and contribute to tumor growth [28]. Alternatively, FMcCs may play a protective role in suppressing tumor growth in pregnant women who developed BC.

The FMcCs are matured and formatted in the mother's body and then they are transferred from mother to baby through breastfeeding. If the mother has a miscarriage or abortion, the FMcCs will not be transferred to the offspring, and these cells will remain in the mother's breast tissue and will have to wait. But, the FMcCs which don't return to the offspring will cause alloimmunity in the mothers' body. These cells, as a result of the mutations or microenvironmental influences, may lose their regulatory controls that normally keep their proliferation and differentiation in check, and thus, they may be converted to cancer cells. FMcCs, similar to spreading cancer cells, are highly proliferative. Tumors may be loaded with FMcCs, for example, suggesting that they might help drive cancer. Breast carcinomas or melanomas have been suspected to be more severe during gestation.

A determinative feature of human cancers is genetic instability. Most human cancer exhibits structural and numerical chromosome anomalies, and there is growing evidence that at least some of these anomalies play an important role in the development of all types of cancer [29]. In the present study, X chromosome aneuploidies were found in 35.68% of the cells examined in BC patients. Sansregred & Swanton reported that high chromosome dissociation errors which are observed in cancer cells from solid tumors, almost always lead to aneuploidy [30]. In a study on the effects of X chromosome aneuploidies in BC patients, it has been shown that increases in the number of X chromosomes were associated with large-sized tumors and advanced histological stages [31]. Moreover, genes involved in carcinogenesis and associated with malignant progression in different tumor types have been reported on the X chromosome [32]. Some studies also reported that chromosomal gain/loss is associated with poor prognosis, metastasis, and tumor progression in breast tumors [33,34]. Such changes may be important for the early detection of cancer and may be a diagnostic criterion for BC as well.

In the present study, there was an increase in the number of X chromosomes in breast cells of patients with increased age, especially in breast cells of those over 71 years of age,

and this increase was found statistically significant (p = 0.020). It has been reported by Jacobs *et al*. that sex chromosome aneuploidies increase with raised age [17]. Additionally, a study involving lymphocyte cultures of healthy men and women showed an increase in sex chromosome anomalies with raised age. Probably the increase in the rate of aneuploidy due to aging may be the result of a high somatic mutation rate or accumulation of these mutations and decreased genome stability capacity due to telomere shortening [35].

A high number of full-term pregnancies is associated with a reduced risk of BC, but the risk of the disease temporarily increases during pregnancy. Despite male fetuses are associated with several factors such as pregnancy toxemia, increased levels of a-fetoprotein (AFP), and increased levels of sex hormone-binding globulin, which are linked to a low BC risk; little attention has been paid to a possible association of BC risk with the sex of offspring. Apparently, the protective effect of pregnancies with male fetuses is limited to women younger than 40 years. Endocrine or other metabolic processes associated with the male fetus rather than the female fetus can provide relative protection against BC during childbearing [36]. In the present study, considering that 67.3% of the patients are already in the menopause period and the average age is 48,818 ± 5,311 (median 49, min. 38 and max. 59), it can be clearly seen that their pregnancies have not protected them against BC. The presence of younger [29,44 ± 5,003 (min. 21- max. 38)] and healthy controls which have at least one male child and have not developed BC yet, supports this fact.

X polysomia was higher in women with 7 or more children as compared with women with 1-2 children in our patient group it was found statistically significant at the border (p = 0.064). This finding led us to question whether the number of children affects BC development. However, the findings in the literature showed that more full-term pregnancies are associated with a reduction in BC risk [37]. Women without children and women who had children later in their lives were reported to be at risk for BC development [38].

