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Expression of messenger RNA Interleukin-1 After Injection of 20 mg of Triamcinolone and 2.5 mg of Bevacizumab Subconjunctival in Pterygium Patients

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Abstract – Pterygium is a conjunctival tissue hyperplastic degenerative process in form of triangular fibrovascular tissue, growing on conjunctival towards and infiltrating corneal surface. Inflammation process on ocular surface was believed to be involved in the recurrence of pterygium tissue post excision surgery. Recent years, the effect of local injection of triamcinolone and bevacizumab in recurrence of pterygium have been studied but remain with unsatisfied results. This study aimed to analyze changes of expression of messenger RNA (mRNA) interleukin-1 (IL-1) after injection of 20 mg of triamcinolone and 2.5 mg of bevacizumab subconjunctiva in pterygium patient. Methods: Fifteen eyes with stage II primary pterygia were included in this study. Patients were randomized into three groups; the triamcinolone group, bevacizumab group and placebo group. Subconjunctival of 20 mg of Triamcinolone or 2.5 mg of bevacizumab or placebo (depend on group) were injected one week before surgery. All subject was done pterygium surgery with autograft technique. Main outcome measures included changes of mRNA IL-1 expression between Triamcinolone group, Bevacizumab group and placebo group. One month follow up was completed in fifteen eyes. Blood level changes of mRNA IL-1 expression in bevacizumab group was 4.09 ± 0.52, triamcinolone group was 3.40 ± 2.62, and placebo group was 1.08 ± 1.48, respectively ($p = 0.04$). Comparison between group, there are significant effect between bevacizumab group and placebo group ($p= 0.00$), and no significant effect in triamcinolone group ($p = 0.06$). Conclusion: Subconjunctival injection of Bevacizumab and Triamcinolone before surgery effective in suppressing inflammation in pterygium.

Keywords: Pterygium, mRNA, Interleukin 1, Triamcinolone, Bevacizumab.

1. Introduction

Pterygium is an a wing-shape fibrovascular external ocular mass that growth from over the perilimbal bulbar conjunctiva onto the cornea [1,2].The exact cause of pterygium still remains unclear; there are some mechanism involved in pathomechanism of pterygium, such as inflammation, viral infection and heredity, oxidative stress, inflammatory mediators, extracellular matrix modulators, apoptotic and oncogenic proteins, loss of heterozygosity, microsatellite instability, angiogenesis and lymphangiogenesis, epithelial-mesenchymal transition, and cholesterol metabolism have been identified as causes [3-5]. Epidemiological study shows that ultraviolet B (UV B) was the major risk factor of pterygium occurrence [6,7], it triggers inflammation and progressive fibrovascular proliferation on ocular surface in long time exposure6. Recurrence rate post excision of pterygium cases were still high (30-90%) and 97% was occurred in one-year period after excision [8]. Inflammation process in conjunctiva is one of factor that involve in

recurrence process in pterygium. The role of corticosteroid in prevent recurrence of pterygium is well known. Intraoperative subconjunctival triamcinolone injection was proved to be effective in inhibiting the recurrence of pterygium [9]. Another factor that involve in recurrence of pterygium is increasing of growth factor, Vascular endothelial growth factor (VEGF). It has been proven to be increased and involve in pathogenesis of pterygium [10]. Bevacizumab is a recombinant humanized murine monoclonal immunoglobulin G1 that inhibit VEGF-A isoform which stimulates angiogenesis. It administration intralesional has been proved to decrease pterygium size up to 14.47% [11]. Gupta RK et al 2017 [9] even revealed that subconjunctival administration of bevacizumab 2.5mg was effective in prevent recurrence of pterygium.

This study is aimed to analyze changes of expression of messenger RNA (mRNA) interleukin-1 (IL-1) after injection of 20 mg of triamcinolone and 2.5 mg of bevacizumab subconjunctiva in pterygium patient.

2. Materials and Methods

2.1 Study Design and subject

We enrolled consecutively 15 eyes of adult's patients (30-45 years old) who had clinically confirmed diagnosis of stage II primary pterygium according to Bhargava et all classification [12].

