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Red blood cell indexes and laboratory findings in stage III of retinopathy of prematurity

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ABSTRACT

Background and Objective: Retinopathy of prematurity (ROP) is a progressive neovascular retinal disorder in neonates. This study investigated the possible correlation of hematological and biochemical laboratory indexes with the plus disease and the neovascularization of the iris (NVI) statuses in stage III ROP patients.

Methods: This case-control study was performed on 124 stages III ROP patients [(a) 58 cases with plus disease and 66 cases without plus disease, (b) 7 cases with NVI and 117 cases without NVI] in the Ophthalmology center of Ayatollah Rouhani Hospital, Babol, Iran. All ophthalmologic examinations were performed according to the international classification of retinopathy of prematurity (ICROP) criteria. The hematopoietic/biochemical parameters, i.e., WBC, Hb, Plt, Na, K, Ca, CRP, T_4, TSH, PCO_2, PO_2, HCO_3, SpO_2, MCV, and MCH, were evaluated based on standard protocols.

Findings: The MCV in patients with and without plus disease were 84.88±22.274 and 94.12±14.419 fl/cell in ROP patients, respectively; (p=0.012). Also, the MCH in patients with and without the plus disease were 30.12±3.649 and 31.99±5.149 pg/cell, respectively (p =0.033). Our results showed that CRP is higher in patients with plus disease (16.11±29.403 mg/L) than patients without plus disease (3.25±2.633 mg/dl); (p=0.033). Also, SpO2 was significantly higher in stage III ROP patients with NVI (64.00±13.730%) compared to non-NVI patients (75.58±16.135%); (p=0.043).

Conclusion: NVI and plus disease statuses in stage III ROP are associated with CRP, SpO_2, MCV, and MCH. It may be related to dysregulated hematopoiesis or transfusion. Other studies are recommended to investigate these associations.

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Introduction

Retinopathy of prematurity (ROP) is a progressive neovascular retinal disorder in neonates causing vision loss and blindness in infants [1, 2]. ROP is a multifactorial disease; many studies have been proved the relationship between pathogenesis of ROP and risk factors such as low birth weight, short gestational age, hypoxia, and other factors [3, 4]. A critical element in ROP is fibro-vascular proliferation that might cause retinal detachment in advanced stages. Many factors are involved in normal vascular growth in the retina; among them, vascular endothelial growth factor (VEGF) has a key role in angiogenesis in the retina. It plays a vital role in the development of ROP, which results in neovascularization, the critical mechanism in the pathogenesis of ROP [5-7].

VEGF has various biologic effects, including neovascularization and angiogenesis. It can also induce macular edema by increasing the permeability of retinal vessels and disrupting the blood-retina barrier, which leads to bleeding propensity [8]. Therefore, VEGF inhibition could be a promising treatment for progressed ROP. According to the results of the clinical trials, periodic intravitreal injections of an anti-VEGF drug into the retina can decrease the new blood vessel growth and swell [2, 9, 10]. Anti-VEGF drugs consist of bevacizumab (Avastin), ranibizumab (Lucentis), and aflibercept (Eylea). In the last two decades, there is much evidence of intravitreal injection of bevacizumab (Avastin) as a full-length, humanized, anti-VEGF monoclonal antibody that improved ocular manifestations in ROP patients [11, 12].

The plus disease and neovascularization of the iris (NVI) are two vascularization indicating factors associated with the most severe vascularization in severe ROP [12]. The presence of plus disease and NVI is clinically associated with a poor prognosis in ROP patients. Thus, detecting any significant difference in laboratory indexes may help better manage ROP in infants [12, 13]. Various studies indicate the possible correlations of hematological/biochemical parameters with the progression of ROP [14]. Akkawi et al. found that low hemoglobin level is reversely correlated with progression of ROP [15]. Ünsal et al. established that hemoglobin and related parameters in ROP infants were significantly lower compared to non-ROP infants [16]. This study investigated the possible correlation of hematological and biochemical laboratory indexes with the plus disease and NVI statuses in stage III ROP patients' candidates for anti-VEGF therapy.

