THE VALUE OF NUCLEAR MORPHOMETRY IN BREAST CARCINOMA

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Abstract

Background: Breast carcinoma is the most important malignant tumor in female population. There are many indications for the use of adjuvant chemotherapy in its treatment, which need a special selection of patients especially those with high risk &this is judged by several prognostic parameters such as patient's age, tumor size, histological grade and others.

Nuclear morphometry has shown to be a good objective, quantitative method for the evaluation of prognosis, in which the Mean Nuclear Area &

Standard Deviation of Nuclear Area showed an increase from the baseline value of normal breast epithelium to invasive carcinoma &found to be strongly correlated with the recurrence rate within 2.5 years.

Objective: To evaluate some prognostic parameters of breast carcinoma by the use of computerized nuclear morphometry.

Material &Methods:

Fifty-four cases of a histologically diagnosed invasive breast carcinoma of ductal type with a known tumor grade, size &patient's age were reviewed. In each case an average of 5-10 microscopical fields were screened &30 consecutive nuclei were determined at x400 magnification by the use of an image analysis system. Statistical analysis was performed using ANOVA, Tukey's and t-test.

Results:

The mean values of nuclear area varied between the three histological grades (grade I, II and III) by using analysis of variance (p>0.01).

These values increased with increasing histological grade, (Tukey's test: p>0.01).

Similarly the mean values of nuclear area were significantly higher in tumors measuring more than 5cm in diameter than those less than 5cm in diameter. (t-test :p>0.01).

On the other hand, the nuclear area in tumors of patients less than 50 years and more than 50 years of age showed no significant difference. (t-test: p<0.01)

Conclusions:

The Mean Nuclear Area was of value in the assessment of the histological grade, in which higher figures seen in higher grades. The same relationship found between tumors more than 5cm and those less than 5cm in diameter, in which higher values seen in tumors more than 5cm in diameter. On the contrary the importance of the Mean Nuclear Area was restricted when comparing tumors in patients less than 50 years and those more than 50 years of age.

In conclusion our data suggest that adapting a nuclear morphometric parameter e.g. nuclear area, which was performed on this study, may be a valuable objective tool in evaluating various prognostic indices.

Keywords: Breast carcinoma, nuclear morphometry , and prognosis.

Introduction:

Breast carcinoma is one of the most common malignancies in female population [1].

There are several indications that early

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systemic adjuvant chemotherapy improves the treatment of patients with primary breast carcinoma. [2, 3, 4] However the side effect of those drugs requires a selection of patient at higher risk for this type of therapy. [5]

Several prognostic parameters are included in this selection such as the patient's age, size of the tumor, histological type, stage, histological& nuclear grade, mitotic index & axillary lymph node metastasis.[6,7,8]However

some of these parameters may have a subjective nature ,like the mitotic index , histological and nuclear grade and according to certain studies the interobserver agreement rarely exceed 80-90%. [9, 10]

The advantage of quantitative methods is that they are objective and reproducible. [11, 12]

Nuclear morphometry has shown a prognostic significance in many studies, [13] and that the mean nuclear area (MNA) & standard deviation of the nuclear area (SDNA) is an important prognostic predictor. [5]

Investigators found a gradual increase in MNA from the baseline value of normal breast epithelium through benign diseases to invasive carcinoma [13], and that patients with larger MNA &SDNA have worse prognosis. [5]

Other studies like Sterkvist, et.al [14] work, reported that mitotic frequency &the variance of the nuclear area (NA) were most strongly correlated with recurrence rate within 2.5 years & since the quantitative microscopical studies of breast carcinoma has an objective nature, so NA measurement has a considerable advantage over the mitotic index study which is a value of subjective nature. [5]

The MNA & variation of NA were also of prognostic significance in cytological specimens, [15, 16] and that the study of automated diagnosis & grading of breast carcinoma have been proved valuable. [17]

Further more a significant correlation found between morphometric features and estrogen receptor status. [18, 19]

In the present study we attempted to evaluate additional prognostic value of the nuclear profile to other prognostic variables.

Materials and methods:

Fifty-four cases of breast carcinoma were selected from the files of the histopathological laboratory at Al-Yarmouk Teaching Hospital in Baghdad, and reviewed later by two other pathologists; as invasive breast carcinoma of ductal type. All the examined samples were fixed in 10% formalin, embedded in paraffin; the sections were cut at 5µm & stained with hematoxylin and eosin. Histological grading was performed according to the modified Bloom-Richardson grading system on the basis of tubule formation, nuclear atypia & mitotic activity (grade I tumors have fairly normal appearance, grade III are poorly differentiated &grade II are intermediate between I and III. [20]

Two other prognostic parameters were also investigated; namely the tumor size or diameter (more or less than 5cm) and patient's age whether (more or less than 50 years).

