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Morphometric Studies of Cytological Specimens in Breast Carcinoma using Computerised Image Analysis System

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ABSTRACT

The value of nuclear parameters were determined using interactive computer image analysis system on 100 preoperative FNAC smears of breast carcinoma where radical mastectomy was performed subsequently. Twenty cases of benign breast lesion were taken as control. The lymph node status was correlated with the nuclear and nucleolar variables. It is found that few nuclear parameters have good discriminatory power. Computerised interactive morphometry proved to be far superior to eye-piece measurements with respect to accuracy, reproducibility and time taken for parameter estimation which leads to the determination of better prognostic indicator.

1. INTRODUCTION

Introduction of computers and image analysis systems are gaining faster momentum to quantify the assessment of cells for diagnosis and prognosis. Substantial research efforts are directed to make diagnotic pathology more objective. The emphasis of the new technological approach today is on information extraction from cell, utilisation of information accessible only by computation and providing diagnostic and prognostic clues to the cyto-pathologist.

Image analysis techniques have been used with some success to separate benign from malignant human breast tumors[1-5]. Several studies have dealt with the extent to which cytology may supply information on the prognosis of breast cancer patients[3-7]. It is known that about one half of breast cancers recur and most of the re-currences appear within 5 to 7 years from primary therapy[8,9]. To date lymph node status is widely known prognostic indicator to predict relapse of breast cancer[10]. Although lymph node metastasis is widely used as high risk indicator, it only predicts prognosis accurately in about 60-65 per cent of patients with breast cancer[6]. Hence there is need to find an alternative prognostic indicator which is cent per cent accurate. It is expected that more accurate and quantitative studies at cellular level and the relation between nuclear morphometric data and lymph node metastasis would throw light in arriving at correct prognosis. In this paper the morphometric studies conducted on cytological specimens in breast carcinoma using computerised image analysis are presented.

2. MATERIALS AND METHODS

FNAC specimens of 100 patients have been used in this study. These cytological specimens were stained by hypochromic Papanicolaou staining procedure which is specially suitable for proper identification of nucleoli. Out of 100 cases, 20 cases belonged to benign group, 20 lymph node negative and 60 lymph node positive cases. Subsequently, radical mastectomy was performed in negative and positive lymph node cases.

An interactive image processing system built in the laboratory is used to determine the morphometric and texture parameters of cell and nucleus. The system is built around PDP 11/23+ computer and Q-bus compatible image processing modules from Imaging Technology Inc., USA. The system is equipped with Carls Zeiss research microscope, CCTV camera and

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monitor for acquisition and display of cell images. The microscope carries a fast scanning stage with motor control unit interfaced to computer. The stage can be moved in steps of one micron or higher, in both x and y directions. The video image captured by the camera is digitised to 512×512 pixels with 8 bit gray scale resolution by A/D converter board. The digitised image is stored in frame buffer for further processing. The digitiser tablet interfaced to the system provides user interaction. It is also used to mark the boundary of cell and nucleus. The detailed system configuration has been discussed and reported elsewhere[11]. The microscope magnification used in the present study is 2000x. The pixel resolution on the monitor screen is 0.1 and 0.075 micron in x and y directions respectively. For accurate measurements of nucleoli, the cell image was further zoomed twice to give final magnification of 4000X.

The user interactive software was developed in the laboratory to isolate the cell using graphic tablet and to compute various morphometric and texture parameters. The typical parameters are area, perimeter, shape, long and short axes, nucleus to cytoplasm ratio, and texture. Texture has 3 parameters: two based on gray level run length code[12] and one based on gray level co-occurence matrix[13].

One hundred nuclei per slide were considered for the purpose of creating the database. Number of nucleoli in each nucleus was also entered to get final count of nucleoli per hundred nuclei Histograms depicting distribution of nuclear and nucleolar variables with respect to number of cells and number of patients were produced. Data were analysed for statistical significance with chi-square test. The differences were considered to be statistically significant when $p < 0.05$.

