Systematic Review

THROMBOCYTOPENIA AS A CLINICAL BIOMARKER OF RETINOPATHY OF PREMATURITY: A SYSTEMATIC REVIEW

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ABSTRACT

Retinopathy of prematurity (ROP) is the leading cause of childhood blindness and occurs due to the underdevelopment of retinal blood vessels in premature infants. Platelets are essential in the regulation of angiogenesis. Hence, thrombocytopenia might aid in the progression of ROP. This systematic review aims to look into the relationship between thrombocytopenia and retinopathy of prematurity. The PubMed and Cochrane Library databases were accessed to include retrospective case-control and cross-sectional studies, following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). In our review, 9 records were analyzed. All research was done in the period between 2017 and 2022. Seven studies have reported the prevalence of thrombocytopenia in infants with retinopathy of prematurity (ROP), ranging from 18.37\% to 71\%. In preterm children without ROP, the occurrence of thrombocytopenia is between 5.71\% and 21\%. Seven studies have significantly identified thrombocytopenia as a risk factor for ROP, with the Odd Ratio (OR) for thrombocytopenia ranging from 2.8 to 6.69. Therefore, thrombocytopenia in premature infants could be thought of as a potential clinical biomarker for Type-1 ROP screening. Additionally, this discovery implied that thrombocytopenia can contribute to the pathophysiology of ROP. The crucial platelet count threshold in ROP requires additional investigations.

Keywords: Thrombocytopenia; retinopathy of prematurity; preterm infant; angiogenesis; childbirth complications

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Highlights:
1. This is the first systematic review investigating thrombocytopenia and its association with retinopathy of prematurity
2. The findings suggest that thrombocytopenia could serve as a potential clinical biomarker for screening ROP, considering its quick, affordable, and widespread availability for examination purposes.

INTRODUCTION

Retinopathy of prematurity (ROP) is a proliferative condition that has impacts on the retinal vasculature of premature newborns (Kim \textit{et al.}, 2018). The most frequent cause of blindness in children worldwide is this disease. Around the world, ROP caused at least 50,000 children to go blind. This blindness brought on by ROP occurs infrequently in developed countries. However, the occurrence of ROP leading to blindness is more common in developing countries due to a higher survival rate among premature infants, inadequate regulation of neonatal intensive care unit (NICU), and the scarcity of funduscopic examinations which restricts the frequency and accessibility to monitor changes in the retinal vasculature (Wood \textit{et al.}, 2021).

Low gestational age (GA) and low birth weight (BW) represent the primary factors increasing the risk of ROP development. Underdeveloped retinal vasculature and neurons are correlated with both low GA and BW due to the delicate retinal structure during birth. However, the study did not establish a statistically significant relationship between BW and severe ROP. This finding might point to the significance of weight increase in slowing the course of ROP. Other risk factors include gender, the need for oxygen therapy and blood transfusions, as well as the
presence of patent ductus arteriosus (PDA), intraventricular extension of hemorrhage, necrotizing enterocolitis (NEC), sepsis, and newborn infections (Hong et al., 2022).

Elevated levels of oxygen pressure in ROP inhibit the formation of new blood vessels, impede the function of existing retinal capillaries, and lead to non-vascularized regions within the retina. When the retina experiences hypoxia due to incomplete vascularization, it stimulates the secretion of angiogenic factors, such as Vascular Endothelial Growth Factor (VEGF) and erythropoietin. These factors contribute to the development of neovascularization, intraocular fibrosis, and retinal detachment. VEGF and vascular permeability in the eye have a significant role in pathological neovascularization (Eldweik et al., 2016). Anti-VEGF medication has been utilized to treat ROP due to the well-established link between vascular endothelial growth factor and ROP development (Wu and Wu, 2018).

A quick and simple test to check for thrombocytopenia can be done using standard blood samples. Additionally, a regularly used treatment is the platelet transfusion procedure (Sola-Visner and Bercovitz, 2016). It may help in the development of ROP screening and treatment to understand the etiology of thrombocytopenia in ROP. A recent study found that thrombocytopenia was linked to a worse prognosis for preterm newborns with neonatal sepsis and NEC (Resch et al., 2018). The main objective of this systematic review is to investigate the correlation between thrombocytopenia and retinopathy of prematurity.

MATERIALS AND METHODS

Search Strategy

This systematic review was carried out adhering to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) criteria (Page et al., 2021). A comprehensive literature search was conducted from September 2012 to September 2022, utilizing the Cochrane Library and PubMed databases. The following keywords and the Boolean operator were used to search the literature: (“Thrombocytopenia” OR “Low Thrombocyte” OR “Thrombocyte” OR “Platelet”), AND (“ROP” OR “Retinopathy of Prematurity”).

