• Clinical Research •

Changes of the peripapillary vascular parameters in premature infants without retinopathy of prematurity using U-net segmentation

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Received: 2023-10-22 Accepted: 2024-04-03

Abstract

● **AIM:** To quantitatively assess the changes in mean vascular tortuosity (mVT) and mean vascular width (mVW) around the optic disc and their correlation with gestational age (GA) and birth weight (BW) in premature infants without retinopathy of prematurity (ROP).

● **METHODS:** A single-center retrospective study included a total of 133 (133 eyes) premature infants [mean corrected gestational age (CGA) 43.6wk] without ROP as the premature group and 130 (130 eyes) CGA-matched full-term infants as the control group. The peripapillary mVT and mVW were quantitatively measured using computer-assisted techniques.

● **RESULTS:** Premature infants had significantly higher mVT (P=0.0032) and lower mVW (P=0.0086) by 2.68 (10^4 cm^{-3}) and 1.85 μm, respectively. Subgroup analysis with GA showed significant differences (P=0.0244) in mVT between the early preterm and middle to late preterm groups, but the differences between mVW were not significant (P=0.6652). The results of the multiple linear regression model showed a significant negative correlation between GA and BW with mVT after adjusting sex and CGA (P=0.0211 and P=0.0006, respectively). For each day increase in GA at birth, mVT decreased by 0.1281 (10^4 cm^{-3}) and for each 1 g increase in BW, mVT decreased by 0.006 (10^4 cm^{-3}). However, GA (P=0.9402) and BW (P=0.7275) were not significantly correlated with mVW.

● **CONCLUSION:** Preterm birth significantly affects the peripapillary vascular parameters that indicate higher mVT and narrower mVW in premature infants without ROP. Alterations in these parameters may provide new insights into the pathogenesis of ocular vascular disease.

● **KEYWORDS:** premature infants; retinopathy of prematurity; retinal vessels parameter; computer-assisted techniques

DOI:10.18240/ijo.2024.08.10

Citation: Liu S, Liu L, Ma CX, Huang LH, Li B. Changes of the peripapillary vascular parameters in premature infants without retinopathy of prematurity using U-net segmentation. *Int J Ophthalmol* 2024;17(8):1453-1461

INTRODUCTION

In humans, the retina is to undergo vascularization in the developing fetus, and retinal vasculature begins to crawl and grow from the optic disc toward the periphery at 14-16wk after conception, developing to the nasal side at 36wk and to the temporal side at 40wk[1]. Preterm infants are born with incomplete vascularization of the peripheral retina and postnatal exposure to relative hyperoxia and inadequate serum insulin-like growth factor levels lead to the arrest of the growth of normally developing retinal vessels *in utero* and the loss of some developed vessels[1-3]. As the infant matures, the metabolism of the non-vascularized retina continues to increase, and the hypoxia is also becoming more and more serious, leading to the occurrence of retinopathy of prematurity (ROP)[1-3].

It is well known that the development of ROP is accompanied by significant changes in vascular morphology in the posterior pole of the retina[4-7]. Mao *et al*[6] quantified the morphological characteristics of the retinal vessels by computer-assisted

techniques and showed that preterm infants with pre-plus or plus ROP had significantly higher vessel curvature, vessel width, vessel density, and fractal dimension than normal preterm infants. Another study indicated that retinal vessel width and tortuosity increased more rapidly in eyes that eventually developed type 1 ROP compared to eyes that did not develop type 1 ROP[7]. Taylor *et al*[5] automated a quantitative severity scale for ROP based on retinal vascular morphology using deep learning and showed that automated image analysis may be used to quantify clinical disease progression and identify infants at high risk for eventually developing treatment-requiring ROP. These findings suggest that preterm infants who develop ROP have significant alterations in vascular parameters in the posterior pole of the retina, and that monitoring quantitative changes in vascular parameters is important for the management of the disease.

Since ROP is one of the common causes of childhood blindness[3,8], researchers studied the vascular characteristics of the posterior pole of the retina in preterm children with ROP systematically and comprehensively. However, to date, only several studies focus on the morphological changes in the posterior pole of the retina in children born prematurely without ROP. Hellström *et al*[9-10] showed that premature children exhibited a higher tortuosity index for arteries and veins compared to controls, independent of the previous history of ROP. Lind *et al*[11] reported higher retinal arterial and venous tortuosity indices in 10-year-old moderate-to-late preterm children without ROP compared to controls. Gishti *et al*[12] showed that preterm children at the age of 6y (no classification of whether ROP occurs) had narrower retinal artery calibers compared to controls, but the difference in vein calibers was not significant between the two groups. These results suggest that a history of preterm birth without ROP may significantly influence the pattern of vascular development in the posterior pole of the retina in children. To date, as far as we know, the effect of preterm birth on the pattern of vascular development in the posterior pole of the retina in infants without ROP has not been reported.

Based on the authors' clinical observations and published studies, we conjecture that the posterior pole vascular parameters of the retina are also significantly altered in infants born prematurely without ROP. The study of changes in vascular parameters in the posterior pole of the retina in preterm children without ROP is critical for gaining a full understanding of the impact of preterm delivery on retinal vascular development, which may aid in understanding the pathophysiology of vascular illnesses. Therefore, the aim of this study was to quantitatively investigate the changes in peripapillary vascular morphology [mean vascular tortuosity (VT) and vascular width (VW)] and their correlation with gestational age (GA) at birth and birth weight (BW) in premature infants without ROP using computer-assisted techniques.

