Original Article

Insulin-like growth factor binding protein-3 in preterm infants with retinopathy of prematurity

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Background: Retinopathy of prematurity (ROP) is the main cause of visual impairment in preterm newborn infants. **Objective:** This study was conducted to determine whether insulin-like growth factor binding protein -3 (IGFBP-3) is associated with proliferative ROP and has a role in pathogenesis of the disease in premature infants. **Materials and Methods:** A total of 71 preterm infants born at or before 32 weeks of gestation participated in this study. Studied patients consisted of 41 neonates without vaso-proliferative findings of ROP as the control group and 30 preterm infants with evidence of severe ROP in follow up eye examination as the case group. Blood samples obtained from these infants 6-8 weeks after birth and blood levels of IGFBP-3 were measured using enzyme-linked immunosorbent assay (ELISA). **Results:** The mean gestation age and birth weight of the studied patients were 28.2±1.6 weeks and 1120.7±197 gram in the case group and 28.4±1.6 weeks and 1189.4±454 gram in the control group (P=0.25 and P=0.44 respectively). The infants in the case group had significantly lower Apgar score at first and 5 min after birth. Insulin-like growth factor binding protein -3 (IGFBP-3) was significantly lower in the patients with proliferative ROP than the patients without ROP [592.5±472.9 vs. 995.5±422.2 ng/ml (P=0.009)]. Using a cut-off point 770.45 ng/ml for the plasma IGFBP-3, we obtained a sensitivity of 65.9% and a specificity of 66.7% in the preterm infants with vasoproliferative ROP. **Conclusion:** Our data demonstrated that the blood levels IGFBP-3 was significantly lower in the patients with ROP and it is suspected that IGFBP-3 deficiency in the premature infants may have a pathogenetic role in proliferative ROP.

Key words: Insulin-like growth factor-1, insulin-like growth factor binding protein-3, retinopathy of prematurity

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Retinopathy of prematurity (ROP) is the main cause of visual impairment in the preterm newborn infants. In recent years, with advances in the neonatal intensive care the survival of extremely low birth weight infants has increased.[1] Retina is incompletely vascularized in the prematurely born infant. The growth of vessels slows or ceases after premature birth. This is the first stage of ROP. In addition to gestational age, the extent of the non-perfusion region of retina in the initial phase of ROP, determines the subsequent degree of neovascularization. Phase II follows when phase I induced tissue hypoxia releases factors to stimulate new blood vessel growth. Aberrant neovascularization is the hallmark of ROP.[2,3]

The two major risk factors of ROP are preterm birth with decreased gestation age and the use of oxygen. Pathophysiologic mechanisms that might contribute to ROP have been investigated in recent years.[4-6] Vascular endothelial growth factor (VEGF), which is an important oxygen regulated factor increases with hypoxia and plays a key role in retinal angiogenesis.[2,3,6-13] Insulin-like growth factor (IGF-1) is a critical oxygen-lacking regulated growth factor for normal retinal vascular development and its reduced levels is associated with the absence of vascular growth and the subsequent proliferative ROP.[2,3,6,11,13,14] IGF binding protein 3 (IGFBP-3) is the most prevalent binding protein in the plasma. However, the absolute role of IGFBP-3 in the angiogenesis and controlling cell growth is not well defined.

IGFBP-3 can increase or inhibit the proliferative effects of IGF-1. In the cell culture, it has been shown that IGFBP-3 promotes both apoptosis and survival; and may enhance or suppress cell growth depending on specific conditions.[15-17] IGFBP-3 regulates the pro-mitogenic and anti-apoptotic functions of IGFs, but with independent functions.

We conducted this study to determine the plasma IGFBP-3 levels in the patients who develop advanced stages of ROP to ascertain whether it has a role in the pathogenesis of proliferative retinopathy of prematurity.

Materials and Methods

All the preterm infants who were born at or before 32 weeks of gestation and had initial and follow-up eye examination for diagnosis of ROP in the neonatal intensive care unit (NICU) of an university teaching hospital, from March 2009 to June 2010 were eligible for inclusion, in this study. The newborn infants with chromosomal anomalies or major congenital malformations were excluded from the study. During the 18 months study period, 200 premature newborn infants were admitted in the NICU, 120 of them underwent a follow-up eye examination at the hospital. We included 30 preterm newborn infants who were diagnosed as proliferative ROP in the case group. The patients with ROP stage I or II were excluded from the study. Forty-one infants with normal ophthalmologic examinations were randomly selected as the control group.

