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RESEARCH ARTICLE

# Correlation among Transforming Growth Factor β, Tumour Necrosis Factor- α, Prostate Specific Antigen, and Testosterone with Prostate Stromal to Epithelial Ratio in Benign Prostate Hyperplasia

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## Abstract

Introduction: Benign Prostatic Hyperplasia (BPH) occurs due to an imbalance between cell proliferation and the apoptotic process. Many factors influence the growth of the prostate. This study aims to determine which factors among TGF-β, TNF-α,, PSA, testosterone, correlate the most the stromal-to-epithelial ratio of the prostate. Methods: The study was conducted in 83 BPH subjects in several hospitals in Denpasar. From all subjects, blood was taken for PSA and testosterone examination. TGF-β and TNF-α were examined from the histopathological examination of prostate tissue specimens. Data were collected, then statistical analysis was done with SPSS 22.0. Results: From all parameters, only PSA was significantly negatively related to stromal-to-epithelial ratio, although the strength was negatively weak (r -0.28). Multivariate analysis showed that the combination of PSA and TGF- levels had an effect of 10.8% on the epithelial-stromal ratio. Conclusion: PSA showed a significant association with the ratio of prostate epithelial stroma and had an effect value of 10.8% when combined with TGF-β

**Keywords:** *Prostate enlargement, Epithelial-stromal ratio, TNF-α, TGF-β, PSA, Testosterone.*

## Introduction

Benign prostate hyperplasia (BPH) is one of the most common diseases found in men [1]. The aetiology of BPH is still uncertain but is said to be associated with a high inflammatory reaction compared to apoptosis [2, 4].

Various growth factors and cytokines are known to play a role in the inflammatory process, affecting the histological composition of the prostate, where occurred the hyperplastic processes of the fibromuscular stromal and glandular epithelial elements of the prostate [2, 4, 8]. Prostate-Specific Antigen (PSA) was found to increase stromal proliferation and prostate volume [9, 10].

On the other hand, testosterone in the form of dihydrotestosterone (DHT) will bind to androgen receptors and trigger prostate cell proliferation. In molecular level, cytokines are found to play a role [2, 11, 13]. Tumor necrosis factor-α (TNF-α), a proinflammatory cytokine produced by macrophages and T cells, stimulates stromal and epithelial cells to produce IL-6 so that epithelial cells proliferate [2, 5, 6, 14]. Also, TNF-α induces transforming growth factor-β (TGF-β) and causes an epithelial-mesenchymal transition (EMT) process so that stromal cells will increase [2, 7, 8, 15, 17]. TGF- β is one of the main growth factors produced by granulation

tissue cells which causes fibroblast migration and proliferation, increases collagen and fibronectin synthesis, thus decreases the degradation of the extracellular matrix so that stromal tissue will increase.

Until now, there was no existing study that compared which among these four parameters that influenced the most in changing of stromal epithelial ratio in BPH patients [2, 14, 17, 20]. Therefore, the aim of this study was to determine the relationship between TGF-beta, TNF-alpha, PSA, and testosterone with the stromal ratio of prostate epithelial in benign prostate hyperplasia and determine which factor was the most influential.

## Methods

This is prospective study conducted in September to October 2018 with a nested consecutive sampling technique, identifying cases in 83 BPH patients aged 50 to 80 years with a prostate weight of 20 to 80 grams through ultrasound examination that underwent TURP. The study protocol was approved by the institutional review board.

All subjects provided a written informed consent to be included in the study. PSA and testosterone levels were examined in patients, then a histopathological examination of prostate tissue specimens was carried out to determine the stromal-to-epithelial ratio, and immunohistochemical examination for TGF-β and TNF-α. From the data obtained, statistical analysis was performed with multiple linear regressions between TNF-α, TGF-ß, PSA, and testosterone with the stromal-to-epithelial ratio to determine which factor was the most significant. Data analysis was carried out using statistical package for social sciences (SPSS) version 21.0. Spearman's correlation test is used to see if there is any correlation between two variables. A p value of <0.05 was considered significant.

## Results

This study involved 83 subjects with the age range between 50 and 84 years old. The mean BMI of the patient is 22.99 kg/m2, with a standard deviation of 3.08. The PSA level of the study subjects ranged from 0.22 to 43.20 ng/dl, with a median value of 5.11 with a p-value of 0.177. The mean of testosterone is 412.4 ng/dl with a standard deviation of 177.05.

TNF-α from the study subjects ranged from 10.76 to 121.70 pg/dl with a median value of 45.99 with a p-value of 0.308. The TGF-β value ranges from 128.99-387.57, the median value is 200.59 with p-value of 0.156. While the stromal-to-epithelial ratio ranged from 0.33 to 4, median 1 with a p-value of 0.185 (Table 1).