SUBJECTS AND METHODS

Ethical Approval The study adhered to the principles of the Declaration of Helsinki and was approved by the Ethics Committee of Anhui Maternal and Child Health Hospital. The IRB number is YYLL2023-01-01-1.0. All guardians signed the informed consent form.

Participant Demographics This was a retrospective, case-control study. We retrospectively studied the records of preterm infants who underwent fundus examinations from April 1st, 2019 to December 31st, 2021. At each examination, the pupil was dilated using compound tropicamide eye drops (SenTen Pharmaceuticals China Ltd.), and the fundus images were recorded using a wide-field fundus imaging system, RetCam III (Clarity Medical Systems, Pleasanton, CA, USA), after the pupil was fully dilated.

Fundus examinations for older preterm and full-term infants have been incorporated into routine newborn screening programs in some areas of mainland China[13]. According to the Chinese Guidelines for Retinopathy of Prematurity Screening (2014) and the actual implementation of fundus examination in our hospital[14], we have formulated the following inclusion and exclusion criteria. The inclusion criteria for the preterm group were: 1) GA at birth less than 37wk; 2) first fundus examination at 4-6wk after birth; 3) the interval of follow-up is determined by the retinal vascularization of the preterm infant: weekly for preterm infants without ROP in zone I; every 2-3wk for preterm infants without ROP in zone II; every 4wk for preterm infants without ROP in zone III until the temporal retina was completely vascularized or corrected gestational age (CGA) up to 45wk; 4) both of the eyes had no ROP at the first screening and throughout the follow-up period; 6) recorded fundus images must meet the criteria for computer image analysis. Exclusion criteria: 1) premature infants with ROP in either eye at first fundus examination or during follow-up; 2) preterm infants without ROP but with superficial retinal hemorrhage or vitreous hemorrhage in the fundus; 3) other ocular diseases (congenital cataract, persistent hyperplastic primary vitreous and familial exudative vitreoretinopathy, *etc*.) are also excluded.

Premature infants who were eligible for the inclusion and exclusion criteria were selected as the premature group. All premature infants without ROP were followed up until the temporal retina was completely vascularized or CGA up to 45wk. The fundus images (Figure 1) recorded at the last follow-up were used to analyze the vascular characteristics around the optic disc. When both eyes met the inclusion criteria, one eye was randomly selected. A total of 133 (133

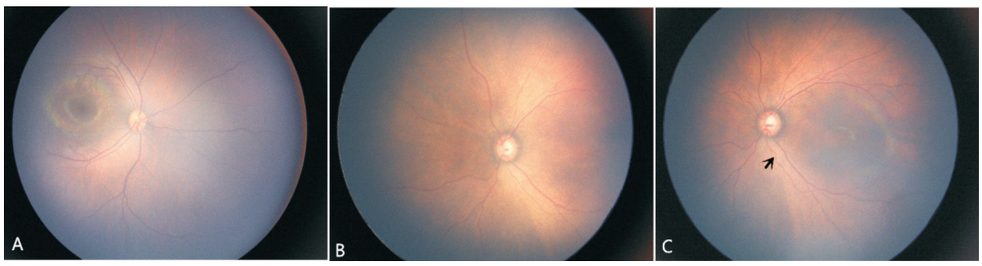

Figure 1 Representative fundus images of normal full-term newborns and premature infants without ROP taken by RetCam 3 A: Fundus image of a full-term neonate born at 41+1wk of gestational age (GA). Corrected gestational age (CGA) 45+1wk. No significant abnormalities in the posterior pole vessels of the retina. B: Fundus of a preterm neonate born at GA 31wk. CGA 31wk, no significant abnormalities in the posterior pole vessels of the retina. C: Fundus image of the same eye as in Figure B. CGA 43+3wk. Although no ROP was found during the entire follow-up period, significant tortuosity of the arteries could be observed in the posterior pole of the retina (black arrow). ROP: Retinopathy of prematurity.

