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Correlation Between Serum and Tissue Deoxyribonuclease Levels in Breast Cancer Patients

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Abstract. *Acid and alkaline DNase levels were determined in the serum of 224 breast cancer patients and in the extracts of 121 breast tissue and 48 lymph node biopsies of the above patients. The DNase levels were compared to the histological diagnoses of the tumors. Serum and tissue DNase levels were found to be significantly increased in malignancies as compared to benign conditions. A close correlation was found between serum and tissue DNase levels. Measurement of DNase levels within tumors could become clinically important i.e. screening of patients for metastatic disease, or* in vitro *testing of tumor cells sensitivity to therapeutic regimes.*

Monitoring of tumor burden remains an important problem in the treatment of most patients with cancer. The measurement of tumor products is, therefore, invaluable in the diagnosis, monitoring of therapy and detection of tumor recurrence.

Although no single sensitive marker has been found for breast cancer, alterations in the level or properties of one or more tumor related substances have been reported in several studies (1-4). We have recently found that cancer patients have increased levels of serum deoxyribonucleases (5-7). Our studies included patients with breast cancer (5) or other malignant neoplasms (6, 7). A similar study also indicated that deoxyribonuclease levels are elevated in benign and malignant neoplasms of female reproductive system compared to normal tissue (8). A comparison between DNase levels and histological parameters in patients with benign and malignant breast disease exhibited a correlation between increased serum DNase levels and malignancy, whereas only a small percentage of patients with benign tumors had increased DNase levels (9). Moreover, a correlation was found between serum DNase levels and level of lymph node involvement on histological grade of the tumor (9).

In the present work DNase levels in breast and lymph node tissue have been measured. It has been found that breast and lymph node malignant tissue have increased DNase levels compared to non-malignant tissue. Moreover, a comparison of tissue to serum DNase levels of the same patient was carried out. In particular, DNase levels were examined in the serum before the removal of the tumor and in the breast or lymph node tissue biopsies. The DNase levels were then compared to the histological findings. A close correlation between serum and tissue DNase levels has been observed.

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Key Words: Serum and tissue deoxyribonuclease, breast cancer.

Materials and Methods

Patients and sample preparations. Two hundred and twenty-four internal cancer patients 19-68 years of age, of the Hellenic Cancer Institute, Athens, were examined in this study. Assessment of all patients included medical history and examination, chest and bone X-rays, routine blood chemistries and nuclear scans of the liver and bone.

Serum was frozen within 2h of venesection and stored at −20°C until assayed within a week. Tumors were sliced finely and homogenized (10 strokes in a Dounce homogenizer) in TEM buffer (10 mM, Tris HCl pH 7.4, 1.0 mM EDTA, 0.5 mM β-mercaptoethanol) containing 0.5 % of the non-ionic detergent Nonidet P-40. After centrifugation at 1000 g for 10 min the supernatant (tissue extract) was used for enzyme and protein determinations.

Enzyme assays. The assays for the serum acid and alkaline deoxyribonucleases have been described in detail elsewhere (5). Enzyme levels were expressed as Units/ml serum. The Unit was defined as that amount of enzyme which resulted in an increase of absorbance at 260 nm of 0.001 per min at 25°C (10). Tissue DNases were assayed in the same way, except that enzyme levels were expressed as Units/mg protein. Protein determinations were performed as described (11). A DNase standard (DNase I, Sigma Chemical Co). was run in parallel with the test sera or extracts. Intra-assay variation of the same sera, tested on 10 cancer patients and 10 controls was always less than 10 %. A less than 10 % intra-assay variation of the same tissue extracts was also found on 5 cancer patients and 5 controls tested.

Histological examination. A total of 835 slides corresponding to one hundred and sixty-nine biopsy samples from breast cancer patients were reviewed. Slides were stained with H & E and all malignant breast cases were graded as described (12).

