Tumor Necrosis Factor and Coagulopathy in Patients with Prostate Cancer

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ABSTRACT

This study was undertaken to evaluate the relationship between serum tumor necrosis factor (TNF) and coagulopathy in patients with prostate cancer. TNF levels in 104 sera obtained from 101 prostate cancer patients were determined using an enzyme immunoassay. Serum levels of fibrin/fibrinogen degradation product E fragment (FDP) and plasma levels of fibrin degradation product D-dimer in patients with elevated serum TNF levels were 1221.95 ± 375.94 ng/ml and 27.34 ± 9.81 ug/ml, which were significantly higher than those (FDP, 94.35 ± 13.17 ng/ml; D-dimer, 1.03 ± 0.20 ug/ml) in patients with undetectable serum TNF levels (P < 0.01). In addition, patients with elevated serum TNF levels showed significant increases in plasma levels of thrombin-antithrombin-III complex and plasmin-α2-antiplasmin inhibitor complex and a significantly higher incidence of positive plasma soluble fibrin monomer complex than those with undetectable serum TNF levels. The percentage of prothrombin time was significantly decreased in the group with elevated serum levels of TNF. Serum levels of TNF were significantly elevated in patients with serum FDP levels of ≥200 ng/ml than in those with serum FDP levels of <200 ng/ml (3.91 ± 0.45 versus 2.17 ± 0.08 units/ml) and in patients with plasma D-dimer levels of ≥2 ug/ml than in those with plasma D-dimer levels of <2 ug/ml (3.82 ± 0.48 versus 2.10 ± 0.06 units/ml). These results suggest that TNF may be one of the pathogenetic factors that could explain the occurrence of coagulopathy in patients with prostate cancer.

INTRODUCTION

TNF was discovered in 1975 to be a cytotoxic factor causing tumor necrosis (1). Later, it was demonstrated that TNF is strikingly similar to cachectin (2), and that TNF is involved in a wide variety of biological processes. Tracy et al. (3) have suggested that TNF may mediate both beneficial and deleterious actions. Sharief and Hengtes (4) have reported that the level of TNF in cerebrospinal fluid in patients with multiple sclerosis correlates with the severity and progression of the disease. It also has been demonstrated that serum levels of TNF are elevated in children with Gram-negative sepsis and purpura fulminans with higher risks (5), in patients with severe falciparum malaria, particularly in those with cerebral malaria or hypoglycemia (6), and in children who had primary dengue virus infection with clinical features of Reye’s syndrome (7). In addition, it has been reported that increased TNF production is associated with cerebral malaria and a fatal outcome (8), inflammatory bowel disease (9), and adult respiratory distress syndrome (10). It also has been demonstrated that i.v. infusion of lipopolysaccharide/endotoxin causes the appearance of TNF in the bloodstream (11). On the basis of these reports, TNF may be involved in the pathophysiology of diverse infectious and inflammatory diseases. Conversely, Balkwill et al. (12) have described that TNF-like activity was detected by ELISA in 50% of serum samples from cancer patients with active disease. The clinical significance of TNF in cancer patients has not, however, been fully elucidated, although a wide range of biological activities of TNF have been discussed in association with acute and chronic inflammatory states. In recent years, TNF has been considered to be involved in pathogenetic mechanisms of disseminated intravascular coagulation associated with sepsis (13). This study was undertaken to investigate the clinical implication of TNF in coagulopathy in patients with prostate cancer.

PATIENTS AND METHODS

Patient Population. A total of 101 patients with prostate cancer, ranging in age from 53 to 91 years, was examined in this study. No patient had evidence of active-infection or inflammatory disease at the time of examination. The diagnosis of prostate cancer was confirmed by the examination of needle biopsy or transurethral resection of the prostate. A total of 22 patients had well differentiated adenocarcinoma, 58 had moderately differentiated adenocarcinoma, and 21 had poorly differentiated adenocarcinoma. The staging procedures included a clinical examination, i.v. pyelography, transrectal ultrasonography, magnetic resonance imaging with endorectal surface coil, bone scanning, and computed tomography scan and/or ultrasonography, and/or magnetic resonance imaging of the abdomen and pelvic cavity. The staging evaluation revealed stage A in four patients; stage B in 22; stage C in 23; and stage D in 52, which include 24 patients with untreated disease, 14 with remission by endocrine therapy, and 17 with relapsed disease.