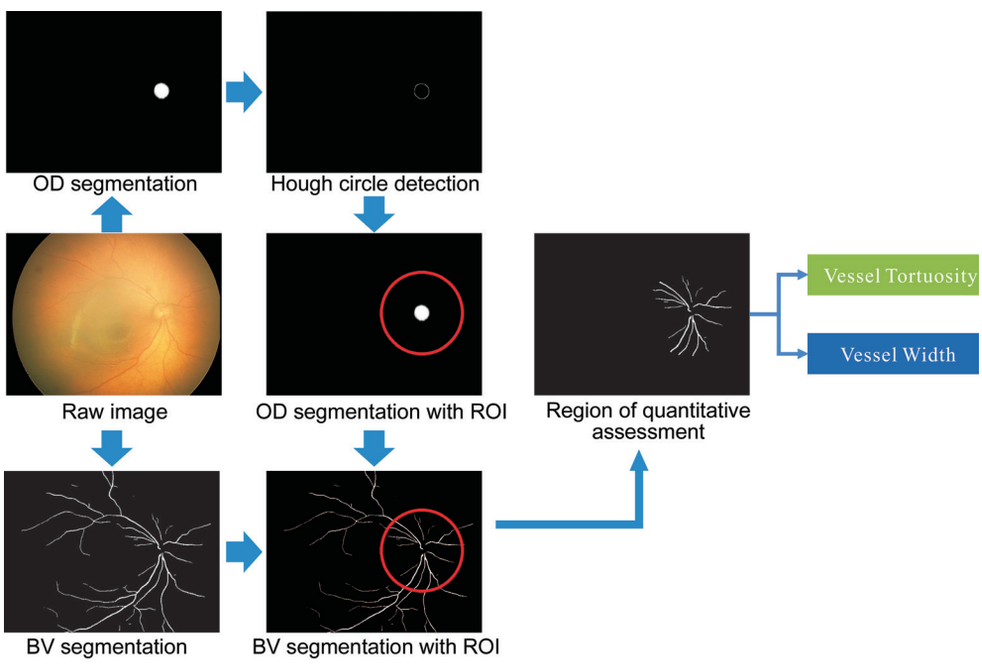

Figure 2 The pipeline of quantitative assessment in premature infants without retinopathy of prematurity OD: Optic disc; BV: Blood vessel; ROI: Region of interest.

eyes) preterm infants who met the criteria were included in this study as the premature group, and 130 (130 eyes) CGA-matched full-term infants were selected as the control group. We excluded infants with superficial retinal hemorrhage (n=9), vitreous hemorrhage (n=1) or other ocular diseases (n=5).

Image Progressing This study has been considerably supported by computer-aided analysis and artificial intelligence (AI) tools, which have addressed several obstacles in the research process. The wide-angle fundus imaging system (RetCam III) was used in this study to obtain the subjects' fundus images, and then a series of AI technologies were used to obtain the analysis area, and finally two important blood vessel characteristics were obtained: the tortuosity of the blood vessels and the width of the blood vessels. We carefully screened the fundus images before the experiment. The following are our screening criteria: 1) The optic disc, blood vessels, and macula are clearly visible in the fundus image; 2) the fundus image meets the image quality standards for assessing the disease.

The entire technique of AI-based blood vessel feature analysis is the same as the paper published by Liu *et al*[15], which is presented in Figure 2. The specifics are as follows: 1) The optic disc and blood vessels of the fundus picture are taken from the original image. The procedure is based on an open-source project (https://github.com/orobix/retina-unet) that first trains two effective optic disc segmentation and blood vessel segmentation models before implementing automated segmentation based on the trained model. This differs from earlier research on manual segmentation[16]. 2) Define the region of interest (ROI) based on the optic disc segmentation results. To achieve an accurate optic disc radius, Hough Circle detection is used to the results of optic disc segmentation. According to earlier research, the distance from the center of the fovea to the center of the optic disc is 4.93 mm, which

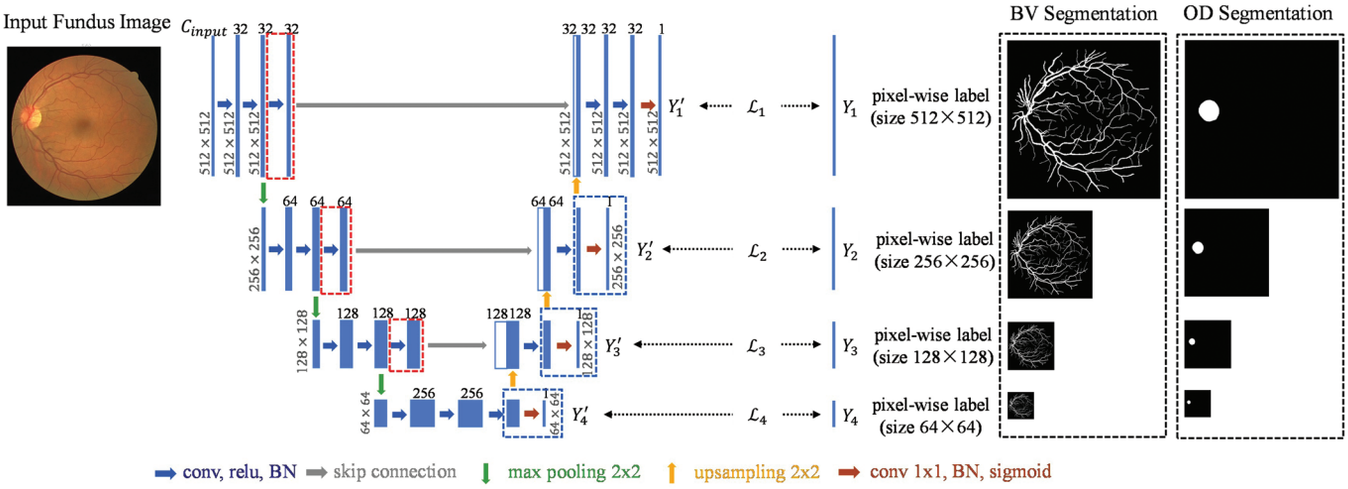

Figure 3 U-net model for BV and OD segmentation OD: Optic disc; BV: Blood vessel.

Table 1 BV and OD segmentation performance on both public datasets DRIVE and STARE

Parameters	Dataset	ACC	Se	Sp	Precision	Dice score
BV Segmentation	DRIVE	0.9564	0.8314	0.9747	0.8275	0.8290
	STARE	0.9751	0.8482	0.9856	0.8310	0.8395
OD Segmentation		0.9992	0.9042	0.9996	0.9256	0.9148

BV: Blood vessel; OD: Optic disc; ACC: Accuracy; Se: Sensitivity; Sp: Specificity.

is approximately 5 times the diameter of the human body's optic disc (1.83 mm). As a result, to encompass the optic disc and macular area while also allowing us to focus on critical blood arteries, as in the previous work[17], we chose a circular area with a radius of 6 times the optic disc radius in the ROI. 3) On the blood vessel segmentation map, apply the defined ROI to produce the blood vessel quantitative assessment area necessary for quantitative analysis. 4) In the domain of vascular quantitative evaluation, two significant characteristics are calculated: VT and VW.