Results

Serum DNase levels in patients with benign or malignant neoplasms of the breast. Two hundred and twenty-four patients of the Breast Clinic of the Hellenic Cancer Institute in Athens, for whom histological analyses of their biopsies were available, were considered for this study. Serum samples had been taken 1-5 days preoperatively and serum acid and alkaline DNase levels were determined. Out of 161 patients with histologically certified malignant tumors, 97 (60.2 %) had increased levels of acid DNase (more than 400 Units/ml serum) (5) and of the 63 patients with non-malignant conditions, 4 (6.3 %) had increased levels of the same DNase activity (fig. 1). Similar results were obtained when serum alkaline DNase levels were studied in the above patients (fig. 2). As observed, out of the 161 patients with malignant tumors, 94 (58.4 %) had increased alkaline DNase levels and of the 63 patients with non-malignant conditions, 2 (3.2 %) had elevated levels of the same DNase activity. These results confirm and extend our previous observations with serum DNases in breast cancer patients (5).

Tissue DNase levels in benign and malignant neoplasms of the breast. We have previously found that benign and malignant neoplasms of female reproductive system have elevated levels of DNases when compared to normal tissue (8). To examine whether this is true for neoplasms of the breast we have measured the levels of acid and alkaline DNases in breast tissue of cancer patients. 78 and 43 breast tissue biopsies were available out of the 161 and 63 patients with malignant and non-malignant conditions respectively. In comparison to the non-malignant breast tissue, increased levels of the acid (fig. 3) and alkaline (fig. 4) DNases were observed.

DNase levels in lymph node tissue of breast cancer patients. The DNase levels in 48 lymph node tissue biopsies available of the above 224 patients were examined. Again increased acid (fig. 5) and alkaline (fig. 6) DNase levels were found in 27 patients with histologically certified malignant lymph node tissues, when compared to the 21 patients with non-malignant histological findings.

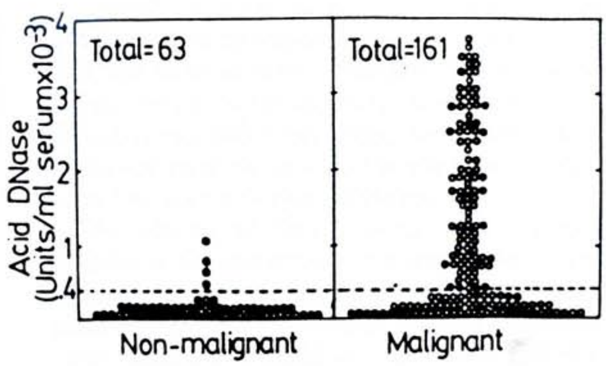

Fig. 1. *Serum acid DNase in breast cancer patients.*

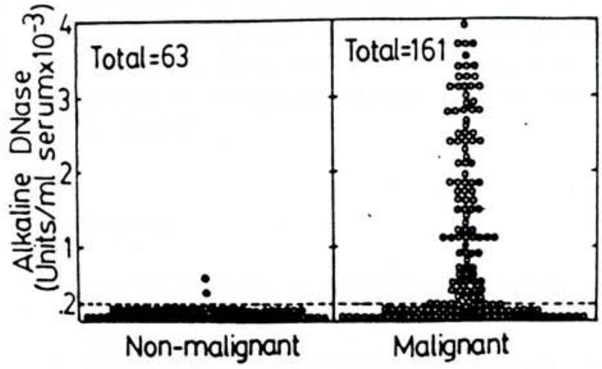

Fig. 2. *Serum alkaline DNase in breast cancer patients.*

Discussion

The objectives of this study were twofold. Initially, to measure the DNase levels in breast and lymph node tissue of patients with benign or malignant neoplasms of the breast. Secondly to determine whether there is any correlation with serum DNase levels measured preoperatively, of the same patient. It has been found that patients with malignant neoplasms have increased tissue DNase levels and a good correlation has been observed between tissue and serum DNase levels.

