

Efficacy of Combination Dutasteride and Lycopene to Reduce Bleeding in Benign Prostate Hyperplasia (BPH) Post- Transurethral Resection of the Prostate (TURP) Overview of Hypoxia inducible factor-1 alpha (HIF-1 α) Expression and Hematocrit Levels

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ABSTRACT

Trans Urethral Resection of the Prostate (TURP) is one of the gold operating standards for people with *Benign Prostate Hyperplasia (BPH)*, but the complications, like bleedings, often occur both in durante and post-surgery. The risk of bleeding in TURP is caused by angiogenesis. *Hypoxia-Induced Factor-1 Alpha (HIF-1 α)* is one of the trigger factors for angiogenesis in BPH. Based on the existing literature, it was found that there was a relationship between lycopene and dutasteride which both had a synergistic effect in inhibiting angiogenesis. However, the mechanism is unknown. This study aims to assess the effectiveness of the combination of dutasteride and lycopene on the expression of HIF-1 α and hematocrit levels in BPH patients undergoing TURP surgery. This research was an experimental study with "*Double-Blind Randomized Controlled Trial Post Test Only Design*". The 22 patients were divided into 2 groups, namely T (dutasteride + lycopene), C (lycopene+plasebo), which were given every 24 hours for a minimum of 30 days until the TURP operation was carried out. The observation of HIF-1 α expression used immunohistochemical methods. Different tests were conducted between the groups with Independent T-test. The result shows the combination of dutasteride and lycopene was not significant in reducing the HIF-1 α expression, indicated by mean rank in the group (C) (= 21.60), group (T) (= 20.00). The results obtained from the Independent T-test were (p = 0.410), meaning the difference in HIF-1 α expression between the 2 groups was not significant: Average Δ Ht group C (= 1.26); group T (= -0,98). The statistical test with the Independent T-test obtained (p = 0.027), where there were significant differences. Giving a combination of dutasteride and lycopene for at least 30 days pre-TURP surgery was not effective in reducing the expression of hypoxia-induced factor - 1 alpha (HIF-1 α) but was effective in reducing hematocrit levels compared to a single administration of dutasteride in BPH patients undergoing TURP surgery.

Keywords: HIF - 1 α ; hematocrit; BPH; dutasteride; lycopene

INTRODUCTION

The main health problem for men over 50 years is prostate enlargement, where the incidence increases with age. In the hospital education center in Indonesia, such as in the Kariadi Hospital Semarang, from 2008 to 2010, where there were patients with prostate enlargement as many as 1,742 cases, consisting of 293 patients who underwent surgery, 229 hospitalized, and 1220 outpatients; Cipto Mangunkusomo Hospital Jakarta about 462 cases; and in Soetomo Hospital Surabaya, where there were 1,948 cases of benign enlargement in the period 1993-2002 (Nugroho *et al.*, 2015).

BPH is a secondary cause of Lower Urinary Track Symptom in men and its incidence will increase with age. These complaints often result in

stress, anxiety, pain, and discomfort that can affect the quality of life of patients. Therefore one of the goals of BPH therapy is to improve the quality of life of patients. Intervention therapy is the golden standard for handling BPH is the *Trans Urethral Resection of the Prostate (TURP)*. TURP is performed on BPH patients who do not respond to medical therapy and prostate size of 30-80mL (Berry *et al.*, 1984, Fitzpatric *et al.*, 2006, Hoffman *et al.*, 2000).

One of the complications of TURP is bleeding, which can occur during surgery and post-surgery. The incidence of hematuria and clot retention after TURP is around 6-11% of cases, this will indirectly increase hospital costs, length of care and patient morbidity and mortality. Several attempts have been made to reduce TURP bleeding including the coagulation of surgical durante, catheter traction, catheter balloon position, drugs, and irrigation. (McVary *et al.*, 2010) At benign

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enlargement of the prostate, angiogenesis in the prostate area can be found which can cause easy bleeding when TURP is performed. Several studies have been developed to look for angiogenesis inhibitors, including using 5 α reductase inhibitors. These 5 α reductase inhibitors can prevent prostate tissue growth and cause prostate cell apoptosis, thereby reducing the size and symptoms of LUTS in BPH cases, while also reducing expression of the *Vascular Endothelial Growth Factor* (VEGF) and *Micro Vascular Density* (MVD) on tissues sub epithelial prostate. (Pujari *et al.*, 2016, Walsh *et al.*, 1998, Foley *et al.*, 2003)

