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ORIGINAL ARTICLE

Research on the association of plasma TGF-β1 level and blood lymphocyte/monocyte ratio with pathological grade, clinical stage and prognosis of prostate cancer

Qi Wang[1], Tongwen Ou[1], Jin Li[1], Xin Cui[1], Jingjin Liang[2]

[1]Department of Urology, Xuanwu Hospital, Capital Medical University, Beijing, China. [2]Department of Infectious Diseases, Peking University Third Hospital, Beijing, China.

Summary

Purpose: *To explore the association of the plasma transforming growth factor-β1 (TGF-β1) level and blood lymphocyte/monocyte ratio (LMR) with the pathological grade, clinical stage and prognosis of prostate cancer (PCa).*

Methods: *A total of 86 PCa patients treated in our hospital were enrolled. The changes in the expression of TGF-β1 were observed in patients with different clinical stages, different Gleason scores and different ages, and with or without bone metastasis. The correlation between blood LMR and clinicopathological features of PCa patients was detected. Moreover, the univariate and multivariate analyses were performed for clinicopathological factors and progression-free survival (PFS) after treatment, respectively.*

Results: *In terms of the clinical stage II, III and IV, the number of patients with high TGF-β1 expression was significantly larger than that with low TGF-β1 expression ($p<0.05$). Among those with Gleason score of 2-4 points, 5-6 points and 7-10 points, the number of patients with high TGF-β1 expression was significantly larger than that with low TGF-β1 expression ($p<0.05$). Among those aged ≥70 years old and <70 years old, there were more patients with high TGF-β1 expression than those with low TGF-β1 expression, but without significant differences ($p>0.05$). There were also more patients with high TGF-β1 expression than those with low TGF-β1 expression regardless of the presence or absence of bone metastasis, showing obvious differences ($p<0.05$). Besides, the association of LMR with depth of tumor infiltration, stage, grade, size and Gleason score was explored, and the results showed that LMR was negatively correlated with the above indexes ($p<0.05$). The univariate analysis was performed for 6 indexes, and the patients were divided into progression group (n=52) and non-progression group (n=34) based on the presence or absence of cancer progression after treatment. Obvious differences were found in the comparison of Gleason score, lymph node metastasis, TGF-β1 level and clinical stage between the two groups ($p<0.05$). It was found in the multivariate analysis that TGF-β1, Gleason score, clinical stage and lymph node metastasis were influencing factors for PFS after treatment ($p<0.05$).*

Conclusions: *The TGF-β1 level is positively correlated with the severity, clinical stage and pathological grade of PCa. LMR is negatively correlated with the depth of tumor infiltration, stage and grade. Clinical stage, TGF-β1, lymph node metastasis and Gleason score are influencing factors for PFS of PCa patients after treatment.*

Key words: *prostate cancer, TGF-β1, pathological grade, prognosis*

Introduction

Prostate cancer (PCa) is a malignant tumor that frequently occurs in elderly men. Among malignant tumors in American males, the morbidity and mortality rates of PCa rank 1st and 2nd, and the patients have mostly been in the late stage when diagnosed due to lack of specific symptoms in the early stage [1]. The morbidity rate of PCa in China is far lower than that in European countries, but it has risen

Corresponding author: Tongwen Ou, MD. Department of Urology, Xuanwu Hospital, Capital Medical University, No.45 Changchun Street, Xicheng District, Beijing 100053, China.
Tel: +86 013681283415, Email: outongwen@sohu.com
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obviously in recent years with the gradual aging of the population, changes in dietary structure and advance of disease diagnosis [2]. In recent years, transforming growth factor-β (TGF-β) and vascular endothelial growth factor (VEGF) have been the research hotspots in the occurrence, mechanism and treatment of PCa [3]. The abnormality of the TGF-β signal transduction pathway plays a role in the occurrence, development and metastasis of tumor, and TGF-β1 exerts dual functions in the formation and development of tumor [4]. TGF-β1 acts as a tumor suppressor in the ground state of normal cells in the early stage of tumor, while its expression gradually increases in the late stage of tumor and serves as a promotor for angiogenesis, cell proliferation, immunosuppression and tumor proliferation [5]. Endocrine therapy is the preferred treatment for advanced PCa, in which the androgen secretion in patients is inhibited with drugs, so that the conversion to dihydrotestosterone is also delayed to exert an inhibitory effect in the binding of androgens to receptors, thus inhibiting the proliferation of PCa cells [6]. In particular, it should be noted that the initial efficacy of endocrine therapy is good in most patients, but once the treatment lasts for a longer time, PCa will easily develop into androgen-independent PCa with poor prognosis, shortening the progression-free survival (PFS) of patients [7]. In this paper, therefore, the clinical data of 86 PCa patients were analyzed, and the association of the plasma TGF-β1 level and blood lymphocyte/monocyte ratio (LMR) with the pathological grade, clinical stage and prognosis of PCa was detected.

