IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 20, Issue 2 Ser.8 (February. 2021), PP 16-23
www.iosrjournals.org

The differences between IL-10 and TGF-β1 levels in Prostate Cancer and BPH at Dr. Saiful Anwar General Hospital Malang

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Abstract: *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 malignancy that occurs in men around the world. Interleukin-10 (IL-10) is an anti-inflammatory cytokine that can suppress pro-inflammatory cytokines. IL-10 is secreted by Th2 cells, and its production is involved in the regulation of the immune and inflammatory responses. TGF-β1 is a multi-function peptide with anti-tumorigenesis affect. TGF-β1 is known to act as an inhibitor in normal growth and early stage diseases and promoters in advanced prostate cancer. In Indonesia, until now there has been no study on the difference between IL-10 levels and TGF-β1 levels in patients with prostate cancer and BPH. This study used an analytic observational design with a cross sectional approach. The control group is the group that does not experience BPH and prostate cancer. Therefore, further study is conducted with the aim of knowing the levels of IL-10 and TGF-β1 in prostate cancer and BPH at Dr. Saiful Anwar General Hospital Malang. Furthermore, peripheral blood samples were taken and the levels of IL-10 and TGF-β1 were measured using the enzyme linked immunosorbent assay (ELISA) method.*

Keywords:

Date of Submission: 05-02-2021 Date of Acceptance: 19-02-2021

I. Introduction

Prostate is the male genital organ with the most common tumors, both benign and malignant tumors. Prostate is located inferior to the bladder, in front of the rectum and enclosing the posterior urethra (Purnomo, 2011). Benign prostatic hyperplasia (BPH) is an enlargement of the prostate gland caused by cellular hyperplasia (Xiaou, 2016). Prostate cancer is a cancer that develops in the prostate gland, caused by mutations of prostate cells so that there is a proliferation of cells that are out of control (Khodijah, 2009). BPH and prostate cancer are the most common prostate diseases, where BPH occurs in at least 70% of men aged 70 years (Parsons, 2010), while prostate cancer is one of the most common malignancies that occur in men around the world, including Asia (Umbas, 2011; Hsing, 2000). However, unlike most cancers, the incidence of prostate cancer increases with age. It has been found 40% in men aged 50 years (Smith, 2013). In Indonesia, the profile of prostate cancer has been studied at Prof. DR. Dr. dr. R.D. Kandou in the 2013 - 2015 period with results from 54 prostate cancer patients in Indonesia, there were 37.0% in the age group of 61 - 70 Years (Valdo, 2016). One of the risk factors in BPH pathogenesis, which also seems to play a role in the development of prostate cancer is inflammation. Inflammation as a risk factor is proven by several studies where BPH progression is obtained into prostate cancer higher in tissues with inflammatory infiltration than without inflammation (Guyatt, 2002). Inflammatory processes are mediated by the immune system both cellular and humoral. The role of the immune system of BPH pathogenesis and prostate cancer in recent years began to be widely studied (Mrakovcic, 2014; Takeuchi, 2016). Chronic inflammation is caused by infectious agents, causing a turn over of the epithelial which increases the risk of malignancy by about 15%.

Interleukin-10 (IL-10) is an anti-inflammatory cytokine that can suppress pro-inflammatory cytokines. IL-10 is secreted by Th2 cells, and its production is involved in the regulation of the immune and inflammatory response (Tingting, 2017). The mechanism of IL-10 in prostate cancer is known to be anti-cancerous by suppressing the growth of cancer cells. The role of IL-10 against pathogenesis of prostate cancer or BPH is clearly still not widely studied. Study by Yoo, et al in 2011 which stated that there is a relationship between IL-10 levels and BPH events (Yoo, 2011). A meta-analysis by Zhao (2015) stated that the increase in IL-10 expression is negatively correlated with the life expectancy of cancer patients so that it has the potential to be a cancer biomarker (Zhao, 2015). However, other studies by Shoa (2012) prove that there is no significant link between IL-10 levels and prostate cancer risk (Shoa, 2012). Based on the above evidence, study on the regulatory role of IL-10 in prostate carcinogenesis is an interesting subject to study (Tingting, 2017).

