**Cukurova Medical Journal**
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2019;44(3):745-752
DOI: 10.17826/cumj.472840

**ARAŞTIRMA / RESEARCH**

# The value of VEGF and IGF-1 in the diagnosis retinopathy of prematurity and follow-up of response to laser therapy

## Prematür retinopatisinin tanısı ve lazer tedavisine cevabın takibinde VEGF ve IGF-1'in değeri

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*Cukurova Medical Journal 2019;44(3):745-752.*

**Abstract**

**Purpose:** Early recognition of the retinopathy of prematurity (ROP), timely and appropriate treatment will contribute to the developmental process of the infant and increase the quality of life by preventing vision loss. We here by aimed to figure out the value of the blood levels of vascular endothelial growth factor (VEGF) and insulin-like growth factor-1 (IGF-1) in infants with severe ROP requiring laser therapy.

**Materials and Methods:** Very low birth weight infants (VLBW, ≤1500 g and gestational age ≤32 weeks) were included in the study. Blood samples for the evaluation of markers were obtained from the cord at birth for all preterms and at the same postpartum corrected age (34 weeks) for ROP and control group.

**Results:** The mean serum VEGF level was 185.6 ± 88.1 pg/mL in the control group and 590.2 ± 97.4 pg/mL before laser treatment in the ROP group . It was decreased to 83.7 ± 25.4 pg/mL at 4 day after laser treatment in the ROP group). No significant difference was existed between the values of IGF-1 before and after laser treatment in infants with severe ROP.

**Conclusion:** Serum VEGF and IGF-1 levels may be suggested as a sensitive marker of severe ROP. Additionally, the decrease in serum levels of VEGF after laser therapy can be used as an indicator for the efficacy of laser treatment.

**Keywords:** insulin-like growth factor-1, retinopathy of prematurity, vascular endothelial growth factor

**Öz**

**Amaç:** Prematüre retinopatisinin (ROP) erken tanınması, zamanında ve uygun tedavisi, bebeğin gelişim sürecine katkıda bulunacak ve görme kaybını önleyerek yaşam kalitesini artıracaktır. Biz burada lazer tedavisi gerektiren şiddetli ROP olan bebeklerde vasküler endotelyal büyüme faktörü (VEGF) ve insülin benzeri büyüme faktörü-1'in (IGF-1) kan düzeylerinin değerini belirlemeyi amaçladık.

**Gereç ve Yöntem:** Çok düşük doğum ağırlıklı bebekler (ÇDDA, ≤1500 g ve gestasyonel yaş ≤32 hafta) çalışmaya alındı. ROP ve kontrol grubu için tüm pretermler için doğumdan itibaren ve aynı doğum sonrası düzeltilmiş yaşta (34 hafta) belirteçlerin değerlendirilmesi için kan örnekleri alındı.

**Bulgular:** Kontrol grubunda ortalama serum VEGF düzeyi 185,6 ± 88,1 pg / mL ve ROP grubunda lazer tedavisi öncesi 590,2 ± 97,4 pg / mL idi. ROP grubunda lazer tedavisinden 4 gün sonra 83,7 ± 25,4 pg / mL'ye düştü Şiddetli ROP'lu bebeklerde lazer tedavisi öncesi ve sonrasında IGF-1 değerleri arasında anlamlı fark yoktu.

**Sonuç:** Serum VEGF ve IGF-1 seviyeleri, şiddetli ROP'un duyarlı bir belirteçleri olarak önerilebilir. Ek olarak, lazer tedavisinden sonra VEGF'nin serum seviyelerindeki azalma, lazer tedavisinin etkinliği için bir gösterge olarak kullanılabilir.