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Gestational age was determined by either the maternal last menstrual period or the first trimester ultrasound examination and confirmed by neonatal examination using the Ballard gestational age scoring.[18] Surfactant was given to the infants who met the clinical and radiologic criteria for respiratory distress syndrome (RDS) as INSURE treatment method within 2-6 h of life. Targeted Spo2 was 90-92%. Patent ductus arteriosus (PDA) was diagnosed based on the presence of the compatible clinical signs associated with echocardiogram findings. Doppler measurements interpreted by a pediatric cardiologist.

The diagnosis of sepsis was made by the presence of the clinical sign and symptoms of sepsis documented by positive blood culture.

Each infant was examined initially by 4th week's chronologic age and repeated examinations were done within at least two-week interval if the retinal vessels had grown only into zone II or every week in the infants with vessels only as far as zone I. All the eye examinations were performed by an ophthalmologist experienced in evaluating the infants for ROP who had no knowledge of IGFBP-3 levels. The follow-up examinations were recommended by him on the basis of retinal findings classified according to the international classification[19] until full vascularization. After pupillary dilation, the retinas were examined through the indirect ophthalmoscope. Retinopathy of prematurity 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).[19] The classification was made according to the most advanced ROP stage observed. Proliferative ROP was defined as stage III or higher. All the infants with ROP stage III or higher were enrolled in the case group. Forty-one premature infants without evidence of ROP at initial and follow-up ophthalmologic examination were randomly selected as control group. The ethic committee of Tabriz University of Medical Sciences approved the study and written informed parental consent was obtained in all the 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°C. The laboratory analysis was done by the one with no idea about patients' group.

IGF-1 and IGFBP-3 concentrations were measured by quantitative sandwich enzymatic immunoassay technique (ELISA) byIBL international GmbH, Hamburg, Germany with a sensitivity of 3.1 μg/L and 10.5 ng/ml respectively. The interassay coefficients of variation at 3.1 μg/L and 10.5 ng/ml were 7.2% and 4.3% for IGF-1 and IGFBP-3 respectively. We calculated the molar ratio of IGF-1 to IGFBP-3 as an indicator of bio-active IGF-1 using the following equivalents for conversion: 1ng/ml IGF-1= 0.130 nmol/L IGF-1, and 1 ng/ml IGFBP-3 = 0.036 nmol/L IGFBP-3.

Statistical analysis was carried out using SPSS package 15. The Student's T test or Mann-Whitney U-test and Chi-squared test were used for quantitative and qualitative variables, respectively. The receiver operating characteristic (ROC) method was used in order to stabilize the optimal cut off point. Then, the sensitivity and the specificity were calculated. $P<0.05$ were considered to be statistically significant.

Results

Seventy one infants were included in this study. The mean gestation age (GA) and birth weight were not significantly different among neonates in the case and control groups (the mean± SD gestation age was 28.2±1.6 vs. 28.4±1.6 weeks and the mean± SD birth weight was 1120.7±197 vs. 1189.4±454 gram in case and control groups $P=0.25$ and $P=0.44$ respectively).

The infants in the case group had significantly lower Apgar score, at first, and 5 min after birth. The mean ± SD first min Apgar score was 4.8±1.35 vs. 6.54±1.4 ($P<0.001$) and at 5 min it was 7.2±1.4 vs. 8.4±1.3 ($P=0.001$), in the case and the control groups, respectively. The need for surfactant replacement therapy was significantly higher for the patients with proliferative ROP than preterm infants with normal eye examination [15 cases (50%) in the case group vs. 11 patients (26.8%) in the control group, $P=0.04$]. Nine patients (30%) in the case group needed ventilator support whereas it was 9 cases (22%) in the control group ($P=.44$). The duration of the need for oxygen was 30.8±24.7 and 25.8±15.9 days in the case and control groups without significant difference. Other risk factors in the studied patients are shown in Table 1.

The patients with stage I or II ROP were not included, in this study. There was not any case of ROP with stage II plus in the case group. Diode laser photocoagulation was done in 21 patients in the case group.

The mean plasma concentration of IGF-1 was not different between the infants with and without ROP but IGFBP-3 and IGF-1: IGFBP-3 molar ratio was significantly different between infants with ROP and control group [Table 2].

