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PATTERN OF TRANSFORMING GROWTH FACTOR-ß1 (TGF-ß1) LEVEL IN BPH AND NON-BPH PATIENTS

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ABSTRACT

The etiology and pathogenesis of Benign Prostatic Hyperplasia (BPH) remains unclearly defined, and one of this unclarity is the reduction of Transforming Growth Factor-β1 (TGF-β1) level. The aim of this study was to disclose the role of TGF-β1 in the pathogenesis of BPH. The study on plasma TGF-β1 level pattern was carried out to BPH patients (group 1), non-BPH patients of more than 50 years of age (group 2), and non-BPH patients of less than 50 years of age (group 3). The samples in these groups comprised 18, 17, and 13 individuals respectively, and the TGF-β1 level in respective groups were ranging between 3.4 to 31.52 (14.72 ± 8.07) ng/ml, 1 to 65.3 (25.42 ±17.97) ng/ml and 4.7 to 69.6 (23.76 ± 19.56) ng/ml. The mean of plasma TGF-β1 level in BPH patients was lower that that of non-BPH group irrespective of their age. There was difference in plasma TGF-β1 between that in group 1 and 2 after being tested with independent sample t 2 test (p = 0.035), probably due to the role of plasma TGF-β1 in BPH pathogenesis directly and indirectly. It is suggested to undertake further studies using tissue sample from BPH patient and normal prostate tissue to prove that TGF-β1 has an important role in BPH pathogenesis.

Keywords: *Transforming Growth Factor-β1 (TGF-β1), Benign Prostatic Hyperplasia (BPH)*

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INTRODUCTION

Benign Prostatic Hyperplasia (BPH) is a condition commonly found in male elderly. BPH itself may not be life-threatening. However, the present clinical symptom, the lower urinary tract symptoms (LUTS), may bother daily activities. LUTS can be found in 30% of men aged more than 65 years old. The symptoms of LUTS are poor stream, frequent voiding, incomplete voiding, and nocturia. Complications that may occur are bladder function regression, urinary tract infection, and acute urinary retention as well as surgery. These symptoms, augmented with the resulting complication, inevitably reduce the quality of the patient's life. Due to the increase of life expectancy, these difficulties will clearly become a health problem (de la Rosette 2001; Liber 2001).

Correlation between age increase and BPH is confirmed by the results of studies revealing that about 70% of males aged more than 60 years and 90% of those more than 80 years have predisposition to BPH (Roehrborn 2002). According to Agency for Health Care Policy and Research at the beginning of 2000, there were 6.5 million males aged 50-79 years old who experience BPH. In Indonesia, the definitive occurrence rate of BPH has not been studied. However, a brief description can be seen from the cases in RSCM and Sumber Waras Hospital, Jakarta, where between 1994 and 1997 (for three years) there was a number of 1,040 cases (Rahardjo 1999). In Dr Soetomo Hospital, Surabaya, the annual BPH cases is found in 250 individuals aged 50-77 years who need surgical procedure.

BPH etiology and pathogenesis has not been clearly understood. Many theories and hypotheses have been proposed to explain BPH mechanism, one of which is the presence of imbalance between prostate cell proliferation and death. The theory suggests that the increase of cell count and volume of an organ is not only caused by the increase of cell proliferation, but also by the reduction of cell death. In aged men, there is a reduction of testosterone that results in the increase of estrogen to maintain homeostasis. Estrogen increase will trigger several growth factors, among which are Fibroblast Growth Factors (FGF) and Epidermal Growth Factors (EGF) that may raise proliferation and hyperplasia of prostatic stromal cells. In addition,

estrogen also has capability to suppress the Transforming Growth Factor-β1 (TGF-β1), one of the cytokines produced by the prostate, whose function is to inhibit epithelial proliferation and induce prostatic cell death. Both lead to proliferation (growth) balance mechanism and the death of prostatic stromal cells become disturbed, which finally leads to the increase of proliferation to become hyperplasia (benign prostatic hyperplasia = BPH) (Matsuda 2001; Roehrborn 2002). The result of study by Soetojo showed that estrogen provision to white rats increased EGF and FGF, and reduced TGF-β1 so that the proliferation of prostatic tissue increased (Soetojo 2004).

Information on plasma TGF-β1 level in Indonesia still unexist. This fact has motivated the authors to investigate the the pattern of TGF-β1 level related with the occurrence of BPH. The findings of this investigation will improve our knowledge on the process of BPH and to assist BPH treatment.