From our previous (5-9) and present studies (see Appendix) we are of the opinion that DNases fulfil the initial requirements of a satisfactory tumor marker. Their levels are frequently increased, often to a high level, and they are more commonly raised in advance than in local disease. Moreover, in follow up of individual patients we have found that changes in abnormal serum DNase levels are

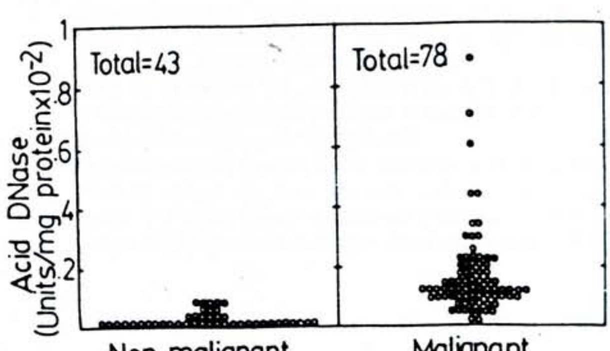

Fig. 3. *Acid DNase in breast tissue biopsies.*

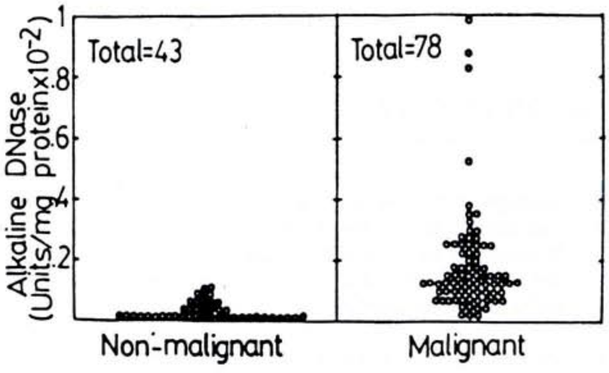

Fig. 4. *Alkaline DNase in breast tissue biopsies.*

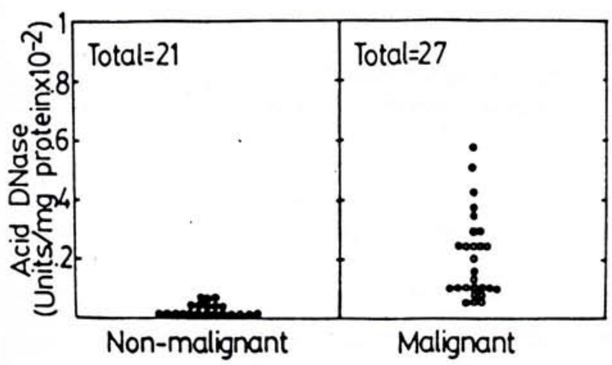

Fig. 5. *Acid DNase in lymph node biopsis.*

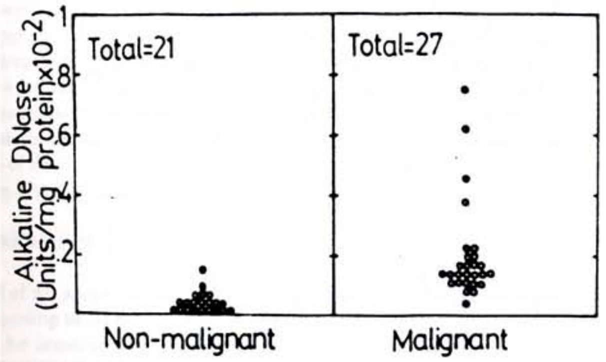

Fig. 6. *Alkaline DNase in lymph node biopsies.*

concordant with clinical change in tumor burden (5, 6 and unpublished results).

Differences in the serum DNase levels provide good evidence of tumor secretion of a marker. However, measurements in tumor extracts could be a more practicable alternative for breast tumors. As described in the accompanying statistical analysis (see Appendix) a close correlation has been observed between the serum and tissue DNase levels. Consequently, patients with high serum DNase levels also exhibit high tissue levels, whereas patients with reduced activity of serum DNases were found to have low levels of tissue DNases.

