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SERUM TRANSFORMING GROWTH FACTOR β1 AND PROSTATE SPECIFIC ANTIGEN AS MARKERS FOR LOCALIZED AND METASTATIC PROSTATE CANCER

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Objectives: To assess any additional benefits of the estimation of serum TGF-ß1 over serum prostate specific antigen (PSA) for differentiating localized from metastatic prostatic carcinoma.

Patients and Methods: Forty-seven prostate cancer patients (23 with and 24 without metastases) and ten controls were included in the study. Serum PSA was estimated using the chemiluminescent immunometric assay, and serum TGF-ß1 was assessed using the enzyme immunoassay.

Results: The mean serum PSA in the localized and metastatic disease groups was significantly higher than that in the control group ($p<0.001$ and $p<0.001$, respectively), while the mean serum TGF-ß1 in the metastatic disease group only was significantly higher than in the control group ($p<0.01$). The mean serum PSA and TGF-ß1 in the metastatic disease group were significantly higher than the values in the localized disease group ($p<0.001$ and $p<0.001$, respectively). Serum PSA was directly correlated with the Gleason score in both patient groups (localized group: $r=0.427$, $p<0.05$; and metastatic group: $r=0.425$, $p<0.05$), while serum TGF-ß1 was directly correlated with the Gleason score in the localized disease group only ($r=0.686$, $p<0.001$). Serum PSA was directly correlated with serum TGF-ß1 in the metastatic disease group only ($r=0.418$, $p<0.05$).

Conclusion: Although we found that both serum PSA and TGF-ß1 are useful markers for metastatic prostate cancer, we could not detect a specific advantage of TGF-ß1 over PSA. In the localized form of the disease PSA is a more reliable marker.

Key Words: TGF-β1, PSA, prostate cancer

INTRODUCTION

Prostate specific antigen (PSA) has been a valuable tool in enhancing our understanding of the prevalence and virulence of prostate cancer. Although PSA is currently the best serum marker for prostate cancer, its low specificity for the detection of prostate cancer, especially in the grey zone of PSA, is a problem[1]. Therefore, more effective tumor markers for prostate cancer are being sought. A potential candidate marker is the transforming growth factor beta (TGF-ß). TGF-ß1 has gained considerable attention as a factor implicated in the induction of chronic fibrotic conditions and enhancing tumorigenicity in some tissues. TGF-ß1 is involved in numerous vital processes including inflammation, stimulation of intracellular matrix formation, production of fibroblasts, and normal healing[2]. Our previous studies[3-6] showed an increase in TGF-ß1 protein expression associated with fibrotic conditions such as Peyronie's disease and priapism.

The expression of TGF-ß1 has been shown to be significantly correlated with the expression of the vascular endothelial growth factor and a poor prognosis in patients with advanced gastric carcinoma[7]. Also, an over-expression of TGF-ß1 has been observed in patients with various cancers including clear cell renal carcinoma, with serum and urine levels correlating inversely with the prognosis[8]. Additionally, the association of TGF-ß1 with myometrial

Table 1: Comparison between Group I (Localized Disease) and the Control Group

	Control Group (Mean ± SD)	Group I (Mean ± SD)	t	p*
Age (years)	59.5 ± 2.31	61.4 ± 0.97	0.075	0.23
PSA (ng/ml)	4.38 ± 0.48	9.64 ± 1.0	4.72	<0.001
TFG-β1 (ng/ml)	38.82 ± 2.46	38.54 ± 2.23	0.07	0.94

*Student's t-test

Table 2: Comparison between Group II (Metastatic Disease) and the Control Group

	Control Group (Mean ± SD)	Group II (Mean ± SD)	t	p*
Age (years)	59.5 ± 2.31	68.7 ± 1.11	4.29	<0.001
PSA (ng/ml)	4.38 ± 0.48	94.4 ± 21.27	2.77	<0.001
TFG-β1 (ng/ml)	38.82 ± 2.46	51.94 ± 2.95	2.74	<0.01

*Student's t-test

invasion of endometrial carcinoma through effects on matrix metalloproteinase has been reported[9].

In the prostate, TGF-ß1 inhibits epithelial cell proliferation in the normal prostate, while prostate tumors express high levels of TGF-ß1 and seem to acquire resistance to its anti-proliferative effects with tumor progression[10].

