COMPARISON OF SERUM AND VITREOUS TGF-β1 LEVELS IN PROLIFERATIVE DIABETIC RETINOPATHY WITH AND WITHOUT PANRETINAL PHOTOCOAGULATION LASER THERAPY

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Abstract:
Background: A long-term diabetic retinopathy will cause an increase of several growth factors expression like Transforming Growth Factor β (TGF-β), a multipotent cytokine that involved in the process of endothelial cell proliferation.
Purpose: This study aims to observe the relationship between TGF-β1 levels in serum and vitreous fluid of Proliferative Diabetic Retinopathy (PDR) patients given Pan Retinal Photocoagulation (PRP) laser therapy.
Method: This was a cross-sectional study involving 14 patients with PDR. TGF-β1 levels of vitreous and peripheral blood were measured using Enzyme linked Immunosorbent Assay (ELISA) method.
Results: Our subjects consisted of 57.1% males with a mean age of 51.8 years, where dyslipidemia was the most common comorbid disease. Mean serum TGF-β1 level was 12,821.43 ± 5,253.16 pg/ml, while the mean value in vitreous was 3,692.86 ± 333.89 pg/ml. Meanwhile, there was no significant difference in serum and vitreous TGF-β1 levels between subjects with and without PRP laser therapy ($p>0.05$).
Conclusion: There were no significant correlation between TGF-β1 levels in proliferative diabetic retinopathy patients with and without pan retinal photocoagulation laser therapy. However, there was a decreasing trend in TGF-β1 levels in the vitreous fluid which indicates that PRP laser therapy has a positive effect on preventing the formation of neovascularization in the eye.

Keywords: TGF-β1 levels, proliferative diabetic retinopathy, panretinal photocoagulation laser

Fundings: None

Introduction

Diabetes Mellitus (DM) is a metabolic disease characterized by chronic hyperglycemia due to the failure of the pancreas to produce sufficient insulin or the occurrence of cell resistance in peripheral tissues.(1) This condition can cause damage to various organs including the heart, kidneys, and eyes, even become a risk factor for death from complications.(2,3) Epidemiological estimates in several studies predict that patients with DM will reach 380 million in 2025 with 4 million being at risk for visual loss due to Diabetic Retinopathy (DR).(4)

Diabetic retinopathy is classified into an early stage, namely Non-Proliferative Diabetic Retinopathy (NPDR), and an advanced stage, called Proliferative Diabetic Retinopathy (PDR).(5) The case of decrease in visual ability occurs due to two mechanisms, namely increased intraretinal vascular permeability which leads to macular edema and narrowing of the capillary blood vessels' lumen, to cause macular ischemia.(6)

The state of DR in the long term causes an increase in the expression of several growth factors such as Vascular Endothelial Growth Factor (VEGF), Platelet-Derived Growth Factor (PDGF), basic Fibroblast Growth Factor (bFGF), and Transforming Growth Factor beta (TGF-β).(7) These biochemical molecules are known to

trigger the occurrence of PDR which leads to the threat of permanent blindness in patients.(8) TGF-β is a multipotent cytokine that works through activin receptor-like kinase-1 (ALK1) and 5 (ALK-5).(9) It is involved in the process of endothelial cell proliferation, formation and degradation of extracellular matrix, as well as chemotactic and apoptotic processes that lead to the thickening of the capillary basement membrane and in impaired regulation of systemic blood vessels.(8–10)

The best treatment for RD patients is to control the blood sugar levels,(11) also, the therapeutic outcome is not aimed at curing or restoring visual function but at slowing the progression of vision loss.(12) Panretinal photocoagulation (PRP) laser therapy is reportedly effective for fulfilling these goals.(13) It is performed when high-risk PDR is found to numb the ischemic area, thereby inhibiting further neovascularization.(14)

This study aims to observe the relationship between TGF-β1 levels in serum and vitreous fluid of PDR patients given PRP laser therapy. The results are expected to form the basis for preventing further complications in DM patients, specifically those already having visual complaints.

Material and methods

Study Design

This was a cross-sectional study involving 14 patients with PDR who had vitrectomy surgery. The history and laboratory tests were investigated to confirm the diabetic status of patients by checking fasting blood sugar and HbA1c levels. Furthermore, routine ophthalmological examinations were carried out including visual acuity test, intraocular pressure (IOP), examination of the anterior segment of the eye with slit-lamp biomicroscopy, as well as the posterior segment using funduscopy. The fundoscopic examination results were stated to be normal for NPDR and PDR, but only patients with PDR were analyzed. Furthermore, an analysis was conducted on the relationship between the duration and number of laser burns given to patients due to changes in TGF-β1 levels examined in the serum and vitreous fluids.