**Table 1: Characteristics of the subjects (N=83)**

| Variables | Results |
|---|---|
| Age (years), mean ±SD | 64.42 ± 8.19 |
| BMI (kg/m$^2$), mean ±SD | 22.99 ± 3.08 |
| PSA (ng/dl), mean ±SD | 5.11 (0.22-43.20) |
| Testosterone (ng/dl), mean ±SD | 412.4 ± 177.05 |
| TNF-α (pg/dl), median (min-max) | 45.99 (10.76-121.70) |
| TGF-β (pg/dl), median (min-max) | 200.59 (128.99-387.57) |
| Stromal-to-epithelial ratio, median (min-max) | 1 (0.33-4) |

SD: standard deviation; BMI: body mass index; PSA: prostate-specific antigen; TNF: tumour necrosis factor; TGF: transforming growth factor

Because the distribution of data is not normal, the relationship between variables (bivariate analysis) was tested by the Spearman Rank test. In Table 2, the correlation among PSA, testosterone, TNF-α, TGF-β, and the stromal-to-epithelial ratio in the form of a matrix is shown. Only PSA is shown to have a significant relationship, although the correlation is negative weak with the stromal-to-epithelial ratio (p=0.010). Testosterone has a positive relationship with the stromal-to-epithelial ratio, but it was still not significant statistically (p=0.45). TNF-α and TGF-β have a negative relationship with the stromal-to-epithelial ratio but not statistically significant with p values of 0.345 and 0.133, respectively (Table 2 and Figure 1). In this study, only two variables, PSA and TGF-□, met the requirements of multivariate analysis with p smaller than 0.25. The combination of PSA and TGF-β levels affects 10.8% of the prostatic stromal-to-epithelial

 

ratio. Both have a negative relationship with the stromal-to-epithelial ratio (Table 3).

**Table 2: Correlation among PSA, Testosterone, TNF-alpha, TGF-beta with prostate stromal-to-epithelial ratio**

| Variables | Prostate stromal-to-epithelial ratio | |
|---|---|---|
| | R | p |
| PSA | -0.28 | 0.010 |
| Testosterone | 0.084 | 0.450 |
| TNF-α | -0.105 | 0.345 |
| TGF-β | -0.166 | 0.133 |