Since the 70s, researches have been conducted on whether breastfeeding has a protective effect against BC and other cancers; while some say it has a protective effect, some argue it is not or has a slight effect against BC development. Various studies used different methodologies and conducted on diverse patient groups having different features (such as the age of menarche, age at first pregnancy, family history of cancer, how many children a woman has had, oral contraceptive use, menopause, and smoking) led to these conflicting results. It was reported by Chowdhury *et al*. that the risk of developing breast carcinoma was reduced by 26% among women who breastfed for more than 12 months as compared with women who did not breastfeed [39]. Likewise, Ip *et al*. reported a decrease in the relative risk of BC in parous women according to breastfeeding history and number of births [40]. They reported the reduced risk of BC in parous women particularly after four or more births. In the present study, almost all BC women (94.9%) with data breastfed their children over 12 months in the past and, their ages at first pregnancy were below 30 years. It was found that X polysomies were higher in the patients who breastfed in the past when compared with patients with no breastfeeding story. Even though both breastfeeding and early gestational age are expected to protect people against breast cancer, the results of the present study showed that having these features may not protect mothers against BC.

The average number of children the patients had was 3.15 ± 1.388 and it was seen that having more children was also not sufficient to protect against BC. Another factor in BC development is the age of menopause. The mean menopausal age of our patients was 48,818 ± 5,311 (median 49, min. 38, and max. 59). It has been reported that especially menopause after the age of 55 increased the risk of ovarian, breast, and uterine cancers [41]. Similarly, the risk of BC increases with an early start of first menstruation before age 12. Surakasula *et al*. explained this situation that longer exposure to estrogen in women increases the risk of BC [41]. The results of our study also showed that the age of menopause was not effective in BC development. Concerning a family history of cancer, almost half of the patients (44, 9%) have a familial history of cancer in the present study. Cumulative effects of mutations inherited from parental generations may have played a role in BC development and the occurrence of X-polysomy. As a result, although it has been said that there is a relationship between breastfeeding and BC in recent years, many factors play a role in cancer formation, much more remains to be determined.

Vargas-Rondon *et al*. reported that chromosome aberrations can occur during the early stages of tumorigenesis [42]. In another study, it has been reported that monosomies of chromosomes 1, 3, and X can be routinely used as positive genetic biomarkers to diagnose cervical cancers [43]. In the present study, X chromosome monosomies were found higher in early-stage breast cancers. Therefore, X chromosome loss can be used as an early marker in BC diagnosis.

Conclusion

This study demonstrated that FMcCs was present in the tumor and surrounding tissues of BC patients who have a son(s). Similarly, neoplastic breast tissue harbors significantly higher levels of male cells than peripheral blood tissue. We speculated that FMcCs could have a general beneficial role against cancer, but it might have triggered cancer development due to allogeneic maternal immune reaction. Therefore, future studies aimed to better understand fetal-maternal Mc and its association with BC development/progression will help clarify the effects of fetal-maternal Mc on BC. We found that the presence of sex chromosome aneuploidy is a common finding in BC patients and the frequency of sex chromosome aneuploidy greatly increases with age. A significantly higher frequency of X monosomies detected especially in the early stage-tumors implies an increased genomic instability during BC progression. Therefore, the presence of sex chromosome aneuploidy is a significant predictor for BC progression and patient survival, and it deserves to be focused on in therapeutic planning.

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References

1. Bray F, Jacques Ferlay ME, Soerjomataram I, Sıegel RL, Torre LA*l*. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A

