

ORIGINAL ARTICLES

Expression of IL-6 and TNF- α in benign prostatic hyperplasia combined with histological inflammation

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Received: January 12, 2016

Accepted: February 15, 2016

Online Published: March 10, 2016

DOI: 10.14725/dcc.v3n1p11

URL: <http://dx.doi.org/10.14725/dcc.v3n1p11>

Abstract

Objective: To detect the expression of TNF- α and IL-6 in simple benign prostatic hyperplasia (BPH) and BPH combined with prostatitis, and to evaluate the effect of inflammation of the development and progression of BPH.

Methods: Specimens of 90 patients with BPH underwent transurethral resection of prostate (TURP) were obtained. All specimens were divided histologically by HE staining into group of 35 cases of simple BPH (A group) and group of 55 cases of BPH combined with prostatitis (B group). Proteins levels of TNF- α and IL-6 were examined by immunohistochemistry. All patients' clinical indicators including PSA, prostate volume, age, urinary flow rate were recorded, and all patients were diagnosed as BPH by pathology.

Results: All patients of B group were found combined with chronic prostatitis. The constituent ratio was 61%. Patients' prostate volume and PSA of B group were significant higher than A group ($p < .05$), but there were no significant differences in urine flow rate and patients' age ($p > .05$). As compared with A group, the expression of TNF- α and IL-6 significantly increased in B group ($p < .05$). The results of analysis were as follows: there existed a moderate correlation between prostate volume and patients' age ($r = 0.430, p < .001$); there existed a moderate correlation between prostate volume and the degree of inflammation ($r = 0.610$ and $r = 0.609, p < .01$); there existed a moderate correlation between PSA and the degree of inflammation ($r = 0.572$ and $r = 0.487, p < .01$); there was not significant correlation between PSA and patients' age ($r = 0.065, p > .1$); controlling influence of the age factor by partial correlation analysis, there was still a significant correlation between the prostate volume and level of inflammation.

Conclusions: Most BPH patients are combined with prostatitis especially chronic inflammation. TNF- α and IL-6 are two-pro-inflammation cytokines, which obtain high expression in the prostate tissue combined with histological inflammation. Patients' prostate volume and PSA of BPH combined with prostatitis are significantly higher than those of simple BPH. However, there are no significant differences in urine flow rate and patients' age. These two cytokines may play an important part in promoting BPH and secretion of PSA.

Key Words: Prostatitis, Benign prostatic hyperplasia, TNF- α , IL-6, Prostate volume, Prostate specific antigen

Benign prostatic hyperplasia (BPH) is one of the common diseases among elderly population. It is mainly characterized by urgency, frequent micturition and dysuria. The quality of life of elderly men is greatly influenced by the

disease. Population aging is the development trend in the world, resulting in lower urinary tract symptoms (LUTS) due to BPH. It may affect the normal life of the patients in less severe cases. Even worse, the disease would influence

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renal function in patients with full bladder in a long-term. Therefore, BPH has attracted more and more attention from the academic community. At the beginning of last century, the global medical workers studied the etiology of BPH, and put forward a lot about the etiology of BPH hypothesis, such as sex hormone imbalance, dihydrotestosterone (DHT) and stem cell hypothesis. However, regardless of the hypothesis, there is no sufficient evidence to explain the pathogenesis of BPH. Recently, scholars have found that inflammation has a significant impact on BPH, and put forward several possible hypotheses, including inflammatory-hormone theory,^[1] oxidative stress theory,^[2] and vitamin D pathway theory.^[3] According to the new classification method by National Institutes of Health (NIH) of the United States,^[4] the patients with prostatic hyperplasia associated with inflammation are attributed to type IV prostatitis, that is, asymptomatic inflammatory prostatitis (AIP). In other words, there is no manifestation of clinical symptoms only in the microscopic examination of inflammatory changes or other signs of inflammation. In order to investigate the relationship between the occurrence and development of inflammation and BPH, we collected 90 specimens of BPH by TURP and the expressions of TNF- α and IL-6 in all samples were detected by immunohistochemical method. The results are reported as follows.