To determine the tortuosity of the vessel effectively, we used the arc-length normalized total square curvature to calculate the tortuosity of each vessel in the vessel assessment area, and then used the average value of all VTs as the tortuosity of the whole picture[18]. The VT calculation formula is as follows:

$$VT = \int_{t_0}^{t_1} k(t)^2 dt \quad (1)$$

Where $k(t)$ denotes curvature.

To compute vessel width effectively, we use the Euclidean distance transform to calculate the distance between each vessel pixel and the nearest background pixel[19]. Using an adaptive thresholding approach, we extract vessel centerlines and determine the width of individual vessels[20]. The average VW is determined as follows:

$$VW = \frac{1}{N}\sum_{j}^{N} W_i \quad (2)$$

W_i is the width of the i-th vessel in ROI.

A U-net-based segmentation model was trained for retinal blood vessel and optic disc segmentation tasks (Figure 3). For the BV segmentation task, the model demonstrated commendable performance after being trained on both public datasets DRIVE and STARE[21] in Table 1. On the DRIVE dataset, the model achieved notable metrics including an accuracy of 0.9564, sensitivity of 0.8314, specificity of 0.9747, precision of 0.8275, and a Dice coefficient of 0.8290. Similarly, on the STARE dataset, it performed admirably with metrics as follows: accuracy of 0.9751, sensitivity of 0.8482, specificity of 0.9856, precision of 0.8310, and a Dice score of 0.8395.

For optic disc segmentation, since no publicly available dataset exists, we constructed a custom dataset and trained a dedicated model. This model attained highly accurate segmentation outcomes, boasting accuracy of 0.9992, sensitivity of 0.9042, specificity of 0.9996, precision of 0.9256, and a Dice coefficient of 0.9148 on our custom dataset in Table 1.

The consistently high performance metrics obtained across both segmentation tasks provide strong assurance for the reliability and validity of any subsequent analyses that rely on accurately segmented retinal vessels and optic discs.

Statistical Analysis Statistical analysis of the data was performed using SAS Statistics software (version 9.4 Cary, NC, USA). The measurement data were expressed as mean±standard deviation. According to the normality test, comparisons of means between two groups were performed using the unpaired *t*-test or Wilcoxon-Mann-Whitney test. We used multiple linear regression models to investigate the relationship between peripapillary vascular parameters and birth GA and BW. To control the effect of confounding factors, the model was adjusted for sex and the CGA of the infant. We

first analyzed GA at birth and BW as categorical variables separately. We then reconstructed the above regression model using birth GA and BW as continuous variables. *P* values less than 0.05 were defined as statistically significant.

RESULTS

Participant Demographics A total of 133 (133 eyes) preterm infants without ROP were included in this study as the premature group, and 130 (130 eyes) CGA-matched full-term infants were selected as the control group. The CGA of the preterm group was 283-332d, with a mean of 305.5±14.1d; the CGA of the control group was 280-334d, with a mean of 305.5±10.0d (*P*=0.9823). The premature group had significantly smaller birth GA and lower BW than the control group (both *P*<0.0001). Additional detailed demographic information is shown in Table 2.

Premature Infants Indicated Increased Vessel Tortuosity Compared with Controls Quantitative analysis showed a significant increase in peripapillary mean vascular tortuosity (mVT) in the preterm group compared to the control group, 78.69±6.47 (10^4 cm^{-3}) and 76.01±8.02 (10^4 cm^{-3}), respectively (*P*=0.0032; Table 3). We further divided the study population in the preterm group into early preterm (EP; GA<224d) and middle to late preterm (MLP; 224≤GA<259d) groups according to GA at birth for subgroup analysis[22]. The results of the subgroup analysis showed that the differences in peri-optic disc mVT between the EP, MLP and control groups were all significant (Table 4). The results suggest that infants born prematurely without ROP have higher retinal vascular curvature.

Premature Infants Indicated Narrower Vessel Caliber Compared with Controls Quantitative analysis showed that peripapillary mean vascular width (mVW) was significantly narrower in the preterm group compared to the control group (*P*=0.0086; Table 3). Further subgroup analysis showed that the differences in peripapillary mVW were significant between the EP group, MLP group, and control group (*P*=0.0092 and 0.0369; Table 4). However, the difference in mVW around the optic disc between the EP group and MLP group was not significant (*P*=0.6652; Table 4). The results suggest that preterm infants have narrower retinal vessel diameters.

Correlations Between Gestational Age and Birth Weight with Mean Vessel Tortuosity and Width We created multiple linear regression models to further explore the correlation between GA at birth and BW with peripapillary mVT and mVW in the premature group. Table 5 shows the relationship between BW and birth GA classification and peripapillary mVT and mVW. The results show that after adjusting for sex and CGA of the infant, smaller birth GA is significantly associated with higher peripapillary mVT (*P* for trend=0.0006). Similar findings were found for BW, with lower BW being significantly associated with greater peripapillary mVT (*P* for trend=0.0211).

