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SERUM DEOXYRIBONUCLEASES AS A BIOLOGICAL MARKER IN CANCER PATIENTS

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INTRODUCTION

A large number of biochemical and immunochemical markers are being used more or less routinely to monitor the course of cancer therapy; some seem in preliminary experiments, to be specific markers for individual tumors, others have been found to be indicators of the presence of cancer (5).

The markers that were prime candidates 5 years ago are carcinoembryonic antigen (CEA) and a-fetoprotein (AFP). Since then numerous biological markers have been used in different forms of cancer. Some of these markers are enzymes which are present in the serum or other biological fluids, and they can be easily assayed (7). However, 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. Towards this end we have studied serum DNAases as an additional biological marker; our previous results have suggested that these enzymes are increased in the serum of cancer patients.

The objectives of this study are: 1. To determine in more detail the levels of serum DNAases in patients with a variety of common cancers, 2. To evaluate the significance of the serum DNAases in the conventional therapy of these tumors and 3. To show whether these enzyme markers can be early signs of recurrence of the disease.

PATIENTS AND METHODS

Blood samples were drawn from cancer patients and healthy blood donors and the serum was kept at -20 C until it was used usually within a week.

The extent of disease was determined by physical examination, liver chemistries, chest and bone roentgenograms, broncho- and or mediastinoscopy and nuclear scans of the liver and bone. Roentgenograms, blood chemistries and nuclear scans were repeated at intervals during therapy.

A complete remission was defined as complete regression of all measurable lesions and disappearance of all other objective parameters of disease. A partial remission was defined as an average decrease of all measurable lesions to a value less than 50% of the pretreatment values. The appearance of a new lesion or a 50% increase in the size of an existing lesion constituted progressive disease.

The enzyme assay for the alkaline deoxyribonuclease involved, in 1.0 ml, 100 g DNA, 0.1 M Tris-Cl buffer pH 8.0 and 25 l serum. The assay for the acid deoxyribonuclease involved, in 1.0 ml, 100 g DNA, 0.1 M sodium acetate buffer pH 5.0, 5mM $MgCl_2$ and 25 l

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 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 C.

RESULTS

We first examined the levels of serum acid and alkaline deoxyribonuclease in patients with a variety of common cancers and in healthy individuals. Only patients with progressive disease or in relapse are included here. More than 400 Units/ml serum of acid DNAase and 200 Units/ml serum of alkaline DNAase were considered as abnormal values. There is a total of 128 patients, 14 with cancer of the colon and rectum, 33 of breast, 13 of lung, 7 of uterus and ovary, 7 of bladder, 9 of nasopharynx and 45 of other sites. Of these patients 85% had increased acid and 81% increased alkaline DNAase levels. All of the 108 healthy individuals examined had low values of serum DNAases.

We next examined the levels of serum acid and alkaline DNAase in cancer patients with metastases. Again only patients with progressive disease or in relapse are included. There is a total of 71 patients, 7 with primary site of cancer in the colon and rectum, 19 in breast, 9 in lung, 3 in uterus and ovary, 3 in bladder, 6 in nasopharynx and 24 in other primary sites. Of these patients 83% had increased acid and 82% had increased alkaline DNAase levels.

The clinical status and disease activity in relation to deoxyribonuclease levels in cancer patients was then examined. Of the 62 patients with fast growing tumors 97% had increased acid and 92% increased alkaline DNAase activities, whereas of the 44 patients with slow growing tumors 70% had increased acid and 66% increased alkaline DNAase activity. Of the 57 patients in partial remission 51% had increased acid and 37% increased alkaline DNAase activity. In 17 patients in complete remission only 12% had increased acid and 6% increased alkaline DNAase; whereas in 15 patients in relapse 87% had increased acid and 93% increased alkaline DNAase. Finally all the 31 patients on adjuvant therapy had normal values of serum DNAases.

The correlation between DNAase levels and altered clinical status in a variety of cancer patients during treatment with chemotherapy was next examined.

The results in a melanoma patient are shown in Figure 1. When the DNAase levels were first assayed the patient was receiving chemotherapy; he also had a local disease. A month later he was in relapse and the DNAase levels were dramatically increased and remained at high levels until his death.

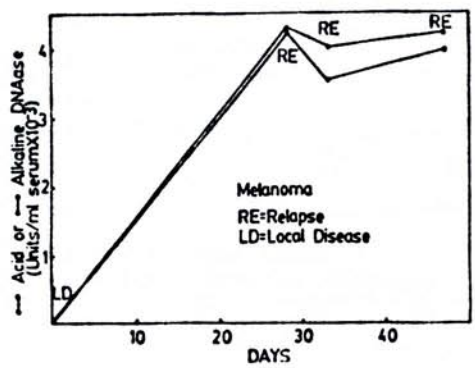

Figure 1. Correlation between serum DNAase levels and clinical status in a melanoma patient.

In Figure 2 are shown the results for a urinary bladder cancer patient during treatment with chemotherapy. High levels of both acid and alkaline DNAases were found in the patient with progressive disease, whereas three weeks later after successful chemotherapy, when the patient was clinically in partial remission, the DNAase levels had dropped significantly.

