The differenceof CD4+ CD25+ FOXP3+ T Cell Regulators in Benign Prostate Hyperplasia Patients, Prostate Cancer, and Control of Healthy Subjects in dr. Saiful Anwar General Hospital

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Benign prostatic hyperplasia (BPH) and prostate cancer are the most common prostate diseases, where BPH occurs in at least 70% of men aged 70 years, while prostate cancer is one of the most common malignancies that occur in men throughout the world, including Asia. The possible role of the immune system in the pathogenesis of BPH and prostate cancer in recent years has begun to be widely studied. Although many studies have focused on T lymphocytes on the development of BPH and prostate cancer, the role of regulatory T cells in the pathogenesis of BPH and prostate cancer is still not well known. The introduction of a regulatory T cell subset in BPH and prostate cancer has just begun. In Indonesia, until now there has been no research on the amount of regulatory T cell levels in BPH and prostate cancer. This study aims to determine the amount of regulatory T cells in prostate cancer and BPH so that it can contribute to the concept of understanding the pathogenesis of prostate cancer and BPH.This study uses an analytic observational design with a cross-sectional approach. In this study, the total sample was 36, with a breakdown of 12 control subjects, 13 subjects prostate cancer group, and 11 subjects BPH group. Furthermore, peripheral blood samples are taken and then the amount of regulatory T cells is calculated. After obtaining data on the amount of CD4+ CD25+ Foxp3+ regulatory T cells in the blood, data analysis was performed between groups of patients diagnosed with prostate cancer, benign prostatic hyperplasia and healthy subjects as controls.The mean amount of regulatory T cells obtained in the control group was 33.01 ± 12.51, the prostate cancer group was 57.19 ± 17.04 and the BPH group 80.94 ± 17.06. One Way ANOVA test results showed that the average amount of regulatory T cells between treatment groups had a significant difference in reg T cells with a p-value (0,000) <0.05.It can be concluded that there is a statistically significant difference in the average number of regulator T cells in the BPH group and the prostate cancer group compared to the control group. In addition there are differences in the average number of regulator T cells in the BPH group compared with prostate cancer with statistically significant differences. Further research is needed regarding the number of regulator T cells CD4 + CD25 + FOXP3 + in prostate cancer patients (grouped according to Gleason score), benign prostatic hyperplasia and control of healthy subjects before and after therapy with bigger and more homogeneous samples.
***Key Word**:Prostate cancer; benign prostate hyperplasia; T cell regulator; CD4.*

I. Introduction

The prostate is a male genital organ located inferiorly from the urinary bladder, in front of the rectum and enveloping the posterior urethra.[1] Benign prostatic hyperplasia (BPH) is an enlarged prostate gland caused by cellular hyperplasia.[2] Meanwhile, prostate cancer is cancer that develops in the prostate gland, caused by mutations of prostate cells resulting in cell proliferation that is out of control.[3]

Benign prostatic hyperplasia and prostate cancer are the most common prostate diseases, where BPH occurs in at least 70% of men aged 70 years,[2] while prostate cancer is one of the most common malignancies that occur in men throughout the world, including Asia.[4,5] One risk factor for the pathogenesis of BPH, which also seems to play a role in the development of prostate cancer, is inflammation. Inflammation as a risk factor is evidenced by several studies where BPH progression to prostate cancer is higher in tissues with inflammatory infiltrates than without inflammation.[6]

The possible role of the immune system in the pathogenesis of BPH and prostate cancer in recent years has begun to be widely studied.[7,8] Chronic inflammation is caused by an infectious agent, causing epithelial turnover which increases the risk of malignancy by around 15%. Acute and chronic inflammation in the urogenital system shows an accumulation of immunocompetent cells in prostate tissue, especially T

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lymphocytes and macrophages. These cells secrete various cytokines, such as IL-2, IFN gamma, IL-6, IL-8, IL-15, which play a role in pathological changes and also lymphocyte activation which is characteristic of BPH and prostate cancer.[7]

