

Variations of Vascular Endothelial Growth Factor and Pigment Epithelial-derived Factor Are Related to Retinopathy of Prematurity in Human Babies

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ABSTRACT

Background: To examine the role of variations in vascular endothelial growth factor (VEGF) and pigment epithelial-derived factor (PEDF) levels and VEGF/PEDF ratio in predicting the occurrence of retinopathy of prematurity (ROP) in extremely premature human babies.

Methods: This is a retrospective hospital based case-control study of 54 preterm neonates born at or before 32 weeks of gestation between 2006 and 2007 at the Neonatal Intensive Care Unit of the First People's Hospital affiliated to Fudan University. Birthweight was less than 1250 g. Eleven diagnosed with ROP were identified as cases. A control group of 43 infants, closely matched for birthweight and gestational age, was selected. The levels of VEGF and PEDF were measured at different time points of postnatal ages. Two-way repeated measure analysis of variance (ANOVA) was performed to examine the time trend.

Results: Vascular endothelial growth factor level in ROP cases showed an increasing trend during the postnatal 35 day age ($p < 0.01$), while it was persistently decreasing in the control group ($p = 0.025$). In contrast, PEDF level in the control group was steadily increasing with postnatal day ages, while it remained approximately at the same level in the study group. On the other hand, the PEDF/VEGF ratio in cases was found to be extremely high at the beginning, then continuously declined during the entire study period, while it remained steady in the control group during the same period.

Conclusion: Increased expression of VEGF levels was found to be associated with older postnatal day age in our study. Monitoring of variations in VEGF level and PEDF/VEGF ratio might be helpful in predicting the occurrence of ROP in premature human babies.

Keywords: Pigment epithelial-derived factor, retinopathy of prematurity, vascular endothelial growth factor ratio

Las variaciones del factor de crecimiento endotelial vascular y el factor derivado del epitelio pigmentario están relacionadas con la retinopatía de los recién nacidos prematuros

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RESUMEN

Antecedentes: Examinar el papel de las variaciones en los niveles del factor de crecimiento endotelial vascular (VEGF, siglas en inglés) y del factor derivado del epitelio pigmentario (PEDF, siglas en inglés), y el cociente VEGF/PEDF en la predicción de la aparición de la retinopatía del prematuro (ROP) en bebés humanos extremadamente prematuros.

Métodos: Se trata de un estudio de casos y controles retrospectivo de base hospitalaria, realizado a 54 neonatos prematuros nacidos en o antes de las 32 semanas de gestación entre 2006 y 2007 en la Unidad de Cuidados Intensivos Neonatales del First People's Hospital afiliado a la Universidad de Fudan. El peso al nacer fue menos de 1250 g. Once de los diagnosticados con ROP se identi-

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ficaron como casos. Se seleccionó un grupo control de 43 neonatos, estrechamente pareados por edad gestacional y peso al nacer. Se midieron los niveles de VEGF y PEDF en diferentes puntos temporales de edad postnatal. Se realizó un análisis de varianza (ANOVA) bidireccional de medidas repetidas con el fin de examinar la tendencia temporal.

Resultados: El nivel del factor de crecimiento endotelial vascular en los casos de ROP mostró una tendencia creciente durante la edad postnatal de 35 días ($p < 0.01$), mientras que en el grupo control se caracterizó por una persistente disminución ($p = 0.025$). En contraste con ello, el nivel PEDF en el grupo control fue aumentando constantemente con las edades postnatales en días, mientras que se mantenía aproximadamente al mismo nivel en el grupo de estudio. Por otra parte, hubo casos en que el cociente PEDF/VEGF fue extremadamente alto al principio, luego disminuyó continuamente durante todo el periodo de estudio, mientras que seguía siendo constante en el grupo control durante el mismo periodo.

Conclusión: Se halló que el aumento de la expresión de los niveles VEGF estaba asociado con una mayor edad postnatal en días en nuestro estudio. El monitoreo de las variaciones del nivel de VEGF y el cociente de PEDF/VEGF podrían ser útiles a la hora de predecir la ocurrencia de ROP en los bebés humanos prematuros.