All patients underwent full ophthalmologic examination before and after surgery. Exclusion criteria if there were ocular surface disease or infection, autoimmune disorders, systemic disease (such as diabetes mellitus and hypertension) and previous ocular/pterygium surgery. Patients were randomized into 3 groups (bevacizumab, triamcinolone and placebo group) consist of 5 eyes each group. Each eye received subconjunctival injection of 2.5 mg of bevacizumab, 20 mg of triamcinolone and placebo (depend on group) 7 days prior to pterygium excision.

Approval of the Institutional Review Board Ethics Committee of the Medical Faculty of Hasanuddin University, Makassar, Indonesia was obtained. Written informed consent for injection, surgery, blood sampling and pterygium sampling was obtained from all participants according to the Declaration of Helsinki.

2.2 Surgical procedures

All 15 patients underwent pterygium excision with conjunctival autograft technique, performed by a single surgeon. The surgical technique featured (1) subconjunctival anesthetic (lidocaine 2%) injection in the area adjacent to the pterygium (5 mm from limbus); (2) excision of the pterygium, starting from its head, followed by pterygium body removal; (3) exposition of a triangular-shaped bare scleral bed of little dimensions (with the base at the level of the limbus and margins of 1 mm each); (4) excision of autolimbal conjunctival graft from superior bulbar conjunctiva (4) conjunctival graft suture with vicryl 8-0 at the end of the procedure.

2.3 Sample Collection and measurement of mRNA interleukin-1 expression

Blood samples were collected 1 week prior to surgery and 1 month after surgery and put in Guanidium thiocyanate L6 buffer. The samples were collected without prior until examined.

Expression of mRNA IL-1 were measured using real-time PCR technique. The technique begins with extraction of nucleic acid and continued with mRNA expression analysis using specific mRNA IL-1 target gene. After that calculation of calibration curve with cycle threshold (Ct) were done along with evaluation of gene target expression relative quantitation using comparative Ct method. The PCR protocol were adjusted according to previous study [13,14,15] and optimized for instrument real-time PCR (CFX Connect System machine, USA). The PCR results were analyzed using Bio-Rad CFX Manager 3.1 software (Biorad, USA)

2.4 Statistical Analysis

All values are expressed as mean □ standard deviation (SD) and all data are presented in table. Statistical analyses were performed using Statistical Package for Social Science (SPSS) version 21.0. Changes expression of mRNA IL-1 using Chi square and Kruskal Wallis test were used. The differences between groups were tested using one-way ANOVA with Post-hoc LSD test. A value of $p<0.05$ was considered a statistically significant result.

3. Results

One month follow up was completed in fifteen eyes. Changes of mRNA of IL-1 expression with Kruskal Wallis test was significant, 0.036. Highest changes in mRNA IL-1 level was found in bevacizumab group. There are significant effect in blood level changes of mRNA IL-1 expression three groups, in bevacizumab group was 4.81 ± 0.52, triamcinolone group was 3.40 ± 2.63, and placebo group was 1.08 ± 1.48, p = 0.04 (**Table 1**).

Group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Bevacizumab	5	4.81	0.52	0.23	4.16	5.45	4.35	5.63
Triamcinolone	5	3.40	2.63	1.17	0.14	6.67	0.17	5.84
Placebo	5	1.08	1.48	0.66	-0.76	2.92	0.00	3.04
Total	15	3.10	2.28	0.59	1.83	4.36	0.00	5.84

Table 1. Level changes of mRNA of IL-1 expression after subconjunctival injection of each agent

In comparison between group, no significant effect in triamcinolone group, 1.40 ± 1.12 (p = 0.06), whereas in bevacizumab group and placebo group, there are significant effect, 3.73 ± 1.12 (p= 0.00). (**Table 2**).

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Bevacizumab	Triamcinolone	1,40	1,12	0,23	-1,03	3,83
	Placebo	3,73*	1,12	0.00	1,30	6,16
Triamcinolone	Bevacizumab	-1,40	1,12	0,23	-3,94	1,03
	Placebo	2,33	1,12	0,06	-0,11	4,77
Placebo	Bevacizumab	3,73*	1,12	0.00	-6.16	-1,30
	Triamcinolone	-2,33	1,12	0,06	-4,77	0,11

*. The mean difference is significant at the 0.05 level.