Although many studies have focused on T lymphocytes on the development of BPH and prostate cancer, the role of regulatory T cells in the pathogenesis of BPH and prostate cancer is still not well known.[7] Regulatory T cells are a population of CD4 T cells with special phenotypic characteristics namely CD4⁺ CD25⁺ FOXP3⁺ which play a role in maintaining cell tolerance and maintaining tissue homeostasis.[9,10]

Cancer cells express antigens that can trigger cytotoxic T cells, Natural Killer (NK) cells and macrophages to destroy these cells.[11,12] But if there is a failure of the immune system, it will result in tumor growth.[13] Regulatory T cells can suppress both immune cells that play a role in the humoral and cellular immune systems, by influencing the activity of surrounding immune cells.[8,14] This suppression of antitumor immunity makes these cells promoters of tumor growth.[8] Obtained increased Regulatory T cells in tumor tissue or cancer patient circulation, is evidence that these cells are involved in the pathogenesis and progression of cancer.[15] Study of CD4⁺ CD25⁺ FoxP3⁺ T cells in prostate cancer, and BPH was investigated by Mrakovcic in 2014. In his research, CD4⁺ CD25⁺ FoxP3⁺ levels were higher in prostate cancer than in BPH.[16]

The introduction of a regulatory T cell subset in BPH and prostate cancer has just begun. In Indonesia, until now there has been no research on the amount of regulatory T cell levels in BPH and prostate cancer. This study aims to determine the amount of regulatory T cells in prostate cancer and BPH so that it can contribute to the concept of understanding the pathogenesis of prostate cancer and BPH. This study will analyze the differences of CD4⁺ CD25⁺ Foxp3⁺ T cell regulators in prostate cancer patients, BPH and healthy subjects in RSUD dr. Saiful Anwar Malang.

II. Material And Methods

The research design was analytic observational with cross-sectional approach. The subjects in this study were divided into 3 groups, namely the BPH group (11 patients), the prostate cancer group (13 patients), and the control group (12 patients). The target population in this study were patients diagnosed with prostate cancer, benign prostatic hyperplasia and healthy subjects at Dr. General Hospital Saiful Anwar in the research period from October 2018 to February 2019.

Inclusion criteria:
1. Men aged 30 to 75 years.
2. Subjects were diagnosed with benign prostatic hyperplasia by PSA, ultrasound TRUS or histopathology (BPH group).
3. Subjects were diagnosed with prostate cancer by PSA, TRUS ultrasound or histopathology, at all stages (prostate cancer group).
4. Subjects who have been diagnosed with prostate cancer who have been treated, but drop out within a period of 6 months, before sampling (prostate cancer group).
5. Men with vital signs within normal limits and leukocyte counts from 4,700-11,300 mg/dl, LEDs within normal limits (control group).
6. Understand the research objectives and research procedures, and be willing to participate in research voluntarily by signing an informed consent agreement sheet.

Exclusion criteria:
1. Subjects suffering from diseases that are likely to affect regulatory T cell levels, such as autoimmune diseases, multiple sclerosis, type 1 diabetes, rheumatoid arthritis.
2. Subjects who have been diagnosed with prostate cancer who have received hormonal, immunosuppressive or radiation therapy for less than 6 months, before sampling.
3. Subjects who received immunosuppressive therapy.

Procedure methodology

All patients who had been the study sample were made complete prostate cancer status, BPH and healthy subjects control and data collection sheets. After history taking and physical examination, a full blood screening is performed to control healthy subjects, as well as documentation. Subsequent samples were examined by flow cytometry in the Biomedical Laboratory of the Faculty of Medicine, Universitas Brawijaya. The results of the data obtained were entered in a data collection sheet, analyzed and determined the amount of CD4⁺ CD25⁺ Foxp3⁺ T cells in prostate cancer, benign prostatic hyperplasia, and healthy subjects.