Methods

Study design and participants

This case-control study was performed in the Ophthalmology center of Ayatollah Rouhani Hospital, Babol, Iran, on 124 ROP patients (gestation age <32 weeks and birth weight <1500 g) from 2010 to 2020. All participants were in stage III of ROP and were a candidate for the treatment of intravitreal anti-VEGF. Overall, 124 stage III ROP patients have participated in this study. In terms of plus disease, they were divided into 58 cases (stage III ROP patients with plus disease) and 66 controls (stage III ROP patients without plus disease). Also, according to NVI, they were divided into 7 cases (stage III ROP patients with NVI) and 117 controls (stage III ROP patients without NVI).

Ophthalmologic examinations

The ophthalmological examinations were performed one hour after the administration of 2.5% phenylephrine and 0.5% tropicamide and funduscopic examinations implemented using a binocular indirect ophthalmoscope, 28D lens, scleral depressor, and pediatric speculum. Then, stage III ROP patients with neovascularization were identified according to the international classification of retinopathy of prematurity (ICROP) criteria (17). Also, the plus disease and NVI status were recorded for each patient. A certain ophthalmologist applied all examinations. Other medical indicators (i.e., fresh frozen plasma (FFP) transfusion, multiple birth statuses (Singleton, twin, etc.), gestation weight, gestation age, and Apgar score) were recorded for each patient.

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Laboratory examinations

The blood samples and laboratory indexes, including white blood cell (WBC), hemoglobin (Hb), platelet (Plt), bilirubin total, sodium (Na), potassium (K), calcium (Ca), C-reactive protein (CRP), pH, thyroxin (T_4), Thyroid-stimulating hormone (TSH), partial pressure of carbon dioxide (PCO_2), partial pressure of oxygen (PO_2), Bicarbonate (HCO_3), the percent saturation of oxygen (SpO_2), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and Lactate were evaluated in laboratory department of Ayatollah Rouhani Hospital, Babol, Iran.

Statistical analysis

Statistical analysis was performed using the SPSS 21.0 software. Quantitative variables are reported with mean ±SD. For parametric and non-parametric quantitative variables, the independent sample T-test and Mann-Whitney were used, respectively. Also, Fisher's exact test was used for qualitative variables. The level of significance was considered for p-value <0.05 for all statistical tests.

Results

Demographic results

The mean±SD of gestation age of 124 stage III ROP patients who participated in this study was 28.87±2.35-year-old. Gestation age was not significantly different between case/control groups (both plus-disease categorization (p=0.231) and NVI categorization (p=0.769)). Also, the mean±SD of birth weight of participants was 1184.20±317.35 gr. Birth weight was not significantly different between case and control groups (both plus-disease categorization (p=0.105) and NVI categorization (p=0.675)), (table 1 and 2).

The correlation of plus disease and NVI with laboratory variables

MCV and MCH were significantly lower in stage III ROP patients with plus disease. MCV in patients with the plus disease was 84.88±22.274 fl/cell, while 94.12±14.419 fl/cell in ROP patients without the plus disease (p=0.012). Also, MCH in patients with the plus disease was 30.12±3.649 pg/cell, while it was 31.99 ±5.149 pg/cell in ROP patients without the plus disease (p=0.033).

CRP level indicates the inflammatory status in patients. Our results showed that CRP is significantly higher in patients with the plus disease (16.11±29.403 mg/L) compared to patients without the plus disease (3.25±2.633 mg/dl) (p=0.033). There was no other significant correlation between other laboratory variables and plus disease. Also, SpO_2 was significantly higher in stage III ROP patients with NVI (64.00±13.730 %) compared to patients without NVI (75.58±16.135 %), (p=0.043). There was no other significant correlation between other laboratory variables and NVI.

The correlation of plus disease and NVI with clinical indicators

Our results showed that the plus disease was significantly correlated with multiple birth statuses (p-value=0.030). In singleton birth, 56.34% of ROP patients (40 in 71 patients) had plus disease. In twin birth, 29.41% of ROP patients (10 in 34 patients) had plus disease, while in ROP patients birthed in triplet birth manner, 42.86% of ROP patients (6 in 14 patients) had plus disease. Therefore, the plus disease is less prevalent in twins.

NVI was significantly correlated with the requirement for FFP transfusion (p-value=0.05). Between 117 patients with NVI, five patients (4.27%) had FFP transfusion, while in 7 non-NVI ROP patients; two patients (28.57%) had FFP transfusion.

