Urol Res (1982) 10:67–70

Urological Research

The Role of Acid and Alkaline DNases as Tumour Markers in Cancer of the Genitourinary Tract

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Accepted: November 3, 1981

Summary. The levels of Acid and Alkaline DNases were measured in the serum of patients with: (a) Cancer of the Genitourinary Tract (confirmed by biopsy), (b) with inflamatory diseases and non-malignant tumours of the Genitourinary tract, (c) healthy blood donors. In the first group the results showed that the Acid DNase level was raised in 62% and Alkaline DNase in 43%. In the second group acid DNase was increased in 30% and Alkaline DNase in 13%. In the third group Acid and Alkaline DNase levels were normal. These results suggest that the measurement of Acid and Alkaline DNases could be considered as malignant diseases markers, in spite of false positive and false negative results in some cases.

Key words: Acid-Alkaline DNases, Tumour markers, Genitourinary cancer.

Introduction

The use of biochemical markers in screening for the presence of cancer and in the initial diagnosis of the disease has not received unqualified support. It has been well established that some malignant cells produce substances which, when released into the circulation, reflect the presence and even the stage of the tumour [6]. However only a few of these are useful in the primary diagnosis of common cancers [2].

Most of the well-defined diagnostic biochemical and immunological tests are related to uncommon types of cancer. In some, enzymes have been used in the diagnosis of cancer and in the prognosis of the disease [5].

DNase and RNase as tumour markers have not previously been investigated in urinary cancers. Kottel [1] has investigated the serum ribonuclease activity in general cancer with good results.

We have studied Serum Doexyribonuclease in malignant and non-malignant cases and our results suggests that this enzyme could be useful in the initial diagnosis and in the follow-up of cancer patients.

Patients and Methods

The patients were divided into three groups: a) Patients with cancer of the genitourinary tract, confirmed by pathological examination; b) Patients with inflammatory diseases and non-malignant tumours of the Genitourinary tract; c) Healthy patients who were blood donors.

In the first group 109 patients with urological cancers, 68 had transitional cell carcinoma of the urinary bladder stages I–IV and Grades I–IV; 18 had prostatic cancer of all stages and Grades; 10 patients had testicular tumours and 13 patients had penile carcinoma,

The second group of 30 patients had non-malignant tumours and inflammatory diseases of the urinary tract such as papilloma, hypertrophy of the prostate, urethritis, cystitis, epididymo-orchitis and pyelonephritis.

The third group of 38 included healthy patients who had come to the hospital as blood donors.

Calf thymus DNA (DI 501 Type I), was purchased from SIGMA Chemical Co. and was used as a substrate. Serum from all the patients was taken and kept at −20 °C until assayed within one month.

The enzyme assay for alkaline deoxyribonuclease involved 1.0 ml 100 μg DNa 0, 1 Sodium acetate buffer PH 5, 0.005 M $MgCl_2$ and 25 μl serum.

In both assays, the mixture was incubated at 24 °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 3,000 rpm for 10 min. The supernatant was kept and its absorbance at 260 nm was measured against a blank which was made in 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 min at 25 °C [3].

DNase and RNase circulate in the serum normally as products of the mitosis of human cells. The level of acid DNase in normals should not be more than 300 units/ml and Alkaline DNase no more than 180 units/ml. In our measurements we accepted as the upper limit of normal for acid DNase 400 units/ml and for alkaline DNase 200 units/ml.

Results

Group I: Urological Cancer (109 Cases)

Increased levels of Acid DNase were found in 62% and increased levels of Alkaline DNase were found in 43% of pa-

0300-5623/82/0010/0067/$01.00

Table 1. Increased[a] acid and alkaline deoxyribonuclease in the serum of a) cancer patients, b) in the serum of patients with benign diseases, c) in the serum of healthy blood donors (control group)

a) Patients with cancer (site of cancer)	No. of patients	No. of patients with increased acid DNAase	No. of patients with increased alkaline DNase
Bladder	68	44 (65%)	31 (44%)
Prostate	18	10 (56%)	6 (33%)
Penis	13	9 (69%)	6 (46%)
Testis	10	5 (50%)	4 (40%)
Total	109	68 (62%)	47 (43%)
b) Patients with benign diseases of Urogenital system	30	9 (30%)	4 (13%)
c) Healthy blood Donors (control group)	38	0[b]	0[a]