Cancer Journal for Clinicians. 2018; 0:1-31.
2. Bayram S, Sumbul AT, Dadas E. A functional HOTAIR rs12826786 C>T polymorphism is associated with breast cancer susceptibility and poor clinicopathological characteristics in a Turkish population: a hospital-based case–control study. Tumor Biology. 2016; 37:5577-5584.
3. Key TJ, Verkasalo PK, Banks E. Epidemiology of breast cancer. Lancet Oncol. 2001; 2(3):133-40.
4. Gadı VK, Nelson JL. Fetal Microchimerism in Women with Breast Cancer. Cancer Res. 2007; 67(19):9035-9038.
5. Kamper-Jørgensen M, Bıggar RJ, Tjønneland A, Hjalgrım H, Kroman N *et al*. Opposite effects of microchimerism on breast and colon cancer. Eur J Cancer. 2012; 48(14):2227-2235.
6. Nelson JL. Maternal-fetal immunology and autoimmune disease: is some autoimmune disease auto-alloimmune or allo-autoimmune? Arthritis Rheum. 1996; 39:191-194.
7. Fugazzola L, Cırello V, Beck-Peccoz P. Fetal microchimerism as an explanation of disease. Nat Rev Endocrinol. 2011; 7(2): 89-97.
8. Galofré JC, Duntas LH, Premawardhana LD, Davıes TF. Advances in Graves' Disease. J Thyroid Res. 2012; 2012: 809231.
9. Hansen KP, Kamper-Jørgensen M. Microchimerism and cancer in women. Ugeskr Laeger. 2017; 179(9):V10160722.
10. Broestl L, Rubın JB, Dahıya S. Fetal microchimerism in human brain tumors. Brain Pathol. 2018; 28(4):484-494.
11. Nemescu D, Ursu RG, Nemescu ER, Negura L. Heterogeneous Distribution of Fetal Microchimerism in Local Breast Cancer Environment. PLoS One. 2016; 11(1):e0147675.
12. Sawaya HHB, Jımenez SA, Artlett CM. Quantification of fetal microchimeric cells in clinically affected and unaffected skin of patients with systemic sclerosis. Rheumatology. 2004; 43:965-968.
13. Srıvatsa B, Srıvatsa S, Johnson KL, Samura O, Lee SL, *et al*. Microchimerism of presumed fetal origin in thyroid specimens from women: a case-control study. Lancet. 2001; 358:2034-2038.
14. O'donoghue K, Sultan HA, AL-Allaf FA, Anderson JR, Wyatt-Ashmead J, *et al*. Microchimeric fetal cells cluster at sites of tissue injury in lung decades after pregnancy. Reprod Biomed Online. 2008; 16(3):382-90.
15. Cha D, Khosrotehranı K, Kım Y, Stroh H, Bıanchi DW *et al*. Cervical cancer and microchimerism. Obstet Gynecol. 2003; 102(4):774-81.
16. Rıske CB, Morgan R, Ondreyco S, Sandberg AA. X and Y chromosome loss as sole abnormality in acute non-lymphocytic leukemia (ANLL). Cancer Genet Cytogenet. 1994; 72:44-47.
17. Jacobs PA, Maloney V, Cooke R, Crolla JA, Ashworth A, *et al*. Male breast cancer, age and sex chromosome aneuploidy. Br J Cancer. 2013; 108: 959–963.
18. Tastemır Korkmaz D, Demırhan O, Abat D, Demırberk B, Tunc E, *et al*. Microchimeric Cells, Sex Chromosome Aneuploidies and Cancer. Pathol Oncol Res. 2015; 21(4):1157-65.
19. BEN-DAVID U, AMON A. Context is everything: aneuploidy in cancer. Nature Reviews Genetics. 2020; 21:44-62.
20. Scheıke O, Vısfeldt J, Petersen B. Male breast cancer. 3. Breast cancer in association with Klinefelter syndrome. Acta Path Microbiol Scand A. 1973; 81(3):352-358
21. Borah V, Shah PN, Ghosh SN, Sampat MB, Jussawalla DJ. Further studies on the prognostic importance of Barr body frequency in human breast cancer: with discussion on its probable mechanism. J Surg Oncol. 1980; 13(1):1-7.
22. Krasna I, Lee H, Sınılow ML, Scıorra P, Eıerman L. Risk of malignancy in bilateral streak gonads: the role of the Y chromosome. J Pediatr Surg. 1992; 27:1376-1380.
23. Mıssıaglıa E, Moore PS, Wıllıamson J, Lemoıne NR, Falconı M *et al*. Sex chromosome anomalies in pancreatic endocrine tumors. Int J Cancer. 2002; 98(4):532-538.
24. Bottarellı L, Azzonı C, Necchı F, Lagrasta C, Tamburını E, *et al*. Sex chromosome alterations associate with tumor progression in sporadic colorectal carcinomas. Clin Cancer Res. 2007; 13(15 Pt 1):4365-4370.
25. Cantú ES, Moses MD, Nemana LJ, Pıerre RV. Sex chromosome loss in adults with haematological neoplasms. Br J Haematol. 2015; 169(6):899-901.
26. Tastemır D, Demırhan O, Gurkan E, Tunc E, Inandıklıoglu N. Applications of Fluorescence in Situ Hybridization (Fısh) for Detecting Genetic Changes in Hematological Malignancies. Journal of Cancer Therapy. 2011; 2(2):125-134.
27. Dhımolea E, Denes V, Lakk M, AL-Bazzaz S, Azız-ZAMAN S, *et al*. High male chimerism in the female breast shows quantitative links with cancer. Int J Cancer. 2013; 133:835-842.
28. Sawıckı JA. Fetal microchimerism and cancer. Cancer Res. 2008; 68:9567-9569.
29. Grıgorova M, Lyman RC, Caldas C, Edwards PAW. Chromosome abnormalities in 10 lung cancer cell lines of the NCI-H series analyzed with spectral karyotyping. Cancer Genet Cytogenet. 2005; 162:1-9.
30. Sansregred L, Swanton C. The Role of Aneuploidy in Cancer Evolution. Cold Spring Harb Perspect Med. 2017; 7(1):a028373.
31. Nakopoulou L, Panayotopoulou EG, Gıannopoulou I, Tsırmpa I, Katsarou S, *et al*. Extra copies of chromosomes 16 and X in invasive breast carcinomas are related to aggressive phenotype and poor prognosis. J Clin Pathol. 2007; 60:808-815.
32. Ross Mt, Grafham DV, Coffey AJ, Scherer S, MCLAY K, *et al*. The DNA sequence of the human X chromosome. Nature. 2005; 434:325-337.
33. Kersemaekers AM, Van DE Vıjver MJ, Kenter GG, Fleuren GJ. Genetic alterations during the progression of squamous cell carcinomas of the uterine cervix. Gene Chromosome Cancer. 1999; 26:346-354.
34. Pıao Z, Malkhosyan SR. Frequent loss Xq25 on the inactive X chromosome in primary breast carcinomas is associated with tumor grade and axillary lymph node metastasis. Gene Chromosome Cancer. 2002; 33:262-269.
35. Sahın E, Depınho RA. Linking functional decline of telomeres, mitochondria and stem cells during ageing.

