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Serum Deoxyribonucleases in Patients with Breast Cancer

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Abstract—*Serum acid and alkaline DNase levels were determined in the serum of* 224 *breast cancer patients and* 110 *healthy individuals. Values above* 400 U/ml *serum for the acid DNase and* 200 U/ml *serum for the alkaline DNase were taken as abnormal. Whereas a percentage of breast cancer patients showed increased DNase levels none of the healthy individuals reached an abnormal value. DNase levels were correlated with the clinical status of the breast cancer patients. The percentage of patients with elevated DNase levels was found to be increased with increasing clinical stage. For the acid DNase it was found to be* 4/63 (6%), 2/11 (18%), 7/25 (28%), 39/80 (49%) *and* 43/45 (96%) *for benign tumors and stages I, II, III and IV breast carcinomas, respectively, whereas for alkaline DNase it was* 2/63 (3%), 2/11 (18%), 8/25 (32%), 42/80 (52%) *and* 42/45 (93%). *All* 20 *of the clinical stage III patients examined* 1 month *after modified radical mastectomy, as well as* 35 *other breast cancer patients on adjuvant therapy, had a normal value of DNase levels. With few exceptions i.e., rheumatic fever and polymyositis, no significant increase in nuclease activity was found in the serum of patients with some other conditions causing inflammatory responses and cell death. These preliminary findings indicate that measurements of serum DNase could be used in diagnosis and in monitoring the response to surgical treatment of breast cancer patients.*

INTRODUCTION

IN THE last few years a large number of biological cancer markers have been claimed to be useful in diagnosis and monitoring the response to therapy of cancer patients [1]. However, most of the well defined diagnostic biological markers are related to rarer forms of cancer [2]. The problem that exists in the diagnosis of cancer is the following: as a tumor grows it reaches a finite size when there is probability of the occurrence of metastases. However, by the time the tumor is large enough for a diagnostic test to be positive the chances of metastases are much greater: and by the time the patient experiences symptoms, there is significant chance that metastases have occurred.

If improved cure rates are a function of treatment before the cancer is spread, then successful diagnostic testing is essential before clinical symptoms occur. Towards these ends we have studied serum deoxyribonucleases in patients with a variety of cancer as an additional biological cancer marker: our previous results have suggested that these enzymes are increased in the serum and tumor biopsies of cancer patients [3, 4]. The objectives of this study are: (1) to determine in more detail the levels of serum DNases in breast cancer patients and (2) to evaluate the significance of the serum DNases in the conventional therapy of these tumors.

MATERIALS AND METHODS

Patients

Blood samples were drawn from internal female cancer patients and healthy blood donors of the Greek Cancer Institute, Athens, and the serum was kept at −20°C until it was used, within a week. No significant decrease in DNase(s) levels of sera kept under these conditions was observed. The extent of disease was determined by physical examination, liver chemistries, chest and bone roentgenograms, broncho- and mediastinoscopy and nuclear scans of the liver and bone. Roentgenograms, blood chemistries and nuc-

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lear scans were repeated at intervals during therapy. The patients for the follow-up were examined at 1–3 week intervals.

Enzyme assays

The deoxyribonuclease assay was performed following the method developed by Kunitz [5]. This is based upon the increased absorption at 260 nm observed during the depolymerization of DNA by DNase. The unit was defined as that amount of enzyme which caused an increase of absorbance at 260 nm of 1.0 per min at 25°C. A standard enzyme preparation (Worthington standard deoxyribonuclease I) was run in parallel with the unknown for standardization of DNA preparations. Calf thymus DNA was used as substrate and it was purchased from Sigma Chemical Co.

The enzyme assay for the acid DNase involved, in 1.0 ml; 100 μg DNA, 0.1 M sodium acetate buffer pH 5.0, 0.005 M $MgCl_2$ and 25 μl serum. The assay for the alkaline DNase involved, in 1.0 ml; 100 μg DNA, 0.1 M Tris–HCl buffer pH 8.0 and 25 μl serum. In both assays, the mixture was incubated at 25°C for 15 min and then 2 ml of 1.5 M perchloric acid was added at 4°C. After 10 min the mixture was centrifuged at 3000 ***g*** 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.

RESULTS

Evidence for serum deoxyribonuclease activity

Deoxyribonuclease activities in the sera of internal breast cancer patients of our Institute and in healthy blood donors was first examined. Preliminary assays showed two peaks of activity; one at pH 5.0 (acid deoxyribonuclease) and a second at pH 8.0 (alkaline deoxyribonuclease). Low levels of acid deoxyribonuclease (0–200 U/ml serum) and alkaline deoxyribonuclease (0–100 U/ml serum) were found in 110 healthy blood donors. However, in 62 of the first 100 breast cancer patients examined serum acid and alkaline deoxyribonucleases were found to be increased (more than a 100% increase) compared to the control healthy blood donors.

