# Plasma Leptin Concentrations in Preterm Infants with Retinopathy of Prematurity (ROP)

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## Abstract

### Introduction
Retinopathy of prematurity (ROP) is a postnatal disorder of retinal vessels that develops in the incompletely vascularized retina of preterm infants. This disorder regresses in most patients but can lead to severe visual impairment. There is evidence that leptin stimulates angiogenesis.
Objective: This study was conducted to determine blood levels of leptin in premature infants with proliferative ROP.

### Materials and Methods
Blood samples were obtained 6-8 weeks after birth from 71 preterm infants born at or before 32 weeks of gestation. These infants consisted of two groups according their eye examination results. The control group consisted of 41 neonates without evidence of ROP and the case group included 30 patients with proliferative ROP at stage III or more. Plasma leptin concentrations were measured using enzyme-linked immunosorbent assay (ELISA).

### Results
The mean gestation age of studied patients at birth were 28.4±1.6 wk and 28.8±1.6 in case and control group respectively (P= .25). The mean post menstrual age of studied patients at blood sampling was 34.9±1.2 wk in the case group and 34.6±1.3 wk in the control group (p=0.33). Mean blood levels of leptin were not significantly different among patients of the two groups (0.64±0.41 ng/ml in case group and 0.79±0.83 ng/ml in control group respectively,p=0.39).

### Conclusion
Our data demonstrated that plasma leptin concentrations were not significantly different in premature infants with proliferative ROP at 6-8 weeks after birth from premature infants without ROP at this age.

### Key words
Retinopathy of prematurity, Preterm infant, Leptin, Angiogenesis

## Introduction
Despite recent advances in neonatal intensive care, the survival of preterm infants increased with no significant reduction in major morbidities such as retinopathy of prematurity (ROP), visual impairment and cognitive disabilities.[1] ROP is a retinal vaso-proliferative disease associated with preterm birth. It is a major cause of blindness in children in the developed and developing countries. In addition to preterm birth and low birth weight, ROP associated with higher risk for respiratory conditions, chronic respiratory disease, intra ventricular hemorrhage and blood transfusion.[2]

Aberrant neo-vascularization is the hallmark of proliferative retinopathy as seen in ROP. Vascular endothelial growth factor (VEGF) is viewed as the major effector for ocular neovascularization.[3,4] Insulin like growth factor-1 (IGF-1) is main factor involved in the fetal tissue growth, including the retina. Premature birth results in a sudden fall in IGF-1 serum levels and development the first stage of ROP.[5,6] Beside these, other mediators such as erythropoietin, angiotensin II, endothelins and leptin have been found to stimulate angiogenesis.[7]
Leptin, a 16 KDa protein of 167 amino acid, is an adipocyte derived hormone first discovered by

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Zhang et al in 1994.[8] Leptin regulates body weight and its serum levels reflect the amount of adipose tissue and positively correlate with the insulin resistance. Leptin is involved in a number of diverse physiologic processes, including endocrine functions, inflammation, immune responses and angiogenesis.[9,12] It up regulates various pro-angiogenic factors such as CC-chemokine ligand Z, while it synergistically stimulates angiogenesis with vascular endothelial growth factor, indicating that leptin may contribute to the promotion of neo vascularization processes.[13] The exact role of leptin in retinal neo-vascularization is still not completely understood. This study aims to determine the relationship between serum leptin levels and the presence of proliferative ROP in preterm newborn infants.

**Material and methods**

All Preterm infants whose gestational age were 32 weeks of gestation or less and were examined for diagnosis of ROP in the neonatal intensive care unit (NICU) of Al-Zahra university teaching hospital, Tabriz, Iran from March 2009 to June 2010 were eligible for inclusion in this study. Newborn infants with chromosomal anomalies or major congenital malformations were excluded from the study. During the 18-month study period,30 preterm newborn infants diagnosed with proliferative ROP were enrolled in the case group. Patients with ROP stage I or II were excluded from study. Forty-one infants with normal ophthalmologic examinations were randomly selected as controls. Gestational age was determined by either the maternal last menstrual period or first trimester ultrasound examination and confirmed by neonatal examination using Ballard gestational age scoring.[14] Each infant was examined initially when aged 4 weeks and repeated examinations were done at 2 week intervals if retinal vessels have grown only into zone II or every week in infants with vessels only as far as zone I. All eye examinations were performed by an ophthalmologist experienced in evaluating infants for ROP who had no knowledge of the study design. ROP was classified according to the international classification and subdivided into stage I (demarcation line), stage II (ridge), stage III (ridge with external fibro vascular proliferation), stage IV (subtotal retinal detachment) or stage V (total retinal detachment).[15] Classification was done according to the most advance ROP stage observed. Proliferative ROP was defined as stage III or more. All infants with ROP stage III or more were enrolled in the case group. The ethic committee of Tabriz University of Medical Sciences approved the study and written informed parental consent was obtained in all cases.

Blood samples were collected at the time of follow up eye examination at 6-8 weeks of birth. The samples were centrifuged at 3000 rpm and stored at -70 degrees centigrade. The laboratory analysis was done by one blinded to the study.

Leptin concentrations were measured by quantitative sandwich enzymatic immunoassay technique (ELISA) by using leptin kits (Mediagnost, Germany) with a sensitivity of 0.2 ng/ml and inter-assay coefficients of 2.55%.

