Morphological features and development dynamics of retinal vessels in preterm infants with retinopathy of prematurity

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Research Article

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Abstract

Purpose: To study the difference in retinal vascular morphology and the developmental dynamics between preterm infants with and without Retinopathy of Prematurity (ROP).

Methods: 226 preterm infants with ROP and 928 preterm infants without ROP were studied. We developed a deep learning-based method to evaluate the vascular morphological features automatically. Stepwise regression analysis was applied to analyze the differences in the retinal vascular morphology between the two groups. Furthermore, analysis of covariance was used to analyze the differences in vascular development dynamics between preterm infants with ROP and without ROP.

Results: Vascular tortuosity, caliber, and branch angle in preterm infants with ROP were significantly higher than those without ROP. Furthermore, preterm infants' vascular density with ROP increased significantly slower with gestational age than preterm infants without ROP.

Conclusion: ROP is a disease closely associated with the vessels in the posterior pole region of retinal. Vascular morphology of preterm infants with ROP is significantly different from that of preterm infants without ROP. The density, which could reflect the proliferation of the vessels, develops significantly slower in preterm infants with ROP. The findings could provide insights into the pathogenesis of ROP.

Introduction

Retinopathy of prematurity (ROP) is a vitreoretinal vascular proliferative disease in preterm infants and is the leading cause of childhood blindness[1–3]. ROP can be viewed as the arrest of normal retinal neuronal and vascular development in preterm infants, ultimately leading to abnormal retinal vascularization[2]. The immature retinal vessels of preterm infants after birth have neovascularization and fibrous proliferation during the development process, leading to traction retinal detachment and causing visual impairment even blindness in preterm infants with ROP[3].

Plus disease is one of the indicators that reflects the progression of ROP to the stage requiring treatment[4], and pre-plus disease is defined as an intermediate state between normal and plus disease. Computer-aided quantitative assessment of plus and pre-plus diseases, such as the assessment of vascular tortuosity and dilatation, facilitates dynamics analysis of the characteristics of ROP progression[5]. Mao et al.[5] found that preterm infants with plus or pre-plus diseases had larger vascular tortuosity, vascular caliber, vascular density, and vascular fractal dimension than normal preterm infants with quantitative analysis of retinal images. The study suggests that the morphology of retinal vessels constantly changes with development of the disease. In a follow-up study on 10 preterm infants with ROP and 31 normal preterm infants, Ghodasra et al.[6] found that the width and tortuosity of retinal vessels of eyes that eventually appeared ROP increased faster than eyes that did not appear. The authors speculated that further kinetic study of retinal vascular changes might help identify ROP earlier. Ji et al.[7] found that preterm infants with ROP had significantly lower levels of retinal vascularization than normal

preterm infants. This study suggests that retinal vessel development dynamics of preterm infants with ROP may be different from normal preterm infants.

Furthermore, using deep learning algorithm, a quantitative ROP severity score can be derived to assess the degree of tortuosity and dilation of retinal vessels in the posterior pole region and monitor ROP progression objectively. Brown et al.[8] used deep learning methods to conduct continuous research on ROP plus disease and divided the fundus image into normal group, pre-plus group, and plus group based on vessel segmentation results. A scoring system was developed with vessel segmentation results to measure the severity of retinal diseases and provide auxiliary guidance for doctors during treatment[9]. The disease severity scoring results, combined with clinical diagnosis, can objectively evaluate whether the sick child needs treatment and the effect after treatment[10].

In summary, quantitative analysis of retinal vascular morphology features of preterm infants with and without ROP is of great significance for revealing the pathogenesis of ROP and monitoring the change of ROP. However, to our knowledge, few studies have examined the differences in vascular morphological features between preterm infants with ROP but without plus or pre-plus and preterm infants without ROP. At the same time, as far as we know, many studies have studied the numerical change of vessel morphology or the change in vessel development brought about by ROP alone, and there is no work to explore both aspects at the same time. The early research on the change on retinal vessels caused by ROP mainly focuses on the vessel around the demarcation line between vascular and avascular areas, and there is a lack of research on the vessels in the posterior pole region. Therefore, our study focused on assessing the influence of ROP on the vascular morphology in the posterior pole region of preterm infants with ROP that did not develop plus or pre-plus diseases. Our analysis included two aspects, including the systematic analysis of retinal vascular morphological features and the dynamics of retinal vascular development. We try to understand the changes that ROP brings to retinal vessels comprehensively.