Statistical analysis

Data were analyzed using SPSS version 17 (SPSS Inc., Chicago, IL). Characteristics of the sample are presented descriptively, using tables and narration. Analysis of data normality with the KolmogorovSmirnov test. If it is normal, then analyze the data with the Annova Test. Value of P <0.05 was considered as the cutoff value or significance.

III. Result

In this study, the total sample was 36, with a breakdown of 12 control subjects, 13 subjects prostate cancer group, and 11 subjects BPH group. The mean age of prostate cancer patients was 63.08 ± 4.92, BPH was 63.55 ± 4.80, while the mean age in the control group was 31.92 ± 1.78. The mean age was tested differently, but before the difference test was carried out, the normality test was done first using the Kolmogorov Smirnov test and homogenity test with the Levene test. Kolmogorov Smirnov test results showed a value of p (0,000) <0.05, so the assumption of normality was not fulfilled. The Levene test results showed a p-value (0.009) <0.05, so the assumption of homogenity was not fulfilled. The comparison test used was the Kruskal Wallis test. Kruskal Wallis comparison test results showed a value of p (0.009) <0.05, it can be concluded that there are differences in age in each treatment group.

Before a different test is performed, the normality assumption test is conducted with the Kolmogorov Smirnov test, if the value of p>(0.05), then the normality assumption is fulfilled. Test the homogeneity assumption with the Levene test, if the value of p>(0.05), then the homogenity assumption is fulfilled.

**Table 1:** Normality and homogenity test for regulatory T cells (%)

Variable	Normality Test		Homogenity Test	
	Kolmogorov Smirnov	p-value	Levene	p-value
Regulator T cells	0.096	0.142	0.647	0.530

The normality test results in Table 1 show the amount of Regulator T cells fulfills the normality assumption of 0.096 (p> 0.05). The homogenity test results in Table 1 also meet the assumption of homogenity (p> 0.05). So that the average analysis of the difference in the amount of regulatory T cells is done using the One Way ANOVA test.
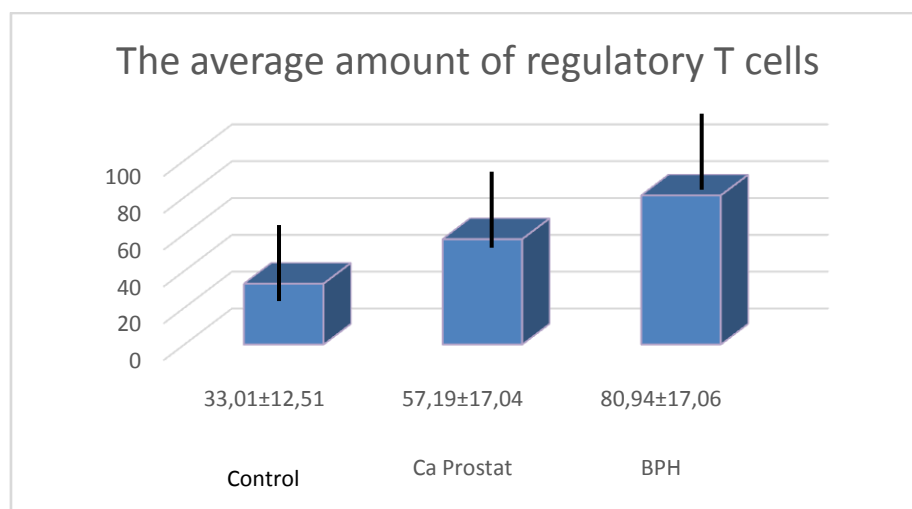
Table 2: The average amountof regulatory T cells

Treatment	Means	p-value
Control	33.01 ± 12.51^a	
Prostate cancer	57.19 ± 17.04^b	0.000
BPH	80.94 ± 17.06^c	