Blood specimens (104) from 101 patients were collected in nonheparinized tubes to measure serum TNF, and sera were separated within 1 h of blood collection. Three patients provided two samples before treatments and after the relapse of the disease.

The hemostatic examination included a platelet count, amount of fibrinogen, and PT in 103 samples; the amount of FDP in 91 samples; the amount of fibrin degradation product D-dimer in 81 samples; the amount of TAT in 52 samples; the amount of PIC in 57 samples; and the amount of SFMC in 66 samples. In the untreated patients, blood samples were obtained before needle biopsy or transurethral resection of the prostate.

Assays. Platelet counts were determined with the use of a flow cytometer. TAT was determined using a commercially available immunoassortment assay kit. FDP was measured by the latex agglutination method (LIPA-1 FDP kit; Diatron, Tokyo, Japan); the plasma concentration of fibrin degradation product D-dimer was measured by the latex agglutination method (LIPA-100; Diatron). The plasma concentration of PIC was determined to assess plasmin generation using commercially available assay kits (Teijin EIA-B, Teijin, Tokyo, Japan). SFMC was measured by a ristocetin precipitation test.

TNF activity was determined with an EIA specific for human TNF (provided by Dainippon Pharmaceutical Co., Ltd.). Details of the EIA procedure have been reported previously (14). In brief, 100 μl of standard solutions containing purified rHu-TNF (0.9–232 units/ml) or serum were incubated with 100 μl of anti-rHu-TNF antibody at 37°C for 1 h. Then, 200 μl of β-galactosidase-labeled rHu-TNF solution were added, and goat anti-rabbit IgG was used for the separation of bound from free labeled antigen. After the addition of substrate solution (2-nitrophenyl-[β]-galactopyranoside), the absorbance at 410 nm was measured, and the concentration of TNF in the samples was determined by the reference to the standard curve. The lower limit of detection with this assay system is 2 units/ml.

Statistical Analysis. Data on platelet count, fibrinogen, PT, FDP, D-dimer, TAT, PIC, and TNF were reported as mean ± SE. TNF levels that were nondetectable were assigned a value equal to the lower limit of detection for the assay. Variables of different groups were compared using the Mann-Whitney test. Spearman’s rank correlation test was used to evaluate the...
correlation between variables. The independence of fit of categorical data was analyzed by the \( \chi^2 \) test. \( P < 0.05 \) was considered statistically significant.

RESULTS

Of the 104 serum samples, 20 (19.2%) had detectable serum levels of TNF. The percentages of positive serum TNF activity in stage A, B, C, and D patients were 0, 9.1, 4.3, and 30.9%, respectively. Among the stage D patients, the patients with relapsed disease showed 82.4% in the positive rate of the serum TNF activity, whereas the untreated patients and the patients with remission by endocrine therapy showed 12.5 and 0%, respectively. The percentages of positive serum TNF activity in patients with well-differentiated, moderately differentiated, and poorly differentiated adenocarcinoma were 9.1, 13.6, and 43.5%, respectively. Serum levels of TNF were elevated significantly in patients with stage D prostate cancer and with poorly differentiated adenocarcinoma \( (P < 0.05) \).