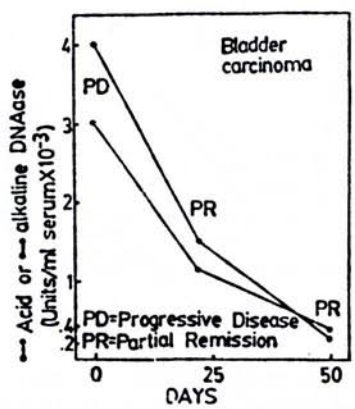

Figure 2. Correlation between serum DNAase levels and clinical status in a urinary bladder cancer patient during treatment with combined chemotherapy.

Figure 3 shows another example with a nasopharyngeal carcinoma patient. The transition from progressive disease to partial remission and relapse was accompanied by an initial drop followed by an increase in the levels of serum acid and alkaline DNAase.

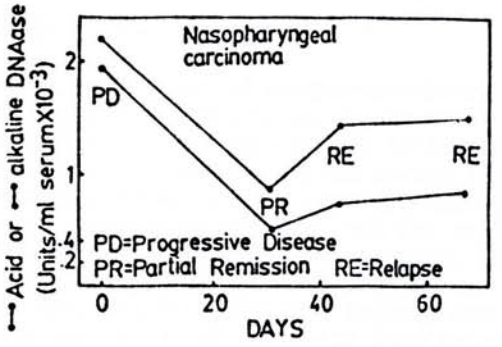

Figure 3. Correlation between serum DNAase levels and clinical status in a nasopharyngeal carcinoma patient during treatment with combined chemotherapy.

In Figure 4 can be seen a similar example with a stage IV breast carcinoma patient. Again the transition from progressive disease to partial remission and relapse was accompanied by an initial drop followed again by an increase in the levels of serum acid and alkaline DNAase.

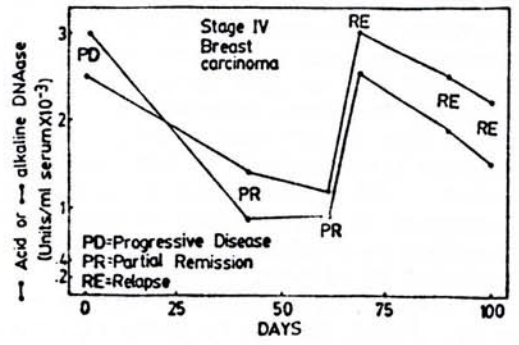

Figure 4. Correlation between serum DNAase levels and clinical status in a breast cancer patient during treatment with combined chemotherapy.

The rate of occurrence of increased serum DNAases in some non-neoplastic disorders was also examined. It was found that serum DNAases are not elevated in viral hepatitis, systemic lupus erythematosus, myocardial infarction, duodenal ulcer, muscular dystrophy, liver cirrhosis, tuberculosis and various non-neoplastic blood diseases i.e. sickle cell anemia, whereas increased values are present in rheumatoid arthritis, polymyositis and in patients with fever of unknown origin.

DISCUSSION

Measurement of cancer-associated biological markers is a useful diagnostic procedure, which, however, is neither absolute nor specific. From our results it can be suggested that, although the assay of DNAases is not always positive in cancer patients, the rate of positivity reported here is obviously higher than other biological markers used for screening of these patients (1) (6). A high rate is observed, close to DNAase level, only when the known biological markers are used simultaneously. In fact, carcinoembryonic antigen has the highest rate of occurrence in patients with some forms of cancer (51%); whereas the a-fetoprotein (AFP), casein and human chorionic gonadotropin (HCG) allowed an increase of the rate of occurrence between 5-28% according to the origin of cancer (1). Study of the rate of occurrence of the various markers does not permit a diagnosis of the tumors, with the exception of AFP, which has a high frequence of occurrence in hepatoma (3) and of HCG, which occurs frequently in trophoblastic tumors (8). Thus DNAases can be used alone or in combination with other known biological markers to provide additional diagnostic information.

The usefulness of the measurements of certain antigens, such as CEA, AFP, and HCG, has already been shown in monitoring treatment by various authors (2), (3), (4). From our results it can be suggested that DNAase levels paralleled changes in tumor status of response or progression during therapy (primarily chemotherapy) for patients with measurable, advanced disease. Also the degree of elevation decreases accordingly in cancer patients in partial or complete remission. Furthermore it is worth emphasizing that none of the patients who were on adjuvant therpay had elevated levels of DNAases in their serum.

It is known that CEA as well as other biological markers have been used to predict early recurrence of some cancers and especially of colon cancer (6). Although our number of patients in relapse is small it can be suggested that DNAase levels can also be used as an early warning of regression of the disease.

Although the mechanism of increased DNAases is not known it might be associated with tissues that undergo a rapid metabolic activity or cell death. It must, however be stressed that serum DNAases, like other biological markers, are elevated at least temporarily in response to a number of stimuli associated primarily with infection (i.e. fever, polymyositis, rheumatoid arthritis) or trauma such as surgery which results in false positive readings. However, all the above conditions can easily be diagnosed by clinical and laboratory investigation. In

addition we believe that in the presence of pathological levels of DNAases one must first of all exclude the above non-neoplastic conditions before ascribing diagnostic significance to the results of the assay. It is however important not to observe high levels of serum DNAases in non-neoplastic liver diseases such as viral hepatitis and liver cirrhosis, in contrast to what has been found with CEA and AFP (6).

In conclusion, further studies of the serum DNAase levels will be necessary to establish the value of these enzymes as an early index of diagnosis, monitoring of therapy and relapse of cancer patients.

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