Nature. 2010; 464:520-528.
36. Hsıeh C, Wuu J, Trıchopoulos D, Adamı HO, Ekbom A. Gender of offsprıng and maternal breast cancer rısk. Int J Cancer. 1999; 81(3):335-8.
37. Keınan-Boker L, Lerner-Geva L, Kaufman B, MEIROW D. Pregnancy associated breast cancer. Isr Med Assoc J. 2008; 10:722-727.
38. Brıtt K, Ashworth A, Smalley M. Pregnancy and the risk of breast cancer. Endocr Rel Cancer. 2007; 14:907-933.
39. Chowdhury R, Sınha B, Sankar MJ, Taneja S, Bhandarı N, *et al*. Breastfeeding and Maternal Health Outcomes: A Systematic Review and Meta-Analysis. Acta Paediatr. 2015; 104(Suppl 467):96-113.
40. IP S, Chung M, Raman G, Chew P, Magula N *et al*. Breastfeeding and maternal and infant health outcomes in developed countries. Evid Rep Technol Assess (Full Rep). 2007; 153:1-186.
41. Surakasula A, Nagarjunapu GC, Raghavaıah KV. A Comparative Study of Pre- And Post-Menopausal Breast Cancer: Risk Factors, Presentation, Characteristics and Management. J Res Pharm Pract. 2014; 3(1):12-18.
42. Vargas-Rondón N, Vıllegas VE, Rondón-Lagos M. The Role of Chromosomal Instability in Cancer and Therapeutic Responses. Cancers (Basel). 2018; 10(1):4.
43. Wang X, Zheng B, Zhang RR, LI S, Chen X, *et al*. Automated analysis of fluorescent in situ hybridization (FISH) labeled genetic biomarkers in assisting cervical cancer diagnosis. Technol Cancer Res Treat. 2010; 9(3):231-42.