

Correlation Between Serum Tissue Polypeptide Specific Antigen Level and Prostate Volume in BPH

by Wahjoe Djatisoesanto

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RESEARCH

6 **CORRELATION BETWEEN SERUM TISSUE POLYPEPTIDE SPECIFIC ANTIGEN LEVEL AND PROSTATE VOLUME IN BPH**

(Kenasaban antara Kadar Tissue Polypeptide Specific Antigen Serum dan Volume Prostat di BPH)

Mahrany Graciella Bumbungan¹, Endang Retnowati¹, Wahjoe Djatisoesanto²

ABSTRAK

Volume prostat menjadi informasi yang penting karena dapat memperkirakan kematian pada Benign Prostatic Hyperplasia (BPH). Volume prostat diukur menggunakan TRUS (Transrectal Ultrasonography) sebagai baku emas namun TRUS mempunyai beberapa kekurangan. Dibutuhkan suatu tolok ukur lain yang dapat memperkirakan volume prostat. Tissue Polypeptide Specific Antigen (TPS) yang terdeteksi di peredaran terdiri dari fragmen sitokeratin yang terdapat dalam jaringan dan menunjukkan status proliferasi. Sel epitel di BPH yang mengandung sitokeratin 18 akan mengalami hiperplasia sehingga dapat terdeteksi dengan pemeriksaan TPS. Tujuan penelitian ini adalah membuktikan adanya kenasaban antara kadar TPS serum dan volume prostat. Subjek penelitian terdiri dari 28 pasien BPH yang datang berobat ke Poli Rawat Jalan Urologi RSUD Dr. Soetomo Surabaya. Volume prostat diukur menggunakan alat TRUS. Kadar TPS serum diukur menggunakan metode ELISA (TPS® ELISA IDL Biotech). Kadar TPS serum berkisar antara 82,45-1771,5 U/L (195,35±349,79 U/L). Volume prostat beragam antara 20,7-87,4 cm (34,70±15,31 cm). Tidak terdapat kenasaban positif yang bermakna antara kadar TPS serum dan volume prostat ($p=0,404$; $r=0,164$).

Kata kunci: BPH, tissue polypeptide specific antigen, volume prostat

ABSTRACT

Prostate volume has become an important information because it can predict morbidity in Benign Prostatic Hyperplasia (BPH). Prostate volume is measured using TRUS (transrectal ultrasonography) as a gold standard; but this has some disadvantages. Other parameters are needed to predict prostate volume. Tissue Polypeptide Specific Antigen (TPS) detected in the circulation consists of cytokeratin fragments contained in the tissue and show the proliferation status. Benign Prostatic Hyperplasia epithelial cells containing cytokeratin 18 will undergo hyperplasia, so it can be detected by TPS examination. The aim of this study was to prove any correlation between the levels of serum TPS and prostate volume. Study subjects consisted of 28 BPH patients from the Urology Outpatient Clinic Dr. Soetomo Hospital Surabaya. Prostate volume was measured using TRUS. Levels of serum TPS were measured using ELISA method (TPS® ELISA IDL Biotech). Levels of serum TPS ranged between 82.45-1771.5 U/L (195.35±349.79 U/L). Prostate volume varied between 20.7-87.4 cm (34.70±15.31 cm). No significant positive correlations between levels of serum TPS and prostate volume were found ($p=0.404$; $r=0.164$).

Key words: BPH, tissue polypeptide specific antigen, prostate volume

INTRODUCTION

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Prostate Hyperplasia (BPH) is the second most common disorder in male patients after urinary tract stones. Guidelines of the American Urological Association (AUA) define BPH as the histopathologic

diagnosis which is proliferation of epithelial cells and prostate stromal cells in the transitional zone of the prostate that cause constriction of the urethra. BPH is common in older males with the incidence increasing with age.

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Prostate hyperplasia or Benign Prostatic Hyperplasia (BPH) is the second most common disorder in male patients after urinary tract stones. Benign prostatic hyperplasia is an uncontrolled and benign growth of prostate cells. American Urological Association (AUA) defined BPH as the histopathologic diagnosis which is proliferation of epithelial cells and prostate stromal cells in the transitional zone of the prostate that cause constriction of the urethra and inhibit urine output. This can cause infection, bladder stones and chronic prostatitis.¹⁻³

Benign prostatic hyperplasia gives complaints and disturbs daily activities even though it is rarely a life threatening condition. This situation is caused by enlargement of the prostate gland or called Benign Prostate Enlargement (BPE) which causes obstruction in bladder neck and urethra also known as Bladder Outlet Obstruction (BOO).^{4,5}

