CATHEPSIN-D AND TNF- α IN BLADDER CANCER

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SUMMARY

In a study of 34 normal healthy controls, 35 patients with urinary tract bilharziasis and 93 bladder cancer patients (62 of them are operable cases and 31 are non-operable ones), serum tumor necrosis factor alpha (TNF- α) and cytosolic Cathepsin-D were estimated. Though both potential markers were elevated in bladder cancer patients, neither Cathepsin-D nor TNF- α showed associations of prognostic value since there were no positive correlations with tumor stages, grades or association of tumors with bilharzial ova or lymph node involvement.

KEY WORDS TNF- α Cathepsin-D Bilharziasis Bladder cancer

INTRODUCTION

Carcinoma of the urinary bladder is the commonest tumor type in Egypt. It represents about 26.3% of all malignant cases registered at National Cancer Institute in Cairo (Mokhtar, 1991). Although radical surgery with preoperative and/or postoperative radiotherapy remain the only treatment modalities, long term survival rate for operable cases is only about 35.0% (El-Sebai, 1983). New ideas are clearly needed to help manage patients with bladder cancer. Tumor markers are of increasing interest for screening, early diagnosis and prognosis of cancer. The Cathepsin-D family of proteases are thought to have important functions in carcinogenesis, facilitating tumor invasion and growth (Goldfarb, 1986), some of them are reported to be secreted more abundantly by cancer cells than normal cells (Matrisian *et al*, 1986). Tumor necrosis factor (TNF- α) is a cytokine released by cells of the immune system in response to various invasive stimuli (Owen-Schaub *et al*, 1988).

This work evaluates those biochemical tumor markers in a group of bladder cancer patients compared with patients suffering from urinary tract bilharziasis and healthy controls.

SUBJECTS AND METHODS

This study included 162 individuals classified into three groups:

Group A. Bladder cancer patients (N=93), 62 operable, 31 inoperable. They were aged 23–71 (mean=42). Seventy were males and 23 females. Twenty-six were heavy smokers

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(more than 15 cigarettes/day), 50 light smokers (less than 15 cigarettes/day) and 17 non-smokers. All patients included in group A were subjected to clinical examination, radiological investigation and cystoscopic biopsy. Histopathological examination classified the 62 operable bladder cases as follows: Squamous cell carcinoma (N=29), Transitional cell carcinoma (N=29), Adenocarcinoma (N=3) and undifferentiated carcinoma (N=1). By surgical stage and histological grade, Stage T1 (N=2), Stage T3 (N=38), Stage T4 (N=22) and Grade G1 (N=1), G2 (N=11) and G3 (N=50). Fifteen of the operable bladder cancer patients had positive lymph nodes while the remaining 47 were negative. In 37, bilharzial ova were found in their tissues while 25 were free.

Group B. Urinary tract bilharzial patients (N=35) were aged from 25–65 years (mean=45). This group included 25 males and 10 females. Ten were heavy smokers, 15 light smokers and 10 nonsmokers. These patients were diagnosed by clinical examination and serological test for bilharzias. Cancer of urinary bladder was excluded by urine cytology and cystoscopy.

Group C. Normal healthy controls (N=34) were aged from 18–65 years (mean=28). This group included 30 males and four females. Nine were heavy smokers, 10 were light smokers and 15 were non-smokers. They were found healthy by clinical examination and all were checked with serological test for bilharzias giving results within the normal range.

Blood Samples. 10 ml. venous blood was withdrawn from all individuals. Serum was obtained by centrifugation at 4000 rpm. divided into aliquots and stored at -80°C until assayed. For the bladder cancer group, blood samples were taken 1–3 days preoperatively.

Tissues Samples. Specimens of bladder tumor tissues were obtained directly at operation. Blood was washed away by ice cold saline. Fat and necrotic tissues were rapidly dissected from the tumor tissue. About 1–2 gm. of tumor tissue was wrapped in aluminium foil and directly frozen at -80°C , while a portion of normal bladder tissue at least 10 cm distant from the tumor was obtained from the same patient to be used as control for the cancer tissue and treated in a similar manner. For isolation of the cytosol and membrane fraction, certain weights of both tumor and control tissues were chopped with a scalpel and scissors. The minced tissues were homogenized, by Teflon glass homogenizer, in 10 volumes of ice cold phosphate buffer. Tissue homogenate was centrifuged at 800 xg for 10 minutes to precipitate the nucleus. The supernatant was separated and ultracentrifuged at 100,000 xg for 30 minutes. The cytosol supernatant was divided into aliquots and the membrane pellet was resuspended in one ml. of the homogenization buffer with the aid of sonicator and ultracentrifuged at 100,000 xg for 30 minutes. The membrane fraction in the supernatant was divided into aliquots. Both cytosol and membrane-enriched fraction aliquots were frozen at -80°C until assayed.