**Anahtar kelimeler**: insülin benzeri büyüme faktörü-1, prematürite retinopatisi, vasküler endotelyal büyüme faktörü

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Geliş tarihi/Received: 20.10.2018 Kabul tarihi/Accepted: 28.12.2018 Çevrimiçi yayın/Published online: 23.08.2019

## INTRODUCTION

Retinopathy of prematurity (ROP) is a pathophysiologic condition of the retina due to abnormal proliferation of retinal vessels and affects particularly preterm infants. Although, its pathogenesis cannot be fully understood, it is believed to be multifactorial. Prematurity with lower birth weight (BW) (<1000g) and gestational age (GA) (<28 weeks) are the best known risk factors[1]. Additionally, longer period of assisted ventilation and oxygen therapy with inappropriate concentration, bronchopulmonary dysplasia (BPD), hyperoxia/hypoxia, hypercapnia/hypocapnia, asphyxia, hypothermia, metabolic acidosis, surfactant therapy, sepsis/meningitis, systemic fungal infections, high blood transfusion volume, cumulative illness severity, hyperglycemia, insulin therapy and early erythropoietin use have been reported to be independently related with high risk factors for ROP as well as genetic predisposition[2].

Growth factors play an important role in retinal neovascularization. Vascular endothelial growth factor (VEGF) is an endothelium-specific mitogen. VEGF is considered to be one of the most important ocular angiogenic factors, especially under hypoxic conditions[3]. And also, VEGF has been determined to be an important pathologic growth factor contributing neovascularization and the development of ROP[4]. One of the other important angiogenic factors involved in ocular neovascularization is insulin-like growth factor-1 (IGF-1). Low levels of IGF-1 in preterm infants are associated with increased risk of developing ROP and are associated with poor vascular development and a large avascular retinal area[5,6].

Early recognition of ROP, timely and appropriate treatment will contribute to the developmental process of the infant and increase the quality of life by preventing vision loss. We here by aimed to figure out the value of the blood levels of VEGF, IGF-1in infants with severe ROP requiring laser therapy.

## MATERIALS AND METHODS

This prospective nested case–control study was conducted in the neonatal intensive care unit (NICU) of Zekai Tahir Burak Maternity Teaching Hospital, Ankara, Turkey, between May 2017 and August 2018. The NICU at our hospital is a referral Level III facility with 130 incubators, treating approximately 4000 newborn patients per year. Ethical approval was obtained from hospital local ethical committee. Informed consent was obtained from all parents of patients who were eligible for the study. Helsinki Declaration principals were followed.

Very low birth weight infants (VLBW, ≤1500 g and GA ≤32 weeks) were included in the study. We excluded patients for whom a parental consent was not obtained and known major congenital defects. Study group included (BW ≤1500g and GA ≤32 weeks) premature newborns applied laser photocoagulation treatment for ROP. Control group was constituted by premature infants of comparable GA born without ROP. Moreover, preterm infants in control group were over 28 days of postnatal age, clinically stable with full oral feeding, non-infected, and no exposed to invasive and non-invasive respiratory support. Blood samples for the evaluation of markers were obtained from the cord at birth for all preterms and at the same postpartum corrected age (34 weeks) for ROP and control group.

General supportive care was applied to all patients according to the NICU's protocol. Demographic and clinical characteristics of the patients included: GA, BW, gender, mode of delivery, 1st and 5th minute Apgar scores, presence of antenatal steroid administration, full enteral feeding time, and length of NICU stay were recorded. Prematurity related outcomes, such as death, respiratory distress syndrome (RDS; surfactant administration), patent ductus arteriosus (PDA; medical or surgery treatment), necrotizing enterocolitis (NEC; staging according to Bell[7]), BPD (moderate/severe; oxygen need at 36 weeks of postconceptional age[8]), intraventricular hemorrhage (IVH; staging according to Papile[9]), ROP (defined according to the International Classification[10]) were also mortality recorded. Zones and stages of ROP were recorded for the study groups.

**ROP classification: zones, stages**

The International Classification of Retinopathy of Prematurity (ICROP) was revised in 2005. A committee for ROP classification proposed an international classification of ROP (ICROP) by dividing the retina into three zones, extending from posterior to anterior retina and describing the extent

of ROP in clock-hours of involvement. ROP severity is described in stages 1 to 5[10].