Methods

Objects and grouping

A total of 86 male patients pathologically diagnosed with PCa in our hospital from January 2014 to December 2018 were retrospectively analyzed. The patients were aged 56-88 years with an average of 67.32±4.12 years, and the course of disease was 2-8 years with an average of 4.65±1.65 years. Among the 86 patients, the LMR was ≥2.7 in 36 cases and <2.7 in 50 cases. The clinical data of patients, including clinical stage, pathological Gleason score and bone metastasis, were collected. This study was approved by the Ethics Committee of Xuanwu Hospital, Capital Medical University. Signed written informed consents were obtained from all participants before the study entry.

Inclusion and exclusion criteria

Inclusion criteria: 1) patients meeting the diagnostic criteria for PCa [8]; 2) those diagnosed with PCa *via* cystoscopy, etc.; and 3) those who and whose families signed the informed consent. Exclusion criteria: 1) patients who quit the study for no reason during the investigation; 2) those who had alcohol dependence; 3) those with obvious discomfort during the investigation; or 4) those complicated with other types of tumors.

Observation indexes

According to the doctor's advice, the 86 patients were treated with castration using the method [9]+ antiandrogen therapy (flutamide). The following detection was performed: 1) changes in the expression of TGF-β1 in patients with different clinical stages; 2) changes in the expression of TGF-β1 in patients with different Gleason scores; 3) expression of TGF-β1 in patients with different ages; 4) expression of TGF-β1 in patients with or without bone metastasis; 5) correlation between blood LMR and clinicopathological features of PCa patients; 6) univariate analysis for clinicopathological factors; and 7) multivariate analysis for PFS after treatment.

Pathological grading and clinical staging criteria

The pathological grade was determined using the Gleason scoring system (5-10 points). Gleason scoring criteria: 2-4 points: well differentiated adenocarcinoma, 5-6 points: moderately differentiated adenocarcinoma, and 7-10 points: poorly differentiated adenocarcinoma. The clinical stage of PCa was determined mainly based on the tumor-node-metastasis (TNM) staging criteria published in 2002 [8] (Table 1).

Measurement criteria

Measurement criteria for TGF-β1: 10 mL of fasting venous blood was drawn from all patients at 6 am on the next day after admission, and centrifuged. The plasma was collected and stored at -20°C until use. Optical density (OD) at 450 nm was carried out *via* enzyme-linked

Table 1. TNM staging

Pathological grade	*Feature*
Grade I	The condition of primary tumor cannot be evaluated.
Grade II	PCa tissues <5% in tumor specimens detected *via* palpation or imaging examination, and confined to the prostate according to biopsy
Grade III	The tumor spreads beyond the prostate, and invades the capsule (lateral or bilateral) and seminal vesicle
Grade IV	The tumor is fixed or invades other adjacent tissue structures other than the seminal vesicle, such as the bladder neck, external urethral sphincter, rectum, levator ani muscle and/or pelvic wall, or there is tumor metastasis

immunosorbent assay, based on which the concentration of TGF-β1 was obtained.

The plasma TGF-β1 concentration ≥5 ng/mL indicated high expression, while that <5 ng/mL indicated low expression.

Statistics

SPSS 20.0 software (IBM, Armonk, NY, USA) was used for the statistical analyses of related data of 86 PCa patients and the expression of TGF-β1. All data were expressed as [n (%)], and chi-square test was performed. Spearman method was used for correlation analysis, and multivariate logistic regression analysis was adopted. P<0.05 suggested statistically significant difference.