Materials And Methods

Data acquisition and participants

Retinal images were collected from the Maternal and Child Health Hospital of Anhui Province between 2013 and 2018. We carefully cleaned the data before the experiment. Our screening criteria include the following three aspects. First, images must be acquired using a commercially available camera (RetCam; Clarity Medical Incorporated, Clarity MSI, Pleasanton, CA, USA). Second, the retinal images were taken from the patients' first examination. Third, images acquired from the posterior pole region were analyzed. The interval between the gestational age and the corrected gestational age was between 4 and 6 weeks for each analyzed infant. Preterm infants with pre-plus or plus disease were not included. The dataset we finally constructed included 226 preterm infants with ROP and 928 preterm infants without ROP. Table 1 describes the characteristics of the dataset. The gestational age of preterm infants with ROP ranged from 181 days to 255 days. Birth weight ranged from 960g to 2700g. The gestational age of preterm infants without ROP ranged from 181 days to 259 days. The birth weight ranged from 800g to 3600g. Significant

differences in gestational age and weight were found between preterm infants with ROP and without ROP. This study adhered to the tenets of the Declaration of Helsinki[11]. All subjects were provided written informed consent.

Table 1
Characteristics of dataset with details of number of patients, age, and weight in these two groups (with ROP *vs* without ROP)

	with ROP	without ROP	p-value
Patients	226	928	
Gestational age (days)	218 ± 13	227 ± 15	**< 0.01**
Gestational age range (days)	181–255	181–259	
Birthweights (g)	1547 ± 365	1860 ± 467	**< 0.01**
Birthweight range (g)	960–2700	800–3600	

Data Preprocessing

Accurate vessel and optic disc segmentation are the prerequisites for evaluating morphological features. We applied MU-Net[12] for vessel and optic disc segmentation. A circular region of interest (ROI) with the center of the optic disc and a radius of 3.5 times the optic disc diameter was applied for each image. In the ROI, we evaluated eight morphological features, including vascular tortuosity, caliber, density, fractal dimension (FD), branch angle (BA), branch coefficient (BC), asymmetry factor (AF), and optimal vascular ratio (OR).

Vascular Morphology Quantitative Calculation

We used arc length-normalized total squared curvature as the measure of tortuosity[13]. Based on the vessel segmentation, we extracted the number of vessels, calculated the tortuosity of each vessel, and then averaged all the vascular tortuosity to get the value of the entire image.

We applied the Euclidean distance transform method to the binarized retinal images to obtain the vascular caliber. The Euclidean distance transform evaluated the distance of each pixel of the vessel to the nearest background pixel[14].

Vascular density was obtained by the ratio of the area of the vessel in the ROI to the area of the entire ROI.

Vascular fractal dimension (FD) of the retinal vessel is an indicator used to measure the complexity of vessels. To measure the fractal dimension, we utilized the sliding box-counting method. The fractal dimension summarizes the branching pattern of the entire retinal vascular tree [15]. Larger values indicate more complex branching patterns.

Branch angle (BA) is the angle between the two sub-vascular at the bifurcation[16]. The branching coefficient (BC) uses three vessels connected at a branch point. We compute branching coefficient based on the definitions proposed by N. Patton et al.[16]. Asymmetry factor (AF) is used to describe the asymmetry at the branch points of the vessels. The asymmetry factor was obtained by the ratio of the squares of the widths of the two daughter vessels at the branch point[17]. Optimality ratio (OR) is defined as:

$$OR = \sqrt[1/3]{\frac{d_1^3 + d_2^3}{2d_0^3}} \quad (1)$$

where d_1 and d_2 are the width of the two daughter vessels and d_0 is the width of the parent vessel. OR is used to measure the relationship between parent and daughter vessels at vascular bifurcations.

Statistical Analysis

Student t-test was used to examine the differences of each vascular morphological feature between preterm infants with ROP and preterm infants without ROP. Then, stepwise regression analysis was performed to re-examine the differences with the correction for the effects of gestational age and weight.

Analysis of covariance was used to analyze differences in vascular development dynamics between preterm infants with ROP and without ROP.

All statistical analysis was conducted using R version 4.1.2 (Foundation for-Statistical Computing, Vienna, Auistria). Student t-test was one-sided and the significance level was set to 0.05. $P < 0.05$ was considered statistically significant.

Results

Vascular morphology features among preterm infants with ROP and without ROP

Vascular tortuosity of preterm infants with ROP was $3.15\times10^{-4}cm^{-3}$ significantly higher than $2.97\times10^{-4}cm^{-3}$ of the preterm infants without ROP ($p < 0.01$). Vascular caliber of preterm infants with ROP was 88.5μm, which was significantly higher than 86.16μm of preterm infants without ROP ($p < 0.01$). Vascular density of preterm infants with ROP was 1.14%, which was significantly lower than the density of 1.21% without ROP ($p < 0.01$). FD of preterm infants with ROP was 1.08, which was significantly lower than FD

of 1.09 without ROP ($p < 0.01$). BA of preterm infants with ROP was 73.09º, which was significantly larger than the normal branch angle of 69.63º ($p < 0.05$).