The purpose of this study was to assess any additional benefits of the estimation of serum TGF-ß1 over serum PSA for differentiating localized from metastatic prostatic carcinoma.

PATIENTS AND METHODS

Forty-seven patients aged between 52 and 83 years (mean age: 65 ± 6.2 years) with pathologically confirmed prostatic carcinoma were enrolled in this prospective study. We only included patients with urinary obstructive and irritative symptoms who on clinical examination had suspicious prostatic nodules that were confirmed by transrectal ultrasonography and biopsy. Patients with carcinoma of the prostate undergoing medical cytoreductive treatment or surgical treatment were not included.

All patients were subjected to bone scanning, plain chest X-ray and abdominal and pelvic CT scanning. The Gleason score was also determined for all patients[11]. According to the results of these examinations and the presence of metastases, the patients were divided into two groups: Group I consisted of 24 patients who did not show any metastases and were, therefore, considered as having localized disease. Twenty-three patients had metastases (bone metastases in 13, lymph node metastases in 7, both bone and lymph node metastases in 2 and bladder metastases in 1 patient) and were considered to have metastatic disease (Group II).

Ten age-matched (mean age: 59.5 ± 2.31 years) apparently healthy males served as controls (Group III). They had no complaints or symptoms related to the urinary system, and digital rectal examination (DRE) revealed prostates of an average size according to their age and no suspicious nodules.

Human PSA was quantitatively estimated in the sera that were kept frozen at 4°C until they were tested using a solid-phase two-site chemiluminescent immunometric assay *(Immulite PSA, Cat. No. LKPS1, DPC, USA)*. The solid phase (a polystyrene bead enclosed within an Immulite Test Unit) was coated with a

Table 3: Comparison between Group I (Localized Disease) and Group II (Metastatic Disease)

	Group I (Mean ± SD)	Group II (Mean ± SD)	t	p*
Age (years)	61.4 ± 0.97	68.7 ± 1.11	4.99	<0.001
PSA (ng/ml)	9.64 ± 1.0	94.4 ± 21.27	4.07	<0.001
TFG-β1 (ng/ml)	38.54 ± 2.23	51.94 ± 2.95	3.64	<0.001

*Student's t-test

polyclonal antibody specific for PSA. The patient's serum sample and alkaline phosphatase-conjugated monoclonal antibody were incubated for ~ 30 minutes at 37°C in the test unit with intermittent agitation. PSA in the sample was bound to form an antibody-sandwich complex. The unbound conjugate was then removed by a centrifugal wash after which the substrate was added, and the test unit was incubated for a further 10 minutes. The chemiluminescent substrate, a phosphate ester of adamantyl dioxetane, underwent hydrolysis in the presence of alkaline phosphatase to yield an unstable intermediate. The continuous production of this intermediate resulted in a sustained emission of light, thus improving precision by providing a window for multiple readings. The bound complex and the photon output (measured by the luminometer) were proportional to the concentration of PSA.

Human TGF-ß1 was quantitatively estimated using a solid-phase enzyme immunoassay method performed on a microtiter plate *(TGF-ß1 EASIA, Cat. No. KAC1681, Biosource, Europe,SA)*. Briefly, a fixed amount of TGF-ß1 labeled with horseradish peroxidase competed with unlabeled TGF-ß1 in the patient serum for a limited number of binding sites on a specific coated antibody. After two hours incubation at room temperature, the microtiter plate was washed to stop the competition reaction. A chromogenic solution was added and the microtiter plate was incubated for 30 minutes before reading. The absorbance was inversely proportional to TGF-ß1 concentration. TGF-ß1 concentrations of were determined by interpolation from a standard curve.

Student's t-test was used to compare the means of numerical values. Simple linear regression was used for studying the correlations between the study groups. The significance level was set at 95%, therefore any difference less than 5% was considered statistically significant.

RESULTS

Forty-seven patients fulfilled the inclusion criteria. Their age ranged from 52 to 83 years with a mean age of 65 ± 6.2 years. Their serum PSA ranged from 3.2 to 419 ng/ml (mean 51.12 ± 12.04) ng/ml. Their TGF-ß1 ranged from 18.7 to 71.6 ng/ml (mean 45.09 ± 2.06) ng/ml.