[a] The test was done prior to any treatment
[b] Less than 400 un/ml for the acid and less than 200 u/ml for the Alkaline DNase

Table 2. Percentage of elevated DNase[a] levels, according to stage of tumours

Stage	Site of tumour – increased DNase			
	Bladder Acid/alkaline	Prostate Acid/alkaline	Testis Acid/alkaline	Penis Acid/alkaline
Stage I	58% – 52%	33% – 33%	33% – 0%	81% – 40%
Stage II	66% – 29%	50% – 25%	50% – 50%	66% – 66%
Stage III	66% – 50%	100% – 100%	50% – 50%	60% – 40%
Stage IV	66% – 53%	63% – 25%	66% – 66%	– –

[a] Increased acid DNase = More than 400 units/ml serum
Increased alkaline DNase = More than 200 units/ml serum

tients (Table 1). In patients with bladder carcinoma increased levels of Acid DNase were found in 65% and of Alkaline DNase in 44%. Of the 18 cases with Prostate carcinoma, ten patients (56%) had increased Alkaline DNase levels. In those with penile carcinoma (13 patients), the Acid DNase level was increased in nine (69%) and the Acid DNase activity was increased in six (46%). Finally in the testis tumour group (ten patients), Acid DNase was increased in five patients (50%), and Alkaline DNase was increased in four patients (40%).

In Group II inflammatory diseases and non-malignant tumours (30 patients), nine (30%) in this group had increased Acid DNase levels and only four (13%) had increased Alkaline DNase levels (Table 1).

The Ratio of Positive Acid and Alkaline DNase Activity According to the Stage of the Tumours (Table 2)

Bladder Carcinoma. In this group the DNase activity was higher in advanced stages.

In patients with Stage I disease, 58% had increased levels of Acid DNase. Alkaline DNase in the same patients was increased in 52%. In more advanced stages (II, III, IV), Acid DNase was increased in 66%, but Alkaline DNase remained almost the same (50%–53%). There was no significant difference in the results of the Alkaline DNase assay for patients with Stage II disease, increased levels being found in 50% and 52% of patients with Stages II and III disease respectively.

Prostate Carcinoma. Table 2 shows that the number of positive results was higher in the advanced stages in Acid DNase assay. In contrast increased levels of Alkaline DNase were found only in the low stage tumours. Acid DNase was increased in 33% of patients with Stage I, and 50%, 100% and 63% in patients with Stages II, III, IV disease respectively. Alkaline DNase levels were increased in 33% of patients with Stage I and 25% of patients with Stages II and IV disease.

Testicular Carcinoma. Increased levels of Acid and Alkaline DNase were commoner in advanced stages of the disease (Table 2). In patients with Stage I disease 33% showed increased Acid DNase activity, while Alkaline DNase was found normal in all cases. In patients with advanced disease,

Table 3. Increased Acid and Alkaline DNase in the group of patients with cancer a) prior to treatment, b) during treatment, c) after treatment

No. of patients and site of tumour		No. of patients with increased acid and alkaline DNase *prior* to treatment	No. of patients with increased acid and alkaline DNase *during* treatment	No. of patients with increased Acid and Alkaline DNase *after* treatment
		Acid/alkaline	Acid/alkaline	Acid/alkaline
Bladder	68	44 (65%) – 31 (44%)	49 (72%) – 29 (43%)	34 (50%) – 24 (35%)
Prostate	18	10 (56%) – 6 (33%)	16 (87%) – 8 (44%)	10 (56%) – 5 (28%)
Penis	13	9 (69%) – 6 (46%)	11 (85%) – 8 (62%)	5 (38%) – 5 (38%)
Testis	10	5 (50%) – 4 (40%)	6 (60%) – 5 (50%)	4 (40%) – 3 (33%)
Total	109	68 (62%) – 47 (43%)	82 (75%) – 50 (46%)	53 (49%) – 37 (34%)

Stages II, III and IV increased levels of Acid and Alkaline DNase were found in 50% and 60%, respectively. The only significant result in this group was the absence of increased levels of Alkaline DNase in patients with Stage I.