Besides IL-10, there are anti-inflammatory cytokines that are thought to play a role in the pathogenesis of prostate cancer, namely transforming growth factor-β1 (TGF-β1). TGF-β1 is a multi-function peptide with anti-tumorigenesis affect. TGF-β1 is known to play an inhibitor role in normal growth and early stage diseases and promoters in advanced prostate cancer.(Zheng, 2015). Study of Ma (2005) also showed that there was a decrease in TGF-β1 levels in BPH patients compared to control (Ma, 2005). Study by Nugroho (2013) stated that the levels of TGF-β1 patients with BPH are lower than non-BPH (Nugroho, 2013). However, some of these studies were conducted in other countries. In Indonesia, until now there has been no study on the difference between IL-10 levels and TGF-β1 levels in patients with BPH and prostate cancer. Therefore, further study was conducted with the aim to find out the levels of IL-10 and TGF-β1 in prostate cancer and BPH at Dr. Saiful Anwar General Hospital.

II. Methods

This study uses analytical observational design with cross sectional approach with the following design: study subjects with prostate cancer, BPH and healthy control. Then, the study subjects will take blood samples and then calculate the IL-10 levels and TGF-β1 levels. After obtaining data on IL-10 levels and TGF-β1 levels in the blood, then the data analysis was carried out on patients diagnosed with prostate cancer, BPH and healthy subjects as controls. This study was conducted at the Dr. Saiful Anwar General Hospital Malang, between August 2019 - August 2020. The target population in this study were patients diagnosed with prostate cancer, BPH and healthy subjects at the Dr. Saiful Anwar General Hospital Malang.

2.1 Inclusion Criteria

2.1.1 Prostate Cancer Criteria

- Male subjects aged 30-75 years
- Subjects diagnosed with prostate cancer either by examination of PSA, USG TRUS or histopathology, at all stages.
- Subjects who have been diagnosed with prostate cancer who have been treated, but drop out within 6 months, prior to sampling
- Understanding the study objectives and study procedures, and are willing to voluntarily participate in study by signing the informed consent form.

2.1.2 Benign Prostatic Hyperplasia Criteria

- Male subjects aged 30-75 years
- Subjects diagnosed with BPH by PSA examination, USG TRUS or histopathology.
- Understand the study objectives and study procedures, and are willing to voluntarily participate in study by signing the informed consent form.

2.1.3 Healthy Control Criteria

- Male subjects aged 30-40 years
- Men with vital signs within normal limits and a leukocyte count of 4,700-11,300mg / dl, ESR within normal limits.
- Understand the study objectives and study procedures, as well as be willing to take part in the study voluntarily by signing the informed consent form.

2.2 Exclusion Criteria

- Subjects suffering from diseases that may affect levels of IL-10 and TGF-β1 levels, such as: autoimmune diseases, multiple sclerosis, type 1 diabetes, rheumatoid arthritis.
- Subjects who have been diagnosed with prostate cancer who have received either hormonal, immunosuppressive or radiation therapy prior to sampling
- Subjects receiving immunosuppressive therapy

2.3 Sample Size

Representation of the population by the sample in study is an important condition for a generalization or inference. Basically, the more homogeneous the value of the variable under study, the smaller the sample is needed, conversely the more heterogeneous the value of the variable under study, the larger the sample needed.
In addition to population representation (representativeness), another thing that needs to be considered in determining sample size is the need for analysis. Some analyzes or statistical tests require a certain minimum sample size to be used.

In different conditions, the method of determining the sample size is also different. Based on the type, observational or experimental study is distinguished. Based on the study objectives or analysis, distinguished descriptive or inferential (estimation or hypothesis testing). Based on the number of population or sample, one population / sample or more than one population / sample is distinguished. This is related to the characteristics of the population or the method of sampling (sampling) which is distinguished by random or non-random sampling. Random sampling is distinguished by simple random, systematic random, stratified random, cluster random or multistage random sampling. Based on the type of data or variables analyzed, proportional or continuous data are distinguished. The things above really determine how to calculate the sample size.
Based on the calculation, the minimum sample size is 10 people. However, it would be better, if the number of samples could be increased, so that the results of the study could be more representative (representative) of the observed population