The control group (Group III) included 10 subjects whose age ranged from 55 to 67 years (mean 59.5 ± 2.31 years). The serum PSA level in this group ranged from 2.1 to 6.7 ng/ml (mean 4.38 ±_0.48) ng/ml. The serum TGF-ß1 in this group ranged from 26.1 to 51.6 ng/ml (mean 38.82 ± 2.46) ng/ml. No statistically significant correlation between the serum PSA and TGF-ß1 levels was found in the control group (p=0.09).

Group I (localized disease) included 24 patients whose age ranged from 52 to 68 years (mean 61.4 ± 0.97) years. The serum PSA level ranged from 3.2 to 21.2 ng/ml (mean 9.64 ± 1.0) ng/ml. Serum TGF-ß1 ranged from 18.7 to 61.0 ng/ml (mean 38.54 ± 2.23) ng/ml. Three of the patients in this group had a Gleason score of (2), 12 had a score of (2-3), 4 had a score of (2-4) and 5 had a score of (3-4). A statistically significant direct correlation between the serum PSA level and the Gleason score was found (p<0.05, r=0.427) (Fig. 1). Similarly, there was a statistically significant direct correlation between serum TGF-ß1 and the Gleason score (p<0.001, r=0.686) (Fig. 2). However, there was no statistically significant correlation between the serum PSA and TGF-ß1 levels (p=0.07).

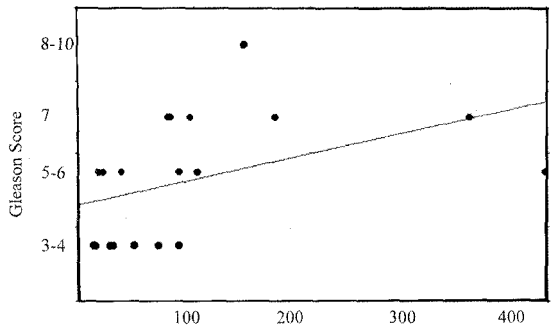

Fig. 1: *Simple linear regression between Gleason score* and serum PSA in Group I (localized disease) [p<0.05, r=0.427]

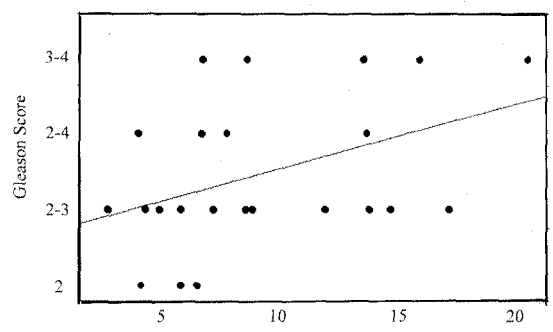

Fig. 2: Simple linear regression between Gleason score and serum TGF-β1 in Group I (localized disease) [p<0.001, r=0.686]

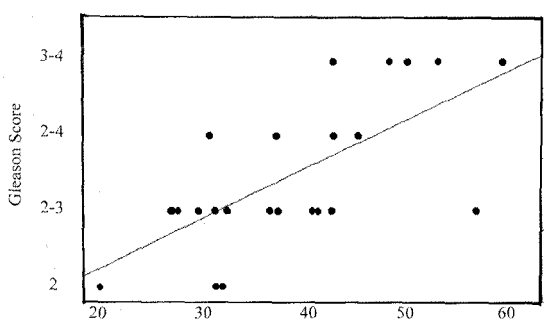

Fig. 3: Simple linear regression between Gleason score and serum PSA in Group II (metastatic disease) [p<0.05, r=0.425]

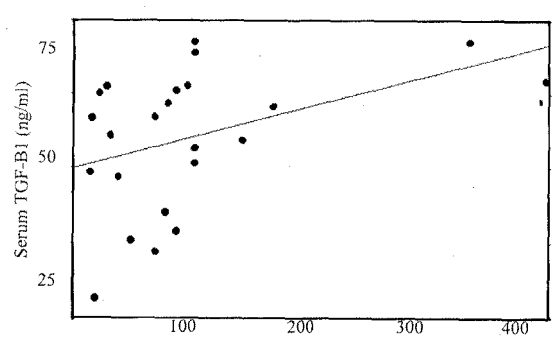

Fig. 4: Simple linear regression between serum PSA and TGF-β1 in Group II (metastatic disease) [p<0.05, r=0.418]

Comparing the localized disease group (Group I) with the control group (Group III) (Table 1), no statistically significant difference was found between the two groups as regards age. However, the serum PSA levels were significantly higher in Group I (9.64 ± 1.0) ng/ml than in Group III (4.38 ± 0.48 ng/ml) (p<0.001). On the other hand, there was no statistically significant difference between the two groups as regards the serum levels of TGF-ß1 (p=0.942).