Paper chromatography of the reaction mixture of both acid and alkaline DNases showed that small amounts of mononucleotides were formed. Further confirmation of the endonucleolytic degradation of the DNA substrate was obtained by gel filtration of the reaction mixture through Sephadex G-200 that excludes polymers of mol. wt 2×10^5 or higher. Eighty per cent of the DNA incubated with the acid DNase and 60% of the DNA incubated with the alkaline DNase was retarded on the column, indicating that hydrolysis did occur. Of the hydrolysis products approximately 60% were only slightly retarded in both cases indicating a mol. wt not much less than 2×10^5.

Relation of deoxyribonucleases to breast cancer

Our early breast cancer patients represent a random sample of a variety of cancers treated or not. Since some of our early breast cancer patients had undergone surgery and they were receiving chemotherapy or radiation a detailed study was needed with patients without any of the above treatments to examine whether DNAses could be used in the initial diagnosis of cancer. we then examined the acid and alkaline DNases in the serum of patients prior to any treatment and the results are shown in Figs. 1 and 2. As shown in the figures, we have classified our patients into two groups according to the histopathological

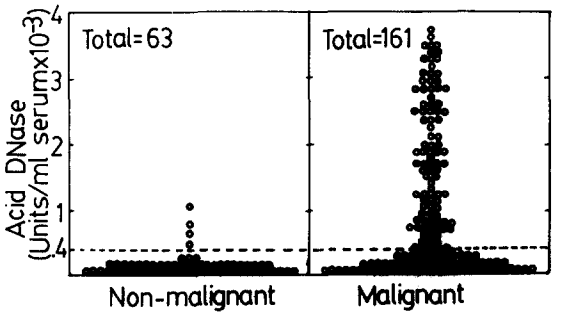

Fig. 1. Serum acid DNase levels in 224 breast cancer patients (63 with non-malignant and 161 with malignant tumors). Each point represents the average of three determinations of the same serum sample of an individual patient. Intra-assay variations were at the level of 1–10% of the average DNase level.

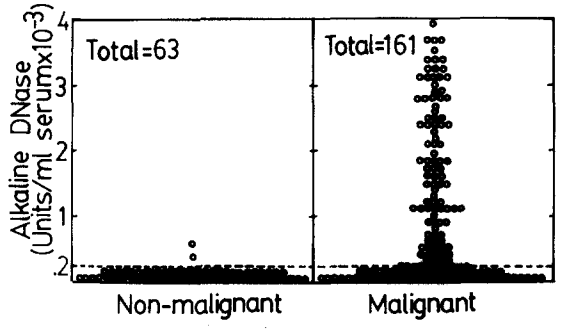

Fig. 2. Serum alkaline DNase levels in the same breast cancer patients as in Fig. 1.

*Table 1. Mean (±S.E.) serum acid and alkaline DNase in healthy blood donors and breast cancer patients**

	Healthy blood donors (patient No.)	Mean ± S.E.	Breast cancer patients (patient No.)	Mean ± S.E.
Acid DNase (U/ml serum)	1	54 ± 5	1	1832 ± 153
	2	125 ± 12	2	3456 ± 317
	3	185 ± 10	3	1540 ± 137
	4	132 ± 2	4	853 ± 78
	5	170 ± 15	5	2247 ± 215
Alkaline DNase (U/ml serum)	1	98 ± 5	1	1528 ± 82
	2	74 ± 4	2	1315 ± 123
	3	65 ± 4	3	3540 ± 347
	4	52 ± 2	4	2810 ± 151
	5	85 ± 8	5	752 ± 45

*Eight determinations of each serum sample were performed.

findings of the tumor biopsies. The first group involves patients with non-malignant (benign) tumors and the second the breast carcinoma patients. As seen in Fig. 1, only 4 out of 63 patients with non-malignant tumors had an increased (more than 400 U/ml) serum acid DNase level. On the other hand 91 out of 161 patients with breast carcinomas had increased acid DNase levels. In Fig. 2 are shown the results for the serum alkaline DNase in the same as above patients. Only 2 out of 63 patients with benign tumors had increased alkaline DNase levels whereas 94 out of 161 with malignant tumors had increased (more than 200 U/ml, and up to 4000 U/ml) serum alkaline DNase levels.

The average age of the 110 healthy blood donors was 34 (range of 20–45), of the 63 patients with non-malignant tumors was 41 (18–71) and of the 161 patients with breast carcinomas was 45 (21–75) yr.

Intra-assay variations of the same serum sample were determined in 30 healthy blood donors and 40 breast cancer patients. The results for 5 representative patients of each group are shown in Table 1. Such variations were found to be at the level of 1–10% of the mean DNase level(s) in all serum samples of the 70 individuals examined.