Several studies on preoperative use of dutasteride in the same timeframe did not affect the MVD of prostate specimens nor on intraoperative bleeding. (Andriole *et al.*, 2004) Other studies also found similar results that pretreatment 2 to 4 weeks with dutasteride did not significantly reduce blood loss during or after TURP compared with placebo, although there was a significant emphasis on intraprostatic DHT. (Rittmaster *et al.*, 1996) Several other studies have reported similar findings and recommended that dutasteride be taken for a longer duration. (Zong *et al.*, 2011) Other studies found a significant reduction in blood loss in the use of dutasteride 0.5 mg for more than 4 weeks before surgery. Vascularity in prostate tissue, especially in the periurethral area decreases after using dutasteride 0.5 mg for 6 weeks. (Hahn *et al.*, 2007)

In addition to drugs class 5 α reductase inhibitors, some substances such as lycopene which are potential compounds as anticancer and have strong antioxidant activity, besides that lycopene can suppress the initiation, progression and metastasis of various tumors by targeting many signaling molecules. In addition, Lycopene can inhibit several signal growth factors, including signaling *Insulin-like Growth Factors* (IGF), *Vascular Endothelial Growth Factor* (VEGF) signals, and *Platelet-Derived Growth Factor* (PDGF) which directly or indirectly result in decreased growth cells by inducing apoptosis, and inhibiting the occurrence of angiogenesis. (Shanmugasundaram *et al.*,) Lycopene substances are abundant in red fruits including tomatoes. (Martov *et al.*, 2008, Semenza *et al.*, 2007)

Some studies have also shown that lycopene can inhibit the expression of HIF-1 in in vivo and in vitro studies. *Hypoxia inducible factor-1 alpha* (HIF 1 α) is involved in many compensation pathways such as angiogenesis, glucose metabolism, survival and tumor development. (Winarsi *et al.*, 2007)

Based on the existing literature it was found that there was a relationship between lycopene and dutasteride which both had a synergistic effect

in terms of inhibiting angiogenesis. The two agents will jointly reduce and inhibit some signal growth factors that directly and / or indirectly result in decreased cell growth by inducing apoptosis and inhibiting the occurrence of angiogenesis. In this study researchers used a combination of dutasteride 0.5 mg and lycopene 30 mg to reduce bleeding in BPH after TURP. One parameter in assessing the effectiveness of these two drugs in reducing blood vessel density and the extent of blood vessels after administration of these agents is HIF-1 α and measuring the difference in reduction in hematocrit levels.

METHODOLOGY

Materials And Method

This research is an experimental study with the design of "*Double Blind Randomized Controlled Post Test Only Design Trial*". It was done by comparing changes in angiogenesis between BPH patients who underwent TURP surgery by assessing the expression of HIF 1 α and levels of Hematocrit after single dutasteride 0.5 mg in the control group and a combination of dutasteride 0.5 mg + lycopene 30 mg in the treatment group for a minimum of 30 days until the TURP operation is carried out.

The research sample was BPH patients who came to polyclinics urology, surgical emergency room, and urology inpatient room at Kariadi Hospital Semarang which was clinically and sonographically diagnosed as BPH and planned to undergo TURP surgery. The size of the sample according to WHO uses a sample size for two sample situations. The minimum sample size for each group is: 40 patients, with *Confidence Interval* (CI) 95%. (Febriansah *et al.*, 2008)

The sample in this study was divided into 2 groups by random allocation:

Group 1 (C) was taken from all male suffered from BPH who came to urology clinic, emergency room, and urology inpatient ward in Kariadi Hospital Semarang which was clinically and sonographically diagnosed as BPH and given dutasteride 0.5 mg once daily for at least 30 days before undergoing TURP surgery (control group).

Group 2 (T) was taken from all male suffered from BPH who came to the urology clinic, emergency room, and urology inpatient ward in Kariadi Hospital Semarang which was clinically and sonographically diagnosed as BPH and given dutasteride therapy 0.5 mg once daily and lycopene capsules 30 mg once daily for at least 30 days before undergoing TURP surgery (treatment group).

