EUROMEDITERRANEAN BIOMEDICAL JOURNAL
2020,15 (34) 138–141
(FORMERLY: CAPSULA EBURNEA)

Original article

PLASMA LEVELS OF ANGIOGENIC AND ANGIOSTATIC FACTORS AFTER PANRETINAL PHOTOCOAGULATION FOR PROLIFERATIVE DIABETIC RETINOPATHY

Bhaskar Gaonkar [1], Krishnananda Prabhu [1], Pragna Rao [1], Asha Kamat [2], Krishna Rao [3], Muralidhar Varma [4]

1. Department of Biochemistry, Kasturba Medical College Manipal, Manipal Academy of Higher Education, Manipal, India
2. Department of Community Medicine, Kasturba Medical College Manipal, Manipal Academy of Higher Education, Manipal, India
3. Department of Ophthalmology, Kasturba Medical College Manipal, Manipal Academy of Higher Education, Manipal, India
4. Department of Medicine, Kasturba Medical College Manipal, Manipal Academy of Higher Education, Manipal, India

ARTICLE INFO

Article history:
Received 25 February 2020
Revised 22 August 2020
Accepted 04 September 2020

Keywords:
Diabetic Retinopathy, proliferative diabetic retinopathy, angiogenic factors, angiostatic factors, panretinal photocoagulation.

ABSTRACT

Panretinal photocoagulation in diabetic retinopathy improves oxygen saturation in the retina which results in regression of neovascularisation. This study is aimed at evaluating the plasma levels of angiogenic and angiostatic factors after panretinal photocoagulation for retinopathy. Thirty controls and 29 proliferative diabetic retinopathy subjects who were advised panretinal photocoagulation were recruited for the study. Plasma was collected both before and four weeks after the last dose of the therapy and analysed for angiogenic and angiostatic factors using ELISA technique. The plasma concentration of HIF-1α and VEGF were significantly decreased after laser therapy compared to baseline levels. The plasma concentration of MMP-9 and PEDF was increased significantly after photocoagulation. A significant improvement in the visual acuity was also observed following laser therapy. Panretinal photocoagulation significantly reduced plasma angiogenic and angiostatic factors. These factors may be used to assess therapeutic outcomes in proliferative retinopathy.

1. Introduction

Proliferative diabetic retinopathy is one of the major causes of visual impairment in diabetic subjects. Hyperglycemia induced oxidative stress that leads to microvascular damage is said to be the major cause for the pathogenesis of diabetic retinopathy **[1]**. Several studies have indicated an imbalance in the angiogenic and angiostatic factors which play a key role in the growth of unwanted, leaky capillary tuft over the retina which renders threat to vision [2]. Previous studies have reported that severe vision loss in the diabetic retinopathy can be successfully tackled using panretinal photocoagulation [3]. The therapy includes ablation of the peripheral retina with photoreceptors that consume more oxygen, using laser burns. Even though there is an irreversible damage to the outer retina, it was reported to improve oxygen saturation by channelling remaining blood flow to the central condensed part of the viable retina. This inhibits hypoxic stimuli and thereby decreases expression of angiogenic growth factors [2,4]. It was reported that intravitreal levels of VEGF-A are closely associated with the degree of retinal hypoxia and, with an effective laser photocoagulation therapy, the levels can be significantly decreased [5].

Several studies reported release of anti-angiogenic factors and downregulation of angiogenic factors in the retinas of diabetic subjects following laser photocoagulation [6-9]. The laser photocoagulation may exerts its beneficial effects by altering angiostatic and/or angiogenic factors, thus decreasing the hypoxia in the retina. The studies reporting plasma levels of angiogenic and angiostatic factors following laser photo coagulation are lacking. With this background, we aimed to assess plasma angiogenic and angiostatic factors in patients undergoing panretinal photocoagulation for proliferative retinopathy.