MATERIALS AND METHODS

This was an observational study using analytic cross-sectional design. Samples were collected between October - December 2005. The study was carried out to BPH and non-BPH patients searching for treatment in Urology Outpatient Clinic, Surgical Division, Center of Emergency Care, or those hospitalized at Urology Wards, Dr Soetomo Hospital, and met the inclusion criteria as follows: clinically diagnosed with BPH (presence of complaints and LUTS symptoms), the presence of radiologically-confirmed prostate enlargement not leading to malignancy, aged more than 50 years, and filled and signed the informed consent of joining the study. The criteria of BPH sample exclusion are: clinically and radiologically lead to prostate malignancy, presence of histopathological diagnosis of prostate malignancy, previous BPH operation, presence of hormonal abnormalities, such as ambiguous genitalia, gynecomastia, hermaphrodite, previous manipulation of the prostate, such as DRE, transrectal ultrasonography (TRUS), sexual intercourse in 48 hours, and catheter installment for less than 48 hours.

The inclusion criteria of non-BPH patients of more than 50 years old were as follows: aged more than 50 years, clinically and radiologically absent of BPH, and filled and signed the informed consent of joining the study. The samples were excluded if the patient had hormonal abnormalities, such as ambiguous genitalia, gynecomastia, hermaphrodite, had previous manipulation of the prostate, such as DRE, transrectal ultrasonography (TRUS), sexual intercourse in 48 hours, and catheter installment for less than 48 hours.

The inclusion criteria of non-BPH patients of less than 50 years old were as follows: aged between 30 and 50 years, clinically and radiologically absent of BPH, and filled and signed the informed consent of joining the study. The samples were excluded if the patient had hormonal abnormalities, such as ambiguous genitalia, gynecomastia, hermaphrodite, had previous manipulation of the prostate, such as DRE, transrectal ultrasonography (TRUS), sexual intercourse in 48 hours, and catheter installment for less than 48 hours.

Sample size was determined using rule of thumb (central limit theorem). Each group required 10 samples, so that 30 samples were required for all groups. Blood sample was put into 4 ml vacutainer tube containing Lithium Heparin. After 30 minutes, the sample was centrifuged with refrigerated centrifuge, in a speed of 3000 rpm for 10 minutes. The separated plasma was kept in -200 degree C in several aliquots until the examination using Enzyme Link Immunosorbent Assay (sandwich) method. Blood specimen taking, plasma separation and preservation, as well as plasma TGF-β1 examination were carried out at the Department of Clinical Pathology, Airlangga University School of Medicine, Dr Soetomo Teaching Hospital, Surabaya.

RESULTS

A precise quality establishment was carried out by examining 19 samples in duplication at the same day in one run. Subsequently, the TGF-β1 level was estimated to obtain the Standard Deviation (SD). The SD found in this study was 1.17 ng/ml. As many as 48 patients with BPH and non-BPH treated at Urology Clinic and hospitalized at Surgical Ward D, Dr Soetomo Hospital met the inclusion and exclusion criteria. These numbers included 18 BPH patients aged more than 50 years (group 1), 17 non-BPH patients aged more than 50 years old (group 2), and 13 non-BPH patients aged between 30-50 years (group 3). The mean age in group 1 was 65.8 years, and the oldest was 82 years. The mean age in group 2 was 59.4 years, the oldest 80 years, and in group 3 the mean age was 37.5 years, and the oldest 47 years.

In BPH patients, the prostate volume was ranging between 23.46 - 79.54 ml. TGF-β1 level was ranging between 3.4 - 31.52 ng/ml with a mean of 14.72 ng/ml. In non-BPH patients aged more than 50 years, the prostate volume was ranging between 12.71-19.78 ml,. TGF-β1 level ranged between 1-65.3 ng/ml. In non-BPH patients of less than 50 years, the prostate volume was ranging between 7.98 - 19.87 ml, TGF-β1 level was

between 4.7 - 69.6 ng/ml. Sample data distribution can be seen in Table 1.