After completion of treatment, the patient will undergo TURP surgery, and before the

Table I. Subject characteristics based on the study group (n = 20)

Variabel	Mean ± SD	Median (min - max)	p
Age	65,35 ± 6,98	66,5 (51 - 80)	0,830 [§]
Prostate Volume	43,66 ± 16,83	37,53 (25,45 - 86,03)	0,597 [‡]
Duration of operation	22,75 ± 8,50	20 (15 - 40)	0,079 [‡]
Duration of drug administration	32,35 ± 2,11	32 (30 - 36)	0,179 [§]
HIF-1 α	20,80 ± 4,21	21 (14 - 28)	0,410 [§]
Amount of irrigation	4600 ± 1187,66	5000 (3000 - 7000)	0,477 [‡]
Duration of catheter	33,15 ± 1,46	34 (31 - 35)	0,345 [‡]
Ht pre	39,43 ± 5,28	39,45 (30,1 - 49,9)	0,735 [§]
Ht post	39,57 ± 5,41	40,5 (29,7 - 47,9)	0,574 [§]
Difference of Ht	0,14 ± 2,33	-0,2 (-4,7 - 4,1)	0,027 ^{§*}

note : * Signifikan; [§] Independent t; [‡] Mann Whitney

Table II. Average Percentage of HIF-1 α in each group

HIF-1 α	Mean ± SD	P
Treatment (T)	20,00 ± 2,83	0,410 [§]
control (C)	21,60 ± 5,30	

note: [§] Independent t

Table III. Average Δ Data on HT levels in each group

Treatment	Mean ± SD	p
C	1,26 ± 1,99	0,027 ^{§*}
T	-0,98 ± 2,16	

note : * Signifikan p < 0,05, [§] Independent t

operation, a hematocrit blood sample is taken. During TURP the prostate scraping tissue was carried out as much as 5-10 periurethra prostate scrapings and then performed anatomical pathology examination at the Anatomical Pathology Laboratory Diponegoro Medical University Semarang.

Immunohistochemical examination of prostate scrapings specimens was performed to assess the expression of HIF 1 α after treatment. HIF 1 α readings are carried out with the Olympus BX-41 light microscope by anatomical pathologists. the TURP procedure uses a 24-F resectoscope (Karl Storz, Tuttlingen, Germany). TURP irrigation uses distilled water and counts the amount of TURP durante irrigation, length of operation, amount of bleeding and the amount of prostate scraping tissue (Table I). Data from research results that have been recorded are collected and processed using the SPSS v.15 program.

RESULT AND DISCUSSION

Result

This study was conducted on 22 BPH patients with urinary retention who will undergo a TURP procedure and have fulfilled the inclusion criteria and exclusion criteria. Where during the study period 2 subjects dropped out of the study, with details; 1 subject did not want to be operated (C group), and 1 subject dropped out because of the results of the Pathology Anatomy examination found a malignancy of the prostate (T group) so that subjects who qualified to continue the study were only 20 subjects.

The results of the statistical test with the Independent T Test in the 2 groups were obtained (p = 0.410) which means that the difference in HIF-1 α between the treatment group (T) and the control group (C) was not significant.

The results of the statistical test with Independent T Test in the 2 groups obtained p = 0.027, which means that the difference in the

decrease in Ht levels pre and postoperatively in the 2 groups was significant.

Discussion

Previous research suggests that agent 5 α reductase inhibitors (dutasteride) are said to reduce the expression of *hypoxia induced factor - 1 alpha* (HIF-1 α) in the sub epithelial tissue of the prostate. (Upadhyay *et al.*, 2009) Theoretically hypoxia is thought to be the most potent stimulant of VEGF, and its expression transcriptional is regulated by HIF - 1. The role of HIF-1 is very important, as a regulator of genomic transcription is widely identified for O₂ hemostasis in response to hypoxic stress. HIF-1 is a heterodimer consisting of HIF-1 α and HIF-1 β subunits. HIF-1 β is expressed constitutively, where HIF-1 α expression is maintained at a low level in most cells under normal conditions. (Ku *et al.*, 2009)