Complaints of BPH patients are Lower Urinary Tract Symptoms (LUTS) consisting of irritative or storage symptoms including frequency, nocturia, urge incontinence and obstructive or voiding symptoms including slow stream, hesitancy, straining, intermittency and incomplete voiding.²

Benign prostatic hyperplasia is more often found in older males with an increasing incidence concomitant with increasing age. One of 4 males in America is estimated as needing treatment for symptomatic BPH when 80 years old. Benign prostatic hyperplasia becomes the second most reason to do surgery in males above 65 years old.⁵ Benign prostatic hyperplasia is also the second rank after urinary tract stones in Indonesia and commonly predicted about 50% males more than 50 years old suffering from BPH.⁶

Physical examinations such as Digital Rectal Examination (DRE) and Transrectal Ultrasonography (TRUS) are important examinations in BPH patients that can predict any prostate enlargement, prostate consistency and nodules.⁷ Information about prostate volume becomes important because it can predict severity of BPH progressivity or BPH outcome such as acute urinary retention and treatment response.^{8,9}

A research by Nickel¹⁰ declared that males with prostate volume ≥ 30 mL have 3 or 4 times a higher risk of mild until severe LUTS, decreased urine flow and urine retention so that prostate volume becomes a more important information because prostate volume can predict morbidity associated with BPH such as urine retention.¹⁰

Prostate volume measurement can be done by DRE and TRUS techniques. All this time, DRE become a reliable method in estimate total prostate volume have low sensitivity, need trained human resources and

have variability between one examiner and others. Transrectal ultrasonography became the gold standard for prostate volume measurement because it has a higher accuracy than DRE, yet routine measurement with DRE is not possible to every patient due to low availability, need an trained expert, high costs and is uncomfortable for patients.^{8,9,11}

Another parameter is needed to predict prostate volume widely named Tissue Polypeptide Specific Antigen (TPS). Polypeptide Specific antigen is a protein that represent M3 epitope of Tissue Polypeptide Antigen (TPA). Tissue polypeptide specific antigen detected in the circulation consists of cytokeratin fragments contained in the tissue and thus show the proliferation status. Benign prostatic hyperplasia epithelial cells containing cytokeratin 18 will undergo hyperplasia. Tissue polypeptide specific antigen is the only test that specifically measures cytokeratin 18 in prostate epithelial cell so it called "proliferation markers".¹²⁻¹⁴

Correlation between TPS and prostate volume in BPH patient hopefully could help to estimate prostate volume especially for clinicians who do not have TRUS nor access to do TRUS. Epithelial cells in BPH consisting cytokeratin 18 will undergo hyperplasia including stromal cells so cytokeratin 18 can be detected by TPS examination. Polypeptide specific antigen is measured by Enzyme Linked Immunosorbent Assay (ELISA) using a high affinity monoclonal antibody to M3, one of 35 TPA epitopes and is an epitope structure in cytokeratin 18 correlating with cell proliferation.¹⁴

The aim of this study was to prove any correlation between the levels of serum TPS and prostate volume and obtain information about correlation between serum TPS and prostate volume in order to establish the diagnosis and estimate prostate enlargement in BPH patients. The usefulness of this research was to increase knowledge about the role of serum TPS and correlation with prostate volume in BPH patients and can be the basic for immunology research to further know the function of TPS in BPH disease.

METHODS

The study design was an observational analytical type with cross sectional design. This study was done in October 2015 until February 2016. Study subjects were patients from the Urology Outpatient Clinic, Dr. Soetomo Hospital Surabaya. The age range was 40-70 years old already established as a BPH patient by an urologist, did not under any alpha blockers

and 5 alpha reductase inhibitors (5-ARI) treatment, also did not suffer from renal disorders such as bladder obstruction, urinary tract infection, cystitis, pyelonephritis and renal failure. The study subjects consisted of 28 samples. Specimens were sera collected and stored at -80°C until the examination was done. Volume of serum was $2 \times 50 \mu\text{L}$. Polypeptide specific antigen was examined with double antibody sandwich Enzyme-Linked Immunosorbent Assay/ELISA method and measured using manual ELISA method (TPS@ ELISA IDL Biotech) with lot number F2636. Optical Density (OD) measured using spectrophotometer at 450 nm with an ELISA reader. Polypeptide specific antigen level were increased if $>80 \text{ U/L}$. Monitoring for accuracy TPS examination was done with positive and negative control reagents. Imprecision test of this tools showed a standard of deviation (SD) 19.61 U/L and Coefficient Variation (CV) 7.9%. All the data that were collected in this study was shown in the form of tables, curves and affidavits. Descriptive data was presented as mean \pm SD. Bivariate normality test was done to know whether the TPS level and prostate volume showed a normal distribution. P value was < 0.05 considered as significant statistically. This study has received approval from the Research Ethics Committees Dr. Soetomo Hospital Surabaya issue 573/Panke.KKE/XI/2015.

