

POTENTIAL ROLE OF PREOPERATIVE CHEMOTHERAPY IN BREAST CANCER

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ABSTRACT:

This is a retrospective and prospective study carried out in the Department of Pathology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi from October 2000 to October 2002. A total of fifty-breast carcinoma specimens were investigated comprising of forty invasive ductal carcinomas and ten invasive lobular carcinomas. Ki-67 antigen was immunostained on formalin-fixed paraffin embedded tissue and the positivity index in tumors of various sizes was determined. Thirty-three cases showed positive nuclear staining. Group-I contained tumors equal to or less than 2 cm in size. In ILC, 03 out of 08 cases and in IDC 07 out of 25 cases, showed a mean Ki-67 index of $13.9 \pm 0.4\%$ and $15.65 \pm 11.65\%$, respectively. Group-II contained tumors between 2.1-5cm in size. In ILC, 02 out of 08 cases and in IDC, 07 out of 25 cases, showed a mean Ki-67 index of $10.9 \pm 3.2\%$ and $14.04 \pm 5.34\%$, respectively. Group-III contained tumors equal to or more than 5.1 cm in size. In ILC 03 out of 08 cases and in IDC 11 out of 25 cases, showed a mean Ki-67 index of $14.2 \pm 9.3\%$ and $17.38 \pm 11.30\%$ respectively. The results of ILC versus IDC in all three groups were statistically insignificant ($P>0.05$). So it is concluded that preoperative chemotherapy is a useful therapeutic strategy for operable breast cancer of any size.

Keywords: Ki-67 Positivity index. Immunostaining. Breast Carcinoma.

INTRODUCTION

The origin of preoperative chemotherapy stems from its use in the treatment of locally advanced inoperable breast cancers where it leads to improvement in local control and survival (DeLena, Zucali, Viganotti *et al.*, 1978; Hortobagyi, Blumenschein and Spanos *et al.*, 1983). The initial aim of preoperative chemotherapy was to achieve tumor shrinkage and to potentially reduce the number of patients requiring mastectomy. This approach was then extended to include large operable breast cancers where mastectomy was the recommended surgical option and patients were likely to be treated with postoperative adjuvant chemotherapy (Goldhirsch, Glick, Gelber *et al.*, 1998).

Conservative surgery is also the treatment of choice whenever possible in the management of larger operable breast cancers prior to surgery to achieve down-staging of tumor (Fisher, Anderson and Remond *et al.*, 1995). Initial trials on selected patients whose tumors were initially not amenable to breast-conserving therapy were able to avoid mastectomy after significant tumor downsizing (Singletary, McNeese and Hortobagyi, 1992).

As the safety and efficacy of this approach was recognized, primary chemotherapy was offered as an alternative to initial surgical treatment for patients with earlier stage disease (Miller and Wilson, 2002). Results from non-randomized studies suggested that by giving preoperative chemotherapy the chance of conservative surgery is increased without

increasing the local recurrence rate (Jacquillat, Weil, Baillet *et al.*, 1990; Smith, Jones, O'Brien *et al.*, 1993; Calais, Berger and Descamps *et al.*, 1994). In a trial using infusional chemotherapy comprising 5-fluorouracil, epirubicin hydrochloride, and cisplatin, eligible patients had to require mastectomy. Following preoperative chemotherapy, only 6% subsequently needed a mastectomy (Smith, Walsh and Jones *et al.*, 1995). Similarly only 15% of 536 patients required mastectomy following preoperative Adriamycin/CMF chemotherapy, although all were considered to require a mastectomy at the time of entry into the study (Bonadonna, Valagussa and Brambilla *et al.*, 1998).

In Institute Curie randomized 414 premenopausal patients with tumors greater than 2 cm to primary chemotherapy followed by local radiation or to initial radiation followed by chemotherapy (Scholi *et al.*, 1994, 1995). In both groups, surgical resection was limited to only those patients with a persistently palpable tumor after radiation. More than 75% of patients in both groups were treated without mastectomy. After a median follow-up of 54 months, the use of primary chemotherapy improved overall survival from 78% to 86%. Trials like these illustrate the potential role of primary chemotherapy as a proving ground for new treatment strategies.

To further document the beneficial role of primary chemotherapy in breast cancers, we evaluated in our study tumors of varying sizes for their proliferative index, as dysregulation of the various components of cell cycle is probably the most prominent feature of cancer cell pathology and is an obvious target of antiproliferation strategies. The antigen Ki-67 is regarded as a marker for proliferating cells. It was identified as a protein (s) (pki-67) which exists free or associated with DNA as evidenced by DNA digestion of cells before or after immunolabelling with Ki-67 (Lopez *et al.*, 1994). As Ki67 identifies the proliferating cells in a tumor, it reflects the percentage of dividing cells (Isola *et al.*, 1990).

MATERIALS & METHODS

This study was performed on formalin fixed paraffin embedded blocks of cases diagnosed as invasive ductal carcinoma and invasive lobular carcinoma of breast with and without lymph node involvement, in the Department of Pathology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi, Pakistan from October 2000 to October 2002.