Table 1. Sample distribution in each category, range, mean, and SD

	Prostate volume (ml)	TGF-β1 plasma (ng/ml)
BPH > 50 yr Group 1 (n=18)		
range	23.46 – 79.54	3.4 – 31.52
mean	42.97	14.72
SD	14.65	8.07
Non-BPH > 50 yr Group 2 (n=17)		
range	12.71 – 19.78	1- 65.3
mean	17.11	25.42
SD	3.25	17.97
Non-BPH < 50 yr Group 3 (n=13)		
range	7.98 – 19.87	4.7 – 69.6
mean	13.83	23.76
SD	4.35	19.56

Normality test on the data, comprising age, prostate volume, and plasma TGF-β1 in all three groups revealed normal distribution with $p > 0.05$. The authors, therefore, could use the existing samples to be evaluated by means of independent sample t 2 test to find the difference of TGF-β1 level in all groups of BPH and non-BPH patients, and by Pearson correlation test to find correlation between plasma TGF-β1 in BPH and non-BPH patients.

Using independent sample t 2 test the plasma TGF-β1 level showed significant difference between BPH patients ($x = 14.72 \pm 8.07$) ng/ml and non-BPH patients of more than 50 years ($x = 25.42 \pm 17.97$) ng/ml in $p < 0.05$ ($p=0.035$), while the result of independent sample t 2 test in group of non-BPH patients aged more than 50 years ($x = 25.42 \pm 17.97$) ng/ml and in non-BPH patients of less than 50 years ($x = 23.76 \pm 19.56$) ng/ml showed no difference. If the samples of non-BPH patients were not sorted into those of more than 50 years and those less than 50 years (only involving non-BPH patients), it would have been obvious that plasma TGF-β1 level in BPH patient was significantly lower than that in non-BPH patients. This can also be seen in Figure 1. It is apparent in Figure 1 that the lowest TGF-β1 level exists in BPH patients. It can also be seen the clear difference of TGF-β1 level between BPH and non-BPH patients aged more than 50 years. Such difference is not apparent in non-BPH patients of more than 50 years old and BPH-patients of less than 50 years old.

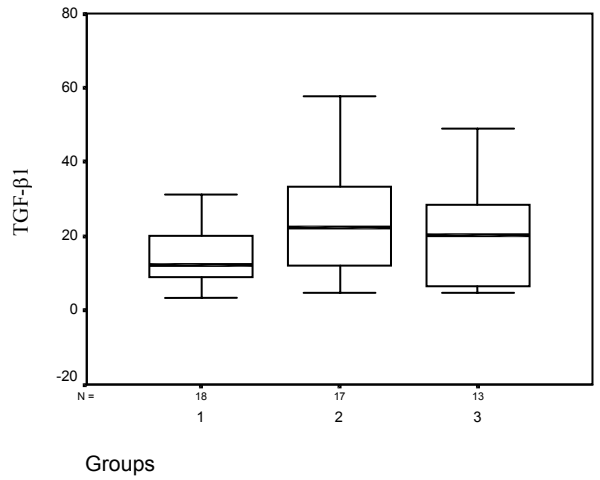
DISCUSSION

In this study we carried out sample examination from 18 BPH patients, 17 non-BPH patients of more than 50 years old and 13 non-BPH patients of less than 50 years old with the result of plasma TGF-β1 as follows: 14.72 ± 8.07 ng/ml in BPH patients; 25.42 ± 17.97 ng/ml for non-BPH patients of more than 50 years; and 23.76 ± 19.56 ng/ml for non-BPH patients of less than 50 years. Plasma TGF-β1 in BPH patients that ranging between 3.4 and 31.52 ng/ml in this study was lower than plasma TGF-β1 in BPH patients found by Wolff et al, which was ranging between 22.3 and 35.7 ng/ml (Wolff 1998). This was due to different samples used. Wolff used serum as sample in his study, while the authors used plasma. TGF-β1 is commonly present in bones and thrombocyte alpha-granule (Pimentel 1994). Each molecule of alpha-granule in the thrombocyte contains 2500 TGF-β1, so that if thrombocyte degranulation in the sample occurs, the TGF-β1 will increase. Therefore, to prevent degranulation, this study used anticoagulant, heparin.

Transforming Growth Factor-b (TGF-b) is a pleotropic growth factor that regulates cell growth and differentiation, extracellular matrix production, cell motility, angiogenesis and immunosuppression. Generally, the main role of TGFb is as inhibitor of epithelial cell growth and growth stimulator of mesenchymal cells (Sintich 1998). There are three types of isoform in mammals, i.e., TGF-β1, TGF-b2, and TGF-b3. TGF-β1 is the most common form found in circulation (Shu 2004). In prostatic stromal cells, there are the expressions of these three TGF-b isoforms, predominating by TGF-β1 (Elliot 2005; Zhou 2003). TGF-β1 and its receptor presents in prostatic epithelial and stromal tissue (Tomlison 2004). TGF-β1 is one of growth factors in the prostate. TGF-β1 inhibits the growth of prostatic epithelial and stromal cells, also induces the death of prostatic epithelial cells. In prostatic stromal cells, the role of TGF-β1 depends on its level. In higher level it inhibits stromal cell proliferation and in lower level it triggers prostatic stromal cells (Lee 2001).