Interassay variations for 55 healthy blood donors examined 30 days after the initial examination were similar to the intra-assay variations, that is at the level of 1–10% of the mean value of serum DNase level(s) for the particular patient examined. However, inter-assay variations for 110 breast cancer patients examined 30 days after the initial examination were in some cases up to 35% higher than the initial value. This could be the result of altered clinical status of the patients.

The distribution frequency by clinical stage of serum deoxyribonucleases in breast cancer patients was next examined. Clinical staging by the TNM system was undertaken on the above 161 breast carcinoma patients in whom sufficient information was available. The staging was done according to U.I.C.C. rules. The results are shown in Table 2. More than 400 U/ml serum for the acid DNase and 200 U/ml serum for the alkaline DNase were considered as abnormal values. As shown in the table the percentage of patients with

Table 2. Distribution frequency by clinical stage of serum deoxyribonucleases in breast cancer patients

Clinical stage	Total No. of patients	Patients with increased DNase (%)	
		Acid*	Alkaline†
Healthy individuals	100	(0)	0 (0)
Non-malignant breast tumors‡	63	4 (6)	2 (3)
Malignant breast tumors‡			
I	11	2 (18)	2 (18)
II	25	7 (28)	8 (32)
III	80	39 (49)	42 (52)
IV	45	43 (96)	42 (93)
Total	161	91 (57)	94 (58)

*More than 400 U/ml serum.
†More than 200 U/ml serum.
‡The classification of non-malignant and malignant tumors was based upon the histopathological findings of tumor biopsies.

*Table 3. Levels of deoxyribonucleases in relation to surgical treatment in breast cancer patients before or after operation**

Days		Total No. of patients	Patients with increased DNase (%)	
Before	After		Acid†	Alkaline‡
−10–0		80	39 (40)	42 (52)
	0–10	26	20 (76)	22 (84)
	11–20	15	6 (40)	9 (60)
	21–30	12	1 (8)	2 (16)
	31	20	0 (0)	0 (0)
Adjuvant		35	0 (0)	0 (0)

*Only patients in clinical stage III were involved in these studies.
†More than 400 U/ml serum.
‡More than 200 U/ml serum.

increased serum acid and alkaline DNase levels increase with advancing stage of disease. For the acid DNase it was found to be 6, 18, 28, 49 and 96% for benign breast tumors and stages I, II, III and IV respectively, whereas for alkaline DNase it was 3, 18, 32, 52 and 93%. None of the 110 normal individuals examined reached an abnormal value of serum acid or alkaline DNase. As a matter of fact, in none of the above 110 patients serum acid and alkaline DNase levels reached levels higher than 200 U/ml serum and 100 U/ml serum respectively.

The levels of deoxyribonucleases in relation to surgical treatment in breast cancer patients before or after operation was next examined. The results are shown in Table 3. Only patients in stage III were involved in these studies. As it was also shown in Table 2, 80 patients were examined before surgical treatment and 49% of these patients had increased acid DNase and 59% increased alkaline DNase. Only 26 of these patients were subjected to surgical removal of the tumor. One to 10 days after the removal of the tumor 76 and 84% of the patients had increased acid and alkaline DNase, respectively. Only some of these patients were further examined for serum DNase levels. Eleven to 20 days after the operation 40 and 60% of the patients had increased DNases, whereas 21–30 days later only 8 and 16% of them showed increased levels. Thirty-one days after operation none of the patients had increased DNase levels. Finally, all 35 patients on adjuvant therapy were found to have normal levels of serum DNases. To monitor the response to surgical treatment of breast cancer patients in more detail the levels of DNase at various time intervals were examined. Two typical examples are shown in Figs. 3 and 4. The patient

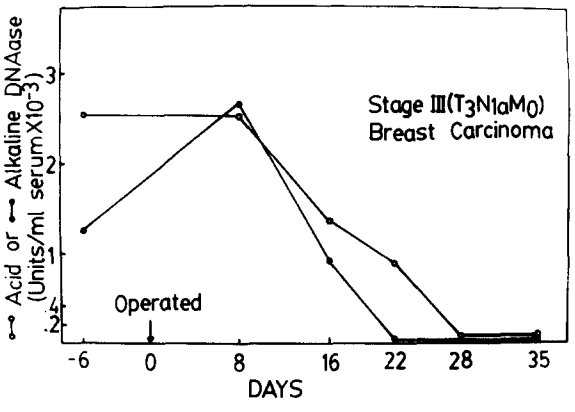

Fig. 3. DNase levels during surgical treatment of a stage III ($T_3N_{1a}M_o$) breast carcinoma patient. Each point represents the average of three determinations.