* *Corresponding author*: Bhaskar Gaonkar, *bskrgnkr@gmail.com*
DOI: 10.3269/1970-5492.2020.15.34

2. Material and methods

Study sample

This study was approved by Institutional ethics committee Kasturba Hospital Manipal and followed the recommendations of the Declaration of Helsinki for human studies. Written informed consent was obtained from each participant before enrollment. Twenty-nine proliferative diabetic retinopathy subjects who were advised for Laser therapy for PDR, in the age group 45 to 75 were recruited for the study.

Out of 29 subjects, 18 were males and 11 were females. The study also involved 30 age matched controls in which 20 were males and 10 were females. Patients with a previous history of laser therapy, cancer, heart diseases, chronic kidney disease, with signs of active infections and ocular diseases other than PDR, were excluded from the study. Both the eyes were examined by an ophthalmologist and retinopathy was confirmed by retinal fundus photographs. On the basis of the characteristics observed in the retinal fundus photographs and the *"International Diabetic Retinopathy Disease Severity Scale,"* the subjects were diagnosed to be PDR [4]. These subjects were advised LASER therapy.

LASER therapy and outcome measure

LASER photocoagulation was carried out in three to four sessions at an interval of ten days each, during which 2,500 burns of about 500 micron size are made on the outer retina in each session. The patients were called one month after the last session of LASER treatment, during which clinical improvement was evaluated by fundus examination and visual acuity assessment.

Visual acuity scores were assessed using the Snellen chart. Visual acuity scores were taken in meters and converted to LogMAR scores for the ease of comparison. Any post LASER change in the visual acuity as compared to pre-therapy was considered as a therapeutic outcome measure.

Collection of blood sample and analysis

After taking written informed consent, a random venous blood sample was drawn from the subjects, plasma was separated, aliquoted into appropriately labelled vials and stored at -70°C until analysis. Similarly, a random venous blood sample was also drawn one month after the last dose of LASER therapy and was used for the analysis. All the angiogenic and anti-angiogenic factors were estimated by using commercially available enzyme linked immune sorbent assay kits (*RayBio®*).

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) version 15 was used for statistical analysis. Paired t-test (for normally distributed data) or Wilcoxon signed rank test (for skewed data) was used to compare pre-therapy levels of variables with respective post-treatment levels in patients with diabetic retinopathy. The values were expressed as Mean±SD or Median (Interquartile range). A p-value less than 0.05 was considered statistically significant.

3. Results

The baseline characteristics indicated that the confounding factors such as, age, duration, HbA1C concentration, gender ratio are matching between the study groups. However, fasting blood glucose concentration was found to be higher in the LASER group (Table 1).

The plasma concentration of angiogenic factor, VEGF-A was significantly (p=0.036) lowered following pan-retinal photocoagulation. Similarly, significant (p=0.011) reduction in the plasma concentration of HIF-1α was also observed in the cases following LASER therapy (Table 2). On the contrary, the MMP-9 level was significantly (0.033) increased after photocoagulation (Table 2). The angiostatic factor PEDF was significantly (p=0.014) increased following photocoagulation (Table 2). The control group did not show any significant (p>0.05) difference in the plasma levels of angiogenic and angiostatic factors during the follow up.

Nevertheless, the angiogenic factors were apparently higher in the cases compared to controls while, the angiostatic factor, PEDF was lower (Table 2).

When the study groups were subjected for visual acuity analysis, it was found that the control group did not show any significant (p=0.178) alteration in the visual acuity scores compared to baseline. However, the cases showed a significant (p=0.036) improvement in the visual acuity following laser photocoagulation (Table 3).