Table 2 Participant demographics mean±SD

Parameters	Control	Premature	*P*
No. of patients	130	133	-
Female/male	59/71	48/85	0.1257[b]
Birth weight (g)	3373.7±369.2	1600.8±347.4	<0.0001[a]
GA (d)	277.6±7.2	219.0±12.5	<0.0001[a]
CGA (d)	305.5±10.0	305.5±14.1	0.9823[a]

[a]Unpaired *t*-test, [b]Chi-square test. GA: Gestational age; CGA: Corrected gestational age; SD: Standard deviation.

Table 3 Vessel parameters between control and premature groups mean±SD

Parameters	Control	Premature	*P*
mVT (10^4 cm^{-3})	76.01±8.02	78.69±6.47	0.0032[a]
mVW (μm)	68.20±6.14	66.35±5.13	0.0086[a]

[a]Unpaired *t*-test. mVT: Mean vessel tortuosity; mVW: Mean vessel width; SD: Standard deviation.

Further multiple linear regression with GA at birth and BW as continuous variables confirmed the results of our analysis above. Table 6 shows that smaller GA at birth and BW were significantly associated with higher peripapillary mVT (*P*=0.0048 and 0.0002, respectively). The regression analysis showed that for each day increase in GA at birth, mVT decreased by 0.1281 (10^4 cm^{-3}) and for each 1 g increase in birth weight, mVT decreased by 0.0060 (10^4 cm^{-3}).

Regardless of classifying birth GA and BW as categorical or continuous variables in multiple linear regression, birth GA and BW were not significantly correlated with peripapillary mVW (*P*>0.05; Tables 5 and 6). These results suggest that GA at birth and BW are significantly negatively correlated with peripapillary mVT in preterm infants without ROP, but not with mVW.

DISCUSSION

In the present study, we used computer-assisted techniques to quantify changes in peripapillary vascular morphology in infants born prematurely without ROP, and showed that a history of prematurity without ROP significantly affected the pattern of vascular development in the posterior pole of the retina. The premature group had higher retinal VT and narrower retinal vessel diameters than the full-term group, and retinal vascular curvature was significantly negatively correlated with birth GA and BW. The findings of this study add to our knowledge of the developmental characteristics of retinal vasculature in the early life of preterm infants.

A previous study in premature children at 4.8 years old showed a significant increase in retinal arterial and venous curvature in children born preterm compared to children born at term, independent of a previous history of ROP[9]. Another

Table 4 Vessel parameters between control and premature subgroups by gestational age mean±SD

Parameters	Control (*n*=130)	EP (*n*=85)	MLP (*n*=48)	$P_{control\ vs\ EP}$	$P_{control\ vs\ MLP}$	$P_{EP\ vs\ MLP}$
mVT (10^4 cm^{-3})	76.01±8.02	79.54±7.16	77.18±4.73	0.0012	0.0445	0.0244[a]
mVW (μm)	68.20±6.14	66.20±4.93	66.61±5.52	0.0092	0.0369	0.6652[a]

[a]Unpaired *t*-test. mVT: Mean vessel tortuosity; mVW: Mean vessel width; SD: Standard deviation; EP: Early premature; MLP: Middle to late premature.

Table 5 Birth weight, gestational age at birth and retinal vessel tortuosity and width mean±SD

Parameters	*n*	mVT (10^4 cm^{-3})	mVW (μm)
Birth weight, g			
First quartile, 800-1350	37	81.94±6.57	66.69±4.49
Second quartile, 1350-1550	30	77.96±6.16	65.85±5.95
Third quartile, 1550-1860	34	77.82±6.50	67.14±5.87
Fourth quartile, 1860-2490	32	76.53±5.37	65.58±4.14
P for trend		0.0006	0.5990
Gestational age at birth, d			
First quartile, 192-210	33	81.55±5.36	65.70±4.98
Second quartile, 210-220	33	79.31±7.38	66.30±5.36
Third quartile, 220-228	29	79.26±7.10	67.75±5.48
Fourth quartile, 228-253	38	77.02±4.80	65.82±4.67
P for trend		0.0211	0.6383

Linear regression models were used to analyzed the association of vessel parameters with the gestational age at birth and birth weight as categorical variables, adjusted for sex and corrected gestational age. mVT: Mean vessel tortuosity; mVW: Mean vessel width; SD: Standard deviation.

Table 6 The correlation of vessel parameters with the GA and BW mean (95%CI)

Parameters (*n*=133)	mVT	*P*	mVW	*P*
GA at birth, d	-0.1281 (-0.2156 to -0.0406)	0.0048	0.0027 (-0.0688 to 0.0743)	0.9402
BW, g	-0.0060 (-0.0029 to -0.0090)	0.0002	-0.0005 (-0.0030 to 0.0021)	0.7275

Linear regression models were used to analyzed the association of vessel parameters with the GA at birth and BW as continuous variable, adjusted for sex and corrected GA. mVT: Mean vessel tortuosity; mVW: Mean vessel width; GA: Gestational age; BW: Birth weight; CI: Confidence interval.

study in MLP children without ROP at 10 years old showed a higher tortuosity index for arteries and veins in MLP children compared to the control group[11]. Our findings in preterm infants without ROP were similar to the previous studies, showing that peripapillary mVT was significantly higher in preterm infants without ROP compared to full-term infants.