The metastatic disease group (Group II) included 23 patients whose age ranged from 56 to 83 years (mean age: 68.7 ± 1.11 years). The serum PSA level ranged from 5.2 to 419 ng/ml (mean 94.40 ± 21.27) ng/ml. Serum TGF-ß1 ranged from 18.7 to 61.0 ng/ml (mean 51.94 ± 2.95) ng/ml. Eight patients had a Gleason score of (3-4), 9 had a score of (5-6), 5 had a score of (7) and one had a score of (8-10). In this group, there was a statistically significant direct correlation between the serum PSA level and the Gleason score (p<0.05, r=0.425) (Fig. 3). However, there was no statistically significant correlation between serum TGF-ß1 and the Gleason score (p=0.184). On the other hand, a statistically significant direct correlation between the serum PSA and TGF-ß1 levels (p<0.05, r=0.418) (Fig. 4) was found.

The patients of Group II were compared with the control group (Table 2). The patients in the advanced disease group were significantly older than the control subjects (p<0.001). Also, the mean serum PSA was significantly higher in Group II (94.4 ± 21.2 ng/ml) than in the control group (4.38 ±

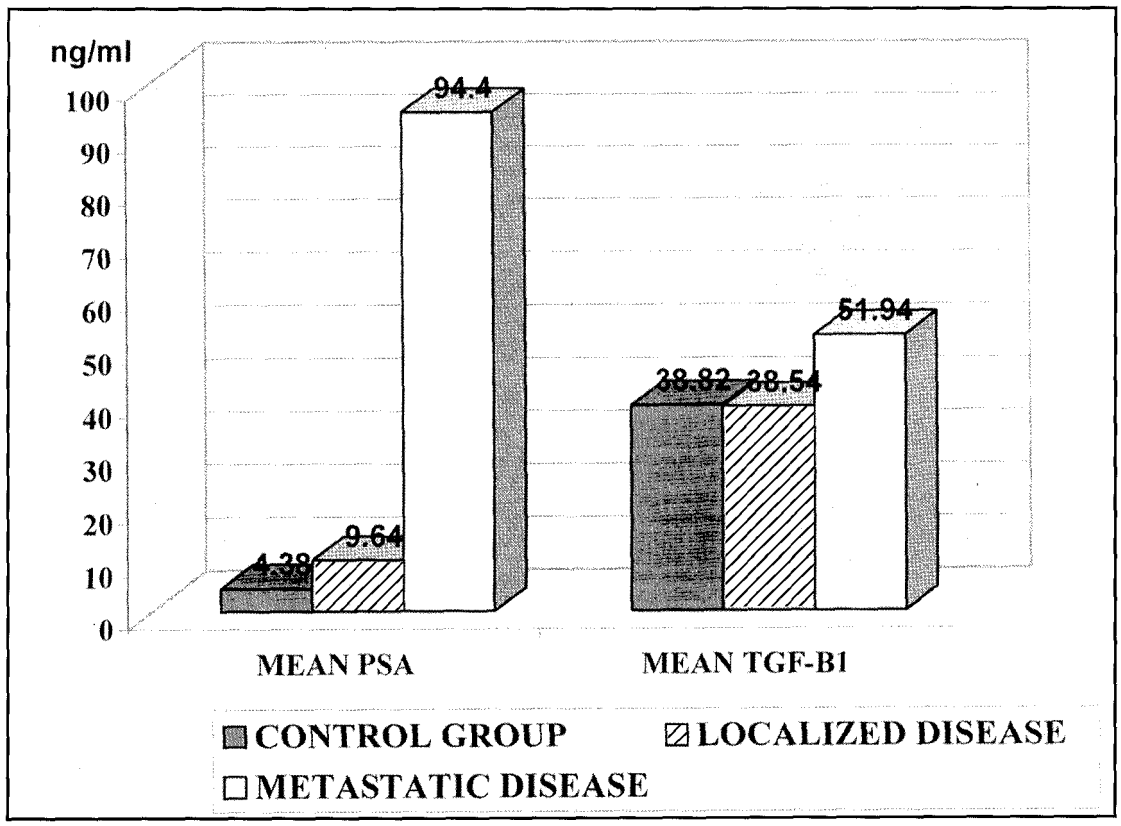

Fig. 5: Comparison between the control group and the patient groups regarding serum PSA and TGF-β1

0.48 ng/ml) ($p<0.001$). Similarly, the mean serum TGF-ß1 was significantly higher in Group II (mean 51.9 ± 2.95 ng/ml) compared to the control group (38.82 ± 2.46 ng/ml) ($p<0.01$).

Both patient groups are compared in Table 3. The mean age of the patients in Group II (metastatic disease) was significantly higher than that of the patients of Group I (localized disease) (68.7 ± 1.11 versus 61.4 ± 0.97) ($p<0.001$). Also, the mean serum PSA in Group II was significantly higher than in Group I (94.4 ± 21.2 ng/ml versus 9.64 ± 1.0 ng/ml) ($p<0.001$). Additionally, the mean serum TGF-ß1 in Group II (51.9 ± 2.95 ng/ml) was significantly higher than in Group I (38.5 ± 2.23 ng/ml) ($p<0.001$). A comparison of serum PSA and TGF-ß1 in the studied groups is shown in Fig. 5.

DISCUSSION

Serum PSA is currently the best serum marker for prostate cancer[1]. The evaluation of the response based on changes in the PSA level is now routinely incorporated into clinical trials as a surrogate end point for response; however, despite the general acceptance of its use as an end point in clinical trials, there is no standardized definition of PSA response[12].