Sample Collection

Vitreous samples were taken using a vitrectomy machine with a volume of 700-1000 µl, while the serum using 3-5 ml blood samples were taken through the median cubital vein. Afterward, they were placed into a vacutainer tube for mobilization and storage.

TGF-β1 Assay

TGF-β1 levels were checked using the human TGF-β1 ELISA reagent kit (Cat. No. MN 55412, R & D Systems, Inc, Minneapolis, USA) where the standard range on the device was 31.2 – 2,000 pg/ml with a detection limit of 4.61 pg/ml.

Processing and Data Analysis

The data were grouped according to the purpose and type of data, then, they were statistically analyzed using SPSS software for Windows ver. 23.0. The normality test showed that the data distribution was abnormal, hence, the Mann-Whitney and the Spearman correlation test were used (Sig. $p \leq 0.05$).

Results

Observations were made to determine serum and vitreous TGF-β1 levels in patients with PDR with or without laser PRP. It was performed on 14 respondents with PDR and had experienced vitrectomy surgery. The univariate data presented in Table 1 shows that the study subjects consisted of 57.1% males and 42.9% females with a mean age of 51.8 years. The most common comorbid disease was dyslipidemia with a prevalence of 50.0%, the mean serum TGF-β1 level was 12,821.43 ± 5,253.16 pg/ml, while the mean value in vitreous was 3,692.86 ± 333.89 pg/ml.

Table 2 shows the comparison of TGF-β1 levels between patients treated with and without PRP laser therapy. The statistical calculations showed no significant association with $p>0.05$ between serum and vitreous TGF-β1 levels in patients with PDR with or without a history of PRP laser therapy. However, there

was a decreasing trend in TGF-β1 levels in the vitreous fluid which indicates that PRP laser therapy has a positive effect on preventing the formation of neovascularization in the eye.

Discussion

The number of male patients in this study was more than females, while the mean age was 51.8 years and the most common comorbid disease was dyslipidemia. According to Jeffrey G et al (2011), this is because the development of DR in women can be inhibited by sex hormone receptors. The PDR development can be inhibited by inhibiting hormone receptors, although this mechanism is still unclear.(15) Meanwhile, the age group above 50 years and a history of metabolic syndrome has been reported to be one of the risk factors in the development of DM and PDR.(16)

The mean value of TGF-β1 levels in both groups of patients with or without PRP laser therapy using a vitreous sample was 3,692.86 ± 333.89 pg/ml, while the mean serum level was 12,821.43 ± 5,253.16 pg/ml (Table 1). However, after therapeutic treatment (Table 2), the vitreous TGF-β1 level in the group treated with laser therapy showed lower values compared to patients who did not receive PSP laser therapy. In contrast, the serum samples showed higher values in patients that received PRP laser therapy. Consequently, it was concluded that the administration of laser therapy has a good effect in reducing the level of TGF-β1 locally in the eye but has no significant effect systemically.(17) Administration of PRP laser therapy improves the hypoxic state of the retina and the levels of cytokines in the vitreous fluid, thereby preventing proliferation and further neovascularization.(18) Shimura et al. (2009) stated that PRP laser therapy before vitrectomy surgery can reduce levels of angiogenic factors such as VEGF, IL-6, and TGFβ.(19)

Serum TGF-β1 levels can be influenced by various other factors such as hypertension and dyslipidemia. In conditions of hyperglycemia, TGF-β1 levels also increase,(20) this is in line with several other studies which stated that elevated levels of TGF-β1 are also found in other systemic diseases such as diabetic nephropathy, lung and autoimmune diseases, cancer, cardiovascular disease, hyperglycemia, and hypercholesterolemia.(21–24)

Conclusion

Based on the results, there was no significant difference in serum and vitreous TGF-β1 levels between subjects with and without PRP laser therapy. However, the trend of TGF-β1 levels in subjects with PRP laser therapy was lower than those without panretinal photocoagulation laser therapy.

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Disclosure

Statement of Ethics

This study protocol was reviewed and approved by The Ethics Committee of Medical Research, Faculty of Medicine, Hasanuddin University with approval number: 515/UN.4.6.4.5.31/PP36/2021.

Conflict of Interest Statement

The authors state there is no conflict of interest in writing this article.

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Data availability statement

Not applicable

Author Contributions

HSM, RZA, AMI: conception or design of the work, performing the medical examination, analysis and interpretation of the data, laboratory examination and drafting the work. BD, JS: Supervision and quality check of the medical examination, caring for patients, performing follow-up after surgery. AS, ICI: project administration, data statistic evaluation, drafting the work and final check for publication.