RESULTS AND DISCUSSION

This study was done in October 2015 until February 2016 with study subjects 28 BPH patients who visited the Urology Outpatient Clinic Dr. Soetomo Hospital, Surabaya. The mean age was 63.18 years old (SD 6.6 years old), with age range between 48 until 70 years old. Benign prostatic hyperplasia patients were mostly between 60–70 years old (82.13%) (see Table 1). This was in accordance with the literature which stated that males ≥ 50 years old have a 6.24 times higher risk than males ≤ 50 years old with study subjects mean 65.90 ± 9.1 years old for case group.¹⁵

Study by Goh *et al.*¹⁶ showed that the mean age of BPH patients was 63.72 ± 9.40 years old with 33.4% patients including in the age group 60–69 years old. This was caused due to an age over 50 years old there was an imbalance between a decrease of testosterone hormones with an increase of estrogen hormones along with increasing of age. The increase of estrogen make androgen receptor sensitivity inside stromal cell in prostate gland. Testosterone is converted to Dihydrotestosterone (DHT) by enzim 5α -reductase and will bound with Androgen Reseptor (AR)

becoming DHT-AR complex in cell nucleus so growth factor protein synthesis will stimulate prostate cell growth.^{16,17}

Table 1. Distribution of BPH patients by age

Age (Year)		Total
40–49	1	1 (3.58%)
50–59	4	4 (14.28%)
60–70	23	23 (82.13%)
Mean		63.18
Standard deviation		6.6

The result of serum TPS level obtained in this study were higher than normal in 13 subjects ranging from 82.45–1822 U/L. Mean \pm SD TPS levels in all subjects were $195.35 \pm 349.79 \text{ U/L}$. Tissue polypeptide specific antigen highest level was found 30–299 U/L in 25 subjects (89.28%) (as can be seen in Table 2).

Table 2. Distribution of the serum TPS levels

Mean TPS Serum Level (U/L)	Total
30–299	25 (89.28%)
300–519	1 (3.57%)
520–739	0 (0%)
740–999	1 (3.57%)
>1000	1 (3.57%)
Mean	195.35
Standard deviation	349.79
Normal value	<80

The mean TPS high levels may be caused by wells that were not washed complete properly so there was a possibility of antigen remaining in the wells and bound to biotin-labeled antibody. High TPS level may also be increased in some diseases such as renal failure, active chronic hepatitis and diabetes mellitus so may result in a false high levels of TPS. Renal failure increased levels of TPS because glomerular epithelial membrane damage so that cytokeratin 18 was released into the circulation. Liver disease either nonmalignant such as chronic hepatitis, autoimmune hepatitis, alcoholic hepatitis and malignancy such as Hepatocellular Carcinoma (HCC) could increase the levels of TPS due to hepatocyte damage and cytolysis process.

Cytokeratin 18 which maintains the hepatocyte's cytoskeleton will released into the blood circulation because of damaged hepatocytes so that it can be

detected by TPS. Increased levels of TPS in DM are mainly in complications of renal and liver dysfunctions.¹⁸

Study from Anitha¹⁹ declared that TPS serum levels only increased in eight research subjects (32%) of the 25 research subjects with $p > 0.1$. This was because in detecting cytokeratin 18, TPS can also detect other cytoskeletal intermediate filaments which were cytokeratin 8 and cytokeratin 19, which was more dominant. Cytokeratin 18, 8 and 19 were synthesized during the process of epithelial and stromal cell proliferation causing cytokeratin protein fragments be released into the circulation.¹⁹

The results of prostate volume measurement obtained in this study were higher than normal in all BPH patients, ranging from 20.7 to 87.4 mL with a mean \pm SD of 34.70 ± 15.31 mL. More than half of the study subjects (64.28%) had a prostate volume greater than or equal to 25 mL (see Table 3).