Table 2 shows the average amount of regulatory T cells in the control group 33.01 ± 12.51, the prostate cancer group 57.19 ± 17.04 and the BPH group 80.94 ± 17.06. One Way ANOVA test results showed that the average amount of regulatory T cells between treatment groups gave a significant difference in regulatory T cells with a p-value (0,000) <0.05. It can be concluded that there are differences in the average amount of regulatory T cells, so we continued the testing with Tukey test.
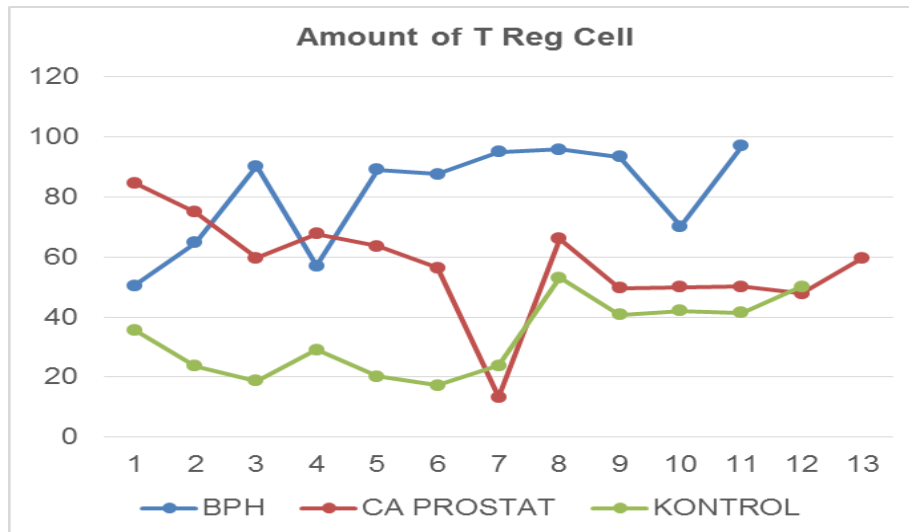
Graph 1 : Amount of T Regulator cell in BPH, Prostat, and Control in this research

Graph 2: The average amountof regulatory T cells

Tukey test results are displayed in the average column, showing different notation amounts in the three groups namely a, b, and c. Based on the notation, it appears that the letters are not the same, so it is concluded that between the treatment groups, and the control groupm there are significant differences in regulatory T cell amount. The mean amount of regulatory T cells in the control group was the lowest and significantly different from the prostate cancer and BPH group. While the mean amount of regulatory T cells in the BPH group was the highest and was significantly different from the prostate cancer and control groups.

IV. Discussion

The role of the immune system in the pathogenesis of BPH and prostate cancer has recently been widely studied.[7,8] Chronic inflammation is caused by an infectious agent, causing an increase in epithelial turnover which increases the risk of malignancy. Research on acute and chronic inflammation of the urogenital system shows, the accumulation of immunocompetent cells in prostate tissue, especially T lymphocytes and macrophages. These cells secrete a variety of cytokines, which play a role in pathological changes and also lymphocyte activation which is characteristic of BPH and prostate cancer.[7]

Regulatory T cells or Regulatory Tare known to play an important role in suppressing the immune response to tumors, preventing autoimmune and balancing immune homeostasis. In cancer, regulatory T cells density in tumors becomes a predictive factor for clinical development towards worsening, therefore it is said that Regulatory T cells play a key role in cancer development.[17] However, the role of regulatory T cells in the pathogenesis of BPH and prostate cancer is still unknown.[7]

To find out the differences in the amount of CD4$^+$ CD25$^+$ FOXP3$^+$ T cells in prostate cancer patients, benign prostatic hyperplasia with healthy subjects, we conducted a cross-sectional study, in which samples were

taken from the blood vein of each subject, then the amount of regulatory T cells was calculated from each sample. In this study, the total sample was 36, namely the control group with 12 subjects, 13 subjects in prostate cancer group, and 11 subjects in the BPH group.