The mean plasma levels of IGFBP-3 were significantly lower in the case group than control group ($P=0.009$). With considering optimal cut off point 770.5 ng/ml for IGFBP-3, a

Table 1: The clinical characteristics of the patients in two groups

	Case group	Control group	*P* value
Blood transfusion, *n* (%)	17 (56.7)	19(46.3)	0.30
PDA, *n* (%)	6 (20)	2 (4.9)	0.06
Sepsis, *n* (%)	5 (16.7)	2 (4.9)	0.12
Post menstrual age, wk	34.9±1.2•	34.6±1.3•	0.65
Infants weight at sampling, gr	1686±338•	1713±327•	0.74

•Mean ± SD, PDA: Patent ductus arteriosus

Table 2: The mean plasma levels of insulin-like growth factor-1 and insulin-like growth factor binding protein-3 in the patients of both groups

	Case group (*n*=30)	Control group (*n*=41)	*P*. value
IGF-1(μg/l)	18.48±11.79•	16.7±13.4•	0.58
IGF-BP3(ng/ml)	592.5±472. 9•	995.5±422.2•	0.009
IGF-1:IGFBP-3 molar ratio	±.0210	±.0039	0.05

•Mean ±SD

sensitivity and specificity 65.9% and 66.7% were determined in the patients with proliferative ROP.

Discussion

Although the mechanisms of retinal neovascularization are not fully understood, several growth factors particularly VEGF, IGF-1 and IGFBP-3 are considered as major contributors. We have demonstrated that the pre term infants with normal initial and follow-up eye examinations had increased levels of IGFBP-3 at 6-8 weeks after birth. Shaw examined the endogenous expression of IGFBP-3 in and its direct effect on retinal endothelial cells.[20] Endothelial cells comprise the critical border between the arterial wall and the blood, and play several key roles in angiogenesis through elaboration of several growth factors. The endothelium regulates metabolism, migration, proliferation and apoptosis of underlying smooth muscle cells, as well as production of extra cellular matrix. Disorders of endothelial function have been closely associated with vascular diseases. There are a few studies on IGFBP-3 in ROP. Franklin and co-workers showed for the first time that IGFBP-3 is a potent inhibitor of VEGF-stimulated proliferation via a mechanism independent of IGF-1 and its receptors.[21] Increasing IGFBP-3 decreases retinal vascular loss, increases vessel re-growth and thereby decreases ROP. The mechanism by which IGFBP-3 acts to prevent vessel loss and improves repair is likely to be multi-factorial. Changes in the plasma levels of the most prevalent growth factor (IGF-1) and its binding protein (IGFBP-3) have been implicated in pathogenesis of the ocular disorders including ROP and the balance between them is vital for tissue and endothelial hemostasis.[20]

Lofgvist and co-workers showed that low levels of serum IGFBP-3 in premature infants 30-35 weeks gestation age are associated with the increased risk of developing proliferative ROP that is compatible with our findings.[16] They have investigated postnatal growth and development of ROP; and concluded monitoring the post natal factors including weight, IGF-1 levels and IGFBP-3 substantially enhances the clinicians' ability to identify the patients who will require treatment for ROP.[22]

Interestingly, we observed that IGF levels were not different among the premature newborn infants who developed proliferative ROP and infants without ROP 6-8 weeks after birth. These findings may be due to this fact that IGF-1 levels changes with gestation age and stage of retinopathy. It has been showed a rapid rise in IGF-1 levels between 3rd and 5th weeks may be related to the development of higher stage of ROP.[23] There was a different molar ratio among our studied patients in two groups, probably due to different levels of free IGF-1 in two groups. Unfortunately, we did not measure free IGF-1 levels in our neonates; therefore we could not analyze relationship between free IGF-1 and molar ratio.

It has been believed for many years that oxygen therapy causes increased risk of ROP in the preterm infants. However, ROP can occur even with careful use of oxygen.[24] In our study, lower Apgar score and severe respiratory distress that needs surfactant replacement therapy were significantly more common in the patients who developed proliferative ROP.

Limitation of our study was the absence of serial measurement of these biochemical markers; and we could not show variation of these parameters over time.

We did not find any differences between our studied ROP and non ROP groups with respect to the need for mechanical ventilation or the need for oxygen supplementation. The patients with RDS that need exogenous surfactant replacement therapy were significantly more common in ROP group, in our study, that signifies the need for strict control of oxygenation after surfactant administration. The lower Apgar score at birth is seen as a strong indicator for the development of ROP and may be explained by altered hemodynamic or vascular stress.

In conclusion, our data demonstrated that blood levels IGFBP-3 was significantly lower in the patients with ROP and it is suggested that IGFBP-3 has a role in the pathogenesis of the disease. Serial measurement of IGFBP-3 and determination of free IGF-1 serum levels in a large number of the patients to clarify its role in ROP is recommended for future studies.

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