There was no significant difference between BC, AF, and OR in the vessel of preterm infants with ROP and without ROP.

As shown in Table 2, after correcting the influence of gestational age and weight, vascular tortuosity, vascular caliber, and BA between preterm infants with ROP and without ROP retained significant differences.

Table 2
Significant differences of vascular morphological features after correction for the influence of gestational age and weight

Vascular Morphological Features	with ROP Mean ± SD	without ROP Mean ± SD	p-value
Tortuosity ($\times 10^4 cm^{-3}$)	3.15 ± 0.93	2.97 ± 0.59	**< 0.01**
Caliber (μm)	88.50 ± 8.20	86.20 ± 4.20	**< 0.01**
Density (%)	1.14 ± 0.02	1.21 ± 0.02	0.27
FD	1.08 ± 0.04	1.09 ± 0.04	0.38
BA (°)	73.09 ± 19.60	69.1 ± 14.33	**< 0.01**
BC	1.49 ± 0.14	1.49 ± 0.12	0.95
AF	1.63 ± 0.30	1.61 ± 0.22	0.50
OR	0.87 ± 0.04	0.87 ± 0.04	0.95

The regression coefficient of each morphological feature in stepwise regression analysis can reflect its contribution to ROP. **Fig. 1** shows the regression coefficients and significant level for each morphological feature. A positive coefficient indicates that the occurrence of ROP is accompanied by an increase in this feature, and a negative coefficient indicates that the occurrence of ROP is accompanied by a decrease in this feature. The larger the value of 1 − p-value, the more significant the difference in the feature between preterm infants with ROP and without ROP.

Vascular Development Dynamics Among Preterm Infants With Rop And Without Rop

The result of analysis of covariance for development dynamics of each morphological feature between preterm infants with ROP and without ROP is shown in Table 3. Only the development dynamics of vascular density showed significant difference between preterm infants with ROP and without ROP.

Table 3
Development dynamic comparison of each morphological features

Vascular Morphological Features	p-value
Tortuosity ($\times 10^4 cm^{-3}$)	0.37
Caliber (μm)	0.43
Density (%)	**0.02**
FD	0.06
BA (°)	0.09
BC	0.86
AF	0.09
OR	0.79

As shown in Fig. 2, the speed in vascular density of preterm infants with ROP is lower than that of preterm infants without ROP.

Discussion

At the early developmental stage, the demarcation line between vascular and avascular areas is the characteristic feature of ROP. However, are there any other vascular differences between the ROP and corresponding group besides this typical feature?

To the best of our knowledge, this is the first study revealing the morphological and developmental differences between preterm infants with and without ROP. The main findings of this study are as follows: (1) The vascular tortuosity, vascular width and vascular branch angle of preterm infants with ROP but without plus disease are significantly larger than those of without ROP. (2) There was no significant difference in vascular density and vascular fractal dimension between preterm infants with ROP and without ROP. (3) Compared with preterm infants without ROP, the vascular development in preterm infants with ROP was slower. All results had excluded the effects of plus disease and pre-plus disease.

Mao et al.[5] found that the vascular curvature, vascular width, vascular density and fractal dimension of preterm infants with ROP with pre-plus and plus disease were significantly higher than preterm infants without ROP. Similarly, we found that the vascular tortuosity and vascular caliber of preterm infants with ROP without plus or pre-plus were significantly larger than those of without ROP. These results suggested that the changes in vascular tortuosity and caliber in the posterior pole region of the retinal are continuous dynamic process in the evolution from the normal state toward ROP, eventually into plus disease, which is consistent with the views of Chiang[4] and other scholars.

We found that vascular density and vascular fractal dimension were significantly different between preterm infants with ROP and without ROP before correcting the effects of gestational age and weight. But after correcting the effect of gestational age and weight, the difference disappeared. This indicates that in the absence of plus or pre-plus diseases, ROP does not bring about changes in density and fractal dimension. But Mao et al.[5] has demonstrated the significant difference between preterm infants with plus diseases and without ROP. This indicated that during development of ROP, the vascular density and vascular fractal dimension of the posterior pole region may not change continuously.