A study in middle-aged adults born prematurely found that retinal artery curvature was significantly higher in the preterm group and was significantly negatively correlated with BW[23]. We reached similar conclusions in the preterm infants without ROP, showing that peripapillary mVT in preterm infants was significantly negatively associated with GA at birth and BW. Regression analysis showed that the mVT decreased by 0.1281 (10^4 cm^{-3}) for each day increase in GA at birth and by 0.00601 (10^4 cm^{-3}) for each 1 g increase in BW. However, our results are inconsistent with Hellström *et al*'s[9] study which concluded that changes in vascular curvature in the posterior pole of the retina did not correlate with GA at birth. In Hellström *et al*'s[9] study, the authors also suggested that increased retinal VT may be a non-specific response of retinal vessels to various stimuli at different ages. The age of patients in our study population is different from Hellström *et al*'s[9], resulting in various conclusions and interesting findings. We can safely draw a conclusion that prematurity may significantly affect peripapillary VT even when ROP does not occur, and this effect may persist from infancy to childhood or even longer.

So far, the mechanism of peripapillary vascular curvature in children born prematurely without ROP is still unclear. Several studies have shown that preterm infants generally exhibit lower serum insulin-like growth factor 1 (IGF-1) levels in the early postnatal period and that serum IGF-1 levels are closely correlated with GA at birth[24-26]. Another study found that neonatal serum IGF-1 levels were significantly negatively correlated with retinal vascular curvature in 10-year-old moderate-to-late preterm children[11]. Based on these studies, we can speculate that lower serum IGF-1 levels in the early postnatal period may be one of the causes of retinal vascular curvature. In the present study, since it is retrospective, we

have not been able to examine serum IGF-1 levels in preterm infants to further clarify the correlation between serum IGF-1 levels and vascular curvature. Further studies are needed to assess the correlation between serum IGF-1 levels and vascular curvature in preterm infants.

There have been several studies on retinal vessel diameters in the preterm and full-term populations, but none of them considered whether ROP occurred. A prospective population-based cohort study found that children born prematurely at age 6y had narrower retinal artery diameters and no significant differences in retinal vein diameters compared to children born at term[12]. Another study showed that at 11 years of age, children born prematurely had smaller retinal artery and vein diameters than children born at term[27]. Hussain *et al*[23] found that middle-aged adults born prematurely had narrower retinal vascular diameter than those born at term, and retinal artery diameter did not correlate with BW after adjusting for age, sex, employment status, marital status, smoking status and cardiovascular disease risk factors. Our findings are similar to these studies in both pediatric and adult populations, suggesting that preterm infants without ROP had narrower retinal vessel diameters than controls, and that vessel diameters did not correlate with GA at birth or BW. Based on the above studies in different age groups, we can see that the effect of preterm birth on retinal vessel diameter may continue from infancy into adulthood.

How does a history of preterm birth without ROP affect the caliber of the retinal vessels? To date, no relevant studies have been conducted. As the human retinal vasculature lacks extrinsic innervation, retinal vessel caliber and local blood flow are usually regulated by non-neural mechanisms intrinsic to the retina[28]. Fahrni *et al*[29] reported a gradual reduction of approximately 30% in retinal artery width around the optic disc from 2 to 144h postnatally in full-term neonates during smooth adaptation to room air. We speculate that, unlike full-term neonates, the immature retinal vasculature of preterm infants may overreact to environmental changes, resulting in preterm infants with narrower retinal vasculature. The ROP-based study further supports our conjecture. Postnatal exposure of preterm neonates to relative hyperoxia and inadequate serum IGF levels would lead to the arrest of the development of immature retinal vessels and loss of some developed vessels, further resulting in retinal vascular occlusion and consequently ROP[1,8,30]. In addition, Kistner *et al*[31] noted in his study that the full-term baby spends the last months of gestation in the relatively stable intrauterine environment, whereas the preterm baby spends the corresponding time period in a markedly different environment, with altered functional and metabolic requirements. This may considerably alter the development of the vascular system. In a word, the inappropriate response of the immature retinal vasculature to the intrauterine environment may be the main reason for the altered morphology of the retinal vasculature in preterm infants without ROP.

The retinal vasculature is the only vessel system that can be observed directly throughout the body, and changes in the morphology of the retinal vasculature may reflect alterations in the general vascular system[32]. Published findings suggest that higher retinal artery curvature and narrower retinal arteries are associated with an increased risk of cardiovascular disease. Tapp *et al*[33] showed that greater curvature of small retinal arteries was associated with higher systolic, mean arterial and pulse pressures. Narrower retinal arteries are associated with higher systolic blood pressure, mean arterial pressure and arterial stiffness index[33], increased risk of stroke and coronary heart disease[34-35] and higher cardiovascular mortality[36]. In the present study, we found that preterm infants without ROP exhibited more curved and narrower retinal vessels. Combined with the published findings, we can infer preterm infants without ROP may be at increased risk of developing cardiovascular disease later in life.

Admittedly, there are some limitations in our study. First, in the present study, the average CGA of premature infants was 43.6wk, so our findings could only reflect the effects of prematurity on retinal vascular development early in life. Further prospective studies are needed to determine the effect of a history of non-ROP premature birth on retinal vascular morphology in childhood and even in adulthood. Second, we could not completely exclude slight ROP that occurred during the follow-up interval. Finally, in regression analysis, we adjusted for only two confounding factors, CGA and sex, leading to possible residual confounding in the observed associations.