Serum levels of FDP and plasma levels of D-dimer in patients with elevated serum TNF levels were 1221.95 ± 375.94 ng/ml \( (n = 20) \) and 27.34 ± 9.81 \( \mu \)g/ml \( (n = 17) \), which were significantly higher \( (P < 0.01) \) than those \( (FDP, 94.35 ± 13.17 \, \text{ng/ml}, n = 71; \, \text{D-dimer}, \, 1.03 ± 0.20 \, \mu \text{g/ml}, n = 64) \) in patients with undetectable serum TNF levels (Figs. 1 and 2). A correlation existed between serum levels of TNF and FDP \( (P < 0.05) \) and between serum levels of TNF and plasma levels of D-dimer \( (P < 0.05) \). In addition, patients with elevated serum TNF levels showed significant increases \( (P < 0.01) \) in plasma levels of TAT \( (19.79 ± 5.58 \, \text{ng/ml}, n = 12) \) and PIC \( (4.3 ± 1.08 \, \mu \text{g/ml}, n = 13) \) than those with undetectable serum TNF levels \( (TAT, 4.07 ± 0.62 \, \text{ng/ml}, n = 40; \, \text{PIC,} \, 1.33 ± 0.08 \, \mu \text{g/ml}, n = 44) \) (Figs. 3 and 4). PT \( (56.05 ± 2.93\%, n = 20) \) in patients with elevated serum TNF levels was significantly lower \( (P < 0.01) \) than was that \( (PT, 71.58 ± 1.30\%, n = 83) \) in patients with undetectable serum TNF levels (Fig. 5). Platelet counts and plasma levels of fibrinogen were 203.55 ± 23.01 \( \times 10^6/\text{ml} \) \( (n = 20; \, P = 0.17) \) and 415.10 ± 38.40 mg/dl \( (n = 20; \, P = 0.10) \) in patients with detectable serum TNF levels which were not significantly different from those in patients with undetectable serum TNF levels (platelet count, 223.35 ± 8.16 \( \times 10^6/\text{ml}, n = 83; \, \text{fibrinogen,} \, 348.74 ± 10.17 \, \text{mg/dl,} \, n = 83) \). Serum TNF levels in patients with elevated serum FDP levels of 200 ng/ml or higher were 3.91 ± 0.45 units/ml \( (n = 18) \), which were significantly higher \( (P < 0.01) \) than those \( (2.17 ± 0.08 \, \text{units/ml,} \, n = 73) \) in patients with serum FDP levels lower than 200 ng/ml (Fig. 6). Serum TNF levels \( (3.82 ± 0.48 \, \text{units/ml,} \, n = 19) \) in patients with
elevated plasma D-dimer levels of 2 μg/ml or higher also were significantly elevated ($P < 0.01$) than those ($2.10 \pm 0.06$ units/ml, $n = 62$) in patients with plasma D-dimer levels lower than 2 μg/ml (Fig. 7). The incidence of positive plasma SFMC in patients with elevated serum TNF levels was 68.8%, which was significantly higher than that (4.0%) in patients with undetectable serum TNF levels (Table 1). In addition, patients with positive plasma levels of SFMC showed a significant increase ($P < 0.01$) in serum TNF levels ($4.10 \pm 0.56$ units/ml, $n = 13$) when compared with those (TNF, 2.16 ± 0.08 units/ml, $n = 53$) in patients with undetectable plasma levels of SFMC.

**DISCUSSION**

In this study, serum levels of TNF were significantly elevated in stage D prostate cancer patients with relapsed disease. Mallmann et al. (15) reported that patients with progressive breast cancer had higher serum levels of TNF than did patients without recurrence. Therefore, it is conceivable that the level of TNF may correlate with the severity
and progression of the disease, although it was reported that no obvious relation was found between disease state or bulk of disease and serum TNF levels in cancer patients (12). Cytokines, including TNF, are thought to play an important role in the regulation of immune response and tumor-host relationship. It has been reported that tumor cells can stimulate macrophages to release TNF (16). Perhaps the spontaneous production of TNF by peripheral blood mononuclear cells of cancer patients reflects a sustained stimulatory effect of the cancer cells similar to the in vitro induction of TNF by certain tumor cells. TNF also causes necrosis and regression of some animal tumors (17). Stovroff et al. (18) investigated the correlation of TNF production with tumor burden and host cachexia in tumor-bearing rats. Their results suggest that host macrophages are activated in response to a malignant tumor before the appearance of clinical signs of cachexia, and that elevated levels of TNF production are associated with both tumor necrosis and host cachexia as the tumor progresses (18). The constitutive production of TNF in cancer patients raises many possibilities about its biological significance—both its beneficial effects and its deleterious effects. It is possible that TNF can represent a host defense mechanism against a progressive tumor in patients with relapsed disease. However, it is also conceivable that TNF may be involved in coagulation disorders as well as hemorrhagic tumor necrosis. Therefore, it appears that some individuals with cancer produce TNF either as part of a host defense to the disease or as part of a pathogenetic process of cancer-associated symptoms.