Plus disease: It is an indicator of severity of the disease and is defined as venous dilation and arterial tortuosity of the posterior pole vessels. Pre-plus disease: It is defined as posterior pole vascular dilation and tortuosity which is more than normal but less than plus disease. Aggressive posterior ROP: Rapidly progressive, form of ROP previously referred to as "rush disease". It is characterized by a posterior location, severe plus disease, and flat intraretinal neovascularization[10].

**Screening for ROP**

ROP screening should start by 31 week post-conceptional age or 4 week after birth, whichever is later[11]. All preterm infants with ≤32 weeks of gestation and ≤1500 g of birth weight were routinely examined for ROP by the same experienced ophthalmologist.

**Examination technique**

The examination technique traditionally involves two steps namely the dilatation of pupil and indirect ophthalmoscopy preferably with a 28D lens. It is preferred to perform pupillary dilatation 45 min prior to commencement of the screening. Dilating drops used are a mixture of cyclopentolate (0.5%) and phenylephrine (2.5%) drops to be applied two to three times about 10-15 min apart. Alternatively, tropicamide (0.4%) may be used instead of cyclopentolate. Diluted cyclopentolate may also be used to reduce probable systemic adverse effects. Use of atropine is to be avoided. The neonatal nurse should be instructed to wife any excess drops from the eye lid to prevent systemic absorption and complications like tachycardia and hyperthermia. If the pupil is resistant to dilatation, it may indicate presence of persistent iris vessels (tunica vasculosa lentis) and must be confirmed by the ophthalmologist before applying more drops[11].

**Laser photocoagulation treatment for ROP**

Following are the treatment stages of ROP.

Threshold ROP: The cryotherapy for retinopathy of prematurity (CRYO-ROP) study[12] stated that treatment should be imparted to eyes with threshold disease, defined as stage 3 ROP in zone I or II, having five contiguous or eight discontiguous clock hours with plus disease. This was the previous "cut off" for treatment.

Pre-threshold ROP: The early treatment for retinopathy of prematurity (ETROP) study[13] redefined these guidelines. They defined the actively treatable and observational types of pre-threshold ROP as "type 1" (high-risk prethreshold ROP) and "type 2" ROP respectively. "Type 1 ROP" is defined as: (1) Any stage of ROP in zone I with plus disease; or (2) Stage 3 in zone I without plus; or (3) Stages 2 or 3 in zone II with plus disease. These are the modified guidelines for treatment. "Type 2 ROP" is defined as stages 1 or 2 in zone I without plus, or stage 3 in zone II without plus. These can be observed and watched at one week or less follow-up. Cases having stages 1 or 2 in zone II require two weekly follow up, while stages 1 or 2 in zone III require three[11].

Before laser treatment, 2.5% phenylephrine (Mydfrin®, Alcon, USA) and 0.5% tropicamide (Tropamid®, Bilim İlaç, Turkey) were locally applied 3 times at 10-minute intervals. After sedation with 0.1 mg / kg midazolam intravenous bolus, infants were intubated by a neonatologist. While vital signs and the level of analgesia were monitoring, intravenous infusion of remifentanil 0.2 to 0.6 mg / kg / min was administered under the supervision of the neonatologist. Subsequently, appropriate sedoanalgesia and pupillary dilation, local anesthesia was performed with 0.5% propancain hydrochloride (Alcaine®, Alcon, USA) prior to laser treatment. A 810 nm diode laser (OcuLight SL; Iridex, Mountain View, CA) attached to binocular indirect ophthalmoscope (Omega®500, Heine Optotechnik, Herrsching, Germany) was used for laser photocoagulation. The laser duration was adjusted to 200 ms and laser power to 150-250 mW for ablation of peripheral retina. The mean number of laser shots for an eye was 1472±478. Following laser therapy, netilmicin or tobramycin drops were applied for anti-microbial treatment, dexamethasone drops for anti-inflammatory treatment and cyclopentolate drops for dilation of pupils during one week.

