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# 13

## Comparison Between DNase Levels and Histological Parameters in Patients with Benign and Malignant Breast Disease

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It is well documented that biological cancer markers are important not only in the initial diagnosis of cancer, but also in monitoring the response to therapy of the disease.<sup>1-4</sup> Although there are a variety of cancer markers, only a few can be used effectively for the above purposes. We have found that serum DNase levels are increased in cancer patients as compared to normal individuals.<sup>5</sup>

The purpose of this work is to find out if there is any correlation between the above DNase levels and the different histological parameters of the disease. For that reason a total of 433 slides of 64 breast cases were reviewed and the results are analyzed in some detail.

### PATIENTS AND METHODS

This study is based upon patients who were treated for breast cancer and other benign conditions at the Breast Clinic of the Greek Anticancer Institute of Athens.

From those patients blood samples were drawn and the serum was kept at  $-20^{\circ}\text{C}$

until it was used, usually within a week. Calf thymus DNA was purchased from Sigma Chemical Co. and it was used as substrate.

The enzyme assay for the acid DNase involved in 1.0 ml was 100  $\mu\text{g}$  DNA, 0.1 M sodium acetate buffer (pH 5.0), 0.005 M  $\text{MgCl}_2$ , and 25  $\mu\text{l}$  serum. The assay for the alkaline DNase involved in 1.0 ml was 100  $\mu\text{g}$  DNA, 0.1 M Tris  $\text{Cl}^-$  buffer (pH 8.0), and 25  $\mu\text{l}$  serum. In both assays, the mixture was incubated at  $25^{\circ}\text{C}$  for 15 min and then 2 ml of 1.5 M perchloric acid was added at  $4^{\circ}\text{C}$ . After 10 min the mixture was centrifuged at 3,000 rpm for 10 min. The supernatant was kept and its absorbance at 260 nm was measured against a blank which was made the same way as above except that the serum was added after the addition of perchloric acid. The unit was defined as that amount of enzyme which caused an increase of absorbance at 260 nm of 1.0 per minute at  $25^{\circ}\text{C}$ .

A total of 433 slides corresponding to 64 breast cases were reviewed. More specifically, 165 slides of 33 benign breast cases

were examined, with an average of five slides per case stained by hemotoxylin and eosin.

The 31 cases of breast carcinoma had 124 slides available for review, with an average of four slides per case, stained by hemotoxylin and eosin. Out of those slides there were three rare types of mammary carcinoma (2 apocrine and 1 mucinous) in which special stains (PAS and mucincarmine) were necessary to classify them. Therefore, six more slides, two for each case, were reviewed.

In 23 cases out of the 31 carcinomas of the breast, a total of 138 slides of axillary lymph nodes were available with an average of six slides per case. Axillary lymph nodes were arbitrarily divided into three levels by borders of the pectoralis minor muscle according to McDivitt *et al.*<sup>6</sup> More specifically, Level I consisted of those nodes below the muscle; Level II, those nodes beneath the muscle; and Level III, the few apical nodes above the muscle.

All malignant breast cases were graded according to Bloom and Richardson<sup>7</sup> who employed as criteria the degree of differentiation of tubular structures, the shape, size and staining properties of the nuclei, the degree of hyperchromatism, and the number of mitoses.

## RESULTS

The correlation between DNase levels and histological examination in patients

with benign breast tumors or other non-malignant conditions was studied. The results are shown in Table 13.1. Among the 33 benign breast cases there were seven breast tumors, nine fibrocystic diseases, and seven different other benign conditions. Only a small percentage (15 and 12%) of the 33 patients examined had increased acid and/or alkaline DNase, respectively.

We next examined the correlation between DNase levels and histological examination in patients with breast carcinoma. The results are shown in Table 13.2. It was found that 21 and 43% of the 14 intraductal cases had increased acid and alkaline DNase, respectively, whereas the acid and alkaline DNase were increased in 50 and 58% of the 26 infiltrating duct cases. Since most of the histological types were of the infiltrating duct carcinoma we also examined the correlation between 20 cases which presented the characteristics of different subtypes of infiltrating duct cancer and serum DNases. The results are shown in Table 13.3. The most common type of invasive carcinoma was the relatively monomorphic one with areas of productive fibrosis, *i.e.*, scirrhous carcinoma. Only 28 and 71% of the 14 cases with scirrhous carcinoma were found to have increased acid and alkaline DNase, respectively. *In situ* carcinoma of the intraductal type was found in 14 cases; in 11 instances out of these it was associated with infiltrating duct cancer and in one case with Paget's disease. In two cases of typical infiltrating lobular

**Table 13.1**  
Correlation Between DNase Levels and Histological Examination in Patients with Benign Breast Tumors or Other Nonmalignant Conditions

Histology	Total No. Patients	Cases of Increased DNase	
		Acid <sup>a</sup>	Alkaline <sup>b</sup>
1. Fibroadenoma	6	2 (33%)	0
2. Cystosarcoma phyllodes	1	1 (100%)	0
3. Fibrocystic disease	19	1 (5%)	1 (5%)
4. Other benign conditions	7	1 (14%)	3 (42%)
Total	33	5 (15%)	4 (12%)

<sup>a</sup> More than 400 units/ml serum.