Coagulopathy is known to be a major complication of prostate cancer (19). Wada et al. (20) report that the activation of immune systems, as shown in increased TNF production, may be important in the onset of DIC. As Edgington suggested (21), it is conceivable that the production and release of procoagulant by tumor cells and the stimulation of procoagulant production by monocytes and macrophages are included in the mechanisms leading to the activation of the coagulation system in association with neoplasia. However, the pathophysiology of coagulopathy in patients with prostate cancer remains unclear.

The major physiological inhibitors of the coagulation system and fibrinolysis are antithrombin-III and α2-antiplasmin inhibitor. TAT is considered to be a sensitive and specific indicator of coagulation activation (22), whereas PIC is thought to reflect fibrinolytic activation (23). SFMC mainly is indicative of coagulation state. FDP is believed to be attributed to the breakdown products of both fibrinogen and non-cross-linked and cross-linked fibrin. In recent years, the specific products of cross-linked fibrin degradation, particularly d-dimer, have been measured. Wilde et al. (24) have evaluated d-dimer levels in patients with carcinoma and have suggested that a hypercoagulable state is a common occurrence under those conditions, although it is not severe enough to manifest as overt DIC in the majority of cases. In this study, plasma levels of d-dimer, TAT, and PIC and serum levels of FDP were elevated significantly in patients with elevated serum TNF levels when compared with patients with undetectable serum TNF levels. It also was demonstrated that the incidence of positive plasma SFMC in patients with elevated serum TNF levels was significantly higher than was that in patients with undetectable serum TNF levels. Conversely, serum levels of TNF were elevated significantly in patients who showed positivity in plasma SFMC or elevated levels of serum FDP or plasma d-dimer when compared with patients without coagulopathy. In this study, activation of the coagulation cascade and accelerated levels of fibrinolysis are demonstrated in prostate cancer patients with elevated serum TNF levels. It seems reasonable to speculate that the coagulation system activated in part by TNF forms fibrin clots, subsequently resulting in the degradation of these clots by accelerated fibrinolysis in patients with prostate cancer.

Kawai et al. (25) have noted fibrin clots in tumor capillaries after administration of TNF, which suggests that the action of TNF is related to microvascular fibrin clots. It also has been documented that TNF can modulate endothelial cell hemostatic properties, leading to the promotion of clot formation by upregulating tissue factor formation (26, 27). Moreover, TNF may stimulate endothelial cells to produce platelet-activating factor, which activates platelets to aggregate (28). It also is suggested that TNF upregulates formation of tissue plasminogen activator inhibitor (29, 30) and downregulates thrombomodulin expression on the cell surface (26, 27, 31), leading to the inhibition of anticoagulant mechanisms. Therefore, the effects of TNF may lead to the development of microvascular clot formation, presumably caused by the marked shift from anticoagulant predominance to procoagulant predominance on the endothelial cell surface. In addition, it is reported that a single injection of TNF elicits a rapid and sustained activation of the common pathway of coagulation, probably induced through the extrinsic route, and it is suggested that TNF plays an important part in the early activation of the hemostatic mechanism in DIC (32) and hemorrhagic necrosis of tumors. Therefore, TNF may be associated with the development of a hypercoagulable state by activating procoagulant activities and inhibiting anticoagulant activities. Although correlation does not prove causation, our findings suggest that TNF may play a possible pathogenetic role in coagulopathy secondary to prostate cancer.

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