Palabras claves: factor derivado del epitelio pigmentario, retinopatía del prematuro, cociente del factor de crecimiento endotelial vascular

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INTRODUCTION

Retinopathy of prematurity (ROP), previously known as retrolental fibroplasias [RLF] (1), is a disorder of neonatal retinal vascularization, accounting for 6–18% of all irreversible blindness in children (2). It is evident from prior studies that proper identification and timely intervention of infants at risk of developing ROP is the most important way of improving visual outcomes and minimizing the long-term negative impact on eye development, due to the availability of preventative measures and advances in treatment modalities in recent times (3–6). Retinopathy of prematurity is an important cause of blindness among premature infants in China. With the increasing survival rates of preterm infants, the incidence of ROP is also rising, causing considerable social and economic burden to individuals and society (7, 8).

Although the underlying mechanism of ROP is not well understood, it is believed that unbalanced expression of angiogenic stimulators and angiogenic inhibitors plays a critical role in pathological neovascularization (9–12). In an ischaemia-induced retinal neovascularization rat model (13), an increased vascular endothelial growth factor (VEGF) and decreased pigment epithelial-derived factor (PEDF) levels and a higher VEGF/PEDF ratio have been observed. Moreover, the duration of VEGF/PEDF ratio change has been found to be correlated with the development and progression of retinal neovascularization. These findings suggest that monitoring changes of serum VEGF/PEDF ratio may be a sensitive indicator of ROP among high-risk premature human babies. However, this has not been established yet.

In the present study, we aimed to examine the variations in blood VEGF and PEDF levels in extremely premature babies, and explored the role of VEGF/PEDF ratio in predicting ROP.

SUBJECT AND METHODS

This retrospective case-control study was conducted in the Neonatal Intensive Care Unit of the First People's Hospital affiliated to Fudan University. Preterm neonates born at or before 32 weeks of gestation during July 2006 to January 2007 and having birthweight (BW) less than 1250 g were included in the study. Gestational age (GA) was estimated by calculating last menstrual period or using prenatal ultrasound. Neonates having congenital syphilis, gonorrhoea, hepatitis B, cytomegalovirus (CMV) infection, congenital deformity, inherited metabolic diseases and haemolytic diseases were excluded. In addition, babies reporting hospital stay of less than 35 days or referred to the hospital after seven postnatal day age were excluded from the final analysis. A signed informed consent was obtained from one of the parents of each eligible infant. Finally, a total of 54 neonates were enrolled; of those, 11 diagnosed with ROP were taken as cases, while 43 neonates were selected for the control group.

All the preterm neonates were managed according to standard neonatal intensive care unit (NICU) protocol. Antenatal, natal and postnatal history as well as demographic information were collected from medical records or attending physicians. Diagnosis of ROP was made by indirect ophthalmoscopy as per recommendations of cryotherapy for retinopathy of prematurity [CRYO-ROP] (14).

This study was reviewed and approved by the Ethics Committee of Fudan University.

Blood sample

One millilitre of peripheral blood sample was collected at time points of P7, P14, P21, P28 and P35, respectively and centrifugated at 3000 rpm for 10 minutes after 30 minutes of

solidification. About 0.2 millilitre of serum sample was isolated and kept at -20 °C for analysis.

VEGF and PEDF measurement

The VEGF and PEDF levels were measured using Quantikine sandwich enzyme linked immunosorbent assays provided by Shanghai senxiong Technology Industrial Co, Ltd. (Quantikine, R&D Systems, Minneapolis, USA). The assays were conducted according to the manufacturer’s guidelines. Each sample was analysed in duplicate.

Statistical analysis

Statistical analyses were carried out using SAS 9.13 (SAS Institute, Cary, NC, USA). Variables were described by frequencies and mean ± standard deviation (SD). Chi-squared test was used for frequency tables. *T*-test was performed for testing mean difference for normally distributed variables and Mann-Whitney U-test was used for categorical variables. Two-way repeated measure analysis of variance (ANOVA) and Bonferroni’s *post hoc* test were used to detect differences between groups at each time point. *p* < 0.05 was considered statistically significant.

RESULTS

A total of 54 premature neonates were included for this analysis, Out of 54 neonates, 29 were males and 25 were females. The mean GA and BW were 29.4 ± 1.56 weeks and of 1123 ± 115.7 g, respectively. The demographic and clinical characteristics of the neonates across groups are presented in Table 1. No significant difference was observed between cases and controls with respect to mean BW, mean GA, male/female ratio and neonatal respiratory distress syndrome (NRDS) occurrence.