<sup>b</sup> More than 200 units/ml serum.

**Table 13.2**  
Correlation Between DNase Levels and Histological Examination in Patients with Breast Carcinoma

Histology	Total No. Patients	Cases of Increased DNase	
		Acid <sup>a</sup>	Alkaline <sup>b</sup>
1. Intraductal	14	3 (21%)	6 (43%)
2. Infiltrating duct	26	13 (50%)	15 (58%)
3. Lobular <i>in situ</i>	0		
4. Infiltrating lobular	2	1 (50%)	0
5. Paget's disease	1	0	1 (100%)
Total	43	17 (40%)	22 (51%)

<sup>a</sup> More than 400 units/ml serum.

<sup>b</sup> More than 200 units/ml serum.

**Table 13.3**  
Correlation Between DNase Levels and Subtypes of Infiltrating Duct Carcinoma

Subtype	Total No. Patients	Cases of Increased DNase	
		Acid <sup>a</sup>	Alkaline <sup>b</sup>
1. Scirrhous carcinoma	14	4 (28%)	10 (71%)
2. Papillary carcinoma	2	2 (100%)	1 (50%)
3. Tubular carcinoma	1	0	0
4. Mucinous carcinoma	1	1 (100%)	1 (100%)
5. Apocrine carcinoma	2	2 (100%)	2 (100%)
Total	20	9 (44%)	14 (69%)

<sup>a</sup> More than 400 units/ml serum.

<sup>b</sup> More than 200 units/ml serum.

**Table 13.4**  
Correlation Between DNase Levels and Lymph Node Metastases

Histology	Total No. Patients	Cases of Increased DNase	
		Acid <sup>a</sup>	Alkaline <sup>b</sup>
Positive	19	6 (31%)	9 (47%)
Negative	4	4 (100%)	4 (100%)
Total	23	10 (43%)	13 (56%)

<sup>a</sup> More than 400 units/ml serum.

<sup>b</sup> More than 200 units/ml serum.

carcinoma we did not find any lobular *in situ*.

The correlation between DNase levels and lymph node metastases is shown in Table 13.4. It seems that lymph node metastases are not required in order to get increased acid or alkaline DNase.

The correlation between DNase levels and axillary lymph node levels involved in breast cancer patients is shown in Table

13.5. Whereas the percentage of patients with increased acid DNase remained constant, alkaline DNase increased steadily. It should be noted that axillary lymph nodes were available in 23 patients, out of which 19 had metastases. The other eight patients were treated by simple mastectomy or by lymphectomy. When patients had positive lymph nodes in all levels, only Level III was evaluated.

**Table 13.5**  
Correlation Between DNase Levels and Axillary Lymph Node Levels Involved in Patients with Breast Cancer

Level	Total No. Lymph Nodes	Cases of Increased DNase	
		Acid <sup>a</sup>	Alkaline <sup>b</sup>
I	5	2 (40%)	2 (40%)
II	5	2 (40%)	3 (60%)
III	10	4 (40%)	7 (70%)
Total	20	8 (40%)	12 (60%)

<sup>a</sup> More than 400 units/ml serum.

<sup>b</sup> More than 200 units/ml serum.

**Table 13.6**  
Correlation Between DNase Levels and Histological Grade in Patients with Breast Cancer

Grade	Total No. Patients	Cases of Increased DNase	
		Acid <sup>a</sup>	Alkaline <sup>b</sup>
I	1	0	0
II	15	6 (40%)	8 (53%)
III	15	9 (60%)	9 (60%)
Total	31	15 (48%)	17 (48%)

<sup>a</sup> More than 400 units/ml serum.

<sup>b</sup> More than 200 units/ml serum.

The correlation between serum DNase levels and histological grade of the primary tumor in patients with breast cancer was examined and the results are shown in Table 13.6. It was found that the percentage of patients with increased DNase was increased with increasing grade; *i.e.*, of the 15 patients with grade II, 40 and 53% had increased acid and alkaline DNase, respectively, whereas of the 15 patients with grade III, 60% for acid and 60% for alkaline were found to have increased DNase.

## DISCUSSION

A comparative study between serum acid and alkaline DNase levels and histological parameters in patients with benign and malignant disease of the breast was carried out. The results are presented in Tables 13.1 to 13.6. Two major conclusions can be drawn from these results.

1. There is a correlation between increased serum DNase levels and malignancy, whereas only a small percentage of patients with benign breast tumors had increased DNase levels.

2. There is a correlation between serum DNase levels and level of lymph node involved or histological grade of the tumor.

