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Comparative Study of Chromosome Aberrations Induced with Aphidicolin in Women Affected by Breast Cancer and Cervix Uterine Cancer

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ABSTRACT: *Blood samples were obtained from 80 women: Twenty of these samples were from women affected by ductal infiltrating breast carcinoma, twenty from women affected by cervix uterine cancer, and forty individuals were screened for a control group. The search for chromosome instability that is known to affect individuals with cancer was performed through chromosome analysis in nontumor cells, intending to establish frequency and different types of numerical and structural aberrations. The results, in regard to spontaneous and aphidicolin induced chromosome aberrations, showed a significantly greater frequency ($p < 0.001$) of chromosome fragility, as well as other numerical and structural aberrations in breast cancer patients when compared to the control group. Similar results were obtained from cervix uterine cancer patients with the exception of certain numerical aberrations in which no significant differences were found. This suggests the existence of a certain degree of chromosomal instability affecting individuals with both types of cancer. The increase in fragility may play an important role in the biologic behavior and progression of cancer.* © *Elsevier Science Inc., 1997*

INTRODUCTION

Cancer is one of the most important causes of death in Ecuador, accounting for more than 5,700 deaths per year [1]. According to epidemiologic data, uterine cervix cancer is the first cause of death and disease among women followed by breast cancer in our country [1, 2].

The relationship between the presence of a high frequency in chromosomal aberrations and predisposition to cancer has been well established in the "chromosomal instability syndromes" [3] and this genomic instability, although it is also present in a low frequency in peripheral blood lymphocytes in individuals affected by several types of cancer [4, 5]. Additionally, Vernole et al [6] have reported an increased frequency of structural chromosomal aberrations in families with high incidences of cancer, affecting both individuals with cancer and those members of the family with no evidence of neoplasia.

The precise relationship between fragile sites and cancer is not well established, even though it has been found that 67% of *in vitro* mutagen induced fragile sites are coincidental with cancer-specific chromosome breakpoints and 72% are coincidental with oncogne loci. So, fragile sites have been proposed to be involved in carcinogenesis [7–12].

Considering that aphidicolin, a diterpenoid micotoxin that specifically inhibits the eukaryotic DNA polymerases alpha and delta primarily involved with chromosomal DNA repair and replication [13, 14], is one of the most important common fragile sites inducers in a highly nonrandom manner in cultured lymphocytes [15], we have done a comparative study of chromosome alterations both spontaneous and induced by aphidicolin in two groups of women. One group of individuals was affected by breast cancer and the other group of women was affected by cervix uterine cancer.

MATERIALS AND METHODS

The subjects of this study were forty women, ranging in age from 28 to 76 years, twenty of them affected by invasive ductal carcinoma of the breast and twenty affected by squamous cell carcinoma of the uterine cervix, and forty healthy women as controls (Table 1). All of these women

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were untreated at the time of examination, and none of them had smoking habits.

Epidemiologic data concerning several factors like age at diagnosis, menopausal status, number of children (stillborns, miscarriages), and age of onset of sexual activity is summarized in Table 1.

Cytogenetics

Two types of culture medium were used with each individual: 1) A normal medium containing RPMI 1640, supplemented with fetal bovine serum (10%), penicillin (10,000 IU/ml)-streptomycin (10 mg/ml), phytohemagglutinin (1mg/ml), L-glutamine (200 mM), and Hepes buffer (1 mM/ml) and 2) the above preparation with the addition of Aphidicolin (AP) in a final concentration of 0.12 μM/ml. Whole blood was cultured for 72 hours at 37°C. Harvesting was performed according to standard techniques. Air-dried chromosome preparations were stained with 5% Giemsa solution. One-hundred metaphases were scored for each culture on precoded slides. The number of metaphases with aberrations (breaks and gaps) per individual and the percentage of damaged cells were evaluated, as well as numerical and structural aberrations. Chromosomal aberrations such as breaks, gaps, and acentric fragments, as well as all the numerical aberrations, were classified according to the Statements of the International System for Human Cytogenetic Nomenclature [16].