TGF-ß1 is a member of an extended gene family involved in the regulation of growth and differentiation. It usually inhibits the proliferation of normal prostatic epithelial cells, whereas cancer cells overexpress TGF-ß1 and become resistant to its growth inhibiting effects[13]. Other biological properties of TGF-ß1 are its role in enhancing prostatic tumorigenicity by promoting angiogenesis[14], stimulating the turnover of the extracellular matrix and enhancing metastasis formation[15]. TGF-ß1 inhibits epithelial cell proliferation in the normal prostate. Prostate tumors express high levels of TGF-ß1 and seem to acquire resistance to its anti-proliferative effects with tumor progression. The biological consequence of these activities is an enhanced tumorigenicity in prostate cancer.

Thus, TGF-ß1 may be a useful tumor marker for prostate cancer. It has been reported that plasma TGF-ß1 concentration is higher in patients with prostate cancer than in patients with BPH or normal prostates[16,17].

We studied the expression of both PSA and TGF-ß1 in localized and metastatic prostatic cancer to evaluate their respective potential in differentiating localized from metastatic prostatic tumors.

The mean serum PSA, but not the mean serum TGF-β1, was significantly higher in Group I (localized disease) than in the control group. This indicates that PSA is a more sensitive predictor of the state of the prostate in the early (non-metastasizing) stage than TGF-ß1. Other investigators reached the same conclusion when they studied metastasis-free, newly diagnosed, untreated prostate carcinoma patients analyzing both tissue (fine needle biopsy) and serum PSA levels. They found that tissue PSA correlated with serum PSA in these patients[18]. Similarly, Pousette et al. found that serum PSA was one of the factors competing with tissue PSA for predicting the time to progression and the time to death in prostate cancer patients[19]. Also Roehrborn et al. reported that serum PSA was a strong predictor of future growth in men with BPH[20].

On the other hand, in the patients with localized disease, both serum PSA and TGF-ß1 demonstrated a significant direct correlation with Gleason score, while no such correlation was detected between serum PSA and TGF-ß1 levels. This finding reflects the association between both serum markers and the histopathologic grade of the tumor in the localized stage. The lack of correlation between serum PSA and TGF-ß1 was also reported by Wolff et al. in their study of serum concentrations of TGF-ß1 in patients with benign and malignant prostatic diseases[21].