We included all pertinent retrospective case-control and cross-sectional studies on thrombocytopenia in premature infants with retinopathy of prematurity. Case reports, letters, editorials, review articles, full texts that were inaccessible, and research that were not published in English were all excluded. Figure 1 shows a flow chart of the research selection.

Extracted Data

The information gathered from the study includes the study’s features (author, year of publication), the participants, the average gestational age, the average birthweight, the threshold for thrombocytopenia, the prevalence of thrombocytopenia in ROP, and the relationship between thrombocytopenia and ROP.

Quality Assessment

A checklist from the Joanna Briggs Institute (JBI) was used to assess the study’s quality and bias risk. The checklist’s items each counted for one point. If a study receives at least half of the maximum possible points, it is said to be of high quality. If it receives fewer than half of the possible points, it is deemed to be of low quality. To prevent bias, two reviewers independently assessed the study’s quality. The disagreement between the two reviewers was settled by consensus.

RESULTS

Study Characteristics

In our initial search, 117 records were found. Nine original papers from those records were used in the research. In Figure 1, the study flowchart is displayed. Based on the JBI checklist, all of the studies are of high quality. Case-control or cross-sectional studies were both included in all of the retrospective investigations. Sample sizes ranged from 9 to 240 individuals. The samples included infants with multiple gestations, Retinopathy of Prematurity type-1, aggressive posterior retinopathy of prematurity (AP-ROP), birth with very low birth or VLBW (very low birth weight), and preterm infants. The mean birthweight of a newborn with ROP was between 585 and 1955 grams. The infants with ROP had a mean gestational age between 24 and 33.25 weeks. Summaries of the studies are included in Table 1.
Cut-off of Thrombocytopenia

The threshold for thrombocytopenia varied from trial to study. Nine research were considered; three had a cut-off of 150,000/mL, two used a cut-off of 100,000/mL, and the other articles did not indicate the cut-off of low thrombocyte.

Prevalence of Low Thrombocyte in ROP

Five studies have reported the prevalence of thrombocytopenia in infants with ROP, ranging from 18.37% to 71%. Several studies (Sancak et al., 2019; Lundgren et al., 2017; Parrozzani et al., 2021; Choreziak et al., 2019; Ozkaya et al., 2022) indicate that lower thrombocyte count is also present in preterm infants without ROP, affecting approximately 5.71% to 21% of cases.

The Relationship of Low Thrombocyte with ROP Progressivity

Seven investigations (Sancak et al., 2019; Cakir et al., 2018; Jensen et al., 2018; Gaber et al., 2021; Yau et al., 2021; Parrozzani et al., 2021; Lundgren et al., 2017) have identified low thrombocyte count as a significant risk factor for ROP. The range of the Odd Ratio (OR) for thrombocytopenia was found to be between 2.8 and 6.69. Most of these studies compared the occurrence of low thrombocyte count between premature infants with type-1 ROP and premature newborns without ROP, finding a significant relationship between the two conditions. However, when analyzing Zone 2 ROP, newborns with gestational age between 35 and 38 weeks, and differentiating stabilized versus progressive ROP, thrombocytopenia did not appear to have any discernible effect.
Table 1. Summary of characteristic findings of included systematic reviews