	Controls	**LASER**
	N =30	**N=20**
Duration (years ± SD)	10.4±6.1	10.4±4.7
HbA1C (g%± SD)	8.8±1.5	9.7±2.7
Age (years± SD)	60.0±9.0	62.0±9.6
Gender n (%)		
- Male	20 (66%)	18 (62%)
- Female	10 (33%)	11 (38%)
Fasting blood glucose (mg/dL± SD)	183.0±74.0	139.0±30.0

Table 1. Baseline characteristics of study population

	Time point	**Controls n=30**	**p-value**	**LASER n=29**	**p-value**
VEGF-A pg/ml	Baseline	162.0 ±13.0	0.241	219.0 ±96.4	0.036
	One month	159.0±14.0		203.0 ±82.0	
HIF-1α pg/ml	Baseline	639.0 ±102.0	0.097	2450.0 ±589.0	0.011
	One month	650.0 ±99.0		2320.0 ±555.0	
MMP-9 pg/ml	Baseline	676.0±76.0	0.231	1248.0 ±370.0	0.033
	One month	684.0±64.0		1563.0 ±683.0	
PEDF μg/ml	Baseline	18.3 ±2.3	0.435	4.2 ±1.8	0.014
	One month	17.9 ±2.3		5.3 ±1.3	
Paired sample t-test, p<0.05 is statistically significant					

Table 2. Plasma concentration of angiogenic and angiostatic factors among cases and controls

	Time point	**Controls n=30**	**p-value**	**LASER n=29**	**p-value**
Visual acuity LogMar scores	Baseline	0.23±0.18	0.178	1.0 (0.8,1.3)	0.036
	One month	0.21±0.18		0.6 (0.6,0.7)	
Paired sample t-test, p<0.05 is statistically significant					

Table 3. Visual acuity differences in cases and controls following LASER photocoagulation

4. Discussion and conclusions

The present study indicated a decline in the plasma concentration of potent angiogenic factors, VEGF-A and HIF-1α following laser therapy. There was also an increase in the angiogenic factor, MMP-9 and angiostatic factor, PEDF in the cases. There are few studies which support these findings. Ishida *et al.* (1998), in their study on cultured retinal pigment epithelial cells, found that the cells, after photocoagulation release TGF-β2, supresses the expression of VEGF by the retinal pigment epithelium. Their in vitro study also showed similar results [7]. Another study showed a reduction in the inflammatory cytokines produced by retinal microglial cells following laser therapy, indicating laser induced deactivation of microglial cells, thereby a decrease in the levels of cytokines[10]**.** Spranger *et al. (*2000) also reported release of angiostatin, an anti-angiogenic factor and a decreased VEGF-A in the subjects with previous photocoagulation [9]. Ogata et al. (2001), in their invitro study, found that following laser photocoagulation there is a transient increase in the levels of angiogenic factors including VEGF [11]. However, the levels come back to baseline within 72 hours of photocoagulation. They have also showed that the laser photocoagulation causes a transient increase in the expression of anti-angiogenic PEDF in cultured human pigment epithelial cells. However, the expression of PEDF was higher, even after weeks in the rat retinas [8]. Most of the available literature regarding laser photocoagulation display study of vasoactive factors in the vitreous of the eye or cultured retinal cells. But, there is one study which indicated the reduction in the serum VEGF concentration following successful laser therapy [12]. Nevertheless, conflicting findings are reported by few studies. Shimura M et al. did not find any significant difference in the ocular VEGF concentration following panretinal photocoagulation when compared with controls [13]. Increase in the MMP-9 following laser therapy may be potentially related to retinal tissue remodelling process. The release of MMP-9 is probably from the pigment epithelial cells near the laser affected area responding to the laser induced tissue damage. But there are no studies reporting effect of laser therapy on plasma/vitreous MMP-9 levels in diabetic retinopathy. A significant improvement in the visual acuity was also noted in the cases following photocoagulation. That would indicate a significant improvement in the retinopathy. However, several studies have reported conflicting results from the current study. Soman et al., (2012) noticed a significant fall in the visual acuity seven days after panretinal photocoagulation, which was regained after three months [14]. But Lee SB et al. did not find any influence of PRP on visual acuity in severe DR subjects [15]. Improvement in visual acuity in the current study may imply an amelioration of the pathological insult caused due to proliferative retinopathy. Several studies have indicated that once the high oxygen consuming photoreceptors are destroyed by laser, the dead tissue is replaced with glial cells which consume less oxygen. Now, the oxygen from the choroid can easily get diffused through the glial cell layer and cater inner viable retina. The improved oxygen saturation suppress expression of angiogenic factors [16, 17].