In conclusion, the present study found that a history of prematurity without ROP significantly affected peripapillary vascular parameters in preterm infants, exhibiting higher peripapillary VT and narrower VW. Furthermore, peripapillary curvature was significantly negatively associated with birth GA and BW. These alterations in retinal vascular morphology may further increase the risk of ocular and even systemic vascular disease.

ACKNOWLEDGEMENTS

Foundations: Supported by the Fundamental Research Funds for the Central Universities (No.WK2100000045); the National Natural Science Foundation of China (No.U19B2044); Hefei Health Care Commission 2022 Applied Medical Research Project (No.Hwk2022yb028); Zhejiang Lab Open Research Project (No.K2022QA0AB04).

Conflicts of Interest: Liu S, None; **Liu L,** None; **Ma CX,** None; **Huang LH,** None; **Li B,** None.

REFERENCES

1 Jang JH, Kim YC. Retinal vascular development in an immature retina at 33-34 weeks postmenstrual age predicts retinopathy of prematurity. *Sci Rep* 2020;10(1):18111.

2 Bai YC, Wu R, Chen SZ, Wei SY, Chen HJ, Chen YC, Feng SF, Lu XH. Efficacy of the WINROP algorithm for retinopathy of prematurity screening in Southern China. *Int J Ophthalmol* 2021;14(1):127-132.

3 Wu FY, Yu WT, Zhao DX, Pu W, Zhang X, Gai CL. Recurrence risk factors of intravitreal ranibizumab monotherapy in retinopathy of prematurity: a retrospective study at one center. *Int J Ophthalmol* 2023;16(1):95-101.

4 Gupta K, Campbell JP, Taylor S, Brown JM, Ostmo S, Paul Chan RVP, Dy J, Erdogmus D, Ioannidis S, Kalpathy-Cramer J, Kim SJ, Chiang MF, Imaging and Informatics in Retinopathy of Prematurity Consortium. A quantitative severity scale for retinopathy of prematurity using deep learning to monitor disease regression after treatment. *JAMA Ophthalmol* 2019;137(9):1029-1036.

5 Taylor S, Brown JM, Gupta K, Campbell JP, Ostmo S, Paul Chan RVP, Dy J, Erdogmus D, Ioannidis S, Kim SJ, Kalpathy-Cramer J, Chiang MF, Imaging and Informatics in Retinopathy of Prematurity Consortium. Monitoring disease progression with a quantitative severity scale for retinopathy of prematurity using deep learning. *JAMA Ophthalmol* 2019;137(9):1022-1028.

6 Mao J, Luo Y, Liu L, Lao J, Shao Y, Zhang M, Zhang C, Sun M, Shen L. Automated diagnosis and quantitative analysis of plus disease in retinopathy of prematurity based on deep convolutional neural networks. *Acta Ophthalmol* 2020;98(3):e339-e345.

7 Hong GJ, Koerner JC, Weinert MC, Stinnett SS, Freedman SF, Wallace DK, Riggins JW, Gallaher KJ, Prakalapakorn SG. Quantitatively comparing weekly changes in retinal vascular characteristics of eyes eventually treated versus not treated for retinopathy of prematurity. *J AAPOS* 2021;25(1):25.e1-25.e7.

8 Fevereiro-Martins M, Marques-Neves C, Guimarães H, Bicho M. Retinopathy of prematurity: a review of pathophysiology and signaling pathways. *Surv Ophthalmol* 2023;68(2):175-210.

9 Hellström A, Hård AL, Chen Y, Niklasson A, Albertsson-Wikland K. Ocular fundus morphology in preterm children. Influence of gestational age, birth size, perinatal morbidity, and postnatal growth. *Invest Ophthalmol Vis Sci* 1997;38(6):1184-1192.

10 Hellström A, Hård AL, Svensson E, Niklasson A. Ocular fundus abnormalities in children born before 29 weeks of gestation: a population-based study. *Eye (Lond)* 2000;14(Pt 3A):324-329.

11 Lind A, Dahlgren J, Raffa L, Allvin K, Ghazi Mroué D, Andersson Grönlund M. Visual function and fundus morphology in relation to growth and cardiovascular status in 10-year-old moderate-to-late preterm children. *Am J Ophthalmol* 2018;195:121-130.

12 Gishti O, Jaddoe VW, Duijts L, Steegers E, Reiss I, Hofman A, Wong TY, Ikram MK, Gaillard R. Impact of birth parameters and early life growth patterns on retinal microvascular structure in children: The Generation R Study. *J Hypertens* 2015;33(7):1429-1437.

13 Tang H, Li N, Li Z, *et al*. Fundus examination of 199 851 newborns by digital imaging in China: a multicentre cross-sectional study. *Br J Ophthalmol* 2018;102(12):1742-1746.

14 Fundus Disease Group of Ophthalmology Society of Chinese Medical Association. Guide for screening retinopathy of premature infants in China (2014). *Chin J Ophthalmol* 2014;50(12):933-935.

15 Liu S, Zhao H, Huang L, Ma C, Wang Q, Liu L. Vascular features around the optic disc in familial exudative vitreoretinopathy: findings and their relationship to disease severity. *BMC Ophthalmol* 2023;23(1):139.

16 Ataer-Cansizoglu E, Bolon-Canedo V, Campbell JP, *et al*. Computer-based image analysis for plus disease diagnosis in retinopathy of prematurity: performance of the "i-ROP" system and image features associated with expert diagnosis. *Transl Vis Sci Technol* 2015;4(6):5.