Serological assay for bilharzias was done using the indirect haemagglutination reagent supplied by Behringwerke AG, Germany. Serum TNF- α was determined according to the principle of Tracey *et al.* (1987) using the reagent supplied by Medgenix Diagnostics, Belgium. It is a solid phase immunoassay on microtitre plate. Cathepsin-D was estimated in the cytosol fraction according to the principle of Predine (1992) using the reagents

supplied by CIS Bio International, France. The Cathepsin-D concentration was expressed per mg tissue protein. Protein concentration was estimated according to the principle of Bradford (1976). It depends on the reaction of protein and coomassie brilliant blue dye which leads to change to colour from blue to green measured colorimetrically at 595 nm.

RESULTS

The findings are presented in Tables 1–6.

DISCUSSION

Schistosomiasis is one of the major public health problems in Egypt. It is one of the predisposing factors for bladder cancer which is the most common malignant tumor among Egyptians (El-Bolkainy *et al.*, 1981).

Tumor markers can be of diagnostic and prognostic value. The ultimate serum concentration of tumor markers reflects a multifactorial dynamic equilibrium between marker synthesis and clearance (Pohl, 1990). Cathepsin-D is an acidic lysosomal aspartyl endoprotease with at least two biologic properties: estrogen and/or growth factor-dependent mitogenic activity and enzymatic activity on proteoglycans, extracellular matrix and basement membrane (Vignon *et al.*, 1986, Briozzo *et al.*, 1988 and Rochefort, 1990). These properties suggest a potential role for Cathepsin-D in the invasion and metastasis of cancer cells (Liotta, 1988). Previous studies reported significant correlations between Cathepsin-D concentration and prognosis in breast cancer. High concentration of total Cathepsin-D was regarded to be a marker for poor prognosis (Thorpe *et al.*, 1989 and Brouillet *et al.*, 1993).

The present work showed a significant elevation in malignant cytosolic Cathepsin-D when compared with that of normal tissue. This agrees with Reid *et al.* (1986), who reported that Cathepsin-D is expressed in bladder carcinoma. This may be attributed to the loss of regulation and overexpression of proteins involved in the control of cell proliferation and migration. Alternatively, a protease acting normally in lysosomes may be secreted excessively from the cancer cells because of an altered processing of its precursor (Capony *et al.*, 1989).

Despite its elevated level in cancer tissue, in the present study, at 100% and 90% specificity, cytosol Cathepsin-D has a sensitivity of only 35.7% and 59.5% respectively. Cathepsin-D level is independent of clinical stage, pathological grade, lymph node involvement and bilharziasis. This is in agreement with Namer *et al.* (1989), Marsigliante *et al.* (1994), Scorilar *et al.* (1994) and Seshadri *et al.* (1994) but contrary to Foekins *et al.* (1993) and Hahnel *et al.* (1994) who found significant correlations between Cathepsin-D level and lymph node involvement with tumor size. We observed that cytosolic Cathepsin-D is more strikingly elevated in transitional cell carcinoma than squamous cell carcinoma; the reason for this is unclear.

Tumor necrosis factors play an important role in the defence against viruses, tumors and bacteria. Evidence is also accumulating that they may play a role in the pathogenesis of several diseases, including autoallergies such as experimental allergic encephalomyelitis and viral infections such as AIDS (Ruddle, 1986). Concerning TNF- α , the present work revealed significant elevations of serum TNF- α in patients with bladder cancer and those with urinary tract bilharziasis. No significant difference was observed either

Table 1. Cytosol Cathepsin-D (fmol/mg Protein) in normal and malignant bladder tissue.