The results of Gupta et al.[10, 18] showed that after anti-VEGF treatment, the severity of vessel alteration in preterm infants with ROP was significantly lower than that before treatment. These findings suggested that hypoxia-induced up-regulation of neovascular factors such as VEGF is one of the main causes of tortuousness and dilation of vessels in the posterior pole region of the preterm infant's retinal. In addition, hypoxia of the retinal leads to block of retinal vessels, which increases the circulatory resistance of the retinal vascular network, and is also one of the reasons for the tortuousness and expansion of retinal vessels. In the process of retinal vascularization in preterm infants, with the retinal hypoxia and the increase of hypoxia-induced neovascularization factors, the vascular tortuosity and vascular caliber in the posterior pole region continue to increase, and patients with severe diseases develop plus disease.

Retinal vascular branch angles become larger, suggesting decreased retinal blood perfusion[19, 20], which could lead to retinal ischemia. Statistical analysis of retinal vascular branch angle showed that the vascular branch angle of preterm infants with ROP but without plus disease was significantly larger than that of preterm infants without ROP, which indicated that retinal blood perfusion in preterm infants with ROP was less than that of preterm infants without ROP. This is consistent with the mechanism of ROP that hypoxia-induced retinal vessel occlusion and vessel-growth-factor-mediated neovascularization can hinder normal retinal vascularization, resulting in less retinal blood perfusion in preterm infants with ROP than in preterm infants without ROP[7, 21].

ROP is a fibrovascular proliferative disorder that affects preterm infants and is characterized by arrest or disruption of normal retinal vascular development[7, 21]. Postnatal hypoxia forms retinal avascular areas. And hypoxia causes spasmodic occlusion of retinal arterioles, which in turn aggravates retinal hypoxia, resulting in retinopathy and delayed retinal vascular development[10, 18]. This fact is consistent with the results of our study, which showed that compared with normal preterm infants, the retinal vascular density of the preterm infants with ROP without plus changed more slowly. That is, the retinal vessel developed more slowly in the preterm infants with ROP. Slower retinal vessel development further aggravates retinal hypoxia, which further promotes the occurrence of ROP.

In summary, during the evolution of normal preterm infants to ROP, the vascular tortuosity and vascular caliber of the retinal posterior pole region continue to increase. When there is a visible tortuousness and expansion, it often indicates that ROP is more serious. When ROP occurs in preterm infants, retinal vascular development is delayed, which further hinders the normal retinal vascularization process. This study is a retrospective study, and no further study was made on the vascular morphological changes

with the disease development in the same patient over time. The research subjects we selected were preterm infants with ROP and without ROP at 4–6 weeks after birth. The vascular development dynamics revealed in this study can only reflect the situation at 4–6 weeks after birth. Next, we will further explore the dynamic changes of retinal morphology over time in preterm infants with ROP and without ROP in order to predict the occurrence of ROP and monitor the progress of the disease through changes in the morphological vessels of the vessels in posterior pole region.

Conclusions

In conclusion, vessels in posterior pole region of preterm infants with ROP are more tortuous and thicker than that of preterm infants without ROP. And vessels of preterm infants with ROP have larger branch angle. Vessels in posterior pole region of preterm infants with ROP develop slower than preterm infants without ROP, indicating differences in the level of vascular proliferation. The findings demonstrate retinal vessels are important markers of ROP-related diseases. The findings could provide insights into the pathogenesis of ROP.

Declarations

Author contribution Q.Wang, Y.Xu and S.Liu equally contributed to data collection and analysis, manuscript preparation and revision as first authors. Y.Yang, Y.Lu and M.Sun analyze the data. H.Liu performed the research and approved the final manuscript.

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Conflicts of Interest The authors declare that they have no conflict of interest with this work.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the Declaration of Helsinki. All subjects were provided written informed consent.