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Table 1. Correlation of Plus disease status with laboratory indexes and demographic data

Variable	Mean ±SD		t	P-value	95% CI	
	Case (Plus-Disease)	Control (non-Plus-disease)			Lower	Upper
Gestational weight (gr)	1147.72 ±306.414	1217.74 ±325.950	-1.205	0.231	-185.134	45.089
Gestation age (weeks)	28.49 ±2.081	29.18 ±2.537	-1.619	0.108	-1.542	0.155
Apgar score	7.50 ±0.577	7.40 ±1.817	0.116	0.912	-2.121	2.321
WBC (cells/µl)	16359.23 ±34105.435	16281.82 ±40665.488	0.011	0.991	-13437.619	13592.444
Hb (gr/dl)	13.09 ±13.548	11.62 ±3.799	0.796	0.428	-2.198	5.142
Plt (cells/µl)	272735.19 ±116401.055	292136.36 ±200762.538	-.629	0.531	-80516.063	41713.706
MCV (fl/cell)	84.88 ±22.274	94.12 ±14.419	-2.573	**0.012**	-16.378	-2.118
MCH (pg/cell)	30.12 ±3.649	31.99 ±5.149	-2.166	**0.033**	-3.583	-0.157
CRP (mg/L)	16.11 ±29.403	3.25 ±2.633	2.253	**0.033**	1.145	24.578
Bilirubin total (µmol/L)	8.01 ±3.823	9.10 ±5.055	-.647	0.522	-4.526	2.345
Bilirubin direct (µmol/L)	0.39 ±0.123	0.99 ±3.097	-0.673	0.506	-2.443	1.230
T_4 (µg/dL)	8.01 ±2.358	11.06 ±13.524	-.863	0.394	-10.249	4.137
TSH (µg/dL)	3.89 ±2.377	5.66 ±3.903	-1.560	0.128	-4.059	0.531
Na (mEq/L)	139.79 ±8.922	139.15 ±6.175	0.323	0.748	-3.391	4.683
K (mEq/L)	6.34 ±9.551	4.14 ±0.758	1.171	0.253	-1.667	6.061
Ca (mEq/L)	1.94 ±3.271	1.42 ±2.383	0.688	0.494	-1.001	2.047
PH	7.27 ±0.467	7.30 ±0.123	-0.418	0.677	-0.181	0.118
PCO_2 (mmHg)	48.30 ±17.764	48.00 ±11.637	0.084	0.933	-6.928	7.536
PO_2 (mmHg)	47.50 ±12.632	45.14 ±17.515	0.587	0.559	-5.658	10.372
HCO_3 (mEq/L)	24.50 ±7.859	22.76 ±3.782	1.016	0.318	-1.760	5.238
SpO_2 (%)	71.94 ±11.358	76.33 ±18.409	-1.052	0.297	-12.726	3.951
Lactate (mg/dL)	2.81 ±5.228	2.17 ±2.077	-0.661	0.511	-2.578	1.299

Table 2. Correlation of NVI status with laboratory indexes and demographic data

Variable	Mean ±SD		Z core	p-value
	Case (NVI)	Control (non-NVI)		
Gestational weight (gr)	1187.72 ±322.025	1127.86 ±241.486	-0.294	0.769
Gestational age (weeks)	28.89 ±2.368	28.50 ±2.258	-0.419	0.675
Apgar score	7.50 ±1.414	7.00 ±1.921	-0.596	0.551
WBC (cells/µl)	14360.62 ±28195.221	48014.29 ±105861.819	-0.028	0.978
Hb (gr/dl)	12.41 ±9.528	9.16 ±2.348	-1.413	0.158
Plt (cells/µl)	283884.96 ±169596.041	275671.43 ±145642.676	-0.420	0.675
MCV (fl/cell)	90.91 ±17.779	81.90 ±29.563	-0.076	0.939
MCH (pg/cell)	31.22 ±4.780	31.88 ±2.346	-1.026	.305
Bilirubin total (µmol/L)	8.65 ±4.675	10.50 ±3.725	-.736	.462
Bilirubin direct (µmol/L)	0.78 ±2.506	0.45 ±1.405	-0.910	0.363
T4 (µg/dL)	9.89 ±10.839	8.60 ±1.980	-0.403	0.687
TSH (µg/dL)	4.75 ±3.310	8.25 ±5.586	-1.310	0.190
Na (mEq/L)	139.67 ±7.185	136.00 ±8.803	-0.753	0.452
K (mEq/L)	4.93 ±6.233	5.94 ±3.294	-1.880	0.060
Ca (mEq/L)	1.64 ±2.758	0.66 ±0.325	-0.250	0.802
pH	7.26 ±0.239	7.56 ±0.645	-1.226	0.220
PCO2 (mmHg)	48.53 ±14.317	43.97 ±10.988	-0.804	0.422
PO2 (mmHg)	46.54 ±15.978	39.60 ±13.202	-1.540	0.124
HCO3 (mEq/L)	23.39 ±5.783	23.80 ±4.093	-0.541	0.589
SpO2 (%)	75.58 ±16.135	64.00 ±13.730	-2.027	**0.043**
Lactate (mg/dL)	2.46 ±3.575	1.56 ±0.518	-0.071	0.944