Table 3. Prostate volume measurement results

Volume (mL)	Total
<25 mL	10 (35.72%)
≥ 25 mL	18 (64.28%)
Mean	34.70
SD	15.31
Normal value	<25

A study in 2011 using a cross-sectional study investigated the changes in the total prostate volume and transitional zone volume in males aged 40–70 years and obtained the result that prostate volume increased 2 times from 5.5 mL in the age group of 40–49 years to 11.1 mL at the age of 60–70 years.²⁰

All study subjects in this study had a prostate volume greater than 20 mL. Some discrepancies in this study were that the study subjects aged 48 years had a prostate volume of 41.05 mL while study subjects who were 67 years old also had a prostate volume of 43.8 mL. This could be due to variability between examiners with different experiences, increasing the length and width of the prostate tended to be stable and only changed slightly at age 40–69 years old because before 60 years old the prostate growth was slower but after 60 years old the prostate will grow faster in length.¹⁵

Bivariate normality analysis test result showed that the data distributed around the diagonal line and no data showed a deviation far from the line so TPS serum level and prostate volume showed a bivariable

7 Normal P-P Plot of Regression Standardized Residual
Dependent Variable: Volume.mL

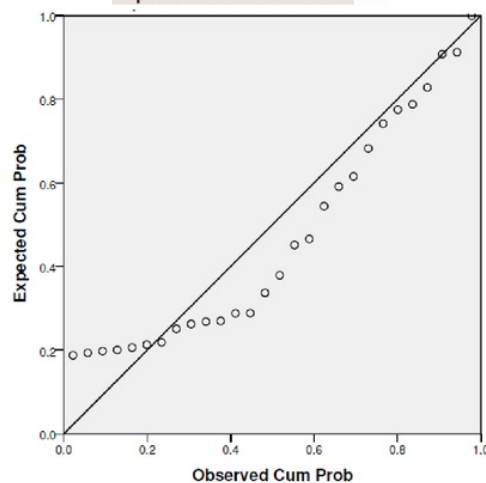


Figure 1. Bivariate normality distribution graphic.

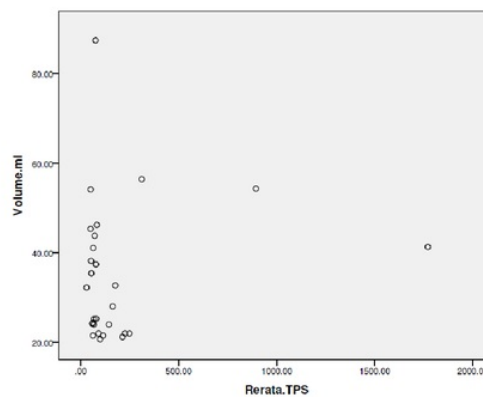


Figure 2. Graphic correlation between mean tps serum value and prostate volume.

normal distribution (Figure 1). Pearson's correlation test showed no significant positive correlation between TPS serum level and prostate volume ($p = 0.404$) with r value = 0.164 (Figure 2).

A study by Zhang¹⁵ about cytokeratin 18 showed histology and morphology in prostate physiologic condition for investigating cytokeratin 18 function by in vivo in the prostate obtained a result that no cytokeratin 18 in experimental animals did not affect normal prostate morphology and histology.¹⁵

The result in this study was contrast with hypothesis. This was due to that cytokeratin 18 had a limited effect on prostate epithelial cells because an excessive increase in other cytokeratins. Cytokeratin

18 that were eliminated in the experimental study by Zhang *et al.*¹⁵ showed a low impact on morphogenesis and growth of prostate epithelial cells for their upregulation of other cytokeratin such as cytokeratin 19 and cytokeratin 8.

The same was stated by Hudson *et al.*⁴ comparing the expression of marker proteins or tissue-specific markers in BPH tissue by *in vivo* and *in vitro* culture media. The *in vivo* results showed no co-expression of cytokeratin 8 and cytokeratin 18 in luminal cells of the prostate, while cytokeratin 19 was also obtained along with cytokeratin 18 so that it could cause cytokeratin 18 detected by TPS did not necessarily increase. The process of *in vitro* cell culture showed prostate luminal cell markers such as cytokeratin 8 and cytokeratin 18 were not always expressed, while cytokeratin 8 was expressed more in cultured prostate epithelial cells than 18 cytokeratin.

CONCLUSION AND SUGGESTION

The TPS serum level did not correlate with prostate volume in BPH and therefore can not be used as a marker or as a prediction of prostate enlargement due to BPH as a physiological process. A further research is needed to assess levels of TPS and its correlation to prostate volume in prostate diseases such as prostate cancer so that the role of TPS measurement in supporting the diagnosis and prognosis of prostate disease can be understood better.

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