**Laboratory analysis**

Obtained blood samples were kept in simple tubes without any separation gel and preservatives, and waited for clotting for 30-45 minutes at room temperature. Subsequently, clotted samples were centrifuged at 3,000 rpm at room temperature for 10 minutes. Supernatants were separated and stored at −80 °C until their measurements. Samples containing a trace amount of hemolysis were

discarded. Serum samples were obtained postnatal week 4 in the control group, and baseline serum samples were drawn one day before laser photocoagulation therapy in the ROP group. And also, serum samples were obtained four days after laser photocoagulation therapy.

Serum VEGF, IGF-1 levels were measured by enzyme-linked immunosorbent assay (ELISA) (ELISA divice, washer-reader Awareness Chromate model, Awereness Stafax 2600 model, Awareness Technology Inc., Palm City, FL, USA), Serum VEGF and IGF-1 concentrations were analysed by an ELISA kits for human anti-VEGF detecting the 121 and 165 isoforms of VEGF (Human VEGF Immunoassay and Human IGF-1; R&D Systems, Minneapolis, MN) according to the manufacturer's instructions.

### Statistical analysis

Statistical analysis was performed by SPSS 15.0 statistical package program (Chicago, IL, USA). The normal distribution of variables was tested with Shapiro-Wilk test. Descriptive statistics were given as mean and standard deviation (SD) or median interquartal range (IQR) and min-max; categorical variables were given as values and percent. Mann–Whitney U test was used for intergroup comparisons of nonparametric variables. To compare dependent groups, Friedman test and Wilcoxon test were used. Values of $P<0.05$ were considered significant.

## RESULTS

We excluded patients for whom a parental consent was not obtained (n = 5), known major congenital defects (n = 3). A final number of 103 subjects were included in analysis. During the study period, 51 infants were identified as the ROP group, and 52 infants were allocated as the control group (Figure 1). A total of 101 eyes of 51 infants (50 bilaterally, 1 unilaterally) were treated in this study.

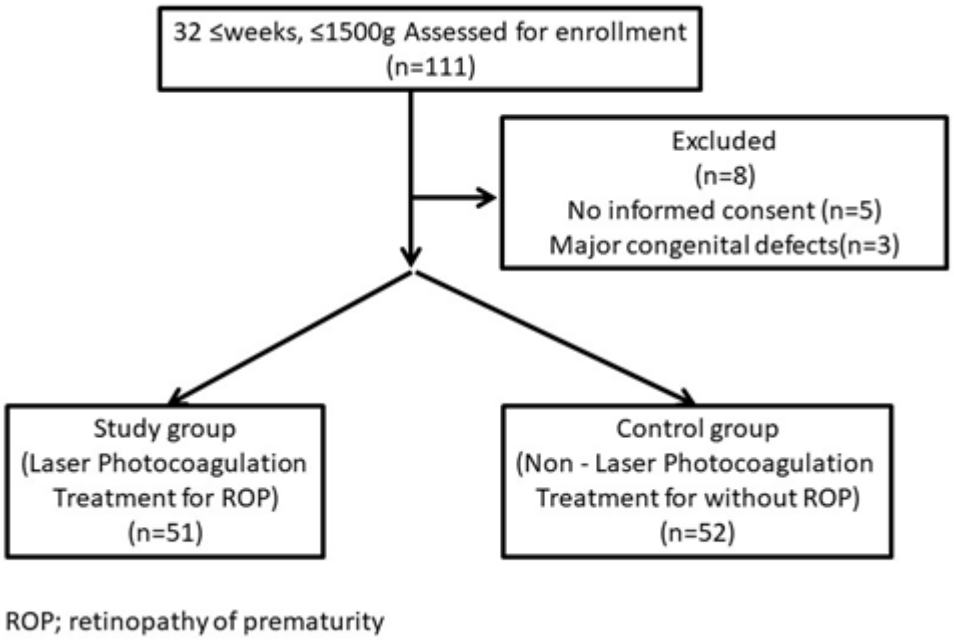


**Figure 1. Flow chart of the study population including ROP and control groups.**

There was no statistically significant difference between the groups in terms of gender, modes of delivery (normal vaginal delivery or cesarean section), resuscitation requirement, antenatal steroid application, mothers' age (year), multiple pregnancy, maternal infection (urinay system infection,