<table>
<thead>
<tr>
<th>No.</th>
<th>References</th>
<th>Type of Research</th>
<th>Total sample subject</th>
<th>Mean of Gestational Age of each research (weeks)</th>
<th>Mean weight at birth of each research’s sample (gram)</th>
<th>Definition of Low Thrombocyte</th>
<th>Prevalence of Thrombocytopenia (%)</th>
<th>Relationship of ROP and low thrombocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sancak et al., 2019</td>
<td>Case-Control</td>
<td>81 Type-1 ROP and 81 premature infants without ROP</td>
<td>27.6±2.1</td>
<td>993±292</td>
<td>&lt;150,000</td>
<td>71</td>
<td>21</td>
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<tr>
<td>2</td>
<td>Cakir et al., 2018</td>
<td>Retrospective</td>
<td>202 VLBW</td>
<td>25.3±1</td>
<td>782±167</td>
<td>&lt;100,000</td>
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<tr>
<td>3</td>
<td>Jensen et al., 2018</td>
<td>Case-Control</td>
<td>100 Type-1 ROP and 100 premature infants without ROP</td>
<td></td>
<td></td>
<td>&lt;150,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Lundgren et al., 2017</td>
<td>Retrospective</td>
<td>9 APROP and 9 Stadium II ROP</td>
<td>24±1</td>
<td>585 (470-700)</td>
<td>&lt;100,000</td>
<td>56</td>
<td>27</td>
</tr>
<tr>
<td>5</td>
<td>Parrozzani et al., 2021</td>
<td>Retrospective</td>
<td>206 ROP and 357 premature infants without ROP</td>
<td>28.72 ± 2.58</td>
<td>1083.8 ± 329.62</td>
<td>-</td>
<td>18.37</td>
<td>5.71</td>
</tr>
<tr>
<td>6</td>
<td>Choreziak et al., 2019</td>
<td>Retrospective</td>
<td>76 ROP with management in ROP and 87 patients with ROP which is</td>
<td>25 ±1.72</td>
<td>830 ±206</td>
<td>-</td>
<td>39.5</td>
<td>32.2</td>
</tr>
<tr>
<td></td>
<td>Authors</td>
<td>Study Type</td>
<td>Participants</td>
<td>Platelet Count</td>
<td>Thrombocyte Count</td>
<td>ROP Type</td>
<td>p Value</td>
<td>Statistic</td>
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<td>7</td>
<td>Ozkaya et al., 2022</td>
<td>Retrospective</td>
<td>40 type-I ROP and 40 premature infants without ROP</td>
<td>26.5 (24–32)</td>
<td>925 (430–1440)</td>
<td>80</td>
<td>&lt;0.05</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Gaber et al., 2021</td>
<td>Retrospective cross-sectional</td>
<td>240 premature infants</td>
<td>33.25±2.74</td>
<td>1955.23±692.43</td>
<td>37</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Yau et al., 2021</td>
<td>Retrospective cross-sectional</td>
<td>153 infants of multiple gestation</td>
<td>30.8±2.4</td>
<td>1284.8±267.4</td>
<td>20</td>
<td>-</td>
<td>-</td>
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</table>
DISCUSSION

Retinopathy of prematurity is a complex vasoproliferative condition that occurs in the retinas of prematurely born infants with inadequate vascularization. It becomes a significant newborn illness that causes about 40% of all childhood blindness worldwide. Because of improvements in neonatal care, more low-birth-weight infants survive, which increases the incidence of ROP (Tan et al., 2022).

According to this study’s comprehensive review, thrombocytopenia increases the likelihood of developing ROP in premature infants. However, the specific mechanism by which thrombocytopenia impacts the stages of ROP and the balance of VEGF is unknown. Existing research has highlighted the crucial regulatory role of angiogenic factors like VEGF (Sancak et al., 2019). Both IGF-1 (Insulin-like Growth Factor 1) and VEGF are transported, stored, and released by platelets (Dai et al., 2021; Guo et al., 2021; Tan et al., 2022).

There are two stages in the pathogenesis of ROP. High oxygen levels in utero during the first phase decrease retinal VEGF expression, which prevents blood from inhibiting blood vessel growth, constricting retinal capillaries, and resulting in avascular regions in the retina. During the second phase, the infant experiences relative hypoxia, which subsequently triggers the upregulation of VEGF by Muller cells and astrocytes. The surge in VEGF results in neovascularization occurring in the retina, which is a pathognomonic feature of ROP. Proliferative retinopathy is associated with increased endogenous IGF-1 production, a consequence of retinal VEGF accumulation during the second phase of ROP pathogenesis. Therefore, thrombocytopenia may contribute to insufficient VEGF sequestration in the developing retina and the subsequent onset or progression of proliferative retinopathy. Investigations into platelet counts and related factors are relevant in both the first and second phases of ROP. Regarding thrombocyte parameters in the first phase, numerous studies did not reveal any differences. Therefore, it is speculated that platelets may be more active in the second phase could cause a rise in neovascularization. As a result, some studies in the literature only include the second phase while others include both the first and second phases. However the precise function of platelets in regulating VEGF is still not well understood (Cakir et al., 2018).

The impact of thrombocytopenia in aggressive posterior ROP (APROP) was investigated by Vinekar et al. They claimed that there was a large discrepancy in platelet counts and APROP. First, they observed the spontaneous regression of APROP in a severely ill patient with thrombocytopenia (21,000/mm³) shortly after the platelet transfusions had corrected the thrombocytopenia (118,000/mm³). They next looked at the other APROP cases. They looked at 21 control subjects and an additional 9 APROP cases. The thrombocyte count between APROP patients and control participants was significantly different. Low platelet counts are likely to prevent preterm children with APROP from clearing excessive VEGF produced by an ischemic, peripheral retina at optimal levels or to a lesser extent (Seliniotaki et al., 2022).