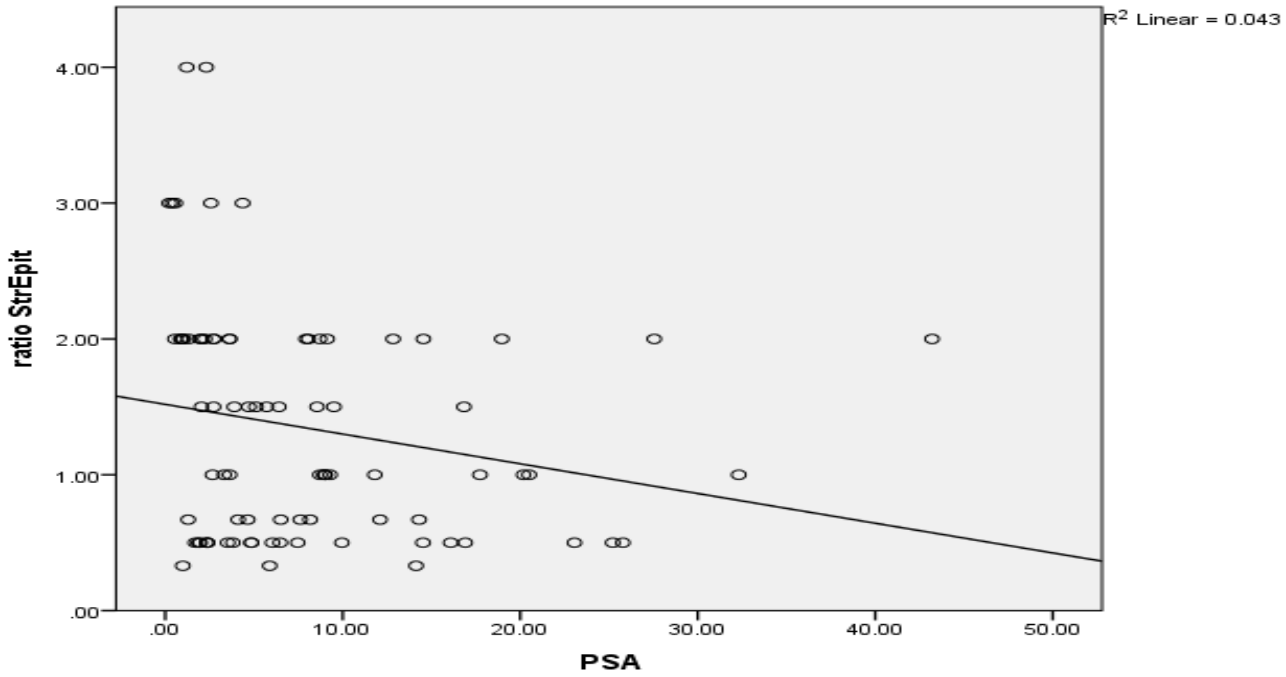


**Figure 1: Scatter plots between PSA Levels and prostate stromal-to-epithelial ratio**

**Table 3: Multiple linear regression of PSA and TGF-□ levels with stromal-to-epithelial ratio**

| Variables | β | 95%CI | p-value | $R^2$ |
|---|---|---|---|---|
| PSA | -0.021 | -0.043-0.001 | 0.065 | 10.8% |
| TGF-ß | -0.003 | -0.006-(-0.001) | 0.018 | |

## Discussion

Mostly, PSA produced by peripheral zone the of the prostate. The transitional zone produces a little amount of PSA. Thus, prostate cancer shows significant higher increased of PSA than BPH. The activity of the PSA is regulated by several factors. Procedure to the prostate, such as prostate surgery and biopsy will increase PSA value. Medicament to reduce prostate size, 5-α reductase inhibitor, will decrease PSA value. PSA is involved in the proliferation of prostate cells. Before entering circulation, stromal prostate was exposed by PSA. It explains that the level of circulating PSA does not directly describe the amount of PSA exposed to the prostate cells in BPH [7, 10, 21, 23].

In normal men, 85-95% PSA in circulation binds to α1-antichymotrypsin or α 2-macroglobulin. When stromal cells are

incubated with PSA, IGF-I, and IGFBP3, an increase in the number of stromal cells is affected by the concentration of PSA in both substances [8, 10, 23, 24]. The results of this study indicate that of all the parameters studied, only PSA levels had a significant negative relationship with the stromal epithelial ratio (r -0.28; p 0.01). This can be caused by PSA can increase systemic bioavailability of IGF-I against IGF receptors of prostate epithelial cells by decreasing the affinity of IGFBP3 so that there is an increase in proliferation of epithelial cells,

 

resulting in lower stromal-to-epithelial ratio [10, 12, 23].

In this study, it was found that TGF-β had a negative relationship with the stromal-to-epithelial ratio, although it was not significant. Cells produce TGF-β as proprotein in latent phase in the extracellular matrix. TGF-β binds to glycoprotein latent TGF-β binding protein.

Activating TGF-β requires retinoic acid and FGF-2 and many other molecules. TGF-β induces anti-proliferative agent in the whole cell cycle, but useful in G1 phase [19, 24, 28].The cycle will be terminated at the terminal of mitotic in G1. Low level of TGF-β induces proliferation of stromal cells; opposite high level of TFG-β induce apoptotic of stromal cells.

TGF-β has a plural function, TGF-□ was known to stimulate mesenchymal cells including fibroblast, but strong inhibitor for epithelial cells, endothelial, hematopoietic and immunologic cells [13, 17, 19, 25, 26].

However, TGF-β also induces epithelial proliferation through EGF and FGF, which acts on fibroblast cells and inhibits glandular prostate cells so that the stromal-to-epithelial ratio is lower even though not statistically significant. Development of prostate controlled by the interaction of stromal epithelial, stromal cells in BPH induce epithelial cells proliferation [17, 25, 26, 29, 30].For TNF-α, it stimulates stromal proliferation with αFGF, increasing stromal cells. Kruslin states that prostate tumour epithelial cells are more sensitive than cells in the normal prostate to TNF-α, where TNF-α induces growth regulation and apoptosis. So that epithelial cells also experience proliferation and apoptosis by the influence of TNF-α. In this study, it was found that testosterone had a positive relationship with the stromal epithelial ratio but was not statistically significant [2, 16, 31, 32].Testosterone which binds to AR is in the form of DHT, while testosterone levels examined in the blood are mostly in association with Sex Hormone Binding Globulin (SHBG) in its inactive form. Enzyme 5alpha-reductase is needed to reduce testosterone to DHT. Sexual hormones regulate epithelial proliferation through a paracrine mechanism that is influenced by various growth factors via epithelial-stromal interactions [33, 36].

From the multivariate analysis, the combination of PSA and TGF β levels affected 10.8% of the epithelial-stromal ratio. Both the glandular epithelial cells and the stromal cells (including muscular fibres) undergoing hyperplasia in BPH, however, the negative relations with PSA and TGF β showed that the epithelial component was more increased in the improvement of these two mediators.

## Conclusion

PSA and TGF-β are protective factors for an increase in the stromal epithelial ratio of the prostate in benign prostate hyperplasia patient. Future research is needed to determine other factors that have a stronger influence on the stromal epithelial ratio of the prostate.

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