1 Materials and methods

1.1 Collection of specimens

From February 2011 to October 2013, 90 BPH tissues of the transurethral resection of prostate (TURP) were collected from our hospital. The patients were ranging in age from 52 to 82 years, with an average of (69.61 ± 7.30) years old and a medical history of 0.5 to 10 years, with an average of 5.2 years. Before admission, 5 patients had acute urinary retention. The incised specimen was quickly sent for the pathological examination when blood clot was removed. The pathology department teacher selected specimens and fixed with 10% neutral formaldehyde solution. The routine wax block was embedded and preserved. The 90 specimens were stained by HE staining and divided into 35 cases (A group) and 55 cases (B group).

1.2 Detection method

Rabbit anti-human IL-6 monoclonal antibody and Rabbit anti-human TNF- α monoclonal antibody were chosen and purchased in Wuhan Doctorate Biological Engineering Co., Ltd.

The paraffin slice of 4 μm was routinely dewaxed with gradient ethanol into the water and incubated with 3% hydrogen peroxide for 5 min, then the slice was washed by phosphate buffer solution (PBS) to repair the thermal antigen.

After blocked by 3% goat serum, anti IL-6 and TNF- α were added and incubated at 4°C for night. The slice was then washed by PBS. Afterwards, the second antibody (HRP labeled Goat anti-mouse IgG antibody) was added and incubated at 4°C in wet box for 20 min. The slice was then washed by PBS, followed by DAB chromogenic reagent, hematoxylin staining, differentiation, back to blue, gradient ethanol dehydration, xylene transparent, and neutral resin mounting.

1.3 The criteria for the determination of immunohistochemistry

Under double blindness, the specimens were observed by two doctors at the middle level and above in the pathology department. Each specimen was randomly selected for 10-15 high magnification (100 or 400 times), and the results were determined by semi-quantitative integration. According to the dyeing strength score, the standard is as follows: colorless (0), light yellow (1), brownish yellow (2), brown (3). According to the percentage of the positive cells score, 0 refers to negative (0%-5%), 1 refers to the number of positive cells accounting for 5%-25%, 2 for 26%-50%, 3 for 51%-75%, and 4 for > 75%. The integral is the sum of the two points above. Classification standard: (1) negative (-): 0-1; weak positive (+): 2-3; positive (++) : 4-5; strongly positive (+++) : 6-7.

1.4 The morphologic standard of histopathology^[5]

The morphological criteria of histology were: 1) infiltration of inflammatory cells around glands; 2) inflammatory cells infiltrated around the glandular canal, 3) the epithelium of glands had different degrees of destruction. It is necessary to employ the three items at the same time to diagnose chronic prostatitis.

1.5 Statistical method

All statistical data were input into the SPSS17.0 statistical software package for analysis, using independent sample variance homogeneity test, two-sample *t* test and Mann-Whitney *U* test. The difference between $p < .05$ was statistically significant. Pearson correlation coefficient and Spearman correlation coefficient described correlation and covariance test.

2 Results

Immunohistochemical results showed that IL-6 was mainly expressed on the basal part of prostatic epithelial cells, and there was a small amount of expression in the acinus. It was

also expressed in prostatic stroma and infiltrating inflammatory cells, which showed brownish yellow granules in cytoplasm and acinus (see Figure 1-1). TNF- α was mainly expressed in the epithelial cells of the prostate, and a large number of brownish yellow granules were found in the cytoplasm (see Figure 1-2).

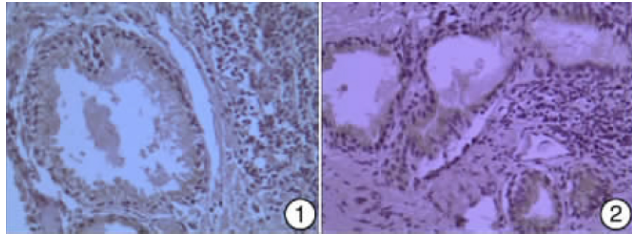


Figure 1: Expression of IL-6 and TNF- α in BPH in inflammatory tissues with BPH

Comparison of expression of IL-6 and TNF- α in the two groups was made (see Table 1).

In Table 1, the expression of IL-6 and TNF- α in B group was significantly higher than A group, the difference was statistically significant ($p < .05$).

The comparison of the differences between the two groups of clinical indicators was shown in Table 2.