In Group II (metastatic disease), both proteins were significantly over-expressed compared to the control group. Previous reports indicated that the plasma TGF-ß1 concentration was higher in patients with prostate cancer than in patients with BPH or normal prostates[16,17]. Many investigators have related the over-expression of TGF-ß1 to invasive and metastasizing tumors rather than to localized ones. Recently, Adler et al. analyzed the levels of several cytokines and growth factors in the systemic circulation of patients with varying stages of prostate cancer compared to controls and found that TGF-ß1 was significantly elevated in patients with clinically evident metastases[22]. In the patients with metastatic disease, there was no significant correlation between serum TGF-ß1 and Gleason score. Wolff et al. demonstrated similar results when they reported that there was no increase in TGF-ß1 concentrations with advancing tumor stage[21].

In this study, there was a statistically significant direct correlation between serum PSA and serum TGF-ß1 in Group II (metastatic disease) only, however we could not detect additional benefits of the estimation of serum TGF-ß1 over serum PSA for the differentiation of localized from metastatic prostatic carcinoma. This agrees with the findings of Adler et al. who stated that TGF-ß1 correlated with increasing serum PSA. In contrast, Wolff et al. did not find such correlation[21].

We conclude that, although we found that both serum PSA and TGF-ß1 were useful markers for metastatic prostate cancer, no specific advantage of TGF-ß1 over PSA could be detected. In the localized form of the disease PSA is a more reliable marker. Both markers may be used simultaneously to monitor metastatic prostate cancer.

REFERENCES

1. Uno H, Koide T, Kuriyama M, Ban Y, Deguchi T, Kawada Y. Prostate specific antigen density for discriminating prostate cancer from benign prostatic hyperplasia in the gray zone of prostate specific antigen. *Hinyokika Kiyo* 1999, 163:457.

2. Terrell TG, Working PK, Chow CP, Green JD. Pathology of recombinant human transforming growth factor β1 in rats and rabbits. *Int Rev Experimen Pathol* 1993, 34:43.

3. El-Sakka AI, Hassoba HM, Pillarisetty RJ, Dahiya R, Lue TF. Peyronie's disease is associated with an increase in transforming growth factor beta protein expression. *J Urol* 1997, 158:1391.

4. El-Sakka AI, Hassoba HM, Chui RM, Bhatnagar RS, Dahiya R, Lue TF. An animal model of Peyronie's-like condition associated with an increase of transforming growth factor beta mRNA and protein expression. *J Urol* 1997, 158:2284.

5. El-Sakka AI, Hassan MU, Nunes L, Bhatnagar RS, Yen TS, Lue TF. Histological and ultrastructural alterations in an animal model of Peyronie's disease. *Brit J Urol* 1998, 81:445.

6. Hassan MU, El-Sakka AI, Yen TSB, Lue TF. Expression of TGF-β1 mRNA and ultrastructural alterations in pharmacologically induced prolonged penile erection. *J Urol* 1998, 160:2263.

7. Saito H, Tsujitani S, Oka S *et al.* The expression of transforming growth factor-beta is significantly correlated with the expression of vascular endothelial growth factor and poor prognosis of patients with advanced gastric carcinoma. *Cancer* 1999, 86:1455.

8. Ananth S, Knebelmann B, Gruning W *et al.* Transforming growth factor beta is a target for the von Hippel-Lindau tumor suppressor and a critical growth factor for clear cell renal carcinoma. *Cancer Res* 1999, 59:2210.

9. Yabushita H, Narumiya H, Hiratake K *et al.* The association of transforming growth factor beta 1 with myometrial invasion of endometrial carcinomas through effects on matrix metalloproteinase. *J Obstet Gynaecol Res* 2000, 26:163.

10. Wikstrom P, Lindh G, Bergh A, Damber JE. Alterations of transforming growth factor beta 1 (TGF-β1) and TGF beta receptor expressions with progression in Dunning rat prostatic adenocarcinoma sublines. *Urol Res* 1999, 27:185.

11. Millikan R, Logothetis C. Update of the NCCN Guidelines for Treatment of Prostate Cancer. *Oncology* 1997, 11:2.

12. Dawson NA. Diagnosis and staging of prostatic carcinoma: what is really necessary? *Urol Int* 1999, 26:57.

13. Steiner MS. Review of peptide growth factors in benign prostatic hyperplasia and urologic malignancy. *J Urol* 1995, 153:1085.