Results

Changes in the expression of TGF-β1 in patients with different clinical stages

In terms of the clinical stage II, III and IV, the number of patients with high TGF-β1 expression was significantly larger than that with low TGF-β1 expression (p<0.05) (Table 2).

Table 2. Changes in the expression of TGF-β1 in patients with different clinical stages

Clinical stage	*Low expression* *n (%)*	*High expression* *n (%)*	x^2	*p*
II	3 (30)	7 (70)	12.801	0.005
III	7 (28)	18 (72)		
IV	13 (25.5)	38 (74.5)		

II: stage T1-T2, III: stage T3, IV: stage T4

Table 3. Changes in the expression of TGF-β1 in patients with different Gleason scores

Gleason score *(points)*	*Low expression* *n (%)*	*High expression* *n (%)*	x^2	*p*
2-4	8 (53.3)	7 (46.7)	20.067	<0.001
5-6	9 (39.1)	14 (66.7)		
7-10	7 (14.6)	41 (85.4)		

Table 4. Expression of TGF-β1 in patients with different ages

Age *(years)*	*Low expression* *n (%)*	*High expression* *n (%)*	x^2	*p*
≤70	15 (30.6)	34 (69.4)	1.526	0.466
>70	9 (24.3)	28 (75.7)		

Table 5. Expression of TGF-β1 in patients with or without bone metastasis

Bone metastasis	*Low expression* *n (%)*	*High expression* *n (%)*	x^2	*p*
No	16 (41)	23 (58.9)	6.554	0.037
Yes	8 (17)	39 (80.8)		

Table 6. Correlation between LMR and clinicopathological features of PCa patients

Dependent variable	*Independent variable*	*r*	*p*
LMR	Depth of tumor infiltration.	-0.301	0.021
	Tumor stage.	-0.327	0.017
	Tumor size.	-0.327	0.039
	Tumor grade.	-0.285	0.033
	Gleason score.	-0.415	0.013

Table 7. Univariate analysis for clinicopathological factors

Factor	*Progression group (n=52)*	*Non-progression group (n=34)*	x^2	*p*
Age (years)				
≥70	27	18	0.133	0.935
<70	25	16		
Gleason score (points)				
≤7	26	19	6.886	0.031
>7	36	15		
Castration method				
Operation/drug alone	22	19	3.983	0.136
Operation + drug	30	15		
Clinical stage				
II	4	6	24.971	<0.001
III	14	11		
IV	34	17		
Lymph node metastasis				
Yes	35	12	8.932	0.011
No	17	22		
TGF-β1				
High expression	20	26	12.166	0.002
Low expression	32	8		

Table 8. Multivariate analysis for PFS after treatment

Influencing factor	*B*	*S.E*	*Wald* x^2	*p*	*OR*	*95%CI*
TGF-β1	0.781	0.391	7.028	0.017	2.981	1.551-7.965
Gleason score	0.765	0.339	6.885	0.021	2.616	1.383-7.319
Castration method	0.338	0.191	2.081	0.161	1.391	1.091-2.535
Clinical stage	0.76	0.326	4.796	0.042	1.782	1.205-3.981
Lymph node metastasis	0.401	0.184	5.022	0.035	1.918	1.321-4.728

Changes in the expression of TGF-β1 in patients with different Gleason scores

Among those with the Gleason score of 2-4 points, 5-6 points and 7-10 points, the number of patients with high TGF-β1 expression was significantly larger than that with low TGF-β1 expression (p<0.05) (Table 3).

Expression of TGF-β1 in patients with different ages

Among those aged ≥70 years old and <70 years old, there were more patients with high TGF-β1 expression than those with low TGF-β1 expression, but without significant differences (p>0.05) (Table 4).

Expression of TGF-β1 in patients with or without bone metastasis

There were also significantly more patients with high TGF-β1 expression than those with low TGF-β1 expression regardless of the presence or absence of bone metastasis (p<0.05) (Table 5).

Correlation between LMR and clinicopathological features of PCa patients

The association of LMR with the depth of tumor infiltration, stage, grade, size and Gleason score was explored, and it was found that LMR was negatively correlated with the above indexes (p<0.05) (Table 6).