In our study, the highest average value or amount was found in the BPH group at 80.94 ± 17.06, while the amount of prostate cancer regulator T cells was below 57.19 ± 17.04, where the three groups differed statistically significantly based on the One Way Anova test with a value of ($p < 0.05$). In the Levene test, the mean amount of regulatory T cells in the control group was the lowest and significantly different from the BPH and prostate cancer group. Based on Tukey's further test results, it was obtained between the treatment groups, and the control group each gave a significant difference in the amount of regulatory T cells. The mean amount of regulatory T cell in the BPH group was the highest and was significantly different from the prostate cancer and control groups.

The increase in Regulatory T cells obtained in the results of this study is in line with research conducted by Miller et al., in 2006 where there are a significant increase in $CD4^+$ $CD25^+$ T cells in tumor tissue and peripheral blood in prostate cancer patients.[18]Increasedamount in regulatory T cells in tumor tissue or cancer patient circulation, is an evidence that these cells are involved in the pathogenesis and progression of cancer.[15]

An increase in Regulatory T cells from BPH patient samples in the results of this study, following research conducted by Norstrom, where a substantial regulatory T cell in BPH also increased. Norstrom also found signs of chronic activation supported by T cell expression that induces activation of co-inhibitor receptors, co-stimulatory receptors, and high frequency of potential regulatory T cells in BPH.[19]

In this study, the amount of regulatory T cells in BPH is higher than the amount of regulatory T cells in prostate cancer with a significant difference. This can be understood, due to the prostate tissue of BPH patients with infiltration of immune cells such as T lymphocytes, NK cells, and HCV, higher than prostate cancer.[7] Where it is known that the main role of regulatory T cells is a suppression of the antitumor immune response.[20]

However, this study found weaknesses, namely where the amount of samples is small so that one variable that has a distorted value will greatly affect the overall average value. In the data obtained one outrange data in the prostate cancer group, which causes the group's average value to be much lower. Therefore, further research needs to be done with larger samples.

Based on meta-analysis and recent studies have proven a strong correlation between the history of chronic prostatitis inflammation with the development of prostate cancer. The causes of prostate inflammation vary, ranging from bacteria that trigger prostatitis, sexually transmitted infections, estrogen imbalance, physical trauma, urine reflux to the prostate gland, and factors such as diet.[21]

In tumors, an increase in Regulatory T cells is consistent with its role in suppressing the antitumor immune response as evidenced in clinical and preclinical studies. This is supported by in vitro studies, where an increase in antitumor immunity after regulator T cells has been removed.[20,22] In other studies, the elimination of $CD4^+$ $CD2^+$Regulatory Ts induces antitumor immunity activity in mice injected with syngeneic tumor cells.[20]

The mechanism of action of regulatory T cells is believed to mediate peripheral tolerance through suppression of self-antigen-reactive T cells. The increase in regulatory T cells in tumor cells aims to inhibit anti-tumor immunity, this is because most tumor antigens are self-antigens (against autoimmune is the primary function of regulatory T cells) and Regulatory T increases to suppress inflammation. It is well known that with the ability of Regulatory T cells to mediate suppression of reactive lymphocytes, this is thought to be a potential mechanism that explains immune failure against antitumors.[20]

Finally, the results of this study showed a significant increase in Regulatory T cells in both BPH and prostate cancer compared to healthy controls. This supports the idea that regulatory T cells in tumor cells play a role in inhibiting anti-tumor immunity. The ability of these cells to mediate suppression of reactive lymphocytes forms the basis of a potential mechanism theory that explains immune failure against antitumor.[20]

V. Conclusion

There was a statistically significant difference in the average number of regulator T cells in the BPH group and the prostate cancer group compared to the group. In addition there were differences in the average number of regulator T cells in the BPH group compared to prostate cancer with a statistically significant difference.

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