3. RESULTS AND DISCUSSION

Table 1 gives the mean and standard deviation values of nuclear morphometric variables for the benign, negative and positive lymph node groups. Shape and texture parameters are in arbitrary units. There is considerable difference in nuclear area and perimeter values reported by Christain and Erik[5]. Their reported values are on the higher side. The discrepancy might be due to the fixation technique employed. Nevertheless the increasing order in the value of nuclear area and perimeter from benign to node negative to node positive groups reported by these authors is contrary to our finding. It is interesting to observe from Table 1 that the mean and standard deviation values of all the nuclear variables except the nuclear texture parameters are

Table 1. Nuclear morphometric variables (mean ± SD)

Group	Area (μ^2)	Perimeter (μ)	Long axis (μ)	Short axis (μ)	Shape	Texture
Benign	48.773 ±10.776	25.470 ±2.869	9.329 ±1.307	6.558 ±0.889	1.039 ±0.028	268.843 ±66.099
LN-VE	95.206 ±34.210	34.842 ±6.219	12.736 ±2.543	8.949 ±1.776	1.083 ±0.441	320.713 ±87.627
LN+VE	81.498 ±28.551	32.494 ±5.525	11.802 ±2.223	8.424 ±1.580	1.048 ±0.112	340.198 ±101.372

LN : Lymph node

Table 2. Nucleolar morphometric variables (mean ± SD)

Group	Area (μ^2)	Perimeter (μ)	Long axis (μ)	Short axis (μ)	Shape	Nucleolar frequency
Benign	2.828 ±0.852	6.218 ±0.904	2.239 ±0.370	1.576 ±0.269	1.054 ±0.026	143
LN-VE	5.621 ±2.683	8.517 ±1.682	3.055 ±0.664	2.198 ±0.464	1.057 ±0.111	165
LN+VE	5.045 ±2.102	8.118 ±1.504	2.894 ±0.595	2.102 ±0.418	1.052 ±0.059	152

LN : Lymph node

higher for lymph node negative group. Similar observation can be made from Table 2 which gives the mean and standard deviation values for nucleolar parameters. The nucleolar frequency is the total number of nucleoli per hundred nucleus counted. These higher mean values of nuclear and nucleoli morphometric variables and larger spread in standard deviation in node negative group are attributed to increased cellular activity representing a sort of dynamic phase. Only nuclear texture parameter has shown increasing trend from benign to node negative to node positive group. Figure 1 gives the histogram of a few nuclear variables for the three groups and the differences in distribution for these groups are statistically significant. The mean and standard deviation values in Table 1 and 2 and the mean values used for histogram in Fig. 1 are produced from the pooled data in each group. When data are pooled in this way it only gives general trend and this need not be true when an individual case is considered for computation. It is worth while to consider the distribution of nuclear parameter vs number of patients in each group. Since the spread in standard deviation is found to be prominent among node negative cases the same was used to produce the histograms in Fig. 2 which represents the distribution of standard deviation of few nuclear variables vs number of patients. The differences in distribution for the three groups are statistically significant. The lymph node negative patients belonging to the overlapping region with respect to lymph node positive distribution may be