2.4 Procedures

2.4.1 Sampling

The study begins with socializing the subject. The socialization will explain the objectives, benefits, procedures, advantages and disadvantages of the study to be carried out. Subjects who agree to take part in the study will have their blood sampled by the laboratory of the Dr. Saiful Anwar General Hospital Malang. The sample was obtained using a purposive sampling method that met the inclusion and exclusion criteria. More complete information about the study will be provided to subjects who are willing to take part in the study and are asked to sign an informed consent after the explanation. After signing, complete identification will be carried out, then a laboratory examination will be carried out with material in the form of blood serum, the blood serum material will be isolated with Ficoll-Hypaque (Pharmacia Biotech, Uppsala, Seden) and stored in the FKUB biomedical laboratory freezer at a temperature of -80 ° C until time will be used for analysis. The stored sample will be measured by Enzyme-linked Immunosorbent Assay (ELISA) and will be analyzed by flow cytometry.

2.4.2 Blood Serum Sampling

Blood serum was taken to check IL-10 levels and TGF-β1 levels using a syringe in the medial cubital vein. The collection was carried out by personnel from the Central Laboratory of Dr. Saiful Anwar General Hospital Malang. The volume of the sample was taken 10 ml and then stored in the serum storage tube.

2.4.3 Examination Procedure Il-10 And TGF-B1 Levels In The Blood

After 30 minutes of sampling blood from peripheral blood in medialis cubiti veins, the sample is centrifuged for 10 minutes at a speed of 2600 rpm. The obtained serum is stored in a tight plastic tube at a temperature of -800C. Measurement of IL-10 levels and TGF-β1 levels in blood was done by Enzyme-linked Immunosorbent Assay.

2.4.4 Data Analysis

Data analysis that has been obtained by the following steps :

- Sample characteristics are presented descriptively, using tables and narration.
- Analysis of data normality with Kolmogorov-smirnof test and homogeneity of data using Homogeneity of Variance test.
- In normality and homogeneous distribution data results will be tested with One-Way ANOVA and Independet T-Test with signification level $p< 0.05$.
- If the data is abnormal and or not homogeneous, it will be analyzed using Kruskal Wallis and Mann Whitney test with significant level $p< 0.05$.
- Analyzes were performed using the Statistical Product and Service Solutions (SPSS) program

III. Results

3.1 Characteristics of Study Subjects

The study that have been conducted in the form of cross sectional study aimed at finding out the difference in IL-10 and TGF-β1 levels in prostate cancer patients, BPH and healthy control. Blood sampling was taken by the venous blood of each subject, then il-10 and TGF-β1 levels were calculated from each sample in the Biomedical laboratory of the Faculty of Medicine, Brawijaya University using *Enzyme Linked Immunosorbent Assay* (ELISA).

Table 1. The Age of Sample

Note	Control	BPH	Prostate Cancer
Mean (±SD)	32,92±1,78	64,55±4,80	64,08±4,92,
Median	32,92	64,55	64,08
Minimum	30	59	59
Maximum	36	72	71

*Results of Descriptive Analysis

In this study, the total sample was 35 men consisting of control group of 11 subjects, prostate cancer group of 13 subjects, and BPH group of 11 subjects. The average age of prostate cancer patients was 64.08±4.92, BPH was 64.55±4.80, while the average age in the healthy control group was 32.92±1.78 (table 5.1). To find out the difference in average age of the three groups, the test was conducted differently from normality and homogeneity test first.Based on kolmogorov Smirnov test and homogeneity test with Levene test.Kolmogorov Smirnov test results showed a value of p (0.000)<0.05, so the assumption of normality was not met.Therefore, followed by a different test Kruskal Wallis.Kruskal Wallis test results showed a value of p (0.009)<0.05, it can be known that there is an age difference in all three groups