Penile Carcinoma. Increased levels of both DNase were commoner in those patients with low stages of the disease. Increased Acid DNase was found in 81% of patients with Stage I disease, but in patients with Stage II and III disease, increased Acid DNase levels were found in 66% and 60% respectively. Alkaline DNase was increased in 40% of patients with Stage I disease, in 66% of patients with Stage II disease, and in patients with Stage III disease increased levels of Alkaline DNase were found in 40%.

A part of this study was to investigate the relation between the increased levels of DNases and the effectiveness of treatment. DNase activity was measured first: (A) before any treatment, (B) during treatment and (C) one month after treatment.

The following results were obtained (Table 3): pretreatment Acid DNase was increased in 43%. During treatment, Acid DNase was increased in 49% of patients, and after treatment Acid DNase was increased in 34% (Table 3).

Further analysis of the results before and after treatment showed that, after treatment, the Acid DNase decreased in 15 patients, ten with bladder cancer, four with penile carcinoma and one with testicular seminoma.

The method of treatment of the above cases was transurethral resection and radiation for bladder tumours, amputation and radiotherapy for penile carcinoma and orchiectomy and radiotherapy for testicular seminoma.

Discussion

Acid and Alkaline DNase activities were increased in the serum of patients with common cancer (breast, stomach, colon, uterus), and in patients with polymyositis, rheumatic fever and other inflammatory diseases. Increased DNase activity has also been observed in the serum of some patients with various types of leukaemia and the level of DNase in the serum of patients who have undergone surgery or successful radiation or chemotherapy returns to normal some days after therapy. Purification of serum DNase, from patients with malignancies showed two peak of Alkaline DNase activity (mMW 170,000 and 100,000 daltons) and two peaks of Acid DNase (MW 200,000 and 65,000 daltons) after the passage of serum throughout a Sephadex G-200 column.

The DNase method is simple, cheap and safe, but it cannot yet be considered as a screening test for cancer. False positive results from rheumatic fever or polymyositis and other inflammatory conditions are easily excluded.

The increased level of DNase in the serum of patients with cancer could be explained by many theories. The most acceptable are 1) overproduction of enzymes by the tumour cells, 2) obstruction of the duct system through which the enzyme passages through the tumour, 3) induction of enzyme by the tumour, 4) change of permeability of the cell membrane allowing leakage of soluble enzyme into the circulation [4]. In addition, it is possible that other unknown factors may be involved in the regulation of serum enzyme activities.

This study was to determine the relationship of DNase activity to cancer of the genitourinary system as regards diagnosis and prognosis. Our results for DNase estimation in tumour detection are comparable with other predictive tests of cancer.

The false positive rate in Group II (inflammatory conditions) was 30%; it can be stated that this ratio is significant for a prediction test. The explanation is in either a massive degeneration of cells or a latent malignancy. This explanation is supported by the fact that in Group III (healthy blood donors) the false positive results were nil. On the other hand, the false negative ratio, which reached 38% for Acid DNase and 57% for Alkaline DNase, could be considered statisfactory for Acid DNase but high for Alkaline DNase. The explanation could be that some kind of tumours, or low stage tumours, produce decreased amounts of DNase or that the method is not yet sufficiently sensitive for the detection of enzymes.

With regard to the prognostic ability of the test in the prediction of progression or regression and the effectiveness

of treatment, our results shows that DNase activity returned to normal in 15 patients. This emphasises the absence of residual tumour after surgical treatment and the absence of metastases.

In spite of the small number of patients in whom DNase returned to normal after treatment and the short follow-up period, we can say that DNase measurement is a useful and safe prognostic test of malignant diseases, and a helpful method in the prediction of micrometastases. A longer follow-up and more experience with the method are required to establish the exact role for this test.

We conclude that the estimation of DNase can be used as a cancer marker after the careful exclusion of other conditions and it is useful for monitoring the progress of individual patients. Further development and evaluation is required to improve the accuracy of the test.

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