The increase of DNase levels in the serum of these patients is not understood at present. For example they could be a metabolic end product of tumor, a result of cell proliferation, cell destruction, specific induction or some other unknown mechanism. The study undertaken here was also directed towards gaining an insight into the mechanisms of the increased DNase levels in the serum of cancer patients. A simple explanation from the presented results could be that the increased serum DNase levels are due to the diffusion of these enzymes from the tumor cells to the blood. However, other explanations, i.e. alterations in the stability of the enzymes in tissue or serum are possible.

The measurement of DNase levels within tumors, as demonstrated in this study, could become clinically important for a variety of reasons, i.e. screening of patients for metastatic disease or *in vitro* testing of tumor cells sensitivity to therapeutic regimes. We are now studying these aspects.

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Appendix

Statistical Analysis of Acid and Alkaline DNase Levels Found in the Serum and Biopsies of Breast Cancer Patients

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Abstract. *A statistical analysis of acid an alkaline DNase levels found in the serum and tissue biopsies of breast cancer patients was carried out. A perfect discrimination was possible for the case [breast tissue], while for the case [lymph node tissue] the discrimination was less clear due to some cancer patients being classified as normals and the converse. This statistical analysis can be of valuable assistance in the diagnosis of a new cancer patient.*

The use of statistical methods in medical problems is widespread. It is well known that statistical analysis in connection with graphical techniques can be of significant assistance in the analysis of medical data. These graphical techniques give a better picture of the data, help in finding a probability model to fit the data and finally can aid the decision which statistical methods must be applied in order to get useful conclusions valuable to the clinician. The rigorous statistical and mathematical analysis of the above techniques is included in this work and is carried out by using discrimination methods.

Graphical Results

Figs 1-6 of the accompanying paper are the one dimensional graphs of the data according to their measurements (Units/ml for serum and Units/mg protein for breast and lymph node tissue DNases).

The MINITAB PACKAGE of the University of Glasgow was used to plot the two dimensional graphs and the normal scores of the logarithms of actual data versus the logarithms of the actual data. The data was first ploted in two-dimensional graphs of six different combinations for case breast (B): e.g. T-AC vs T-AL, T-AC vs S-AC, T-AC vs S-AL vs S-AC, T-AL vs S-AL and S-AC vs S-AL where T=Tissue, S=Serum, AC=Acid and AL=Alkaline. A representative example is shown in fig. 1. These two-dimensional graphs help us to examine whether there is any clear discrimination between patients with malignant histological findings referred to subsequently as cancer patients (C) and patients with non-malignant histological findings referred to as normals (N).

We have also plotted the data as graphs of the normal scores of the natural logarithms of the data (case tissue B) versus the natural logarithms of the data. A representative example is shown in fig. 2. It is suggested that the normal approximation of the log-transformation of the data is acceptable.

The corresponding representative graph for the case lymph (L) is given in fig. 3, whereas the graph in fig. 4 was used to check the normality of the log-data.

Probability Model

As shown above when the normal scores of the natural logarithms of the data were plotted versus the natural logarithms of the data, almost straight lines were obtained. This is in accordance with the analysis of variance. This is a clear indication that the log-transformation of the data follows a normal distribution (1). An alternative approach to verify whether the data followed a log-normal distribution was the chi-square test (2). Such an analysis was also in agreement with the above observations.

Discrimination Methods-Statistical Analysis

From the two-dimensional graphs (Breast Case) it was shown that fig. 1 gives a perfect discrimination (3) when we reject the outlier indicated by an arrow.