As seen from Table 2, there was a significant difference in PSA level and volume between the two groups ($p < .01$). However, there was no significant difference in age and urine flow rate between the two groups ($p > .05$).

The relationships between IL-6, TNF- α , age and BPH volume, PSA were shown in Table 3.

As seen from Table 3, IL-6 and TNF- α were positively correlated with prostate volume and PSA level, while age was weakly correlated with prostate volume but not related to PSA level.

Controlling influence of the age factor by partial correlation, correlation of IL-6, TNF- α and prostate volume was shown in Table 4.

Table 1: Comparison of expression of IL-6 and TNF- α in the two groups

Groups	Cases	IL-6			p-value	TNF- α			p-value
		+	++	+++		+	++	+++	
A group	35	21	12	2	< .05	23	11	1	< .01
B group	55	18	29	8		17	31	7	

Table 2: Differences between the two groups of clinical indicators

Clinical indicators	A group	B group	p-value
Cases	35	55	
PSA/ $\mu\text{g}\cdot\text{L}^{-1}$	8.08 \pm 7.26	4.28 \pm 3.78	< .01
Urine flow rate/ $\text{ml}\cdot\text{s}^{-1}$	4.83 \pm 2.39	5.11 \pm 2.56	> .05
Age/years	69.37 \pm 6.87	69.76 \pm 7.61	> .05
Volume/ml	103.61 \pm 35.14	160.07 \pm 78.16	< .01

Note. Mann Whitney U test was used

Table 3: Relationship between IL-6, TNF- α , age and BPH volume, PSA

Test value	IL-6		TNF- α		Age	
	PSA	Volume	PSA	Volume	PSA	Volume
r-value	0.572	0.610	0.487	0.609	0.065	0.430
p-value	.000	.000	.000	.000	.544	.000

Note. The correlation was significant when the confidence level was 0.01

Table 4: Correlation of IL-6, TNF- α and prostate volume after controlling influence of the age factor by partial correlation

Indicator		IL-6		TNF- α	
Inflammation	correlation	1.000	0.565	1.000	0.552
	significance		0		0
	df	0	87	0	87
Prostate volume	correlation	0.565	1.000	0.552	1.000
	significance	0		0	
	df	87	0	87	0

Note. The cell contains the zero order (Pearson) correlation

It could be seen from Table 4 that after controlling influence of the age factor, IL-6, TNF- α and prostate volume was positively correlated.

3 Discussion

BPH is a common disease in the Department of Urology. Even today, few scholars have been able to define the etiology and pathogenesis of them. Although the pathogenesis of BPH is not clear in molecular biology, previous studies by epidemiologic researchers believe that age and androgen dependence are the two necessary conditions for the onset of BPH.^[6] Recently, scholars have suggested that besides the two factors of androgen and aging, inflammation may be another risk factor in its pathogenesis.^[7]

A total of 3,942 patients with BPH were analyzed retrospectively by Silverio's team,^[8] and found that 1,700 of the BPH patients were associated with chronic inflammation.

According to the finding in the rat model, the proliferation of epithelial cells could be induced by stimulating the prostatic prostate in rats with inflammatory factors, and its proliferative lesions were similar to BPH nodules.^[9] Although physiological and pathological changes of BPH are not clear, the symptoms of lower urinary tract will be significantly aggravated when BPH patients are combined with prostatitis.^[10] In recent years, through epidemiological studies, some scholars^[11] discovered that BPH patients combined with prostatitis had more severe symptoms. Pathologically, it was found that the symptoms of BPH patients were progressing rapidly and the average score of IPSS was increased. In more than 8,000 cases of BPH patients by REDUCE for a period of 4 years of research,^[12] it was detected that more than 78% of patients were with chronic inflammation, and the IPSS score of the inflammation group was significantly higher than the patients with BPH ($p < .001$), but the correlation between IPSS scores and inflammation was weak. The nonsteroidal anti-inflammatory drug, rofecoxib combined with finasteride, was compared with the simple use of finasteride in Silverio's study,^[13] and followed up for 24 weeks. In the first 4 weeks, the IPSS score ($p = .0001$) in the combined treatment group was significantly improved as well as the maximum urinary flow rate (Qmax) ($p = .03$), while the finasteride group did not change. At the 24th weeks, there was no significant difference in the improvement of symptoms between the two groups. It was concluded that early use of anti-inflammatory drugs could better improve the symptoms of BPH. 5 α reductase inhibitors would play an important role when BPH is dominated by hormone after inflammatory control.^[13]