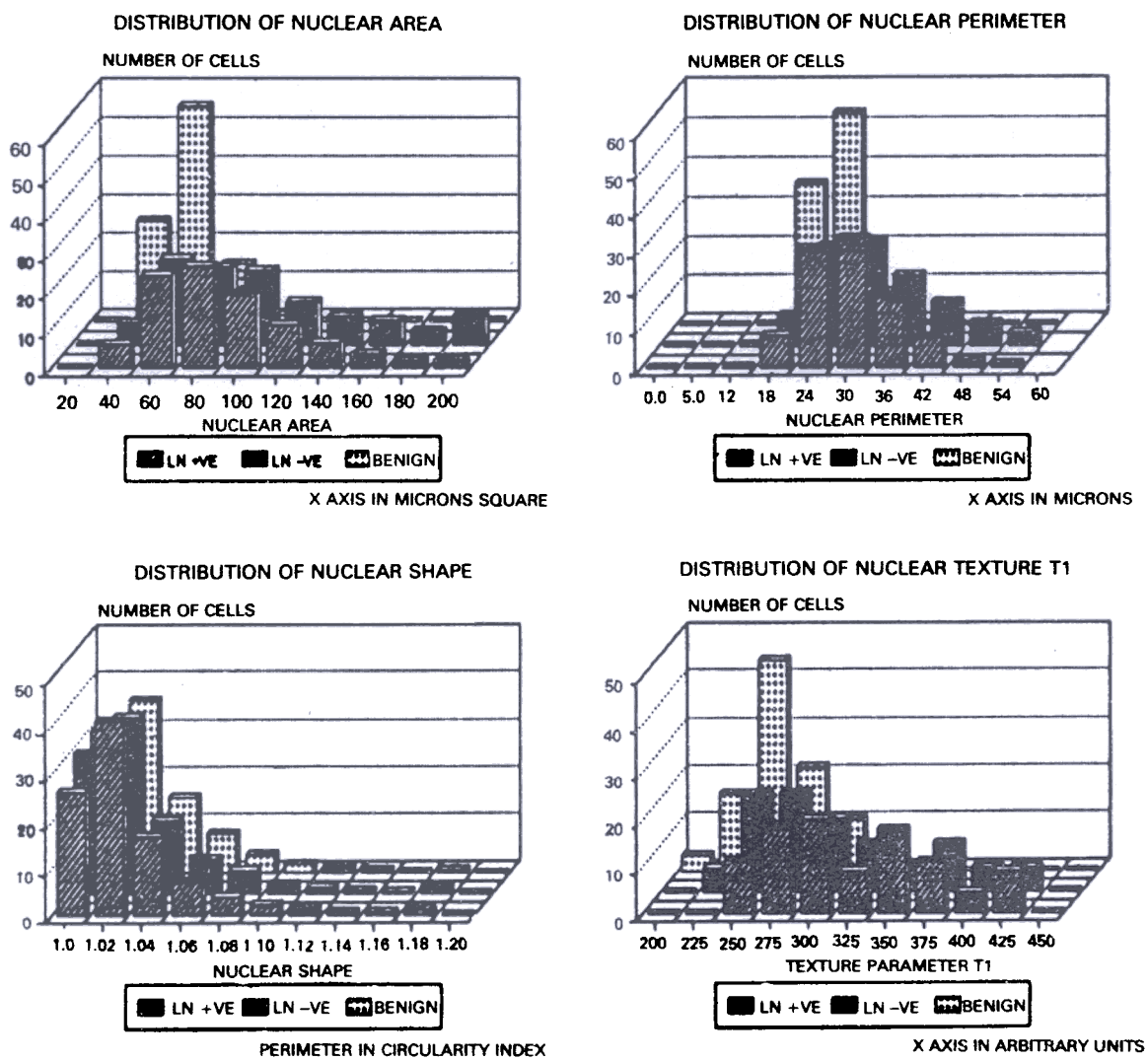

Figure 1. Distribution of nuclear parameters vs frequency of occurrence of number of cells.