Univariate analysis for clinicopathological factors

The univariate analysis was performed for 6 indexes, and the patients were divided into progression group (n=52) and non-progression group (n=34) based on the presence or absence of cancer progression after treatment. Obvious differences were found in the comparison of Gleason score, lymph node metastasis, TGF-β1 level and clinical stage between the two groups (p<0.05) (Table 7).

Multivariate analysis for PFS after treatment

Multivariate analysis showed that TGF-β1, Gleason score, clinical stage and lymph node me-

tastasis were influencing factors for PFS after treatment ($p<0.05$) (Table 8).

Discussion

PCa is one of the genital organ tumors greatly threatening the men's health, and when it grows to the bladder neck, it will block the urethra of patients and cause lower urinary tract obstruction and irritation symptoms, so PCa patients have been mostly in the middle-late stage when diagnosed [10]. TGF-β1 is a member of the TGF-β family, which promotes cell differentiation and inhibits epithelial cell proliferation in normal tissues [11]. In cancer tissues, TGF-β1 has abnormal activity, loses the inhibitory effect on cancer cells, stimulates oncogenes, enhances the tumor angiogenesis, invasion and metastasis, and raises the tumor growth and metastasis rate [12]. PCa grows in dependence on androgen, so endocrine therapy is an effective and conservative method for PCa patients, in which the drug will suppress the androgen secretion, thereby inhibiting the proliferation of cancer cells [13]. However, with the treatment time prolonged, PCa may develop into androgen-independent disease, seriously affecting the prognosis of patients. Therefore, early diagnosis of cancer is extremely important for selecting the subsequent therapeutic regimen [14].

In this study, the expression of TGF-β1 was assessed, and it was found that the number of patients with high TGF-β1 expression was larger among those in clinical stage II-IV, with the Gleason score of 2-4 points, 5-6 points and 7-10 points, aged ≥70 years old and <70 years old, and with or without bone metastasis. A previous study has confirmed that the expression of TGF-β1 in T3 and T4 PCa patients is significantly higher than that in T1 and T2 PCa patients, proving that TGF-β1 plays a negative regulatory role in the progression of disease, which is one of the causes of tumor growth [15]. It has also been proved that TGF-β1 plays a role in regulating the integrin secretion, and due to the abnormal expression of TGF-β1, PCa cells have adhesion as well as enhanced invasion ability [16]. Moreover, TGF-β1 is able to inhibit tumors in the early stage of disease, while it will exert opposite effects with the growth of tumor, promoting the increase of cancer cells and leading to immune dysfunction or loss [17]. A study has shown that under the influence of TGF-β1, tumor angiogenesis is accelerated, immune function is also inhibited and tumor growth is obviously facilitated, and the expression level of TGF-β1 can be used as a key index for judging the clinical efficacy and prognosis [18]. TGF-β1 accelerates tumor proliferation and metastasis through stimulating tumor angiogenesis and impeding the host immune function, and its expression level can also be used as one of the observation indexes for clinical effectiveness [19], consistent with the results in this study.

In this study, the correlation between LMR and pathological features of PCa patients was observed, and both univariate and multivariate analyses were performed. The results manifested that the LMR was negatively correlated with 5 clinical indexes of tumor, and clinical stage, TGF-β1, lymph node metastasis and Gleason score were influencing factors for PFS after treatment. It is reported in the literature that the Gleason score can be used for the grading of PCa tissues and as a reference index for the treatment of PCa [20]. In the endocrine therapy for PCa, androgen-independent PCa patients should be screened based on the TGF-β1 level, Gleason score, clinical stage and lymph node metastasis, and promptly treated with other treatments such as chemotherapy, so as to improve the prognosis.

Conclusions

In conclusion, the TGF-β1 level is positively correlated with the severity, clinical stage and pathological grade of PCa. LMR is negatively correlated with the depth of tumor infiltration, stage and grade. Clinical stage, TGF-β1, lymph node metastasis and Gleason score are influencing factors for PFS of PCa patients after treatment.

Conflict of interests

The authors declare no conflict of interests.

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