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NUCLEAR DNA CONTENT AND DNA PLOIDY ANALYSIS IN BREAST CANCER

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ABSTRACT

To investigate the patterns of DNA ploidy and proliferating activity in breast cancer and relate them to other prognostic factors, paraffin blocks of 53 cases of breast carcinoma were studied. Cancer cells obtained by mechanical tissue disaggregation were examined for DNA content, ploidy and S-phase fraction. DNA assay was done using a CAS interactive image analyzing system.

All of the cases showed high degrees of proliferation. The rate of aneuploidy was 77% in invasive breast carcinomas. S-phase fractions were correlated with the grades of the tumors ($p<0.05$). There was no statistically significant correlation between S-phase fractions and other prognostic factors; this was also true about the pattern of ploidy and other prognostic factors.

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INTRODUCTION

Breast cancer is the most common malignant tumor and the leading cause of carcinoma death in women.[8] In spite of major advances in surgery, radiotherapy, chemotherapy and hormonal therapy,

Until recently breast cancer prognosis has traditionally been based on grading and staging according to WHO criteria; however, the biological behavior of individual tumors may vary considerably in spite of identical typing, grading and staging. Biological parameters such as tumor DNA ploidy and cell proliferation are becoming increasingly useful as prognostic factors.

In many human malignant tumors there is a correlation between aneuploidy and high microscopic grade, high clinical stage, poor prognosis and clinical outcome, and response to chemotherapy.[8] The aim of this study was to find a correlation of ploidy and S-phase fraction with other prognostic factors such as histological grade, size of the tumor, lymph node status, age of the patient and the clinical course.

MATERIALS AND METHODS

Microscopic sections from 63 cases of female breast carcinoma from the files of pathology departments of Shiraz University affiliated hospitals were reviewed. These sections were from modified radical mastectomy specimens. Age range of the patients was 24 to 78 years. All the slides were reviewed and grading was done using the Nottingham modification of the Bloom-Richardson system.[8] The sections (blocks) with no or the least amount of necrosis were chosen. According to the technical requirements of the image analyzing system used in this study (CAS 100), DNA quantitation should be performed on intact tumor cell nuclei prepared as cytological smears.[2] Mechanical tissue disaggregation of the cells from paraffin blocks, homogenization, smear preparation, and Feulgen staining were done. Ten cases were omitted from the study due to inadequacy of the smears prepared.

The stained DNA was quantitated using the CAS Image Analyzer. Special calibration slides supplied in the commerical DNA staining kit were used for calibrating the system by taking the coefficient variation below five (c.v.<5).[2] With the interactive CAS image analyzing workstation using the "Quantitative DNA Analysis" software, the nuclear DNA content, ploidy, S-phase and S/G2M phase fractions of 100 intact randomly selected tumor cell nuclei were evaluated. The overlapping and fragmented nuclei were omitted. According to the DNA histograms, the DNA content values were expressed as the "absolute nuclear DNA mass" (in picograms) and the ratio of the average amount of DNA for a given cell population to

Table I. Image analytical data of 53 cases of breast carcinoma separated by type and grade.

Histologic type	Grade	No.	DNA diploid(%)	DNA aneuploid(%)	Proliferation (%)	Median S-phase(%)
Invasive ductal carcinoma	I	13	8%	92%	>8	36.2%
	II	31	26%	74%	>8	45.7%
	III	3	0	100%	>8	69.0%
Total No.		47				
Medullary carcinoma	I	2	0	100%	>8	29.7%
	II	1	100%	0	>8	55.8%
Total No.		3				
Intraductal carcinoma	I	1	0	100%	>8	62.4%
Total No.		1				
Invasive lobular carcinoma	I	1	100%	0	>8	16.3%
Total No.		1				
Invasive papillary carcinoma	II	1	0	100%	>8	60%
Total No.		1				

DNA content of a normal diploid cell as "DNA Index" (DI).

DNA histogram interpretation

A DNA Index (DI) of 1.0 (10%) indicate a diploid (2 N) cell population. Aneuploidy, any deviation from an exact multiple of the haploid number of chromosomes, whether fewer or more is indicated by a DI of greater or less than 1.0 (±10%).[7]

Diploid (2 N)= 1.0±10%

Aneuploid= >1.0-10%

or <1.0+10%

G_0/G_1 indicates the number of cells in resting state; S/G_2 M indicates cells which are either synthesizing DNA(S) or have already duplicated DNA (G_2M). Greater than 8% of cells in S/G_2M indicates a high degree of proliferation.[7]

It is necessary to point out that the medical charts of the patients were reviewed but no sufficient clinical information including follow up records was found.

Table II. Mean and standard deviation of S-phase, size of the tumor and age of 53 cases of breast carcinoma separated by grade.

Grade	S-Phase (mean)	Size (cm)	Mean age (year)
I	35.8%	4.9	50.6
Std. Dev.	20.1	1.4	12.3
II	46.4%	4.5	48.6
Std. Dev.	11.4	2.0	14.4
III	69.0%	3.7	45.3
Std. Dev.	27.6	2.1	7.6

RESULTS

From fifty-two patients with invasive carcinoma, forty cases (77%) had aneuploid DNA content, and twelve patients (23%) had diploid DNA content (Table I). All (100%) of the cases had high degrees of proliferation (Table I). The difference of mean S-phase fractions between grade I and II, II and III as well as I and III was statistically significant (p= 0.0036). Duncan test with significant level (0.05) showed that the difference of mean S-phase fractions between grades II and III was significantly higher than that of grades I and II.

No significant correlation was found between S-phase fraction and size of the tumors, and age of the patients (Table II). Our study also showed no statistically significant correlation between ploidy and grade, size, age or axillary lymph node status.

Because the hospital charts of the patients were incomplete, no sufficient follow up record chould be found for studying the correlation of ploidy and S-phase fraction with disease free survival.

DISCUSSION

Results of the present study showed a significant correlation between S-phase fraction and grades of breast carcinomas. The prognosis of breast cancer depends on many factors such as age of the patients, presence or absence of invasion, size of the tumor, histological grade and axillary lymph node status.[7] DNA ploidy and the fraction of tumor

cells synthesizing DNA (S-phase fraction) are also important prognostic factors in breast cancer,[3,4,9] so they can be used as an adjunct with histological studies to plan for a better patient management.

Image analysis and flow cytometry have comparable DNA ploidy results with both fresh and formalin fixed paraffin-embedded tissue; however, sensitivity for detection of DNA aneuploidy is somewhat greater by image analysis.[2,10] Using paraffin blocks produces comparable results with studies on fresh tissues, but it is a time consuming procedure. Besides, mechanical tissue disaggregation can have degenerative effects on the tumor cell nuclei. The latter problem could be overcome by applying CAS 200 in which there is no need to disaggregate the tissues in paraffin blocks; however, it is preferred to use fresh tissues by preparing touch slides or scrapings.

The rate of aneuploidy in invasive breast carcinomas in this study corresponds with results of most previous studies which have reported 40%-76.1% aneuploidy rates.[4,6,9,11,12] Many other similar studies have shown correlation of aneuploidy and S-phase fraction with disease free survival and other prognostic factors.[3,5,9] Our study showed a significant correlation between S-phase fraction and grades of breast carcinoma, which is one of the prognostic factors in breast cancer. No statistically significant correlation was found between S-phase fraction and factors such as size of the tumor, lymph node status, or age of the patients.

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