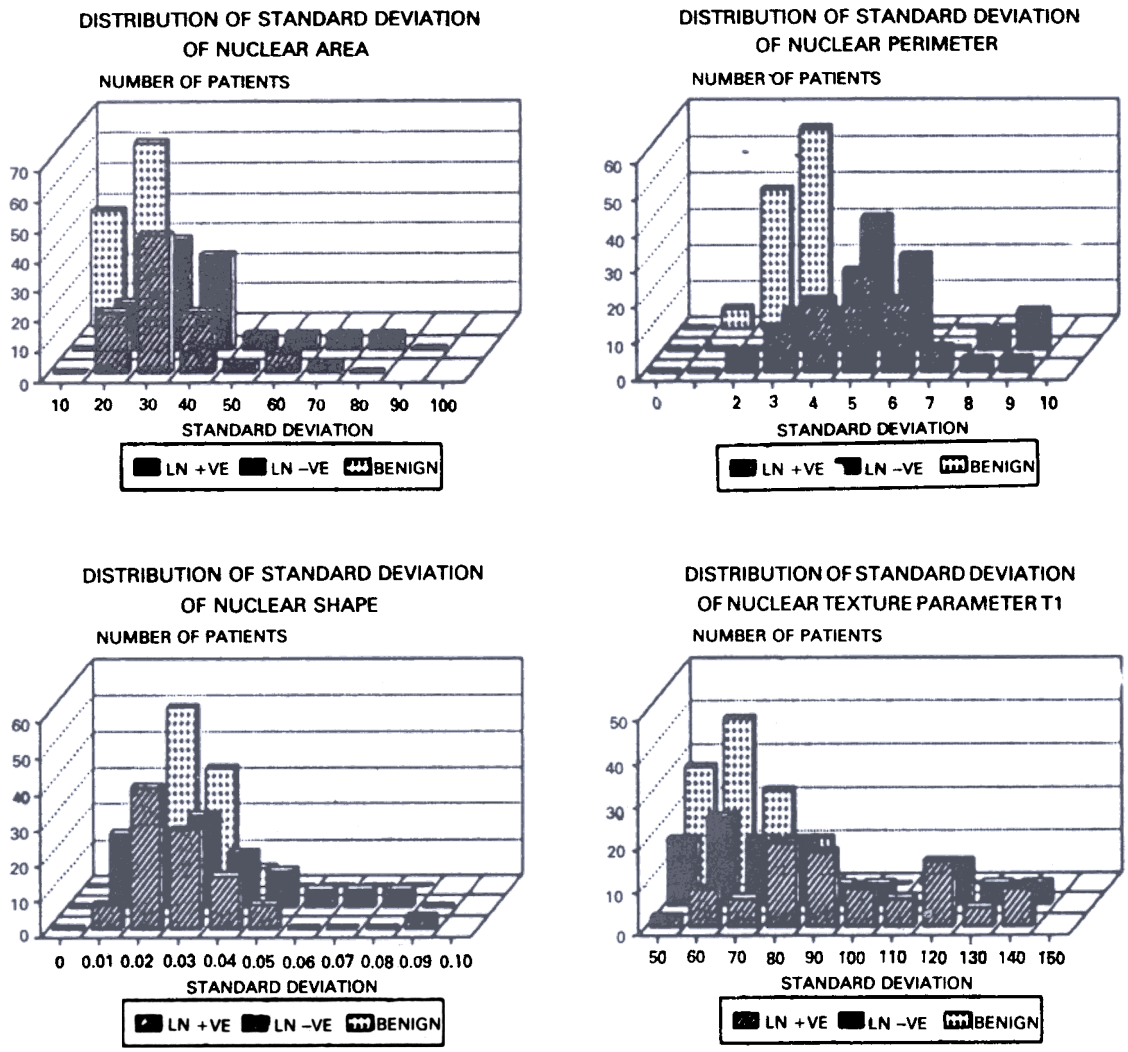

Figure 2. Distribution of standard deviation of nuclear parameters vs frequency of occurrence of number of patients.

considered as potentially high risk group for relapse of breast cancer. Only extensive studies with follow up data of survival of patients would help in proving or disproving this hypothesis. It is concluded that computerised image analysis approach to determine quantitatively the morphometry of cells on smear is far superior, reliable and reproducible compared to classical approach, and appears to hold tremendous promise in providing diagnostic and prognostic clues to cytopathologists in times to come.

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