Statistical analysis was performed using Student's t-test and U Mann-Whitney test to compare data from affected women with their corresponding controls in both types of culture medium.

RESULTS

In relation to the epidemiologic data (Table 1), our results show that 75% of breast cancer patients are older than 40 years and the average age is 52 years for the sample. We have found two age peaks in the affected individuals, one between 36 and 48 years and the other around 60 years. In cervix uterine cancer, 45% of the individuals studied ranged between 36 and 50 years. In relation to menopausal status, breast cancer patients were predominantly postmenopausal women (70%), the other 30% were in the premenopausal stage. In cervix uterine cancer 60% of women were postmenopausal, 20% were premenopausal, and 20% were undergoing their fertile age.

Breast cancer patients showed an average of 3 children per woman, and 60% of them presented with at least one spontaneous abortion. In cervix uterine cancer 85% of the patients showed more than 3 pregnancies and 45% presented with at least one spontaneous abortion.

We have found that 10% (2 individuals) of affected women with cervix uterine cancer started their sexual activity around 15 years of age, 9 individuals (45%) between 16 and 20 years of age, and 4 individuals (20%) between 21 and 25 years of age. This data was not available for 5 individuals included in the affected group with cervix uterine cancer.

Chromosomal aberrations were scored in 8,000 metaphase plates for each type of cancer, breast and cervix, 4,000 metaphases for the affected group and 4,000 metaphases for the control group in each type of cancer. Table 2 summarizes the cytogenetic findings of chromosome fragility and altered metaphases. The aberrations are expressed both in absolute numbers and percentages. The statistical analysis of chromosome fragility and altered metaphases showed significant differences ($p < 0.001$) between the affected individuals and their corresponding control groups in both types of culture mediums and in both types of cancer. A similar behavior was found in relation to the frequency of both parameters when the results from the two types of culture mediums were compared within the affected groups both in cervix uterine and breast cancer. However, the control group in both types of cancer did not show statistical differences between the normal RPMI medium and the medium with the clastogen AP. Acentric

Table 1 Epidemiological data of the affected individuals

Type of cancer	Ind. no.	Age	Sibl/aborts	Menopausal status	Sex/life onset
Breast	14	64	3/1	M	–
Breast	15	34	4/–	NM	–
Breast	25	40	2/–	M	–
Breast	27	32	3/1	NM	–
Breast	29	48	–/–	M	–
Breast	33	63	4/–	M	–
Breast	34	56	6/3	M	–
Breast	36	61	8/1	M	–
Breast	45	42	1/1	NM	–
Breast	48	50	3/–	M	–
Breast	49	70	1/–	M	–
Breast	68	51	6/1	M	–
Breast	69	52	4/4	M	–
Breast	95	40	2/–	NM	–
Breast	103	41	1/–	NM	–
Breast	105	68	7/1	M	–
Breast	116	39	3/3	NM	–
Breast	127	62	1/2	M	–
Breast	130	62	3/2	M	–
Breast	131	58	6/1	M	–
Cervix	5	76	8/–	M	18
Cervix	6	40	11/–	NM	20
Cervix	11	70	13/–	M	–
Cervix	12	32	3/1	NM	22
Cervix	18	28	4/–	NM	15
Cervix	19	75	1/–	M	–
Cervix	22	50	6/–	M	25
Cervix	27	32	5/1	NM	17
Cervix	32	44	9/–	NM	18
Cervix	43	46	2/1	M	–
Cervix	53	50	12/2	M	–
Cervix	66	51	8/1	M	20
Cervix	80	65	7/–	M	16
Cervix	83	52	19/4	M	–
Cervix	84	37	6/4	NM	17
Cervix	104	42	6/–	NM	25
Cervix	115	56	9/–	M	20
Cervix	118	42	8/2	NM	17
Cervix	128	63	9/–	M	23
Cervix	135	50	11/–	M	15

Abbreviations: Ind, individual; Sibl, siblings; M, menopausal; NM, nonmenopausal.