IL-6 is an important pro-inflammatory factor. Tissues produce IL-6 and IL-8 through autocrine or paracrine ways, and then recruit the accumulation of T cells to produce an inflammatory response. The space left by the inflammatory

response in prostate tissue is filled up with fibromuscular nodules by Th0/Th3 immune process.^[14] Royuela et al.^[15] found that IL-6 located in the matrix and luminal epithelial cells of BPH tissues by immunohistochemistry analysis. It was found that IL-6 was mainly expressed in the cytoplasm and nucleus of the epithelial cells of the prostate, and as well as in the stromal cells. Moreover, the expression level of IL-6 in the inflammatory group (B group) was significantly higher than that in the simple group (A group) ($p < .01$), indicating that the proinflammatory factor IL-6 was highly expressed in the BPH glands with inflammation.

In 2005, in the long-term large-scale research of MTOPS, 1,197 patients with BPH underwent prostate biopsy, and 43% of them had prostatitis. The results also demonstrated that the prostate volume of BPH patients with medical history of prostatitis was larger, and the level of PSA was higher.^[16] The results of this study were similar to those of MOTPS, in the inflammatory group, expression of IL-6 was more obvious. The urine flow rate was not affected by the inflammatory factors in the patients, and the PSA level was not affected by age. The volume of BPH and the level of PSA were positively correlated with the degree of inflammation. A total of 676 patients with BPH were studied by Schenk et al.^[17] and he found that the effect of IL-6 on BPH was more obvious in patients younger than 65 years old. In that case, they believed that the inflammation on BPH may be affected by age. Regardless of age factor, our study discovered the positive correlation between inflammation and the volume of BPH and the level of PSA. Results also showed strong correlation between volume of BPH and IL-6 and PSA levels. Therefore, inflammation may be a risk factor for the impact of BPH.

Sun GH et al.^[18] showed that TNF- α was expressed in BPH, prostate cancer and normal prostate tissue through immunohistochemical experiments. While in BPH tissues, the expression of TNF- α was the most obvious, and the difference was statistically significant ($p < .05$). They believed that the high expression of TNF- α in prostate tissue of BPH patients might be due to the inflammatory reaction caused by hyperplasia of prostate tissue and destruction of tissue structure. It was proved that chronic inflammation played an important role in BPH. Nadler et al.^[19] also confirmed that changes of TNF- α numbers were positively correlated with the degree of inflammation in patients with inflammatory chronic pelvic pain syndrome and chronic abacterial prostatitis. The results of this study verified that there were obvious differences in the expression of TNF- α in the simple group and the inflammation group ($p < .01$). Chen YY et al.^[20] conducted a placebo control experiment on BPH patients. TNF- α was found significant different in serum, indicating that the inflammatory response of BPH patients was more severe than that of non BPH patients. And the IPSS score was positively correlated with TNF- α . Bouraoui et al.^[21] studied the relationship between prostatic inflammatory cytokines and serum PSA level. It was found that TNF- α in

BPH group was only expressed in patients with PSA < 20 $\mu\text{g/L}$. In this study, the expression of TNF- α could be seen in all sections on the slice through by immunohistochemistry. Results demonstrated that TNF- α and BPH volume were positively correlated with PSA. Therefore, we could draw a conclusion that inflammation may have a positive effect on the volume of BPH and PSA.

Prostatitis may be an important cause of the production of BPH, but its causality is not yet clear. The body maintains T cell activity through the autoimmune mechanism. The stim-

ulation of inflammatory cells promotes the proliferation of prostate stromal cells and epithelial cells, and develops into benign prostatic nodules due to inflammatory tissue injury and subsequent repeated healing process. Finally, it leads to nodular hyperplasia of the prostate.^[22] It may be one contributor to the pathogenesis of BPH.

Conflicts of Interest Disclosure

The authors have no conflicts of interest related to this article.

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