In each case, the most cellular areas were looked for at the periphery of the tumor. Necrotic and inflamed areas were avoided. An average of 5-10 high power microscopical fields was screened & 30 consecutive tumor cells with clear nuclear boarders were outlined. Overlapping nuclei were not measured .In each case these nuclei were examined at x400 (x40 objective magnification, x10 camera ocular), by the use of an image analysis system run by global lab image 2 software GLI2 (data translation Inc., USA). The system composed of personal computer PC with frame grabber (DT3120k-1data translation Inc., USA) attached to the PC and a microscope (Olympus BH, Japan) with a video camera (KGB, cc-8603, Taiwan).

The images from the sections were obtained at 800X600 pixels resolution in BMP format. The digitalized images of the nuclear profile were outlined on the monitor screen using a computer mouse. The morphometric feature assessed was the nuclear area (NA).

The system was calibrated with a micrometer slide before each measurement. The data were transferred to a Microsoft® excel work sheet and were expressed in terms of micrometers

and the differences in terms of morphometric measurement between the three groups studied were statistically tested using analysis of variance, and Tukey's(HSD) and t-test.

Results:

The mean values of nuclear area varied between the three histological grades (grade I, IIand III) by using analysis of variance (p>0.01). (Table -1)

These values increased significantly with increasing histological grade, (Tukey's(HSD) test: p>0.01) (table -4). Similarly the mean values of nuclear area were significantly higher in tumors measuring more than 5cm in diameter than those less than 5cm in diameter (t-test: p>0.01). (Table-3)

On the other hand, the nuclear area in tumors of patients less than 50 years & those more than 50 years of age showed no significant difference. (t-test: p<0.01) ,(table-2)

Table 1
ANOVA test for the nuclear area of the three grades

Source of variation	Sum of squares	Degree of freedom	Mean squares	F calculated
Between groups	19288.706	2	9644.353	31.877
Within groups	138266.862	457	302.553	
total	157555.569	459		

F tabulated =4.652

Table 2
The mean values of the nuclear area in different age groups (expressed as *mean ± one standard deviation*)

Age group	Mean nuclear area
Less than 50 years old	49.1±20
More than 50 years old	50.21±18.82

Table 3
The mean values of the nuclear area in different tumor size (expressed as *mean ± one standard deviation*)

Tumor size	Mean nuclear area
Less than 5 cm	46.68±17.29
More than 5 cm	54.32±21.89

Table 4
The mean values of the nuclear area in the three histological grades (expressed as *mean ± one standard deviation*)

Histological grade	Mean nuclear area
Grade I	43.75±14.63
Grade II	54.35±18.28
Grade III	67.5±15.86

Discussion:

Breast carcinoma is one of the most important causes of death in female population and in order to predict the tumor's behavior many prognostic parameters have been introduced, some of which are subjectively judged, as the mitotic index, histological and nuclear grade with an interobserver agreement that rarely exceeds 80-90%.

From here the advantage of quantitative analytic methods has been introduced as an objective &reproducible tool for the evaluation of the tumor's behavior.

In this work we attempted to study the value of nuclear morphometry in the evaluation of some of the conventional prognostic parameters, namely; tumor's size, patient's age and histological grade.

There was a significant difference in the value of the MNA between the three histological tumor grades (I, II and III) and between those tumors more than 5cm and those less than 5cm in diameter, while there was no significant difference of the MNA between tumors of (more than 50 years and less than 50 years of age).

This showed that the MNA was of value in the assessment of the histological grade, in those higher figures seen in higher grades. The same relationship found between the tumors more than 5cm and those less than 5cm, in which higher values seen in tumors more than 5cm in diameter. On the contrary the value of the MNA was restricted when comparing tumors in patients less than 50 years and those more than 50 years of age. One explanation for the latter finding might be explained by the improper age recording of the examined patients.

Similar results were obtained by other studies indicating the value of the MNA as an objective morphometric index. [13,21]

In conclusion our data suggest that adapting a nuclear morphometric parameter e.g nuclear area, which was performed on our samples, may be a valuable objective tool in evaluating various prognostic indices.

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