References

1. Deshmukh CD, Jain A. Diabetes Mellitus : A Review Diabetes Mellitus : A Review. Int J Pure App Biosci [Internet]. 2015;3(3):224–30. Available from: https://www.mendeley.com/catalogue/d1c2dc46-7937-3005-ba9e-11b39195dab5/?utm_source=desktop&utm_medium=1.19.8&utm_campaign=open_catalog&userDocumentId=%7Bd45e14ea-873a-428d-a1a6-5c5d48436ea7%7D
2. Paul D, Paul K. Diabetes mellitus and its complications: A review. Int J Curr Pharm Res [Internet]. 2012;4(2):12–7.
3. Alam S, Hasan MK, Neaz S, Hussain N, Hossain MF, Rahman T. Diabetes Mellitus: Insights from Epidemiology, Biochemistry, Risk Factors, Diagnosis, Complications and Comprehensive Management. Diabetology. 2021;2(2):36–50.
4. Forouhi NG, Wareham NJ. Epidemiology of diabetes. Med (United Kingdom) [Internet]. 2014;42(12):698–702. Available from: http://dx.doi.org/10.1016/j.mpmed.2014.09.007
5. Wang W, Lo ACY. Diabetic retinopathy: Pathophysiology and treatments. Int J Mol Sci. 2018;19(6).
6. Kern TS, Antonetti DA, Smith LEH. Pathophysiology of Diabetic Retinopathy: Contribution and Limitations of Laboratory Research. Ophthalmic Res. 2019;62(4):196–202.
7. Zafar MI, Mills K, Ye X, Blakely B, Min J, Kong W, et al. Association between the expression of vascular endothelial growth factors and metabolic syndrome or its components: A systematic review and meta-analysis. Diabetol Metab Syndr [Internet]. 2018;10(1):1–17.
8. Sorrentino FS, Matteini S, Bonifazzi C, Sebastiani A, Parmeggiani F. Diabetic retinopathy and

endothelin system: Microangiopathy versus endothelial dysfunction. Eye [Internet]. 2018;32(7):1157–63.

9. Bonfiglio V, Platania CBM, Lazzara F, Conti F, Pizzo C, Reibaldi M, et al. Tgf-β serum levels in diabetic retinopathy patients and the role of anti-vegf therapy. Int J Mol Sci. 2020;21(24):1–16.

10. Osaadon P, Fagan XJ, Lifshitz T, Levy J. A review of anti-VEGF agents for proliferative diabetic retinopathy. Eye [Internet]. 2014;28(5):510–20. Available from: http://dx.doi.org/10.1038/eye.2014.13

11. Tosi GM, Orlandini M, Galvagni F. The controversial role of tgf-β in neovascular age-related macular degeneration pathogenesis. Int J Mol Sci. 2018;19(11):1–16.

12. Sinclair SH, Schwartz SS. Diabetic Retinopathy–An Underdiagnosed and Undertreated Inflammatory, Neuro-Vascular Complication of Diabetes. Front Endocrinol (Lausanne). 2019;10(December):1–14.

13. Gonzalez VH, Wang P wen, Ruiz CQ. Panretinal Photocoagulation for Diabetic Retinopathy in the RIDE and RISE Trials: Not “1 and Done”. Ophthalmology [Internet]. 2021;128(10):1448–57. Available from: https://doi.org/10.1016/j.ophtha.2019.08.010

14. Mansour AM, El Jawhari K, Arevalo JF. Role of peripheral pan-retinal photocoagulation in diabetic macular edema treated with intravitreal ziv-aflibercept. Clin Ophthalmol. 2019;13:685–94.

15. Gross JG, Glassman AR, Jampol LM, Inusah S, Aiello LP, Antoszyk AN, et al. Panretinal photocoagulation vs intravitreous ranibizumab for proliferative diabetic retinopathy: A randomized clinical trial. JAMA - J Am Med Assoc. 2015;314(20):2137–46.

16. Deák M, Lasca M, Vereşiu IA. Risk factors for diabetic retinopathy progression. Rom J Diabetes, Nutr Metab Dis. 2015;22(2):159–65.

17. Kim KS, Park JM, Kong TH, Kim C, Bae SH, Kim HW, et al. Retinal angiogenesis effects of TGF-β1 and paracrine factors secreted from human placental stem cells in response to a pathological environment. Cell Transplant. 2016;25(6):1145–57.

18. Seymenołlu RG, Ulusoy MO, Başer EF. Safety and efficacy of panretinal photocoagulation in patients with high-risk proliferative diabetic retinopathy using pattern scan laser versus conventional YAG laser. Kaohsiung J Med Sci. 2016;32(1):22–6.