17 Huang F, Dashtbozorg B, Zhang J, Bekkers E, Abbasi-Sureshjani S, Berendschot TT, Ter Haar Romeny BM. Reliability of using retinal vascular fractal dimension as a biomarker in the diabetic retinopathy detection. *J Ophthalmol* 2016;2016:6259047.

18 Hart WE, Goldbaum M, Côté B, Kube P, Nelson MR. Measurement and classification of retinal vascular tortuosity. *Int J Med Inform* 1999;53(2-3):239-252.

19 Saito T, Toriwaki J. New algorithms for euclidean distance transformation of an n-dimensional digitized picture with applications. *Pattern Recognition* 1994;27(11):1551-1565.

20 Wellner PD. Adaptive thresholding for the DigitalDesk. Rank Xerox Ltd.: Cambridge, UK, 1993.

21 Sun K, Chen Y, Chao Y, Geng J, Chen Y. A retinal vessel segmentation method based improved U-Net model. *Biomedical Signal Processing and Control* 2023;82:104574.

22 Nath N, Beltrano W, Haynes L, Dewey D, Bray S. Long-term effects of preterm birth on children's brain structure: an analysis of the adolescent brain cognitive development (ABCD) study. *eNeuro* 2023;10(6):ENEURO.0196-22.2023.

23 Hussain SM, Kähönen M, Raitakari OT, *et al*. Impact of fetal growth and preterm birth on the retinal microvasculature in mid-adulthood. *Microcirculation* 2015;22(4):285-293.

24 Hellström A, Engström E, Hård AL, Albertsson-Wikland K, Carlsson B, Niklasson A, Löfqvist C, Svensson E, Holm S, Ewald U, Holmström G, Smith LE. Postnatal serum insulin-like growth factor I deficiency is associated with retinopathy of prematurity and other complications of premature birth. *Pediatrics* 2003;112(5):1016-1020.

25 Hellström W, Hortensius LM, Löfqvist C, Hellgren G, Tataranno ML, Ley D, Benders MJNL, Hellström A, Björkman-Burtscher IM, Heckemann RA, Sävman K. Postnatal serum IGF-1 levels associate with brain volumes at term in extremely preterm infants. *Pediatr Res* 2023;93(3):666-674.

26 Anvekar A, Athikarisamy S, Rao S, Gill A, Nathan E, Doherty D, Lam G. Time to regain birth weight - a marker to predict the severity of retinopathy of prematurity? *BMC Pediatr* 2021;21(1):540.

27 Wei FF, Raaijmakers A, Zhang ZY, van Tienoven TP, Huang QF, Yang WY, Thijs L, Struijker-Boudier HAJ, Verhamme P, Allegaert K, Staessen JA. Association between cognition and the retinal microvasculature in 11-year old children born preterm or at term. *Early Hum Dev* 2018;118:1-7.

28 Lam HC, Lee JK, Lu CC, Chu CH, Chuang MJ, Wang MC. Role of endothelin in diabetic retinopathy. *Curr Vasc Pharmacol* 2003;1(3):243-250.

29 Fahrni R, Thalmann J, Weber J, von Muralt G, Sidiropoulos D, Bracher D. Central retinal arteries in the full-term newborn: decrease in width and tortuosity during uneventful adaptation. *Helv Paediatr Acta* 1981;36(2):107-121.

30 Zhang L, Buonfiglio F, Fieß A, Pfeiffer N, Gericke A. Retinopathy of prematurity-targeting hypoxic and redox signaling pathways. *Antioxidants (Basel)* 2024;13(2):148.

31 Kistner A, Jacobson L, Jacobson SH, Svensson E, Hellstrom A. Low gestational age associated with abnormal retinal vascularization and increased blood pressure in adult women. *Pediatr Res* 2002;51(6):675-680.

32 Huang Y, Cheung CY, Li D, Tham YC, Sheng B, Cheng CY, Wang YX, Wong TY. AI-integrated ocular imaging for predicting cardiovascular disease: advancements and future outlook. *Eye (Lond)* 2024;38(3):464-472.

33 Tapp RJ, Owen CG, Barman SA, Welikala RA, Foster PJ, Whincup PH, Strachan DP, Rudnicka AR. Associations of retinal microvascular diameters and tortuosity with blood pressure and arterial stiffness: United Kingdom biobank. *Hypertension* 2019;74(6):1383-1390.

34 Wong TY, Klein R, Sharrett AR, Duncan BB, Couper DJ, Tielsch JM, Klein BE, Hubbard LD. Retinal arteriolar narrowing and risk of coronary heart disease in men and women. The Atherosclerosis Risk in Communities Study. *JAMA* 2002;287(9):1153-1159.

35 Aschauer J, Aschauer S, Pollreisz A, Datlinger F, Gatterer C, Mylonas G, Egner B, Hofer D, Steiner I, Hengstenberg C, Schmidt-Erfurth U. Identification of subclinical microvascular biomarkers in coronary heart disease in retinal imaging. *Transl Vis Sci Technol* 2021;10(13):24.

36 Yusufu M, Chen Y, Dayimu A, Bulloch G, Jin S, Vingrys AJ, Zhang L, Shang X, Shi D, He M. Retinal vascular measurements and mortality risk: evidence from the UK biobank study. *Transl Vis Sci Technol* 2024;13(1):2.