In order to define *discrimination* it is required initially to define the bivariate normal distribution. The bivariate variable

$\underline{z} = \binom{x}{\psi}$ follows a bivariate normal

distribution with mean vector $\underline{\mu}$ and matrix variance Σ (denoted $\underline{z}$-N($\underline{\mu}$,Σ) when its probability density function is:

$$f(\underline{z}) = \frac{1}{2\pi|\Sigma|^{1/2}} \exp\left|-\frac{1}{2}(\underline{z}-\underline{\mu})^T\Sigma^{-1}(\underline{z}-\underline{\mu})\right| \qquad 1$$

where $|\Sigma|$ is the determinant of the matrix Σ, $(\underline{z}-\underline{\mu})^T$ is the tranpose of the vector $(\underline{z}-\underline{\mu})$ and Σ^{-1} is the inverse of the matrix Σ(4).

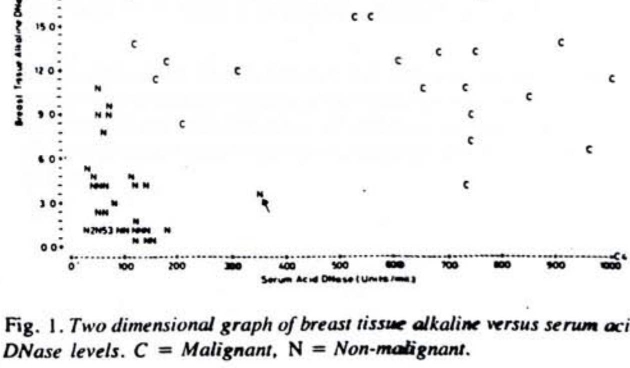

Fig. 1. *Two dimensional graph of breast tissue alkaline versus serum acid DNase levels. C = Malignant,* N *= Non-malignant.*

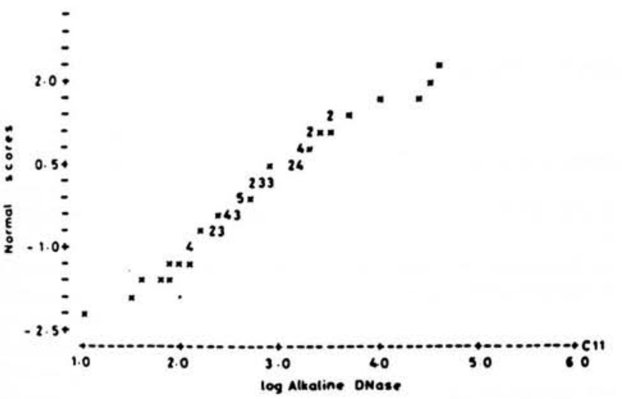

Fig. 2. *Graph of the normal scores of the natural logarithms of the data for breast tissue alkaline DNase versus the natural logarithms of the data.*

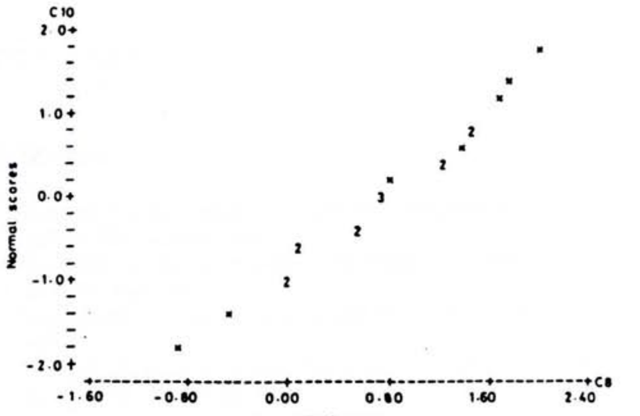

Fig. 4. *Graph of the normal scores of the natural logarithms of the data for lymph node tissue alkaline DNase versus the natural logarithms of the data.*

Fig. 3. *Two dimensional graph of lymph node alkaline versus lymph node acid DNase levels. C = Malignant, N = Non-malignant.*