19. Shimura M, Yasuda K, Nakazawa T, Abe T, Shiono T, Iida T, et al. Panretinal photocoagulation induces pro-inflammatory cytokines and macular thickening in high-risk proliferative diabetic retinopathy. Graefe’s Arch Clin Exp Ophthalmol. 2009;247(12):1617–24.

20. Batlle E, Massagué J. Transforming Growth Factor-β Signaling in Immunity and Cancer. Immunity. 2019;50(4):924–40.

21. Kubiczkova L, Sedlarikova L, Hajek R, Sevcikova S. TGF-β - an excellent servant but a bad master. Vol. 10, Journal of Translational Medicine. 2012.

22. Huang CY, Chung CL, Hu TH, Chen JJ, Liu PF, Chen CL. Recent progress in TGF-β inhibitors for cancer therapy. Biomed Pharmacother [Internet]. 2021;134(December 2020):111046. Available from: https://doi.org/10.1016/j.biopha.2020.111046

23. El-Mesallamy HO, Gad MZ, Sallam AAM. The association of TGF-β1, angiotensin II and oxidative stress with diabetic nephropathy in type 2 diabetic patients. Int J Diabetes Metab. 2008;16(2):63–8.

24. Wan YY, Flavell RA. TGF-β and regulatory T cell in immunity and autoimmunity. J Clin Immunol. 2008;28(6):647–59.

Table 1 Descriptive data of samples

Characteristics	Variable	N	(%)	Description
Gender	Male	8	57.1	
	Female	6	42.9	
Age	<50 years	3	21.4	
	≥50 years	11	78.6	
Comorbidity	Dyslipidemia	7	50.0	
	Hypertension	6	42.9	
	No comorbid	1	7.1	
Serum TGF-β1 levels				
With PRP Laser Therapy	≤12000 pg/ml	3	21.4	Mean of serum TGF-β1 levels of all patients (laser and non-laser): 12,821.43 ± 5,253.16
	>12000 pg/ml	5	35.7	
Without PRP Laser Therapy	≤12000 pg/ml	4	28.6	
	>12000 pg/ml	2	14.3	

Table 2. Comparative Analysis of Serum and Vitreous TGF-β1 Levels

Variable	Laser History	n	Mean	SD	p*
TGF Serum	Yes	8	14,187.5	5,338.9	0.245
	No	6	11,000.0	4,987.6	
TGF Vitreous	Yes	8	3,587.5	352.3	0.104
	No	6	3,833.3	273.3	

*Mann-Whitney test

COVER LETTER

Date: 23rd February 2022

To
The Editor,
Bali Medical Journal
I am enclosing herewith a manuscript entitled:

COMPARISON OF SERUM AND VITREOUS TGF-β1 LEVELS IN PROLIFERATIVE DIABETIC RETINOPATHY WITH AND WITHOUT PANRETINAL PHOTOCOAGULATION LASER THERAPY

for possible evaluation and also publication in Journal of the Bali Medical Journal. The aim of this paper is to observe the relationship between TGF-β1 levels in serum and vitreous fluid of Proliferative Diabetic Retinopathy (PDR) patients given Pan Retinal Photocoagulation (PRP) laser therapy. We really sure that it is very interesting for your journal readers and also matched to your journal scope.

Submitted manuscript is an Original Article. The corresponding author of this manuscript is Andi Muhammad Ichsan (am_ichsan@med.unhas.ac.id) and contribution of the authors as mentioned below:

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With the submission of this manuscript, I would like to undertake that:

1. All authors of this paper have directly participated in the planning, execution, or analysis of this study;
2. All authors of this paper have read and approved the final version submitted;
3. The contents of this manuscript have not been copyrighted or published previously;
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6. There are no directly related manuscripts or abstracts, published or unpublished, by any authors of this paper;
7. My Institute's Department of Ophthalmology, Hasanuddin University, Makassar, Indonesia representative is fully aware of this submission.

Best regards,

Andi Muhammad Ichsan

Contribution Details:

	Contributor 1 Muhiddin H.S.	Contributor 2 Z.A. Rosmiaty	Contributor 3 Budu	Contributor 4 Sirajuddin J.	Contributor 5 Seweng A.	Contributor 6 Islam I.C	Contributor 7 Ichsan A.M
Concepts	√	√					√
Design	√	√					√
Definition of intellectual content	√	√	√	√			√
Literature search		√			√	√	
Clinical studies			√	√			
Experimental studies	-	-	-	-	-	-	-
Data acquisition	√						
Data analysis	√						√
Statistical analysis					√	√	
Manuscript preparation	√	√					√
Manuscript editing					√	√	
Manuscript review			√	√			√
Guarantor			√	√			