In a scientific experiment, platelet transfusions were found to prevent the formation of vascular in a mouse animal model of oxygen-induced retinopathy. The affected mice had lower platelet counts compared to mice grown in normal conditions. Another investigation revealed that in the mouse model of ROP, platelet depletion at the onset of neovascular tuft formation increased retinopathy, while platelet transfusion decreased it. Researchers analyzed the average weekly platelet counts of mice and discovered a statistically significant difference between those with severe ROP and those with no or milder ROP (Cakir et al., 2018).

In newbons, thrombocytopenia is a frequent hematologic condition that can cause death and morbidity. According to estimates, thrombocytopenia affects 1-5% of all newborns, 12% of preterm infants, and 20-40% of infants under intensive care. The prevalence of thrombocytopenia increases while the GA and BW decrease. By disturbing the state of balance of the substances that regulate angiogenesis, such as vascular endothelial growth factor, endostatin, and thromboxane (TXA2), low thrombocyte count may have a role in the formation of ROP. The findings of this study are consistent with a recent analysis by Seliniotaki et al., which noted that severe ROP has been linked to thrombocytopenia (Sahinoglu et al., 2020).

According to some studies (Cakir et al., 2018; Sancak et al., 2019; Kumawat et al., 2021), due to the removal of extra VEGF during the vascularization stage, thrombocytes have an anti-angiogenic effect on the formation of the retina. Thus, more serious ROP is associated with thrombocytopenia. According to research by Lundgren et al. (2017), low VEGF-A levels are correlated with thrombocytopenia.

Research has shown that platelets serve as a storage and transportation system for various pro- and antiangiogenic regulators. These regulators include IGF-binding protein 3, which is the primary serum binding protein for IGF-1, as well as VEGF, IGF-1, and platelet-derived growth factor. All of these regulators are found in platelet alpha granules. In a case study, it was observed that severe thrombocytopenia improved significantly in a patient with APROP after receiving serum platelet infusions. Another study identified a link between type-1 ROP patients in zone 1 and thrombocytopenia. These findings suggest that thrombocytopenia could be considered a risk factor for Zone 1 ROP (Cakir et al., 2018; Holinstat, 2017).
According to Sharda and Flaumenhaft (2018), platelets contain three distinct types of secretory granules that store bioactive molecules. These include dense granules, alpha-granules, and lysosomes. Among these granules, the most common ones are angiostatin, platelet factor 4, thrombospondin-1, 2-macroglobulin, endostatin, and plasminogen activator inhibitor 1. Despite containing both pro-angiogenic and anti-angiogenic substances, the majority of research on the effects of platelets on endothelial cells has focused on their pro-angiogenic or pro-proliferative properties (Rubio and Adamis, 2016).

On the other hand, a separate study discovered a link between severe retinopathy of prematurity (ROP) and thrombocytopenia during the period from birth to 34 weeks of postmenstrual age (PMA). Although none of the newborns required platelet transfusions, and their platelet counts did not drop to levels indicative of thrombocytopenia, notable variations in platelet count were observed between infants who developed ROP and those who did not. Platelets have a pivotal role in controlling angiogenic factors like VEGF and Platelet Derived Growth Factor (PDGF) by storing, transporting, and releasing them. Hence, platelets serve as crucial regulators for these proteins (Jensen et al., 2018; Ozkaya, 2022).

**Strength and limitations**

This study’s main strength lies in being the first comprehensive review of the relationship between ROP and thrombocytopenia. Unlike a previous study that only consisted of an article review, this study encompasses a wider range of research. However, there are a few drawbacks to consider. Firstly, two of the research included in the study were retrospective cross-sectional, and the remaining studies were retrospective. This can affect the reliability and validity of the evidence presented. Moreover, the study population is heterogeneous and thrombocytopenia has a specific cutoff value which may vary across individuals. Additionally, different centers may have used varying instruments to evaluate platelets, leading to potential variations in precision across studies. Lastly, some studies did not control for confounding variables properly which could result in over- or underestimations of results.

**CONCLUSION**

Retinopathy of prematurity is a prevalent cause of blindness among newborns, and research on its risk factors is currently underway. Many studies have identified thrombocytopenia as a risk factor for ROP. Consequently, low thromocyte levels in premature infants could potentially serve as a clinical biomarker for Type-1 ROP detection. Moreover, this finding suggests that thrombocytopenia may play a role in the development of ROP. However, further research is needed to determine the critical threshold of platelet count with ROP.

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**Conflict of interest**

None.

**Funding disclosure**

None.

**Author contribution**

IWES, NMAS, and PAA collected the data and wrote this research manuscript. PD and SA checked the final article result.

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