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DEOXYRIBONUCLEASE LEVELS IN BENIGN AND MALIGNANT NEOPLASMS OF FEMALE REPRODUCTIVE SYSTEM

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It has been shown that cancer patients have increased levels of serum deoxyribonucleases (1).

These enzymes could be used in the diagnosis as well as in monitoring the response to therapy of a variety of carcinomas. In order to get an insight into the mechanism of the increased DNAase levels in the serum of cancer patients we have examined the enzyme levels in extracts of malignant, benign and normal tissue of the female reproductive system. We have found that malignant and benign tissues have elevated levels of DNAases when compared to normal tissue.

MATERIALS AND METHODS

One hundred biopsy samples were obtained from ovarian, cervical and uterine carcinomas as well as benign conditions and normal tissue. These samples were finely minced and 0.2 - 0.4 gr of the resulted tissue was incubated for 15 min. at room temperature, in 1 ml of the extraction solution. This consisted of 0.09% NaCl solution in dd H_2O containing 0.5% of the non-ionic detergent Nonidet P-40. After centrifugation at 3000 rpm for 10 min. the supernatant (tissue extract) was used for enzyme and protein determinations.

The enzyme assay for the alkaline deoxyribonuclease involved, in 1.0 ml 100 µg DNA, (calf thymus from Sigma Chem. Co.), 0.1 M Tris-Cl buffer, pH 8.0 and 100 µl of tissue extract. The assay for the acid deoxyribonuclease involved, in 1.0 ml, 100 µg DNA, 0.1 M sodium acetate buffer pH 5.0, 0.005 M $MgCl_2$ and 100 µl of tissue extract. 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 rpm for 10 min. The supernatant was kept and its absorbance at 260 nm was measured against a blank made as above except that the serum was added after perchloric acid.

The unit of DNAase was defined as that amount of enzyme which caused an increase of absorbance at 260 nm of 1.0 per minute at 25°C.

Protein determinations were done as described(2).

The DNAase levels were expressed as units/mg protein.

RESULTS

The results of deoxyribonucleases levels in histologically examined neoplastic and normal tissue of the female reproductive system are shown in Table 1. Only

TABLE 1. Deoxyribonuclease levels in neoplastic and normal tissues of female reproductive system

Tissue	Total No. of cases	No. of cases with increased DNAase (%)	
		Acid[1]	Alkaline[1]
Malignant	9	2 (22)	5 (56)
Benign	44	12 (27)	29 (66)
Normal	47	4 (9)	13 (28)

[1]More than 3 Units/mg protein.

tissues with more than 3 units/mg protein were considered as positive.

As shown in the Table malignant and benign tissues have higher DNAase levels than normal tissue. In Table 2

TABLE 2. Deoxyribonuclease levels in neoplastic and normal tissues of female reproductive system

Tissue	Total No. of cases	No. of cases with increased DNAase (%)	
		Acid[1]	Alkaline[1]
1. Uterus			
a. Malignant	2	0	1
b. Benign	28	5	16
c. Normal	6	1	5
2. Ovary			
a. Malignant	2	0	1
b. Benign	2	1	1
c. Normal	25	3	8
3. Cervix			
a. Malignant	5	1	2
b. Benign	14	5	10
c. Normal	4	0	0

[1]More than 3 Units/mg protein.

the above results are shown in more detail. Most of the cases are malignant cervical tissue, benign cases of uterus and normal cases of ovarian origin.

To further examine whether high levels of DNAases are characteristic of malignant and benign tissues we next considered as positive only the tissues having more than 5 units of DNAase/mg protein. The results are shown in Table 3.

TABLE 3. Deoxyribonuclease levels in neoplastic and normal tissues of female reproductive system.

Tissue	Total No. of cases	No. of cases with increased DNAase (%)	
		Acid[1]	Alkaline[1]
Malignant	9	2 (22)	3 (33)
Benign	44	10 (23)	19 (43)
Normal	47	2 (4)	7 (15)

[1]More than 5 Units/mg protein.

As shown in the table (22%) and (33%) of the malignant cases have increase DNAase levels whereas in benign tissues they dropped to (23%) and (43%) and in normal to (4%) and (15%) for the acid and alkaline DNAase respectively.

Finally we have considered as positive only the tissues having more than 10 units of DNAase/mg protein. Table 4 shows that (22%) and (33%) of the malignant cases

TABLE 4. Deoxyribonuclease levels in neoplastic and normal tissues of female reproductive system.

Tissue	Total No. of cases	No. of cases with increased DNAase (%)	
		Acid[1]	Alkaline[1]
Malignant	9	2 (22)	3 (33)
Benign	44	8 (18)	9 (20)
Normal	47	0 (0)	2 (4)

[1]More than 10 Units/mg protein.

have increased DNAase levels whereas in benign they dropped to (18%) and (20%), and in normal to (0%) and (4%) for the acid and alkaline DNAase respectively.

DISCUSSION

Over the past few years different individual markers have been developed for the detection and evaluation of tumour status in cancer patients (3,4,5).

However the need for sensitive methods in detecting the presence of a tumour, 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 and our previous results have suggested that these enzymes could be used in the diagnosis as well as in monitoring the response to therapy of a variety of carcinomas (1).

In order to get an insight into the mechanism of the increased DNAase levels in the serum of cancer patients, we have examined the levels of DNAase activities in benign, malignant and normal tissue of the female reproductive system.

These, our preliminary results, indicate that benign and malignant neoplasms contain increased levels of DNAases when compared to normal tissue.

The data taken together with our previous findings (1) might suggest that increased serum DNAases in cancer patients could partly be the result of increased DNAase in the malignant tissue compared to normal tissue.

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