corioamnionitis), PPROM (preterm premature rupture of membranes), maternal preeclampsia (P> 0.05). The mean GA was 26.2±1.6 weeks (range, 24-28 weeks) in the ROP group and 29.2 ± 1.8 weeks (range, 25-30 weeks) in the control group, respectively. The mean GA was found to be lower in infants with diagnosed ROP (P <0.05). When BW was evaluated between both groups, it was 898 ± 245 g (range, 585-1620 g) in infants with ROP which was significantly lower than those in the control group (1290 ± 3215 g; range, 640-1850 g) (P <0.05). Moreover, no significant differences were found between ROP and control groups related to co-mordities involving PDA, RDS, IVH, NEC (P>0.05), except BPD (P<0.05). Furthermore, duration of mechanical ventilation therapy, duration of oxygen therapy and duration of hospitalization were significantly higher in the ROP group (P<0.05) (Table 1).

**Table 1. Demographic variables of the groups**

| **Variables** | | **Control (n=52)** | **ROP (n=51)** | **P** |
|---|---|---|---|---|
| Gender,[a] | Male | 20 (38.5) | 21 (41.1) | 0.512 |
| Birth weight, (g),[b] | | 1290 ± 315 | 898 ± 245 | **<0.001*** |
| Gestational age, (week),[b,c] | | 29.2 ± 1.8 (25-30) | 26.2 ± 1.6 (24-28) | **<0.001*** |
| Modes of delivery,[a] | NVD | 21 (40.4) | 22 (43.1) | 0.611 |
| | C/S | 31 (59.6) | 29 (56.9) | |
| APGAR 1st Min.[d] | | 6 (1) (3-8) | 5 (1) (2-6) | **<0.001*** |
| APGAR 5th Min.[d] | | 8 (1) (5-9) | 7 (1) (3-8) | **<0.001*** |
| Mothers'age, (year),[b] | | 27.6 ± 5.6 | 28.4 ± 8.1 | 0.201 |
| Multiple pregnancy, [a] | | 6 (11.5) | 8 (15.6) | 0.122 |
| Resuscitation, [a] | Performed | 31 (59.6) | 35 (68.6) | 0.187 |
| Antenatal steroid, [a] | Performed | 32 (61.5) | 27 (53) | 0.318 |
| Maternal infection, [a] | | 20 (38.4) | 23 (45.1) | 0.219 |
| PPROM, [a] | | 6 (11.5) | 5 (9.8) | 0.427 |
| Maternal preeclampsia, [a] | | 8 (15.4) | 8 (15.6) | 0.841 |
| Mechanical ventilation therapy (day),[b] | | 5.2 ± 2.4 | 22.1 ± 12.4 | **0.001*** |
| Oxygen therapy (day),[b] | | 20.5 ± 12.4 | 78.2 ± 30.9 | **<0.001*** |
| Duration of hospitalization (day),[b] | | 61.5 ± 24.7 | 109.4 ± 29.8 | **0.001*** |
| Co-morbidities, | PDA | 25 (48.1) | 26 (51) | 0.561 |
| | RDS | 30 (57.7) | 35 (68.6) | 0.117 |
| | IVH | 10 (19.2) | 11 (21.5) | 0.613 |
| | BPD | 17 (32.6) | 30 (58.8) | **0.034** |
| | NEC | 7 (9.5) | 9 (12.3) | 0.242 |

[a] n (%), [b] mean ± SD, [c] min-max, [d]median (IQR) (min-max), *P<0.05 was considered statically significant. NVD, normal vaginal delivery; C/S, cesarean section; PDA, patent ductus arteriosus; RDS, respiratory distress syndrome; IVH, intraventricular hemorrhage; BPD, bronchopulmonary dysplasia; NEC, necrotizing enterocolitis; ROP, retinopathy of prematurity; PPROM, preterm premature rupture of membranes; IQR, interquartile range; SD, standard deviation.