Fifty cases were selected comprising of 10 cases of invasive lobular carcinoma (ILC) and 40 cases of invasive ductal carcinoma (IDC) and were subjected to immuno-staining for Ki-67 positivity. Five-micron thick sections were retrieved for H&E staining. Extra slides were prepared for immuno-staining by cutting 4µm thick sections from representative paraffin embedded blocks and were applied to already positively charged slides. Antigen retrieval was done by trypsin digestion (Zymed Cat No. 00-3003) followed by heat induced antigen recovery. Specific staining is accomplished by localizing the Ki-67 antigen with Ki-67 polyclonal antibody. The antigen/antibody complex is then identified using the LAB-SA biotinylated secondary antibody detection method. A streptavidin enzyme is then added which binds to the biotinylated secondary antibody. A substrate solution is then added that forms a coloured deposit in the presence of the enzyme that is complexed to the antigen. The location of the antigen is then revealed by the presence of the colored deposit that forms around it. Any nuclear staining was regarded as positive. Positivity index of Ki-67 was determined by counting the number of positively stained nuclei in 1000 tumor cells in at least five representative high power fields across the slide.

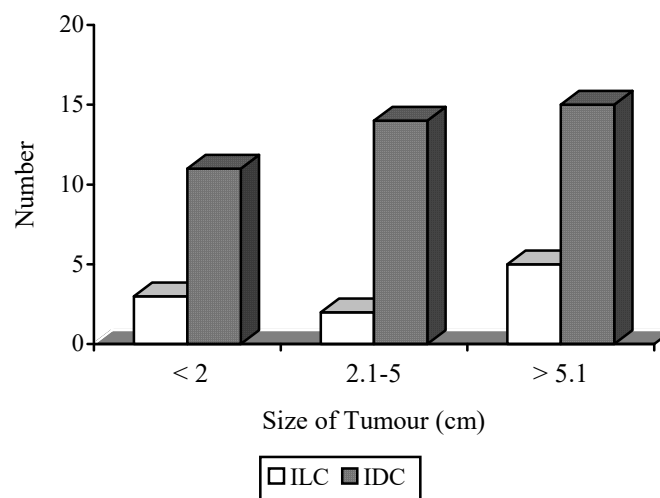
STATISTICAL ANALYSIS

The computer package "Microsoft Excel" was used for data feeding and "EPI-INFOR" was used for statistical analysis. The results were given in the text as number and

Table-1
Distribution of cases of invasive lobular carcinoma and invasive ductal carcinoma of breast according to the size of tumour

S. No.	Size of Tumour (cm)	ILC		IDC		Total	
		No.	%	No.	%	No.	%
1.	≤ 2	03	06%	11	22%	14	28%
2.	2.1- 5	02	04%	14	28%	16	32%
3.	≥ 5.1	05	10%	15	30%	20	40%
	Total	10		40		50	

Key: LN = Lymph node
ILC = Invasive Lobular Carcinoma
IDC = Invasive Ductal Carcinoma



Key: ILC = Invasive Lobular Carcinoma
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Figure 1: Distribution of cases of invasive lobular carcinoma and invasive ductal carcinoma of breast according to the size of tumour.

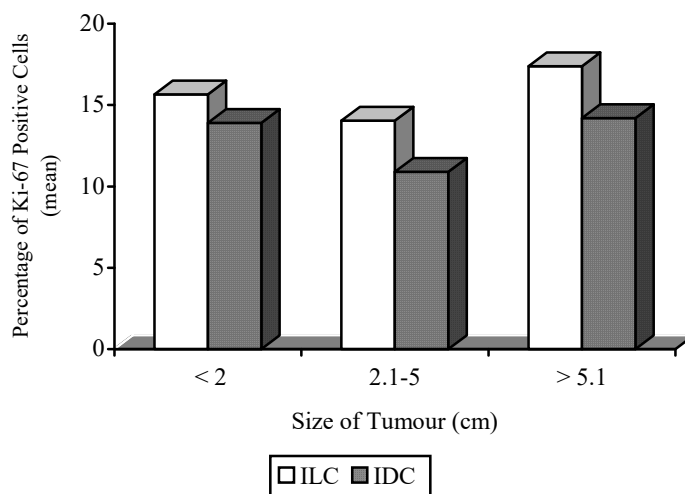
percentage for qualitative variables and mean and standard deviation for quantitative data. To compare the difference between two means, Student t-test was employed. For the

comparison of more than two means Analysis of variance (F-test) was performed. In all statistical analysis, only 'P' values less than '0.05' were considered significant.