Table 1: Characteristic of premature neonates by study groups

	ROP group (n = 11)	No ROP (n = 43)	<i>p</i>
GA (weeks)	29.2 ± 1.73	29.7 ± 1.28	0.79
BW (g)	1116.7 ± 172.14	1133.9 ± 88.03	0.55
Gender (M/F)	5/6	24/19	0.54
RDS (yes/no)	6/5	22/21	0.84
Oxygen exposure time ¹	14.1 (1–35)	12.9 (1–35)	0.67
Asphyxia (yes/no)	7/4	14/29	0.06

GA and BW were presented as median with range

GA: gestational age; BW: birthweight; RDS: respiratory distress syndrome.

Data on VEGF level in both groups are presented in Table 2. Analysis of variance analysis revealed a marked variation in VEGF level with older postnatal day age (*p* < 0.01), and a significant interaction was also observed between day ages and groups (*p* < 0.01), suggesting a different time trend in both groups.

As shown in Table 2, VEGF level in cases remained at a low level before P21 and no obvious fluctuation was seen, and started to increase steadily and peaked at P28. Although a slight decrease was observed at P35, *post-hoc* test indicated it was still significantly higher than those at P7–P21. In contrast, VEGF level in the control group started at a markedly higher

Table 2: Vascular endothelial growth factor level, by study group and postnatal day age (pg/mL)

Group	P7	P14	P21	P28	P35
ROP	101.4 ± 86.4	98.3 ± 44.9	114.9 ± 21.7	212.7 ± 137.3 ^a	189.6 ± 114.9 ^a
Non ROP	280.8 ± 222.7	230.4 ± 155.3 ^b	199.0 ± 146.4 ^b	149.5 ± 114.5 ^b	147.7 ± 118.3 ^b
<i>P</i> ^c	0.04	0.01	0.03	0.52	0.75

a: *p* < 0.05 when compared to P7 in ROP group. b: *p* < 0.05 when compared to P7 in Non ROP group. c: *p*-value for comparison between ROP and Non ROP group at each time point, Mann-Whitney U-test.
ROP: retinopathy of prematurity

level than their day age matched counterparts and then continuously declined to an approximately equal level to that in study group at P28 and P35. Figure 1A depicts the time trend in both groups; there is a marked disparity at the beginning which gradually converges between P21 and P28 and then separates again after P28.

In contrast to VEGF, PEDF level in the control group started at the lowest level at P7 then persistently increased to the highest level at P35, showing a significantly increasing trend during the time course (ANOVA, *p* = 0.025). However, *post-hoc* test did not find any statistical difference between time points. In cases, PEDF level remained at nearly the same level through all time points and no obvious time trend was observed (ANOVA, *p* = 0.94), as well as interaction between time points and groups (*p* = 0.75) was not statistically significant (Table 3 and Fig. 1B).

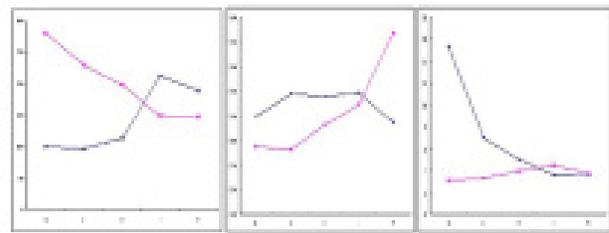


Figure: The role of variations in vascular endothelial growth factor (VEGF), pigment epithelial-derived factor (PEDF) levels and VEGF/ PEDF ratio. 1a: The time trend displays a marked disparity at the beginning and then gradually converges between P21 and P28 and then separates again after P28. 1b: The level of PEDF through all time points in all groups, and their interaction between time points and groups. 1c: The PEDF/VEGF ratios in both groups show an opposite time trend compared to the VEGF level.

Table 3: Pigment epithelial-derived factor level by study group and postnatal day age (pg/mL)

Group	P7	P14	P21	P28	P35
ROP	560.1 ± 17.8	569.1 ± 69.2	567.9 ± 38.7	569.5 ± 29.7	557.5 ± 10.6
Non ROP	547.6 ± 56.254	6.6 ± 50.155	6.3 ± 44.65	64.9 ± 29.9	593.7 ± 66.7
<i>P</i>	0.72	0.50	0.68	0.81	0.13

P: for comparison between ROP and Non ROP group at each time point, Mann-Whitney U-test.
ROP: retinopathy of prematurity.

As shown in Table 4, PEDF/VEGF ratio in cases was dramatically higher at the beginning and then persistently decreased during the postnatal days, while it was relatively stable in the control group. Statistical analysis indicated a marked time trend (ANOVA, $p < 0.01$) and time-group interaction ($p = 0.013$). The ratio in the case group before P21 was lower than that in the control group, and no obvious difference was observed after P21. As depicted in Fig. 1c, PEDF/VEGF ratios in both groups showed an opposite time trend compared to the VEGF level.