During the follow-up, 51 patients were recognized ROP and underwent laser photocoagulation therapy. We obtained blood samples to assess serum levels of VEGF and IGF-1 for both groups (control and ROP), and compared the results. The mean cord VEGF levels was 30.2 ± 9.8 pg/mL in the control group and 59.8 ± 24.7 pg/mL in the ROP group (p<0.001). The mean serum VEGF level was 185.6 ± 88.1 pg/mL in the control group and 590.2 ± 97.4 pg/mL before laser treatment in the ROP group (p=0.001). It was decreased to 83.7 ± 25.4 pg/mL at 4 day after laser treatment in the ROP group (p<0.001). No significant differences were observed between infants receiving laser photocoagulation bilaterally and unilaterally in terms of plasma VEGF. The mean cord blood IGF-1 level of infants in the ROP group was 23.2 ± 4.2 µg / L, and it was 31.5 ± 13.4 µg / L in the control group (p=0.08). The mean level of IGF-1 was 40.1 ± 3.3 µg/L in infants control group, and it was 26.4 ± 2.9

μg/L in infants with severe ROP before laser treatment (p=0.001) (Table 2). Moreover, our results determined that no significant difference was existed between the values of IGF-1 before and after (26.4 ± 2.9 μg/L vs 27.4 ± 2.1 μg/L) laser treatment in infants with severe ROP (p=0.554).

**Table 2. Biochemical (VEGF and IGF-1) markers evaluated and compared between ROP and control groups**

| Variables | Control (n=52) | ROP (n=51) | P |
|---|---|---|---|
| VGEF (pg/mL),[a] (Cord level) | 30.2 ± 9.8 | 59.8 ± 24.7 | <0.001* |
| IGF-1 (μg/L),[a] (Cord level) | 31.5 ± 13.4 | 23.2 ± 4.2 | 0.08 |
| VGEF (pg/mL),[a] (Before laser treatment level) | 185.6 ± 88.1 | 590.2 ± 97.4 | 0.001* |
| IGF-1 (μg/L),[a] (Before laser treatment) | 40.1 ± 3.3 | 26.4 ± 2.9 | 0.001* |

[a] mean ± SD, *P<0.05 was considered statically significant.; VEGF, vascular endothelial growth factor; IGF-1, insulin-like growth factor-1; ROP, retinopathy of prematurity; SD, standard deviation.

## DISCUSSION

In our study, the average GA and BW in the ROP group were lower. More over, the present resultes showed that the proportion of morbidities such as BPD was higher in the ROP group. These findings supported previously known data. Although many etiologic factors have been considered in the development of ROP, the best known risk factors are low BW and GA. It is known that the incidence of retinopathy is significantly increased in infants born less than 1000 g and early gestational age like less than 28 weeks[2,14]. Additionally, more severe forms of ROP in infants with BPD develope more frequently than non-BPD infants, and a good relation between ROP and BPD has been determined[15].

Retinopathy of prematurity has two separate postnatal phases. In phase 1, the nutrients and growth factors provided by maternal-fetal interface are lost with birth. Subsequently, in addition to inhibiting retinal vascularization due to more hyperoxic extrauterine enviroment, some of the advanced vessels are also lost[2]. In phase 2, retina is poorly vascularized and hypoxic due to postnatal development of oxygen and nutrient demanding neurons[16,17]. Hypoxia stimulates expression of the oxygen-regulated factors such as VEGF, which stimulate retinal neovascularization. VEGF is a hypoxia-induced cytokine and known as a vascular endothelial cell mitogen. VEGF should be present for normal vascular development in the retina. Therefore, VEGF has an important role in phase II during neovascularization[18,19]. Serum concentrations of VEGF in infants with severe ROP has been found to be high when the ROP was first detected[20]. The relationship between VEGF level in cord blood and serum plasma specimens and development of ROP was investigated in many studies. However, there are some conradictory results. Our results provided that cord blood VEGF levels were higher in infants with severe ROP in addition to increased circulating VEGF levels in infants with severe ROP required laser therapy. Our findings supported the evidence that VEGF levels were an important contribuiting factor in the pathogenesis of severe ROP. However, further studies may be need to figure out the relationship between ROP and VEGF levels.