Table-2
Ki-67 positivity in cases of invasive lobular carcinoma and invasive ductal carcinoma of breast versus size of tumour

S. No.	Size of Tumour (cm)	IDC		ILC		P Value
		No. of Cases	Ki-67 (Mean $\pm$ SD)	No. of Cases	Ki-67 (Mean $\pm$ SD)	
1.	≤ 2	07	15.65 $\pm$ 11.65	03	13.9 $\pm$ 0.4	P>0.05
2.	2.1 - 5.0	07	14.04 $\pm$ 5.34	02	10.9 $\pm$ 3.2	P>0.05
3.	≥ 5.1	11	17.38 $\pm$ 11.30	03	14.2 $\pm$ 9.3	P>0.05
4.	Total	25		08		
5.	P Value		P > 0.05		P > 0.05	

Key: ILC = Invasive Lobular Carcinoma
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Figure 2: Ki-67 positivity in cases of invasive lobular carcinoma and invasive ductal carcinoma of breast versus size of tumour.

OBSERVATIONS AND RESULTS

In this study, 50 diagnosed cases of human breast carcinoma including invasive lobular carcinoma (ILC) and invasive ductal

carcinoma (IDC) were subjected to immuno-histochemical staining for Ki-67 antigen. Out of 50 cases, 10 were invasive lobular carcinoma and 40 were invasive ductal carcinomas.

Table-1 and Figure-1 show distribution of 50 cases of ILC and IDC of breast according to the size of tumor. All 50 cases had been divided into three different groups. Group-I composed of tumors equal to or less than 2 cm in size. Out of 50 cases, 03 (06%) cases were of ILC and 11(22%) cases were of IDC with a total of 14 (28%) cases.

In Group-II, consisting of tumor between 2.1 to 5 cm in size, out of 50 cases 02 (04%) cases were of ILC and 14 (28%) cases were of IDC with a total number of 16 (32%) cases.

Group-III reveals the tumors which were equal or more than 5.1 cm in size. Out of 50 cases 05 (10%) were of ILC and 15 (30%) were of IDC with a total number of 20 (40%) cases.

Table-2 and Figure-2 are showing Ki-67 positivity index in 33 cases of ILC and IDC of breast according to size of tumour. Group-I contained tumours equal to or less than 2 cm in size. In ILC, 03 out of 08 cases were present in this group with a mean Ki-67 index of $13.9 \pm 0.4\%$. In IDC, 07 out of 25 cases were present in group-I with a mean Ki-67 index of $15.65 \pm 11.65\%$.

Group-II contained tumors between 2.1-5 cm in size. In ILC, 02 out of 0.8 cases were present in this group with a mean Ki-67 index of $10.9 \pm 3.2\%$. In IDC 07 out of 25 cases were present in this group with a mean Ki-67 index of $14.04 \pm 5.34\%$.

Group-III contained tumors equal to or more than 5.1 cm in size. In ILC 03 out of 08 cases were present in this group with a mean Ki-67 index of $14.2 \pm 9.3\%$. In IDC, 11 out of 25 cases were present in this group with a mean Ki-67 index of $17.38 \pm 11.30\%$. The results of ILC versus IDC in all three groups were statistically insignificant ($P>0.05$).

DISCUSSION

Breast is not only accessible to clinical and radiological examination but also to serial

sampling by fine-needle aspirate or Tru-Cut biopsy. There is the potential to identify predictive biological markers of response and long-term outcome. As the number of tumor cells increases, the possibility of the development of chemo-resistant clones increases (Goldie and Coldman, 1979). This would favour the early administration of chemotherapy and may in addition account for the failure of adjuvant chemotherapy in some instances. Assuming that a delay in systemic treatment allows expansion of drug resistant clones, the early initiation of systemic therapy might improve overall survival (Skipper, 1971).

The gross size of invasive breast carcinomas is a sensitive indicator of prognosis, second only to the presence of nodal metastasis in importance, and it is a prominent anatomic feature in staging. In patients with pathologically negative axillary nodes, tumor size remains the single most important prognosticator for survival (Joensuu, Toikkanen and Klemi, 1990). Tumor size has a controversial association with the Ki-67 labelling index (Brown and Gatter, 1990). Most of the previous studies have not shown a significant relationship between Ki-67 labelling index and size of the tumour (Leonardi, Girlando, Serb *et al.*, 1991; Silvestrini, Veneroni, Daidone *et al.*, 1994; Keshgegian and Cnaan 1995; Markiewski and Domagala 1996). Lelle (1989) reported a weak positive relationship to tumor size, while Barzanti *et al.* (2000) found a significant relationship of Ki-67 positivity index to size of the tumor.

In the current study, we did not find any statistically significant relationship between Ki-67 positivity index and size of the tumor. However, tumors equal to or greater than 5.1 cm in size did show high Ki-67 labelling indices i.e., $17.38 \pm 11.3\%$ in IDC and $14.2 \pm 9.3\%$ in ILC as compared to tumors equal to or less than 5 cm in size.

CONCLUSION

Proliferative activity in breast cancer is independent of size of the tumor. So preoperative chemotherapy can be considered for patients with primary operable breast cancer of any size that is amenable to breast-conserving surgery.

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