The histological data support our previous clinical findings on serum DNase in a variety of carcinomas (from the gastrointestinal tract, lung, nasopharynx, breast, and other organs<sup>8</sup>), in 150 breast cancer patients compared with 108 healthy individuals,<sup>9</sup> and in our laboratory findings from 100 biopsy samples obtained from ovarian, cervical, and endometrial carcinomas compared with normal tissues of the female reproductive system.<sup>10</sup>

Rosen *et al.*<sup>11</sup> in a relevant study carried out a detailed pathological review of 333 breast lesions that were analyzed for estrogen receptor protein (ERP). They made a correlation of a series of morphological features with the ERP results. They found a higher frequency of positive ERP in lobular carcinoma than in duct or other types. All "benign" tissues were negative for ERP. From the fibroadenomas examined only 11% were positive for ERP. The presence or absence of axillary metastases and the histological grade of the primary tumor were factors unrelated with ERP levels. However, positivity of ERP was increased with increasing level of lymph node involvement.

During the past few years the application of individual or multiple biomarkers in body fluids has been reported for the detection, diagnosis, and determination of the recurrence of breast cancer.<sup>1,12</sup>

In two other studies, determination of ERP levels in homogenized tissue using multiple tumor specimens from individual patients with breast cancer was carried out. There is an agreement in both reports that a degree of variation exists in ERP levels when a primary tumor is compared with a

subsequent metastasis to regional lymph nodes or to distant, simultaneous or later, metastatic sites.<sup>13, 14</sup>

It is widely accepted that this marker represents a helpful guide for predicting response to hormonal therapy in patients with metastatic breast carcinoma. However, only 60% of those with positive receptor sites do in fact respond.<sup>15</sup>

Since we didn't find from the available literature any specific biomarker that is elevated even in all patients with advanced mammary carcinoma, we have studied serum DNases towards this end.

We believe that this preliminary investigation should be expanded in a variety of tumor tissues of a larger number of cases in order to unravel in more detail several aspects of the mechanism and significance of increased serum DNases in cancer patients. We are now pursuing these studies to that effect.

#### References

1. Franchimont, P., Zangerle, P. F., Nogareda, J., Bury, J., Molter, F., Renter, A., Hendrick, J. C., and Collette, J.: Simultaneous assays of cancer-associated antigens in various neoplastic disorders. *Cancer* 38:2287-2295, 1976.
2. Louich, J. J.: Tumor markers: hormones, antigens and enzymes in malignant disease. *Oncology* 35: 54-51, 1978.
3. Schwartz, M. K.: Laboratory aids to diagnosis enzymes. *Cancer* 37:542-548, 1976.
4. Vaitukaitis, J.: Peptide hormones as tumor markers. *Cancer* 37:567-572, 1976.
5. Spandidos, D. A., and Hadjisotiriou, G.: Serum deoxyribonuclease in diagnosis of human cancer. *Hellenic Oncology* 14:218-222, 1978. (In Greek).
6. McDivitt, R. W., Steward, F. W., and Berg, J. N.: Tumors of the breast. In *Atlas of Tumor Pathology*. Armed Forces Institute of Pathology (A.F.I.P.), Washington D.C., 1968, p. 112.
7. Bloom, H. J. G., and Richardson, W. W.: Histological grading and prognosis in breast cancer: a study of 1409 cases of which 359 have been followed for 20 years. *Br. J. Cancer* 9:359-377, 1957.
8. Spandidos, D. A., and Stathopoulos, G.: Serum deoxyribonuclease as a biological marker in cancer patients. Proceedings of the 8th International Symposium on the Biological characterisation of human tumours. *Excerpta Medica* 5:68-73, 1980.
9. Spandidos, D. A., Ramandanis, G., Garas, J., and Kottaridis, S. D.: Serum deoxyribonucleases in patients with breast cancer. *Eur. J. Cancer* 16: 1615-1619, 1968.
10. Kalogeropoulou, P., Bonikos, D., and Spandidos, D. A.: Deoxyribonuclease levels in benign and malignant neoplasms of female reproductive system. Proceedings of the 5th International Symposium on the Biological characterisation of human tumours. *Excerpta Medica* 5:74-77, 1980.
11. Rosen, P. P., Menendez-Botet, C. J., Nisselbaum, J. S., Urban, J. A., Mike, V., Fracchia, A., and Schwartz, M. K.: Pathological review of breast lesions analysed for estrogen receptor protein. *Cancer Res.* 35:3187-3194, 1975.
12. Waalkes, T. P., and Tormey, D. C.: Biologic markers and breast cancer. *Sem. Oncol.* 5:434-444, 1978.
13. Rosen, P. P., Menendez-Botet, C. J., Urban, J. A., Fracchia, A., and Schwartz, M. K.: Estrogen receptor protein (ERP) in multiple tumor specimens from individual patients with breast cancer. *Cancer* 39:2194-2200, 1977.
14. Webster, D. J. T., Bronn, D. G., and Minton, J. P.: Estrogen receptor levels in multiple biopsies from patients with breast cancer. *Am. J. Surg.* 136:337-338, 1978.
15. Jensen, E. V.: Estrogen receptors in hormone dependent breast cancer. *Cancer Res.* 35:3362-3364, 1975.