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Figures

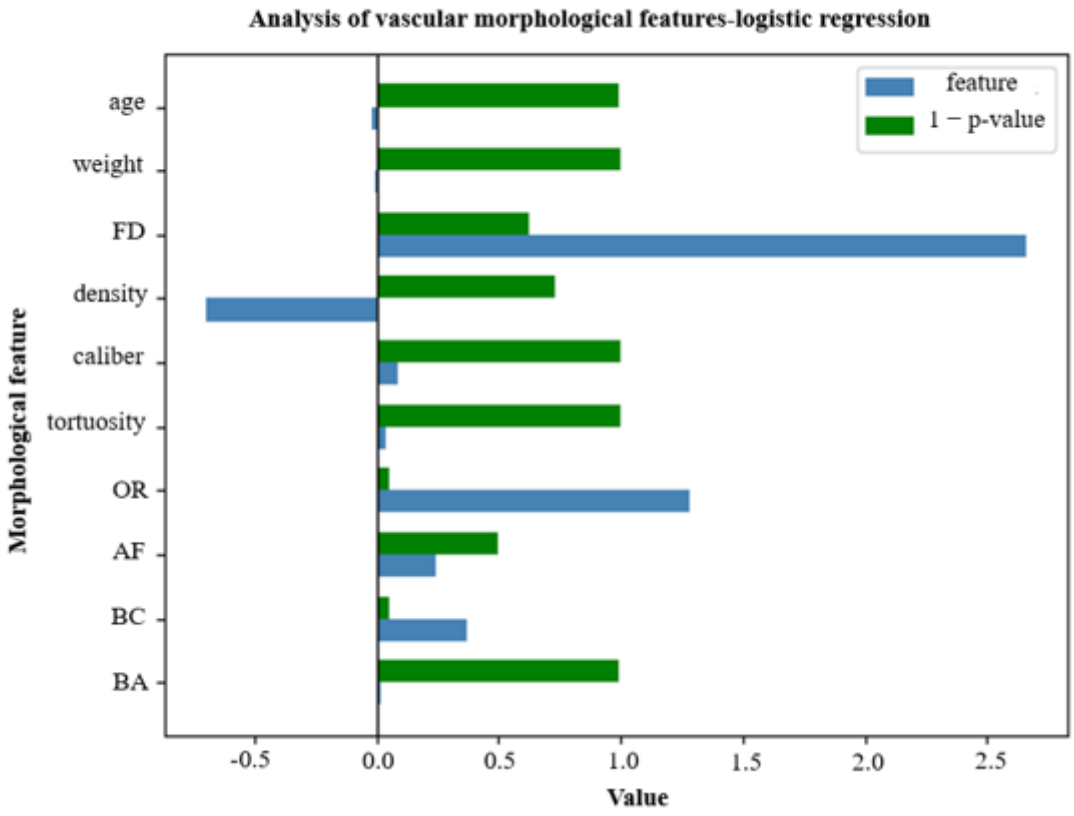

Figure 1

Regression coefficients and significant level for each morphological feature

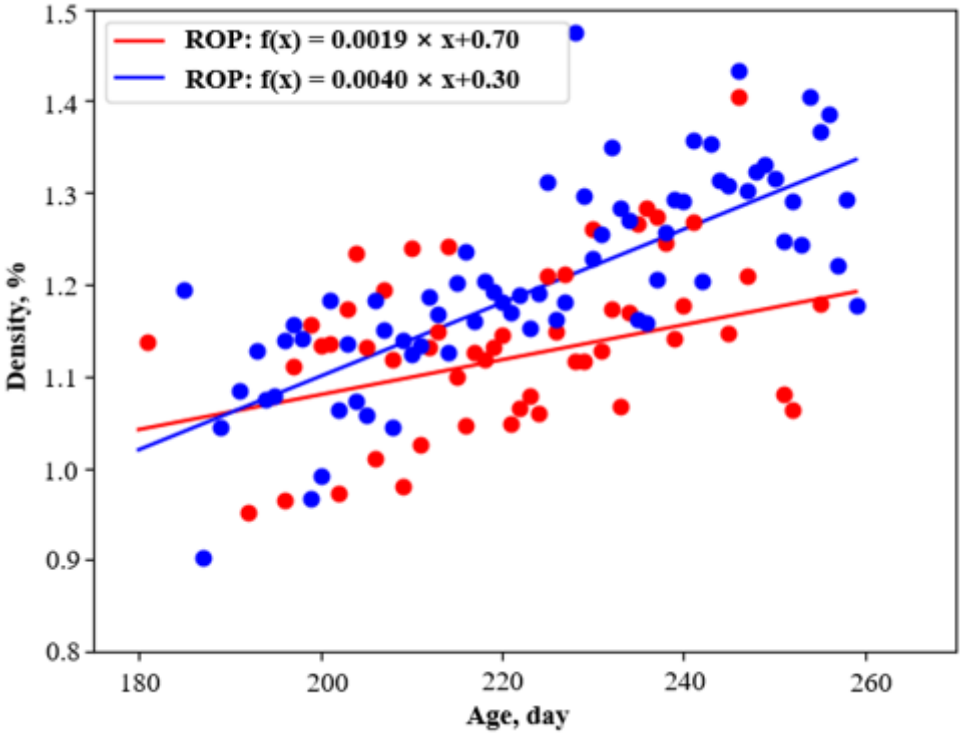

Figure 2

Dynamic comparison of vascular density development (with ROP *vs* without ROP)