Insulin-like growth factor 1 is very important for somatic growth, bone, neural and vascular development during both fetal and postnatal life. Unfortunatelly, IGF-1 serum levels drop fastly immediate after preterm birth because of interruption of the maternal-fetal connection[21]. The role of IGF-1 in retinal angiogenesis is supported by the observation that IGF-1 is required for maximum VEGF activation of vascular endothelial cell proliferation and survival pathways[22]. IGF-1 concentrations increase slowly from extremely low concentrations after preterm birth to concentrations high enough to allow activation of VEGF pathways. This hypoxic phase of ROP can lead to retinal detachment and blindness[16]. IGF-1 regulates retinal revascularization by controlling the activation of VEGF. Low IGF-1 levels in serum of preterm infants are found to be related to the development and severity of ROP[5,6]. In our study, cord blood IGF-1 levels were lower in infants with severe ROP, and circulating IGF- 1 values were also found to be lower in the patients with severe ROP at the time of diagnosis. This result supported that IGF-1 values, which were low from birth, were effective in the development of ROP. It has been shown that minimal IGF-1 levels are required for VEGF release and that low levels of IGF-1 inhibit vascular growth despite VEGF presence[5].

Plasma and serum VEGF levels have been reported to be decreased in infants with advenced ROP after laser therapy[23,24]. In our study, all infants with serious ROP were treated with laser therapy. It was seen that the levels of VEGF which was high before the laser treatment decreased after the treatment. Our results suggest that VEGF can be used for the following of efficacy and response to treatment.

In conclusion, current evidence suggests that VEGF are associated with pathophysiology in the pathogenesis of ROP. In this study, the efficacy of markers such as VEGF and IGF-1 in preterm infants were investigated. We determined that serum VEGF and IGF-1 levels may be suggested as a sensitive markers of severe ROP. Additionally, the decrease in serum levels of VEGF after laser therapy can be used as an indicator for the efficacy of laser treatment. However, additional prospective and controlled studies including larger series are required.

**Yazar Katkıları:** Çalışma konsepti/Tasarımı: CT; Veri toplama: ÖÖ; Veri analizi ve yorumlama: UÇ; Yazı taslağı: CT; İçeriğin eleştirel incelenmesi: UÇ; Son onay ve sorumluluk: CT, UÇ, ÖÖ; Teknik ve malzeme desteği: ÖÖ; Süpervizyon: CT; Fon sağlama (mevcut ise): yok.
**Bilgilendirilmiş Onam:** Katılımcılardan yazılı onam alınmıştır.
**Hakem Değerlendirmesi:** Dış bağımsız.
**Çıkar Çatışması:** Yazarlar çıkar çatışması beyan etmemişlerdir.
**Finansal Destek:** Yazarlar finansal destek beyan etmemişlerdir.
**Author Contributions:** Concept/Design : CT; Data acquisition: ÖÖ; Data analysis and interpretation: UÇ; Drafting manuscript: CT; Critical revision of manuscript: UÇ; Final approval and accountability: CT, UÇ, ÖÖ; Technical or material support: ÖÖ; Supervision: CT; Securing funding (if available): n/a.
**Informed Consent:** Written consent was obtained from the participants.
**Peer-review:** Externally peer-reviewed.
**Conflict of Interest:** Authors declared no conflict of interest.
**Financial Disclosure:** Authors declared no financial support

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