A study by Bretland et al. (2001) in prostatic stromal cell culture from BPH tissue revealed that in TGF-β1 level of 0.01 ng/ml there was an increase of cell growth, and in the level of > 1 ng/ml the growth is reduced. Similar results were also found in a study by Zhou et al (2003). In TGF-β1 level between 0.001 and 0.01 ng/ml, the prostatic stromal cell growth is triggered, and in higher level (between 1.0 and 10 ng/ml), it triggers the cease of growth. It is hypothetically stated that TGF-β1 may play a role in BPH pathogenesis (Zhou 2003). In

aging process, there is a reduction of testicular Leydig cell count, so that the production of testosterone is reduced. To maintain homeostasis, the estrogen is increasing. Estrogen suppresses TGF-β1 in inducing gene expression and directly triggering prostatic epithelial cell proliferation (Matsuda 2001). A study by Soetojo in rats rendered to become older showed that estrogen administration to white rats increased Epidermal Growth Factor (EGF), Fibroblast Growth Factor (FGF) and reduced TGF-β1 so that proliferation of prostatic tissue also increased (Soetojo 2004).



Group 1: BPH,
Group 2: Non-BPH > 50 years
Group 3: Non-BPH < 50 years

Figure 1. The distribution of TGF-β1 level in BPH patients, non-BPH patients of more than 50 years, and non-BPH patients of less than 50 years.

In this study, using independent sample t 2 test, it was found that the plasma TGF-β1 level in BPH patients (x = 14.72 ± 8.07) ng/ml was lower than the level in non-BPH patients of more than 50 years old (25.42 ± 17.97) ng/ml. Further studies using tissue samples from BPH patients and from normal prostate tissue may be needed to prove the hypothesis that TGF-β1 has an important role in BPH pathogenesis. In this study, using independent sample t 2 test, plasma TGF-β1 level in non-BPH patients of more than 50 years was not different from the level in those less than 50 years old. In addition, the mean of TGF-β1 level in non-BPH patients of more than 50 years old was lower than that in non-BPH patients of less than 50 years old. This was likely due to the difference of estrogen level in samples that affected the level of TGF-β1. Further studies are needed to determine the estrogen level related to intraprostatic TGF-β1 level to find the effect of estrogen, either directly or indirectly, on TGF-β1.

To date, the etiology of BPH remains unknown. Various factors involve in affecting BPH occurrence. These factors are generally divided into intrinsic and extrinsic one. The intrinsic factor was interaction between prostatic epithelial and stromal cells. The extrinsic factors include testicular factors, other somatic factors, environmental and genetic factors. Testicular factors comprise estrogen and non-androgen factors. Other somatic factors comprise non-testicular hormones, neurotransmitters, and immunology. The environmental factors are diet and organism infection. The genetic factor is homeobox genes, inherited disease. External factors that may likely trigger BPH occurrence are alcohol, cigarette smoking, certain diet, and obesity. According to Kirby and Roehrborn, alcohol may be able to reduce plasma testosterone production and level, as well as increase testosterone clearance. It was found that BPH incidence in Japan is high among male consuming much milk and less green and yellow vegetables. Some of yellow vegetables and other elements present in Japanese diet, including soybean, have been found to contain phytoestrogen and they possibly provide the protective effect, although convincing evidence proving that diet has a role in BPH pathogenesis has not been found (Kirby 1997; Roehrborn 2002).

CONCLUSIONS

The mean value of plasma TGF-β1 level in BPH patients is lower than that in non-BPH patients, regardless of their age. This likely results from direct and indirect effect of TGF-β1 in the enlargement of prostate volume that leads to BPH. Further studies using samples from the tissue of BPH patients and from normal prostate may be needed to prove the hypothesis that TGF-β1 has an important role in BPH pathogenesis. Studies are also needed on testosterone and estrogen in BPH patients, and also concerning other factors, such as diet and obesity.

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