14. Yang EY, Moses HL. Transforming growth factor B1 induced changes in cell migration, proliferation and angiogenesis in chicken chorioallantoic membrane. *J Cell Biol* 1990, 111:731.

15. Newman MJ. Transforming growth factor beta and the cell surface in tumor progression. *Cancer Metastasis Rev* 1991, 12:239.

16. Ivanovic V, Melman A, Davis-Josef B, Valcic M, Geliebter J. Elevated levels of TGF-B1 in patients with invasive prostate cancer. Nature Med 1995, 1:282.

17. Ivanovic V, Melman A, Davis-Josef B, Geliebter J. Elevated levels of TGF-B1 in plasma of patients with advanced stages of prostate carcinoma. *J Urol* 1995, 153:269A.

18. Stege RH, Tribukait B, Carlström KA, Grande M, Pousette AH. Tissue PSA from fine-needle biopsies of prostatic carcinoma as related to serum PSA, clinical stage, cytological grade and DNA ploidy. *Prostate* 1999, 229:183.

19. Pousette A, Grande M, Carlström K, Stege R. Tissue PSA is the best predicting variable for the outcome of endocrine treatment of prostatic carcinoma. *Scand J Clin Lab Invest* 1999, 229:27.

20. Roehrborn CG, McConnell J, Bonilla J *et al.* Serum prostate specific antigen is a strong predictor of future prostate growth in men with benign prostatic hyperplasia. PROSCAR long-term efficacy and safety study. *J Urol* 2000, 163:13.

21. Wolff JM, Fandel TH, Borchers H, Jakse G. Serum concentrations of transforming growth factor-beta in patient with benign and malignant prostatic disease. *Anticancer Res* 1999, 19:2657.

22. Adler BL, McCurdy MA, Kattan MW, Tinime TL, Scardino PT, Thompson TC. Elevated levels of circulating interleukin-6 and transforming growth factor beta 1 in patients with metastatic prostatic carcinoma. *J Urol* 1999, 161:182.

RESUME

Le Seum Transforming Growth Factor β1(TGF-β1) et le PSA comme Marqueurs des Cancers de Prostate Localisés ou Métastatiques

Objectifs: Déterminer le bénéfice additionnel du dosage du TGF-1 sérique par rapport au PSA dans la différenciation des cancers de la prostate localisés et métastatiques. **Patients et Méthodes**: Quarante sept patients présentant un cancer de la prostate (23 avec métastases et 24 sans métastases) et 10 contrôles étaient inclus dans cette étude. Le taux de PSA était estimé en utilisant le dosage immunométrique luminescent et le taux de TGF-β1 sérique était déterminé par dosage immunoenzymologique. **Résultats:** La moyenne de PSA sérique était significativement plus élevée chez les patients présentant un cancer de prostate localisé ou métastatique que chez le groupe contrôle ($p<0.001$, $p<0.001$ respectivement), tandis que le taux moyen de TGF-β1 sérique chez les patients présentant des métastases n'était significativement plus élevée que par rapport au groupe contrôle ($p<0.01$). La moyenne de PSA était significativement plus élevée chez les patients présentant des métastases que chez les patients avec une tumeur localisée ($p<0.001$, $p<0.001$ respectivement). Le taux de PSA sérique était directement corrélé au score de Gleason dans les deux groupes (groupe à tumeur localisée: $r=0.427$, $p<0.05$; et groupe à métastases: $r=0.425$, $p<0.05$), tandis que le taux sérique de TGF-ß1 était directement corrélé au score de Gleason uniquement chez les patients ayant un cancer localisé ($r=0.686$, $p<0.001$). Le taux de PSA était directement corrélé avec le taux sérique de TGF-ß1 unique-

ment chez les patients à cancer métastatique (r=0.418, p<0.05). **Conclusion**: Bien que nous ayons trouvé qu'aussi bien le taux sérique de PSA et de TGF-ß1 soient des marqueurs utiles du cancer métastatique de la prostate, nous ne pouvions affirmer l'existence d'avantage spécifique du TGF-ß1 sur le PSA. Dans les cancers localisés le dosage du PSA a plus de valeur.

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