3.2 Data Analysis of IL-10 Levels

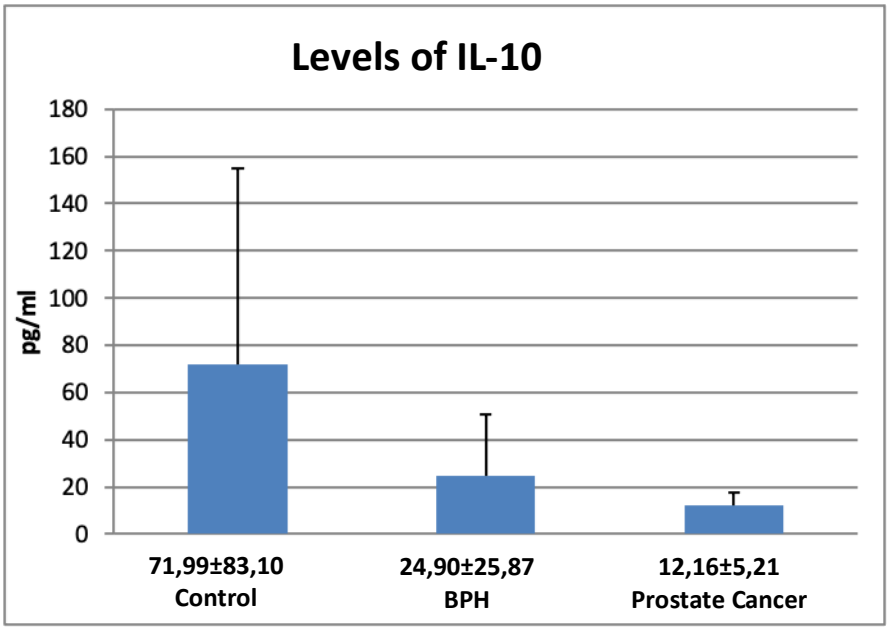

Fig. 1 Results of Mean IL-10 Levels

The mean of IL-10 levels obtained in the control group was 71.99 ± 83.10 pg / mL in the BPH group was 24.90 ± 25.87 pg / mL and the Cancer group was 12.16 ± 5.21 pg / mL.

3.2.1 Normality and Homogeneity of IL-10 Levels

Table 2. Normality and Homogeneity Test of IL-10 Levels

Variable	Normality Test	Homogeneity Test
IL-10	0,000	0,015

The normality and homogeneity test results in table 5.2.showed iL-10 levels indicating an abnormal distribution of p=0.000 (p<0.05) and not homogeneous p=0.015 (p<0.05).Therefore, data analysis using non-parametric statistics namely Kruskal Wallis test

3.2.2 Uji Kruskal Wallis dan Mann Whitney

Table 3. Test For Different Levels Of IL-10

Group	Mean of IL-10 Levels (pg/mL)	Value p
Control	71,99±83,10[a]	
BPH	24,90±25,87[b]	0,020
Prostate Ca	12,16±5,21[b]	

In table 3, the Kruskal Wallis test results show that the mean levels of IL-10 between the prostate cancer and BPH groups were significantly different from the control (p = 0.001; p <0.05). Based on these results, it was found that there were differences in the mean levels of IL-10 in the three groups, so it was continued with the Mann Whitney test.

Mann Whitney's test results were shown in an average column, showing different notation numbers between the control group and the BPH and Prostate cancer groups.Based on the nortasi shows that the control group differs significantly with the BPH column. In addition, the control group also differed significantly with the prostate cancer group.