Table 2 Metaphases with chromosome fragility in all analyzed groups (affected and not affected)

Type of cancer	Group	Medium type	Number of analyzed metaphases	Number of metaphases with chromosome fragility	Percentage
Breast	Af	N	2,000	606	30.3
	Af	Ap	2,000	970	48.5
	C	N	2,000	36	1.8
	C	Ap	2,000	50	2.5
Cervix	Af	N	2,000	502	25.1
	Af	Ap	2,000	738	36.9
	C	N	2,000	38	1.9
	C	Ap	2,000	48	2.4

Abbreviations: Af, individual with cancer; Ap, RPMI medium with aphidicolin; C, control individual; N, RPMI normal medium.

fragments were not very common in either of the studied groups (Table 3).

Three types of numerical aberrations were scored for (Table 4): Hypodiploidy or losses of one or two chromosomes that showed a significant increase ($p < 0.001$) in the affected individuals from both types of cancer when compared to the control group. Hyperdiploidy or gain of one or more chromosomes, which in breast cancer patients was found exclusively in the affected group, in cervix uterine cancer patients there were no statistically significant differences with the corresponding control group. Polyploidies or gain of complete sets of chromosomes, which in breast cancer patients showed a significant increase ($p < 0.001$) when compared to healthy individuals, was not significant in cervix uterine cancer patients in relation to the control group.

Chromosomal gains and losses are detailed only for the group of affected individuals because they were present in considerable amounts.

In breast cancer the chromosomal group C was preferentially affected by hypodiploidy, followed by groups E, G, F, and D. In cervix uterine cancer the highest frequency

Table 3 Chromosome aberrations per group analyzed

Type of cancer	Group	Medium type	Number of analyzed metaphases	G		B		ACE
				CT	CS	CT	CS	
Breast	Af	N	2,000	448	674	34	14	34
	Af	Ap	2,000	692	840	50	24	12
	C	N	2,000	13	29	3	3	–
	C	Ap	2,000	19	32	4	2	1
Cervix	Af	N	2,000	150	462	38	28	4
	Af	Ap	2,000	298	738	70	58	4
	C	N	2,000	13	23	6	2	–
	C	Ap	2,000	11	31	8	4	–

Abbreviations: Af, individual with cancer; Ap, RPMI medium with aphidicolin; C, control individual; N, RPMI normal medium. ACE, acentric fragment; B, break; CS, chromosome; CT, chromatid; G, gap.

Table 4 Numerical aberrations per group analyzed

Type of cancer	Group	Medium type	Number of analyzed metaphases	HYPODIP		HYPERDIP	POLYP
				44 cs	45 cs	(47–57 cs)	(92 ± cs)
Breast	Af	N	2,000	150	242	48	40
	Af	Ap	2,000	134	328	44	22
	C	N	2,000	2	2	–	8
	C	Ap	2,000	1	2	–	4
Cervix	Af	N	2,000	76	122	10	15
	Af	Ap	2,000	92	134	18	7
	C	N	2,000	2	5	2	–
	C	Ap	2,000	2	4	4	1

Abbreviations: Af, individual with cancer; Ap, RPMI medium with aphidicolin; C, control individual; cs, chromosomes; HYPERD, hyperdiploidy; HYPODIP, hypodiploidy; N, RPMI normal medium; POLYP, polyploidy.

Table 5 Comparative statistical analyses of chromosome fragility found in the different groups, affected and control, using Student t test ($p < 0.05$)

Type of cancer	AfN/AfAp	AfN/CN	AfAp/CAp	CN/CAp
Breast	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p > 0.04$
Cervix	$p < 0.05$	$p < 0.001$	$p < 0.001$	$p > 0.05$

Abbreviations: Af, individual with cancer; Ap, RPMI medium with aphidicolin; C, control individual; N, RPMI normal medium.

of chromosomal losses corresponds to group D, followed by group G, C, E and A.

Chromosome gains in the group of patients with breast cancer, affected mainly chromosomes in groups C, E, D, G and B. In cervix uterine cancer the main chromosomal group involved in hyperdiploidy was group C, followed by groups G, E, F, and D. Tables 5 and 6 show statistic comparative analysis of groups.

