Chromosome Breaks and Gaps in Breast Cancer Patient

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Breast cancer is the most frequent tumour among of western countries women, (Sandberg 1990). In India, it is the second most common cancer. The rate per 100,000 of breast cancer is 20.2 (Jussawala 1973). This malignancy can be divided into various stages as follows (Walter and Israel 1987):

Stage I The tumour were confined to the breast including the skin immediately over it.

Stage II Tumour involved the axillary lymph nodes on the same side, but both breast and nodes are mobile.

Stage III Tumour spread to the chest wall on the supra claviclar nodes on the same side.

Stage IV More spread then stage III and included blood borne metastases.

There are various studies where chromosomal changes were seen as an important feature responsible for this cancer (Cruciger *et al.* 1976, Pathak 1980, Gebhart *et al.* 1986, Pandis *et al.* 1993, Thompson *et al.* 1993, Hainsworth *et al.* 1991, Rohen *et al.* 1995) and also some form of genetics instability of an individual might be a predisposing factor (Atkin 1976, German 1974).

In the present study we report the significant higher rate of chromosomal breaks and gaps in the lymphocyte of stage II breast cancer cases as compared to other next stages and their normal controls.

Material and methods

Peripheral blood (PB) was collected from 15 patients with breast cancer (3 stage II, 8 stage II and 4 stage IV). Five normal women were also studied as age matched controls. Smokers, alcoholics and patients taking any kinds of drug were excluded from this study. The intra-veinous blood was collected at 9.00 am. and cultures were put at 10.00 am.

Whole blood cultures were set up by conventional method (Husain and Bamezai 1988) i.e., 0.2 ml of heparinized whole blood was added to 6.00 ml RPMI-1640 medium (Bios, Bombay, India) in a sterile culture bottle under aseptic conditions. To this 0.3 ml of phytohemagglutinin-M (PHA-M, Gibco) was added and the cultures was shaken and incubated at 37°C for 72 hr. Colchicine (0.2 ml–10 µl/ml, Bios) was added at 68 hr i.e., at 4 hr before termination of the cultures to arrest the cells at metaphase, because 4 hr exposure in our system gives better results. The cultures were harvested at 72 hr and slides were prepared by routine procedures, and stained with Giemsa stain (Watt and Stephen 1986) and slides were observed under the microscope. The same slides were observed by two persons to confirm the breaks and gaps.

Only chromosomal breaks and gaps were scored according to the criteria laid down by ISCN (1985) which is as follows:

Fig. 1. Partial Photograph representing with arrow; Chromosome gap (left); only chromatid break (right).

Table 1. Average rates of chromosome gaps and breaks occurring/cell in lymphocytes of patients with various stages of breast cancers and their normal controls

S. No.	Subject	Total no.	Average rate of chromosome gaps and breaks.
1.	Breast cancer		
	(a) Stage II	3	1.61
	(b) Stage III	8	1.04
	(c) Stage IV	4	1.20
2.	Normal Controls	5	1.36

GAP

Chromatid gap (ctg) Chromatid gap is a non-staining region (achromatic lesion) of a single chromatid in which there is a minimum misalignment of the chromatid.

Chromosome gap (csg) Chromosome gap is a non-staining region (achromatic lesion) at the same locus in both chromatids of a single chromosome in which there is minimal misalignment of the chromatid (Fig. 1-left).

BREAK

Chromatid break (ctb) Chromatid break is a discontinuity of a single chromatid in which there is a clear misalignment of one of the chromatids (Fig. 1-right).

Chromosome break (csb) Chromosome break is a discontinuity at the same locus in both chromatids of a fragment and an abnormal monocentric chromosome.

Because no significant difference was observed between breaks and gaps so the data of breaks and gaps was clubbed together.

The Chi-square test was applied to find out any significant difference (Snedecor and Cochran 1980) between the patients of different groups and normal control and among patients themselves.

Results

Table 1 depicts the average rate of occurrence of chromosome gaps and breaks which were 1.61, 1.04 and 1.20 in stage II, III and IV, respectively of breast cancer cases and 1.36 in normal controls.

Table 2 presents the complete data of breaks and gaps of each individual case.

Apparently the incidence of spontaneous chromosomal aberrations i.e., breaks and gaps were higher in stage II breast cancer case.