Assuming that the vector $\underline{z} = \left(\begin{matrix}\log T\ (Alk) \\ \text{Log } S\ (AC)\end{matrix}\Big/ C\right)$ is a bivariate variable with components the natural logarithms of the measurements of Tissue-Alkaline and Serum-Acid conditioned on C. Then relation 1 can be written as follows:

$$f(\underline{z}) = \frac{1}{2\pi|\Sigma_c|^{1/2}} \exp\left|-\frac{1}{2}(\underline{z}-\mu_c)^T\Sigma^{-1}{}_c(\underline{z}-\mu_c)\right| \qquad 2$$

similarly we get

$$f(\underline{z}) = \frac{1}{2\pi|\Sigma_N|^{1/2}} \exp\left|-\frac{1}{2}(\underline{z}-\underline{\mu}_N)^T\Sigma^{-1}{}_N(\underline{z}-\underline{\mu}_N)\right| \qquad 3$$

where $\underline{z} = \left(\begin{matrix}\log T\ (Alk) \\ \log S\ (AC)\end{matrix}\Big/ N\right)$

The conditional variable indicates whether a person with corresponding measurements log T (Alk) and log S (Ac) is from a Cancer Sample or from a Normal Sample.

For a new patient with measurements given by the bicariate variable $\underline{X}$, the "odds" ratio can be defined using the Bayers formula

$$\frac{P(C/\underline{X})}{P(N/\underline{X})} = \frac{P(C)}{P(N)} \frac{P(\underline{X}/C)}{P(\underline{X}/N)} \qquad 4$$

where $P(C/\underline{X})$, $P(N/\underline{X})$ are the probabilities that the new patient has Cancer or is Normal.

Taking the natural logarithm of the relation 4 and making use of the relations 2 and 3 we obtain

$$\log \frac{P(C/\underline{X})}{P(N/\underline{X})} = \log \frac{P(C)}{P(N)} + \frac{1}{2} \log \frac{|\Sigma_c|}{|\Sigma N|} + \frac{1}{2} (\underline{X}-\underline{\mu}_N)^T\Sigma^{-1}(X-\underline{\mu}_N) - \frac{1}{2}(\underline{X}-\underline{\mu}_c)^T\Sigma^{-1}{}_c(\underline{X}-\underline{\mu}_c) \qquad 5$$

In defining the problem by "discrimination analysis" the following rule may be applicable: A patient with vector measurements $\underline{X}$ is a cancer patient if:

$$\log \frac{P(C/\underline{X})}{P(N/\underline{X})} > 0 \qquad 6$$

Results and Discussion

Breast Case. From relations 5 and 6 the unknown parameters involved can be estimated as follows: A patient with measurements X[=log Alk (Tissue)] and Y[=log Acid (serum)] is a cancer patient if:
$-0.2834X^2 + 2.7386X + 0.4731XY - 7.8443Y + 1.2386Y^2 - 1.0715 > 0$. 7

Lymph Node Case. In this instance it is necessary to modify our rule because discrimination regarding cancer patients and normals we see that a Normal (N) and a Cancer patient (C) (indicated by arrows fig. 3) could be taken to be Cancer patient and Normal respectively. Our modified rule has as follows:

I. A new patient with measurements X and Y, as above, is a cancer patient if:

$$-1.2355X^2 + 4.3558X + 1.4683XY + 3.0213Y - 0.8314Y^2 - 10.5611 + \text{Const } 1 > 0 \quad 8$$

where the Const 1 has been added to ensure that every cancer patient satisfies equation 8. II. "Deside that a new patient with measurements X and Y is normal if:

$$-1.2355X^2 + 4.3558X + 1.46833XY + 3.0213Y - 0.8314Y^2 - 10.5611 + 9 \text{ Const } 2 > 0. \quad 9$$

where the const 2 has been added to ensure that every normal satisfies equation 9".

If a new patient satisfies both equations 8 and 9, then it is not possible to reach any conclusion whether he is a cancer patient or not.

In this case Const 1 and Const 2 can be substituted with the values 0.50 and -1.90 respectively.

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