Table 2. Comparison of mRNA IL-1 blood level changes between group

4. Discussion

In this study, we found as result in Table 1, that level changes of mRNA of IL-1 in Bevacizumab group is higher than in Triamcinolone and placebo group, there are significant changes of mRNA expression of IL-1

in Bevacizumab group. Bahar et al, 2008 [16] also reported that subconjunctival bevacizumab injection was not resulted in long term vascular regression in cornea for recurrent pterygium. Razeghinejad, 2010 [17] reported that intra-operative subconjunctival bevacizumab was not effective in recurrence of pterygium. This result could support our result since IL-1 was a cytokine that play important role in inflammation that being a factor for pterygium recurrence. Our study was give triamcinolone before surgery whereas Kheirkhah et al, 2013 [18] was give intraoperative triamcinolone injection and showed that was not significantly reduced conjunctival inflammation. This result could also support our study, which triamcinolone treatment was not reduced expression of mRNA IL-1 that lead to inflammation and pterygium recurrence. Our results were also contrary with other studies about bevacizumab and triamcinolone. Castañeda,2015 [19] report a single subconjunctival injection of 2.5 mg/ml Bevacizumab with pterygium surgery using conjunctival autograft procedure was able to prevent pterygium recurrence when compared to a control group. Nuzzi, 2017 [20] shows that bevacizumab injection subconjunctival, 1 week prior to surgery have lower recurrence rate compare to control. Even though this result seems to be not supported our result, but in their study, they also still reported 7.14% recurrence that still exist after bevacizumab injection. It means that the inflammation process could still be happen after bevacizumab injection and so with IL-1 as a cytokine. Gupta RK, 2017 [9] revealed that triamcinolone and bevacizumab are equally effective in reducing the rate of recurrence of pterygium when used as an adjunct to conjunctival autograft, although this was statistically not significant. Mpyet, 2000 [21] also found that combined subconjunctival and mitomycin C intraoperative was effective in preventing recurrence up to 14 months follow up. Our different results with other studies possibly because we just could have tested changes of mRNA IL-1 expression in blood not in conjunctival tissue because we do not have baseline for pterygium tissues. As we know that many factors could influence systemic condition and this was subject for biases.

In comparison between group, as result in Table 2, there are significant effect between Bevacizumab and placebo group, $p<0.05$. Bevacizumab can inhibit VEGF, and inhibit proliferation of fibrovascular tissue. VEGF, a key factor in remodeling wound healing tissue [22] Pro-inflammatory mediators (IL-1) mainly affects inflammatory processes, and various immune, degradative, and properties of growth-promote, IL-1 play an important role in tumor-mediated angiogenesis and blocking their function may suppress tumor progression [23,24]. Angiogenesis plays a critical role in wound healing, it can be induced in response to injury, if there is a physiological balance between angiogenesis stimulators and inhibitors, it can suppress vascular growth [25,26]. In our study, we used triamcinolone subconjunctival that can inhibit inflammatory and bevacizumab inhibit angiogenesis, and found that expression of mRNA IL-1 in bevacizumab group is higher than triamcinolone. Thus, bevacizumab seems more effective than triamcinolone to prevent recurrence after excision. That one reason why, injection of 20 mg Triamcinolone and 2.5 mg of Bevacizumab subconjunctiva before surgery effective in suppressing inflammation in pterygium patients.

Because pterygium recurrence was highly local related, our results could not support the effect of both triamcinolone and bevacizumab in reducing recurrent risk of pterygium after excision because we observed only until one month after surgery.

5. **Limitations**

The results reported there was considered in some of limitations. Need to evaluate long time to follow up because in this study, we only until one month after surgery, and no get recurrence in this period. We suggest to compare with pterygium tissue before and after treatment.

6. Conclusion

Expression of mRNA IL-1 was increased after injection of 20mg Triamcinolone and 2.5mg of Bevacizumab

subconjunctiva, administrated of these injections before surgery effective in suppressing inflammation in pterygium.

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9. References

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