Table 4: Pigment epithelial-derived factor/vascular endothelial growth factor ratio, by study group and postnatal day age

Group	P7	P14	P21	P28	P35
ROP	15.37 ± 19.1	97.06 ± 4.47 ^a	5.02 ± 0.65 ^a	3.63 ± 2.35 ^a	3.59 ± 1.60 ^a
Non ROP	3.12 ± 2.86	3.28 ± 2.16	3.98 ± 2.93	4.46 ± 4.75	3.78 ± 1.91
<i>P</i>	0.04	0.11	0.90	0.43	0.85

a: $p < 0.05$ when compared to P7 in ROP group; p -value for comparison between ROP and Non ROP group at each time point, Mann-Whitney U-test. ROP: retinopathy of prematurity.

DISCUSSION

This study revealed a significant variation in protein level as expressed in VEGF and PEDF levels and PEDF/VEGF ratio among premature neonates diagnosed with ROP during their 35 day ages. Our data supported the previous findings from the rat model (13, 15, 16) that indicated that an imbalance between angiogenic stimulators and inhibitors might contribute to retinal neovascularization, suggesting that surveillance on the time trend of VEGF and PEDF levels among extremely premature infants might be helpful in predicting the occurrence of ROP.

It has been widely accepted that ROP is a two-phase disease consisting of an initial phase of vessel growth retardation followed by a second phase of vessel proliferation (17, 18). The first phase of ROP is characterized by a decreased level of VEGF induced by hyperoxic exposure to oxygen-rich environment, occurring from birth to postmenstrual age approximately 30–32 weeks (17). The second phase is characterized by an up-regulated expression of VEGF stimulated by hypoxic conditions due to increasingly metabolically active non-vascularized retina. Consistent with this theory, we observed extremely lower VEGF levels in neonates predisposed to ROP from P7 to P21 compared with their GA and BW matched counterparts. Given the mean GA was 29 weeks in the case group, the time duration of decreased level of VEGF was similar to previous reports. Moreover, an overall increasing trend of VEGF level was observed in the case group only. This suggested that VEGF, as shown in previous studies (13, 19), might play a critical role in mediating the pathological neovascularization in the occurrence of ROP.

Pigment epithelial-derived factor is a potent angiogenic inhibitor endogenously expressed in the retina (20). However, the regulatory mechanism of PEDF expression, as well as its role in pathological neovascularization, has not been well understood yet. Gao *et al* (13) reported a negative correlation be-

tween PEDF levels and retinal neovascularization in their rat model, indicating that PEDF was up-regulated by hyperoxic conditions and down-regulated by hypoxic conditions. However, we did not find any changes of PEDF level in the ROP group during the entire study period. Discrepancies observed across studies, in addition to the difference in study subjects, might also be ascribed to the disparity associated with the environment and accompanying risk factors as well as medication. In contrast, PEDF showed a significant increasing trend with day age in the control group, implicating that aggressive progression of retinal neovascularization might be associated with PEDF deficiency compared to PEDF reduction.

As expected, PEDF/VEGF ratio in the case group displayed a continuous declining trend while it remained stable in the control group. This disequilibrium of angiogenic stimulators and angiogenic inhibitors level might disturb the regulation of angiogenesis homeostasis and favoured the process of retinal neovascularization and thus increasing the risk of ROP. This was consistent with the findings in a previous study (13), wherein an increasing trend of VEGF/ PEDF ratio was observed in an ischaemia-induced retinal neovascularization rat model. Both studies demonstrated the role of PEDF/ VEGF ratio in predicting ROP occurrence.

There were some limitations in this study. First, we recruited a comparatively small number of ROP cases, which might have decreased the reliability of the study results. Therefore, further research is needed to better understand the role of PEDF and VEGF variations in the occurrence of ROP. Second, keeping in mind the ethical consideration, we could not select term babies as control, which could have definitely increased the strength of our findings. For the same reason, we conducted a retrospective study design and could obtain information up to age 35 days from the participants.

In conclusion, VEGF and PEDF level might play a critical role in the process of retinal neovascularization. Monitoring the variation in VEGF level and assessment of PEDF/ VEGF ratio may be important in predicting the occurrence of ROP in extremely premature neonates.

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