Under hypoxic conditions, HIF-1 α releases proteosomal degradation and is then translocated to the nucleus. The process formed as a result of the inhibition of oxygen-dependent prolylhydroxylase activity modifies 564 and 402 residues, and the subsequent process is mediated by cell nucleus localization signals. This enzymatic modification of HIF-1 α is needed to bind the von hippel-lindau protein, which recognizes the component of the ubiquitin E3 protein ligase which is the target of HIF-1 α for proteosomal degradation. In contrast to the regulation of oxygen dependence from degradation of HIF-1 α , it is reported that factors that induce synthesis of HIF-1 α proteins via signal transduction pathway lead to tyrosine kinase to PI3K receptors to AKT and FRAP serine / threonine kinase. (Upadhyay *et al.*, 2009, Ku *et al.*, 2009)

The results of statistical tests showed that there was no significant difference in the expression of HIF-1 α in the treatment group (P) given a combination of dutasteride and lycopene with the control group (K) which was only given dutasteride alone (table II). Previous studies have found that preoperative use of dutasteride over a period of 2-4 weeks did not affect the MVD of prostate specimens nor in intraoperative bleeding. (Zong *et al.*, 2011) Other studies also found similar results that pretreatment 2 to 4 weeks with dutasteride did not significantly reduce blood loss during or after TURP compared with placebo, although there was a significant emphasis on intraprostatic DHT.15 Several other studies have reported similar findings and recommended that dutasteride be taken for a longer duration. (Shanmugasundaram *et al.*, 2007).

TURP (*Transurethral Resection of Prostate*) is still the gold standard for handling cases of BPH. The most common complications are intra and perioperative bleeding. However, the bleeding is expected to be reduced by administering a combination of lycopene and dutasteride. The results of statistical tests regarding the differences in hematocrit levels pre and postoperatively between groups were found in the treatment group (P) there was less decline when compared to the control group (K) (Table III).

There are several factors that can affect the reduction of hematocrit in post-TURP surgery in this study, including the duration of action and fluid balance. Research conducted by Ather *et al.*, . Found that bleeding that occurred during TURP was influenced by length of operation and prostate size. Research conducted by Kirolos *et al.*, also suggested that the size of the prostate affected bleeding during TURP surgery. (Kirolos *et al.*, 1997) Research conducted by Miyao *et al.*, found that there were differences in hematocrit in the group with TURP syndrome with asymptomatic ones. This decrease in hematocrit is assumed to occur due to hemodilution during TURP surgery which is also affected by the duration of surgery. (Miyao *et al.*, 2001)

Pastore *et al.*, found a significant reduction in hematocrit for dutasteride for 6 weeks. However, in a study conducted by Hahn RG *et al.*, there was no significant difference in blood loss between the dutasteride group and the untreated group. (Winarsi *et al.*, 2007) Results conducted by Boccon-Gilbod L *et al.*, concluded that differences in blood loss in patients given dutasteride previously for 4 weeks compared to the untreated group after TURP showed insignificant results. The insignificant results are assumed to occur because they are influenced by the duration of administration of therapy which affects the decrease in hematocrit in TURP surgery. (Boccon *et al.*, 2005).

CONCLUSION

Giving a combination of dutasteride and lycopene for at least 30 days pre TURP surgery was not effective in reducing the expression of *Hypoxia Induced Factor-1 alpha* (HIF-1 α) in BPH patients who underwent TURP surgery but significantly reduced post-TURP hematocrit levels when compared with single dutasteride. There were no smaller HIF 1-alpha expressions in the group given a combination of dutasteride and lycopene than the group given dutasteride single. There were differences in the smaller post TURP hematocrit

values in the group given a combination of dutasteride and lycopene compared to the group given single dutasteride.

SUGGESTION

It is recommended to extend the duration of treatment before the TURP procedure, increase the number of samples, use a combination of effective dutasteride and lycopene doses, see long-term side effects on the combination use of dutasteride and lycopene, see the effectiveness of decreasing angiogenesis from a combination of dutasteride and lycopene against other angiogenic receptors, and see a relationship between factors that influence TURP procedure for postoperative bleeding.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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