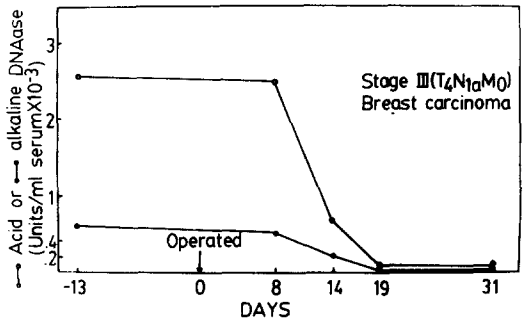

Fig. 4. DNase levels during surgical treatment of a stage III ($T_4N_{1a}M_o$) breast carcinoma patient.

in Fig. 3, a 68-yr-old woman, with a stage III tumor ($T_3N_{1a}M_o$) was subjected to modified radical mastectomy. As seen in the figure high levels of both acid and alkaline DNases were found before surgical treatment of the tumor. Eight days after the operation the alkaline DNase level increased whereas the acid DNase remained constant. Within the next 3 weeks the levels of both DNases dropped to normal values.

The patient in Fig. 4, a 55-yr-old woman, with a stage III tumor ($T_4N_{1a}M_0$) was subjected to modified radical mastectomy. Whereas acid DNase levels reached high values before and 8 days after the operation, alkaline DNase levels were only slightly increased. Nineteen days after the operation DNase levels had dropped to normal values.

DNase activity in the serum of non-cancer patients

The level of serum acid and alkaline DNase activities was examined in patients with conditions causing inflammatory responses and cell death. The conditions examined and the number of patients involved are as follows: liver cirrhosis, 7; systematic lupus erythymatosus, 2; viral hepatitis, 5; myocardial infarction, 3; rheumatic fever, 12; polymyositis, 3; muscular dystrophy, 3; duodenal ulcer, 2; various non-neoplastic blood diseases, i.e., sickle-cell anemia, 11. Only 9 patients with rheumatic fever and all 3 with polymyositis exhibited increased DNases in their serum whereas all other patients had normal values.

DISCUSSION

The need for sensitive methods in detecting the presence of a tumor, its growth and metastases as well as an effective treatment is well understood.

Over the past few years different individual markers have been developed for the detection and evaluation of tumor status in cancer patients [6–8]. Of those biomarkers available to date several appear to have promise while differences of opinion exist in many others.

Because in all the work done so far no single marker has been found to be elevated in advanced breast cancer patients this study was undertaken to that effect. Increased levels of acid and alkaline DNases in the serum of breast cancer patients were found. The increased enzyme levels were correlated with the clinical stage of patients. The results suggested that serum DNases could be used not only in the diagnosis but also in monitoring the response to therapy of breast cancer patients.

The DNase assay described above is simple, inexpensive, accurate, not time consuming and it could be used as a mass screening test for cancer, since other diseases with increased levels of DNase activity in the serum like rheumatic fever or polymyositis could be easily diagnosed.

The increase of acid and alkaline DNases in the serum of the above patients is not well understood. Perhaps it is a metabolic end product of tumor, a result of cell proliferation or distribution or some other unknown mechanism. Based on the above results of this preliminary investigation we can postulate that the utilization of these markers can be of significant clinical value. We are now studying these DNases toward these ends.

REFERENCES

1. J. J. Lokich, Tumor markers: hormones, antigens and enzymes in malignant disease. *Oncology* **35,** 54 (1978).
2. M. K. Schwartz, Laboratory aids to diagnosis—enzymes, *Cancer (Philad.)* **37,** 542 (1976).
3. D. A. Spandidos, M. Papamichail and G. Stathopoulos, Serum deoxyribonucleases as a biological marker in cancer patients. Eighth International Symposium on the Biological Characterization of Human Tumors. *Excerpta med. (Amst.), Sect. XVI,* in press (1979).
4. P. Kalogeropoulou, D. Bonikos and D. A. Spandidos, Deoxyribonuclease levels in benign and malignant neoplasms of female reproductive system. Eighth International Symposium on the Biological Characterization of Human Tumors. *Excerpta med. (Amst.), Sect. XVI,* in press (1979).
5. M. Kunitz, Crystalline deoxyribonuclease. I. Isolation and general properties. *J. gen. Phys.* **33,** 349 (1950).
6. T. P. Waalyss and D. C. Tormey, Biologic markers and breast cancer. *Semin. Oncol.* **5,** 434 (1978).
7. A. M. Neville and E. H. Cooper, Biochemical monitoring of cancer. *Ann. clin. Biochem.* **13,** 282 (1976).
8. P. Franchimont, P. F. Zangerie, J. Nogarede, J. Eury, F. Molter, A. Reuter, J. C. Hendrich and J. Collette, Simultaneous assays of cancer-associated antigens in various neoplastic disorders. *Cancer (Philad.)* **38,** 2287 (1976).