3.3 Data Analysis of TGF-β1 Levels

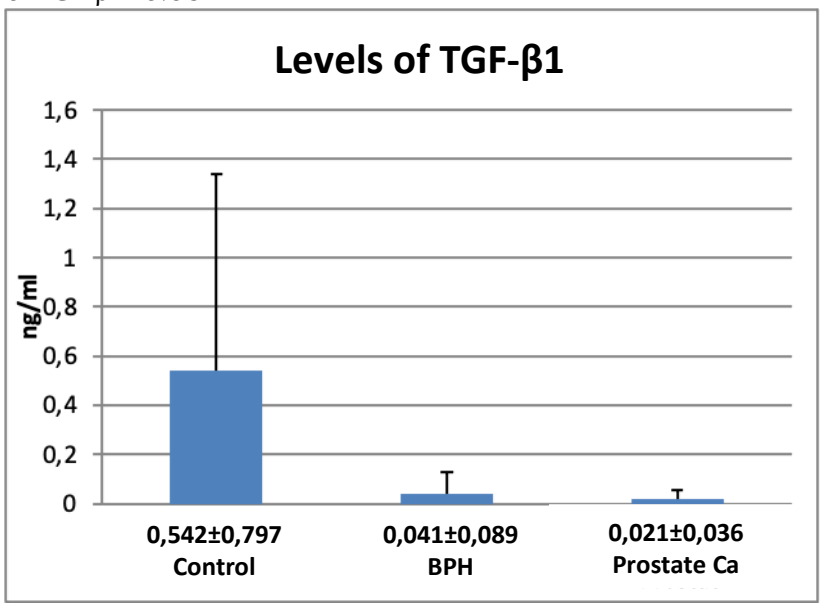

Fig 2. Results of Average TGF-β1 levels

The mean of TGF-β1 level obtained in the control group was 0.542 ± 0.797 ng / mL in the BPH group was 0.041 ± 0.089 ng / mL and the prostate control group was 0.021 ± 0.036 ng / mL.

3.3.1 Normality and Homogeneity Test of TGF-β1 Levels

Table 4. Normality and Homogeneity Test of TGF-β1 Levels

Variable	Normality Test	Homogeneity Test
TGF-B1	0,000	0,014

The results of the normality and homogeneity test are in table 5.4. showed that the TGF-β1 level showed an abnormal distribution of p = 0.000 (p <0.05) and not homogeneous p = 0.014 (p <0.05). Therefore, the statistical data was continued with the Kruskal Wallis test.

3.3.2 Kruskal Wallis and Mann Whitney Test

Table 5. Difference of TGF-β1 Levels Test

Group	Mean of TGF-B1 Levels (ng/mL)	P Value
Control	0,542±0,797[a]	
BPH	0,041±0,089[b]	0,001
Prostate Ca	0,021±0,036[b]	

In table 5.5. It can be seen that the Kruskal Wallis test results showed that the mean TGF-β1 levels between the prostate cancer and BPH groups were significantly different from the control group (p value = 0.001; p <0.05). Based on these results, it was found that there were mean levels of TGF-β1 in the three control groups, so it was continued with the Mann Whitney test.

The results of the Mann Whitney test displayed in the mean column, showing different notation numbers between the control group and the BPH and prostate cancer groups. Based on the nortation, it showed that the control group was significantly different from the BPH and prostate cancer groups. .

IV. Discussion

4.1 Differences of IL-10 Levels

Inflammatory mediators play an important role in the pathogenic malignancy of the prostate. IL-10 and TGF-β1 are inflammatory mediators produced by immune cells used to lysis tumors. Some cells that have the ability to synthesize IL-10 include CD25 Foxp3 , Tr1 CD4 , T cells, B cells, macrophags, mast cells, eosinophils, and dendrite cells. Regulatory T cells and T helpers are the mobile immunity that most synthesize IL-10 for immunosuppressive effects (Dennis K, 2013).

IL-10 is known to be the main immunosuppressive factor that serves to induce tolerance through inhibition of T helper 1 response and cytotoxic T cells. IL-10 inhibits the proliferation and production of

cytokines. Increased levels of IL-10 are known to have cytotoxic effects on tumors and otherwise inhibition in IL-10 causes rejction of transplanted organs. Therefore, the role of IL-10 that plays an important role in cancer needs to be further studyed (Dennis K, 2013).

The study aims to find out the difference in IL-10 and TGF-β1 levels in prostate cancer patients, BPH, and healthy control, then conducted study with cross-sectional design. In this study using the number of prostate cancer study subjects, BPH , and healthy control respectively 13 men, 11 people, and 11 people. The subject then took venous blood to detect il-10 and TGF-β10 levels.

In this study, IL-10 levels were obtained in the highest healthy control group with a score of 71.99±83.10 pg/ml, followed by BPH group of 24.90±25.87 pg/ml, and Prostate cancer 12.16±5.21 pg/ml. Normality and homogeneity test results obtained the three groups abnormal and homogeneous distribution. Therefore, followed by kruskal Wallis and Mann Whitney test with statistical test results showed there was a difference between the three groups ($p<0.05$).In Mann's statistical test, whitney found significant differences between the control group with BPH and Prostate cancer. Meanwhile, there is no significant difference between BPH and Prostate cancer.