Several studies have reported a decline in the expression of angiogenic factors and an increase in the anti-angiogenic factors in the vitreous following panretinal photocoagulation. Results of the current study showed that the same is reflected in the plasma of the patients following panretinal photocoagulation. This indicates that these factors may be used to assess the treatment outcome following laser therapy.

References

1. Eshaq RS, Aldalati AM, Alexander JS, Harris NR, Diabetic retinopathy: Breaking the barrier. Pathophysiology. 2017;24(4):229-241.
2. Heng LZ et al. Diabetic retinopathy: pathogenesis, clinical grading, management and future developments. Diabetic Medicine. 2013;30(6):640-50.
3. Malleswari B, Jyothi NA, Macular thickness changes following panretinal photocoagulation in patients with severe diabetic retinopathy. Journal of evolution of medical and dental sciences. 2018; 7(48):5145-9.
4. Ciulla TA, Amador AG, Zinman B, Diabetic retinopathy and diabetic macular edema: pathophysiology, screening, and novel therapies. Diabetes Care. 2003;26(9):2653–64.
5. Aiello LP et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. The new England journal of medicine. 1994;331(22):1480–7.
6. Yoshimura N, Matsumoto M, Shimizu H, Mandai M, HataY, Ishibashi T, Photocoagulated human retinal pigment epithelial cells produce an inhibitor of vascular endothelial cell proliferation. Investigative ophthalmology & visual science. 1995;36(8):1686-1691.
7. Ishida K, Yoshimura N, Yoshida M, Honda Y,Upregulation of transforming growth factor-beta after panretinal photocoagulation. Investigative ophthalmology & visual science. 1998;39(5):801-807.
8. Ogata N, Tombran-Tink J, Jo N, Mrazek D, Matsumura M,Upregulation of pigment epithelium-derived factor after laser photocoagulation. American journal of ophthalmology. 1998;132(3):427-429.
9. Spranger J, Hammes HP, Preissner KT, Schatz H, Pfeiffer AFH, Release of the angiogenesis inhibitor angiostatin in patients with proliferative diabetic retinopathy: association with retinal photocoagulation. *Diabetologia.* 2000;43(11):1404-1407.
10. Midena E, Micera A, Frizziero L, Pilotto E, Esposito G, Bini S, Sub-threshold micropulse laser treatment reduces inflammatory biomarkers in aqueous humour of diabetic patients with macular edema. Scientific reports. 2019;9(1):1-9.
11. Ogata N, Ando A, Uyama M, Matsumura M, Expression of cytokines and transcription factors in photocoagulated human retinal pigment epithelial cells. Graefe's archive for clinical and experimental ophthalmology. 2001;239(2):87-95.
12. Manaviat MR, Rashidi M, Afkhami-Ardekani M, Mohiti-Ardekani J, Bandala-Sanchez M, Effect of pan retinal photocoagulation on the serum levels of vascular endothelial growth factor in diabetic patients. International ophthalmology. 2011;31(4):271-275.
13. Shimura M et al. Panretinal photocoagulation induces pro-inflammatory cytokines and macular thickening in high-risk proliferative diabetic retinopathy. Graefe's Archive for Clinical and Experimental Ophthalmology. 2009;247(12):1617.

14. Soman M, Ganekal S, Nair U, Nair KGR. Effect of panretinal photocoagulation on macular morphology and thickness in eyes with proliferative diabetic retinopathy without clinically significant macular edema. Clinical Ophthalmology (Auckland, NZ). 2012;6:2013.