The statistical analysis showed a significant difference in the incidence of chromosomal gaps and breaks when comparison were made between total cancer cases and normal control ($P < 0.05$), stage II breast cancer cases and normal controls ($P < 0.001$), stage II breast cancer cases and stage III breast cancer cases ($P < 0.05$).

Table 2. Cancer cases alongwith normal control having chromosomal gaps and breaks as well as metaphase plates with chromosomal gaps and breaks

S. No.	Subjects	Stage of Cancer	Patient No.	Total no. of cases with gaps and breaks studied	Total cell scored	Chromosome Gaps and breaks in chromosome breaks Groups					Total gaps/ breaks scored	
						A	B	C	D	E		
1	Breast Cancer	(a) Stage II	GC-4		16	4	4	7	2		17	
			GC-12		1	—	—	1	—	—	1	
			GC-34		49	15	11	35	25	2	88	
					3	66	19	15	43	27	2	106
		(b) Stage III	GC-28		13	4	4	4	2	1	15	
			GC-29		1	—	—	1	—	—	1	
			GC-31		14	3	3	4	—	1	11	
			GC-35		2	1	1	—	—	—	2	
			GC-56		11	3	1	5	1	1	11	
			GC-64		3	1	—	1	1	—	3	
			GC-79		1	—	—	1	—	—	1	
			GC-84		3	2	—	1	—	—	3	
							8	45	14	9	17	4
		(c) Stage IV	GC-36		2	1	—	2	—	—	3	
			GC-50		6	1	3	2	—	—	6	
			GC-58		6	1	2	3	2	—	8	
			GC-75		1	1	—	—	—	—	1	
					4	15	4	5	7	2	—	18
2. Normal Control												
	1				36	23	9	20	3	—	55	
	2				1	—	—	01	—	—	1	
	3				10	3	1	7	—	—	11	
	4				1	—	—	1	—	—	1	
	5				08	2	2	2	2	—	8	
				5	56	28	12	31	5		76	

Discussions

So far as sufficient literature is not available about the spontaneous chromosomal aberrations such as gaps and breaks in stage specific breast cancer cases. In the present investigation we observe that the rate of breaks and gap in stage II breast cancer is significantly higher as compared to stage III and IV breast cancer cases and normal control in lymphocytes.

An increased frequency of chromosomal aberrations in lymphocytes of patients with various cancers such as retinoblastoma (Czeizel *et al.* 1974, de Nunez *et al.* 1984), renal carcinoma (Wang *et al.* 1982), Cervix cancer (Murthy *et al.* 1985) and many other cancer has already been reported. Although, there is no such report in breast cancer cases. The frequencies of gaps in chromosomes have previously been observed to double in patients as compared to controls (Brown *et al.* 1985). It has also been suggested that the chromosome instability leads to breaks occurring at many different locations which increase the chances of damage and could activate an oncogene to initiate to build up of a clone with the chromosome rearrangement that triggered the neoplastic transformation (Brown *et al.* 1985).

The present study suggests that the chromosome breaks and gaps be one of the primary events of the chromosomal instability. However, the biological meaning of the chromosomal

instability in the development and progression remains unclear (German 1980).

The increased frequency of spontaneous chromosomal aberrations in stage II breast cancer in the present investigation thus may indicate that during the stage II of breast cancer the factors searched by neoplastic cells exerts more pressure for the higher number of breaks and gaps which help in the alteration of chromosomes. However, in the next stage probably there are no much gaps and breaks because altered chromosomes are already stable to produce the required factors for growth of malignant tissues, but this needs an extensive study of breast cancer cases.

Summary

Chromosomal aberrations such as gaps and breaks were studied in 15 cases of breast cancers (i.e., stage II–3 cases, stage III–8 cases and stage IV–4 cases) and their 5 normal age and sex-matched controls.

The average rate of chromosome gaps and breaks were 1.61, 1.04 and 1.20 in stage II, III and IV breast cancer cases respectively, and 1.36 normal age and sex-matched control.

Chi-square comparison showed a significant difference between total breast cancer patients and normal controls ($P < 0.05$), a higher difference between stage II breast cancer cases and normal control ($P < 0.01$) and slightly higher difference between stage II and stage III breast cancer cases ($P < 0.05$).

The result so obtained clearly indicate that probably stage II breast cancer is a crucial stage for maximum chromosomal alteration in this malignancy.

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