The results of this study showed that the IL-10 levels of BPH patients and prostate cancer were lower than the controls (table 5.3). IL-10 is secreted by cell T Helper 2 to inhibit cytokine expression of NK cells and cytotoxic T cells (Zheng, 1996). This inhibition effect can inhibit tumorigenesis in prostate cells (Zheng, 1996). IL-10 is known to inhibit tumorigenesis by lowering levels of VEGF, IL-1b, TNF-α, IL-6, and MMP-9, as well as nuclear factor-KB (NF-KB) translocation (Sheikhpour, 2018).IL-10 stimulates lymphocytes to release Fas L and TRAIL that can bind to epithelial cell receptors so that the epithelial cells die (Sheikhpour, 2018).

The results of this study are consistent with the study of Yoo et al. in 2011 which results that there is a relationship between IL-10 levels and BPH occurrences (Yoo, 2011). IL-10 levels are influenced by cigarette exposure (Dwivedi, 2014) and not influenced by genetic variation of IL-10 polymorphisms (Dwivedi, 2015). However, genetic variation is known to be associated with the recurrence of prostate cancer (Dluzniewski, 2012).

The results of this study differ from study by Shoa (2012) which proves that there is no significant relationship between IL-10 levels and the risk of prostate cancer (Shoa, 2012). Study by Zou (2011) also shows that genetic variation of IL-10 does not affect prostate cancer incidence (Zou, 2011). Therefore, further study is needed on the relationship between genetic variation with IL-10 levels and the incidence of BPH and prostate cancer in Indonesia.

4.2 Differences of TGF-β1 Levels

The levels of TGF-β1 differed between the control group with the BPH and prostate cancer groups (table 5.5). TGF-β1 is a mediator that functions for differentiation homeostasis, apoptosis of epithelial cells and prostatic stroma. Disruption of TGF-β1 can cause carcinogenesis in the prostate. Increasing the level of TGF-β1 in the stroma can inhibit the proliferation of tumor cells (Ahel, 2019).

In this study, the highest levels of TGF-β1 were obtained in the control group with a value of 0.542 ± 0.797 ng / ml, followed by the BPH group, namely 0.041 ± 0.089 ng / ml, and prostate cancer 0.021 ± 0.036 ng / ml. The results of normality and homogeneity tests showed that the three groups were not normally distributed and homogeneous. Therefore, it was continued with the Kruskal Wallis and Mann Whitney tests with statistical test results showing that there were differences between the three groups ($p <0.05$). In the Mann Whitney statistical test, it was found that there were significant differences between the healthy control group with BPH and prostate cancer. Meanwhile, there was no significant difference between BPH and prostate cancer.

These results support the study of Gao Y (2020) which shows that there are differences in TGF-β1 levels between advanced, recurrent prostate cancer compared to controls (Gao Y, 2020). Study of Ma (2005) also showed that there was a decrease in TGF-β1 levels in BPH patients compared to controls (Ma, 2005). In addition to being affected by TGF-β1 levels, polymorphisms also affect the progression of prostate cancer. Several studies have shown that mutilation of TGF-β1, polymorphisms in C-509T, and kodon 10 lowers prostate cancer risk (Shah, 2008; Li, 2004; Ma, 2005).

V. Summary

Based on the results of cross sectional study aimed at finding out the difference in IL-10 and TGF-β1 levels in prostate cancer patients, BPH and healthy control, which can be concluded as follows :

- There were differences in the mean levels of IL-10 and TGF-β1 in the BPH group compared to healthy controls.
- There were differences in the mean levels of IL-10 and TGF-β1 in the prostate cancer group compared to healthy controls.
- There was no difference in the mean levels of IL-10 and TGF-β1 in the BPH group compared to prostate cancer.

- There is a decrease in IL-10 and TGF-β1 levels in patients with BPH and prostate cancer.
- Decreased levels of IL-10 and TGF-β1 are biomarkers of the increasing degree of prostate proliferation in patients with BPH and prostate cancer.

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