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Discussion

In this study we investigated the possible correlation of hematological and biochemical laboratory indexes with the plus disease and the neovascularization of the iris (NVI) statuses in stage III ROP patients. In brief, MCV and MHC in patients with the plus disease were lower than ROP patients without the plus disease. Also, our results showed that CRP is higher in patients with plus disease than patients without plus disease. Furthermore, SpO_2 was significantly higher in stage III ROP patients with NVI compared to non-NVI patients. These findings establish relative hypochromic microcytic RBC status in stage III ROP patients with the plus disease compared to those without. On the other hand, SpO_2 was lower in the arterial blood of stage III ROP patients with the plus disease than stage III ROP patients without the plus disease, but this difference was insignificant. It may show that the hypoxic condition is an ancestral point between the hypochromic microcytic status of RBCs (indicated by lower MCV and MCH level) and severe neovascularization (the presence of neovascularization). Regarding our results, Teoh et al. showed that the duration of oxygen therapy is significantly correlated with ROP progression [22]. Also, regarding our results, in the study of York et al., a lower arterial level of PO_2 was introduced as a risk factor of ROP [23], but in our study, there was no significant importance for PO_2 for the severity of neovascularization of ROP. A study on lower prematurity stage and very low birth weight infants by York et al. may be a reason for this difference in results. In a study by Fleck et al., the beneficial effect of oxygen saturation was examined in plus disease-present ROP patients [24]. Also, VanderVeen et al. established the reverse correlation between the oxygen saturation and severity of ROP in ROP patients without the plus disease [25].

Our results showed that CRP is significantly higher in patients with plus disease than patients without the plus disease. CRP is an inflammatory protein, which high serum level indicates the presence of inflammation in the body. CRP is a membrane of the pentraxin family which triggers the inflammatory status via activation of innate immunity by classical complement activation [26]. In a study by Borțea et al., there was no correlation between ROP severity and CRP level [27].

Various conditions in infants suspect the FFP transfusion requirement in premature infants [28]. Our results showed that the presence of NVI in stage III ROP patients was significantly correlated with the requirement for FFP transfusion. The need for FFP transfusion and NVI presence may have an ancestral point. For this aim, we suggest a study to investigate the possible correlation between the NVI in ROP patients and the reason for FFP transfusion.

Current investigations have demonstrated that both TSH and T_4 have a pro-angiogenic impact on neovascularization through boosting VEGF signaling pathway, increasing basic fibroblast growth factor, bradykinin, angiotensin-II, aminopeptidase, and endothelial cell motility [29, 30]. Our results showed that the serum levels of TSH and T_4 were not significantly different between stage III ROP patients with plus disease-or-NVI compared to stage III ROP patients without plus disease-or-NVI.

The comprehensive profiling of laboratory parameters (i.e., hematological/biochemical parameters), incomplete medical records in patient's history, and single-centrality of this study were limitations of this experiment. Moreover, we suggest a follow-up study on the role of laboratory indexes in the reduction/enhancement of neovascularization of stage III ROP patients after receiving anti-VEGF medications.

Conclusion

Finally, in this study, we found that the severity of neovascularization in ROP patients, indicated by plus disease and NVI, correlates with RBC morphologic condition (lower MCV and MCH) and higher CRP. It may be related to dysregulated hematopoiesis or transfusion. Other studies are recommended to investigate these associations.

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Ethical Code

This research has been registered with the ethical code of IR.MUBABOL.REC.1399.373 in the Ethics Committee of Babol University of Medical Sciences.

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Conflict of interest

There is no conflict of interest in this manuscript.

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