Statistical analyses were carried out using SPSS package version 15. Data were expressed as mean± SD and number (%).Continuous variables were compared using standard nonparametric statistics for paired and unpaired samples t-test. Frequency of data were compared using the chi-square test. P-values less than 0.05 were considered to be statistically significant.

**Results**

Seventy-one infants were included in this study, including 41 preterm neonates without ROP (control group) and 30 patients with proliferative ROP (case group). The mean gestation age (GA) and birth weight in case group was 28.2±1.6 weeks and 1120.7±197 gram and 28.4±1.6 weeks 1189.4±454 gram in control group p=0.25 and p=0.44 respectively. Some of demographic characteristics of studied patients are showed in Table 1.

Twelve patients in the case group (40%) and eight neonates in the control group (20%) were twins (p=0.05). Regarding respiratory management, there were not significant differences among patients of either group, except the need for surfactant replacement therapy. Surfactant replacement therapy was done for 11 (26.8%) patients in the control group and 15 (50%) of neonates in the case group (P=0.04). The mean duration of oxygen therapy was 30.8± 24.7 days in the case group and 25.8± 15.9 days in the control group (p=0.31). The mean serum leptin concentration in neonates who had RDS was 0.64± 0.06 ng/ml which was not statistically different from neonates without RDS 0.77± 0.12 ng/ ml (p= 0.44). Although serum leptin levels were lower in neonates with bronchopulmonary dysplasia than neonates without BPD (0.53±0.03 vs. 0.83± 0.13 ng/ml) the difference was not statistically significant (p=0.08).

The mean plasma levels of leptin in control group

was 0.79±0.83 ng/ml (range: 0.3- 5.2ng/ml) and in the case group was 0.64±0.41 ng/ml (range: 0.2-1.9 ng/ml) without significant difference (p=0.39). Diode laser photocoagulation was performed in 21 patients with proliferative ROP.

**Discussion**

Ischemia induced retinal neo-vascularization often results in loss of vision in the final stage of various ocular disease including diabetic retinopathy (DR), retinal vein occlusion, and ROP.

Studies in experimental animals have shown that leptin promotes neo-intimal growth in mice and stimulates migration of vascular smooth muscles .[12,16] Leptin may also activate adult human endothelial progenitor cells and promote angiogenesis.[12]

Suganami and coworkers designed a study to elucidate the pathophysiological role of leptin in the progression of retinal neo-vascularization. They demonstrated more pronounced retinal neo-vascularization in 17 day-old transgenic mice over-expressing leptin. They concluded that leptin stimulates the ischemia induced retinal neo-vascularization possibly through the up regulation of VEGF .[17]

There are observations suggest that leptin may played a role in the development of DR and its plasma concentrations are elevated significantly in patients with proliferative DR relative to those with non-proliferative retinopathy.[18] Vitreous leptin concentrations are higher in patients with proliferative DR or retinal detachment .[19] However, whether leptin has as a causative role in DR or ROP is currently undefined. In our study plasma leptin concentrations was not significantly different among neonates with proliferative ROP and preterm infants without ROP. Interestingly, leptin concentrations were positively correlated with lung weight on the 7th day of life in lambs who received intravenous leptin that suggest its potential role in lung growth.[20] While clinical studies based on mouse model observations, indicate the critical role of leptin on lung function in various conditions including asthma, heart failure, the pathophysiogical significance of leptin regarding respiratory function in humans remains to be clarified.[21] We did not find a significant difference among patients with RDS or BPD and infants without these respiratory problems with respect to serum leptin levels.

Table 1: Demographic characteristics of patients in two groups

| | Case group N=30 | Control group N=41 | P-Value Gestation age at birth |
|---|---|---|---|
| Gestation age at birth, wk | 28.4±1.6• Range:26-32 | 28.8±1.6• Range: 26-34 | 0.25 Birth weight |
| Birth weight, g | 1120. ±196. • Range: 780-1720 | 1189. ±454. • Range: 800-1750 | 0.44 Sex male |
| Sex male, n (%) | 12 (40) | 22 (53.7) | 0.25 PMA at sampling |
| PMA at sampling, wk | 34.9±1.2• Range: 33-37 | 34.6±1.3• Range: 33-38 | 0.65 Apgar score 1min 5min |
| Apgar score 1min | 4.8±1.4• Range: 2-7 | 6.5±1.4• Range: 2-9 | <0.001 |
| 5min | 7.2±1.4• Range: 3-9 | 8.4±1.3• Range: 6-10 | 0.001 |

Note: • mean± (SD)
PMA=post-menstrual age

In our study lower Apgar score, multiple pregnancy and severe respiratory distress needing surfactant replacement therapy were significantly more common in patients who developed proliferative ROP. It is suggested there is need for strict control of oxygen use in neonatal resuscitation and after surfactant administration for management of respiratory distress syndrome.

A limitation of our study was the absence of serial measurements of leptin in different postnatal ages; and we couldn't elucidate any variation of leptin over time.

In conclusion, our data demonstrated that plasma leptin concentrations were not significantly different in premature infants with proliferative ROP at 6-8 weeks after birth from premature infants without ROP at this age. Future studies with a larger number of neonates and at different post-natal ages to elucidate the role of leptin in pathogenesis of ROP are required.

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