Groups	Normal Bladder Tissues	Malignant Bladder Tissues
Cath.-D (fmol/mg Protein)		
Range	913–11770	4356–17880
Mean±SE	4514.5±569.4	9533±847.6*

* Significant different from normal group at p=0.01

Table 2. Sensitivity of cytosol Cathepsin-D in bladder cancer patients at different specificity.

	Specificity (%)	
	100%	90%
Cut-Off Value (fmol/mg Protein)	11770	8333
Sensitivity (%)	35.7%	59.5%

Table 3. Mean value and sensitivity of cytosol Cathepsin-D according to cancer type, pathological stage, grade, involvement of lymph nodes and bilharziasis.

	Sensitivity (%)		Mean±SE (fmol/mg Protein)
	100%	90%	
Squamous cell carcinoma	40.3	60.1	7990±1080
Transitional cell carcinoma	38.2	59.3	11099±1303*
Stage II & II	45.0	61.0	10520± 882
Stage IV	55.2	67.5	9188±1793
Grade II	40.0	55.0	9179±1400
Grade III	52.4	63.6	10107±1064
+ ve Lymph node	40.0	53.0	9445±2488
- ve Lymph node	46.0	57.5	9912± 832
+ ve Bilharziasis	59.5	64.4	10525±1171
- ve Bilharziasis	38.8	51.5	8737±1201

*Signnificant different from squamous cell carcinoma at p=0.05

Table 4. Serum TNF- α (pg/ml.) in the different studied groups.

Groups	Control	Bilharzial	Bladder Cancer	
TNF- α (pg/ml.)			Operable	Non-Operable
Range	0-95	7-1200	10-1500	20-1500
Mean $\pm$ SE	20.56 $\pm$ 3.4	114.4 $\pm$ 36.0*	95.0 $\pm$ 25.6*	269.0 $\pm$ 79.6*

* Significant different from control group at p=0.01

Table 5. Sensitivity of serum TNF- α in bladder cancer patients and false positivity urinary tract bilharzial patients.

	Sensitivity%		False Positivity%	
	100% Specificity	90% Specificity	100% Specificity	90% Specificity
Cut-Off value (pg/ml.)	95	40	95	40
Operable bladder cancer	17%	55%	-	-
Non-operable bladder cancer	48%	77%	-	-
Urinary tract bilharzial patients	-	-	26%	57%

Table 6. Mean value and sensitivity of serum TNF- α according to cancer type, stage, grade, involvement of lymph nodes and bilharziasis.

	Sensitivity (%) Specificity (%)		Mean $\pm$ SE (pg/ml.)
	100%	90%	
Squamous cell carcinoma	20%	57%	85.5 $\pm$ 18.07
Transitional cell carcinoma	18%	53%	112.9 $\pm$ 51.8
Stage II & III	24%	50%	105.1 $\pm$ 39.8
Stage IV	31%	59%	68.0 $\pm$ 18.6
Grade II	17%	64%	67.1 $\pm$ 27.8
Grade III	19%	56%	95.2 $\pm$ 30.6
+ ve Lymph node	17%	51%	58.6 $\pm$ 21.3
- ve Lymph node	20%	58%	107.0 $\pm$ 33.0
+ ve Bilharziasis	24%	64%	100.4 $\pm$ 14.1
- ve Bilharziasis	14%	43%	88.4 $\pm$ 20.2

between cancer and bilharzial groups or between operable and non-operable cases of bladder cancer. As a tumor marker, serum TNF- α showed the highest sensitivity for non-operable cases. This may be attributed to the highly advanced cancer state of those patients. The high "false positivity" obtained with urinary tract bilharzial patients may be related to the excessive release of TNF- α by cells of the immune system in response to various invasive stimuli. These results agree with Balkwill *et al.* (1987) and Owen-Schaub *et al.* (1988). Our findings also suggest that serum TNF- α is independent of cancer type, pathological stage and grade, involvement or not of lymph nodes and association of the tumor with bilharzial ova or not.

We conclude that TNF- α can not be used for the differential diagnosis between bilharzial and bladder cancer patients. Overexpression of Cathepsin-D was found in tumor tissue compared to normal tissue of the bladder. Nevertheless, neither Cathepsin-D or TNF- α were of prognostic value since there were no positive correlations with tumor stage, grade, of association of tumors with bilharzial ova or lymph node involvement.

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