DISCUSSION

The evaluation of some epidemiologic data, as age distribution, menopausal status, number of children, individual parity, and the onset of sexual activity, in the individuals affected by breast and cervix uterine cancer (Table 1) were similar to other series of studies [17–24].

The results of this investigation show a highly significant difference between affected individuals and their controls concerning both spontaneous aberrations (normal culture medium) and induced alterations (medium with the clastogen aphidicolin), which may indicate the existence of a genetic instability phenomenon that appears to be one of the characteristics of nontumor cells of cancer patients [7, 25, 26]. This leads us to conclude that chromosome fragility and frequency of altered metaphases in peripheral blood lymphocytes of breast cancer and cervix uterine cancer patients can be considered as a reliable marker for genetic predisposition to both types of human neoplasia as has been previously reported by other authors [5, 7, 25]. However, this is not consistent with several previous studies in breast cancer patients [27] that didn't show statistical differences in chromosome fragility and frequency of altered metaphases among affected and control individuals and, therefore, do not support the idea of the existence of genetic instability in individuals affected by breast cancer.

The evidence of a significant increase in chromosome aberrations due to the presence of aphidicolin in the culture medium, can be explained by the fact that this clastogen, a diterpenoid mycotoxin that acts as an eukaryotic DNA polymerases alpha and delta inhibitor, is directly involved with the DNA synthesis and replication processes [28, 29]. Thus, the presence of AP is likely to inhibit DNA repair mechanisms leading to the maintenance of a series of chromosome aberrations caused by chromosome instability. This seems to be even more evident when we consider that the control group showed no statistical differences in chromosome fragility and altered metaphases for both types of culture mediums, which may be explained by the fact that control individuals are genetically stable so that repair inhibitors such as aphidicolin have no particular effect on chromosomal DNA.

In regard to numerical aberrations, hypodiploidy has been demonstrated to be a reliable marker for both types of cancer. Losses of one or two chromosomes were quite common in the affected group, however, they seem to involve specific groups of chromosomes for each type of cancer. In breast cancer patients chromosomal losses of group E could be related to the frequent loss of heterozygosity affecting the whole chromosome 17 already found in studies on tumoral cells [30, 31]. For cervix uterine cancer several studies on solid tumors have reported chromosomal losses affecting groups B, D, and G [32], which are also observed in our study. These results may lead us to conclude that these are critical chromosomal sites for the development of each type of human cancer.

As with hypodiploidy, chromosomal gains or hyperdiploidy presented a nonrandom distribution among groups, specially in breast cancer, where groups C and E were the most frequently involved in these alterations. The few studies available for this type of cancer in peripheral blood lymphocytes do not consider numerical aberrations except for a study by Barrios, et al [25], which reports the presence of a high frequency of trisomy of the X chromosome, which has also been related to a high frequency of breast cancer among individuals affected by Klinefelter syndrome [25].

Sreekantaiah et al [33] showed specific gains of chromosomes in tumor cells in cervix uterine cancer. These results are similar to ours if we consider that some of the

Table 6 Comparative statistical analysis of the numerical aberrations in all the groups

Type of cancer	Type of Ab	Afn/AfAp	AfN/CN	AfAp/CAp	CN/CAp
Breast	Hypod.	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p > 0.05$
	Hyperd.	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p > 0.05$
	Polyp.	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p > 0.05$
Cervix	Hypod.	$p > 0.05$	$p < 0.001$	$p < 0.001$	$p > 0.05$
	Hyperd.	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$
	Polyp.	$p > 0.05$	$p < 0.05$	$p > 0.05$	$p > 0.05$

Abbreviations: Af, individual with cancer; Ap, RPMI medium with aphidicolin; C, control individual; N, RPMI normal medium; Ab, aberration; Hypod, hypodiploidy; Hyperd, hyperdiploidy; Polyp, polyploidy.

most frequent groups that gained chromosomes in our study were C, E, and F.

Polyploidy was quite infrequent in patients studied, however, its presence even in the normal culture medium could be considered as additional evidence of chromosome instability.

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