Original Research Article

https://doi.org/10.53339/aimdr.2025.11.3.8
E-ISSN: 2395-2822 | P-ISSN: 2395-2814

Comparative analysis of retinopathy of prematurity staging and zone distribution in single versus multiple birth preterm neonates

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Abstract

Background: Retinopathy of prematurity (ROP) is a major cause of preventable childhood blindness. Differences in ROP staging and zone distribution between single and multiple birth neonates remain understudied, especially in low-resource settings. This study aimed to compare the staging and zone distribution of ROP among single and multiple-birth preterm neonates in Bangladesh.

Methods: This cross-sectional analytical study included 82 preterm neonates (gestational age ≤35 weeks, birth weight ≤2000 g), categorized into two groups: single birth (Group A) and multiple birth neonates (Group B). Neonates underwent ophthalmic examination using indirect ophthalmoscopy. ROP was classified by stages (1–5) and zones (I–III). Data were analyzed using Statistical Package for the Social Sciences 23, employing Chi-square, Fisher's exact, and Student's *t*-tests, with significance set at $P < 0.05$.

Results: Demographic characteristics, including age, birth weight, gestational age, and gender, were similar between groups ($P > 0.05$). No significant difference was found in ROP staging distribution between groups ($P > 0.05$), though Stage 2 ROP occurred more frequently in multiple births (63.4% vs. 43.9%, $P = 0.076$). However, significant differences emerged in ROP zone distribution, with Zone II ROP being significantly more prevalent in multiples (65.9% vs. 31.7%, $P = 0.002$) and Zone III more common in singletons (58.5% vs. 29.3%, $P = 0.008$).

Conclusion: Multiple birth neonates had significantly higher Zone II ROP prevalence, whereas single birth neonates exhibited more Zone III involvement. Tailored screening protocols considering birth plurality may enhance early ROP detection and improve neonatal ophthalmic outcomes.

Keywords: Multiple births, preterm neonates, retinopathy of prematurity, retinopathy of prematurity staging, zone distribution

Introduction

Retinopathy of prematurity (ROP) is a leading cause of preventable childhood blindness worldwide, affecting preterm infants due to incomplete retinal vascularization at birth. The global prevalence of ROP varies significantly, with incidence rates ranging from 31.9% in low-income settings to 7.5% in high-income countries.[1] The increasing survival rates of very low birth weight and extremely preterm

neonates, particularly in low-resource settings such as Bangladesh, have contributed to a rising burden of ROP-related visual impairment.[2] Although neonatal intensive care advancements have improved survival outcomes, disparities in screening programs and treatment accessibility have led to persistent challenges in ROP management, particularly in developing regions.[3] The International Classification of ROP categorizes the disease based on staging (Stages 1–5) and zone distribution (Zone I, II, and III) to facilitate screening, risk stratification, and treatment planning.[4] ROP staging determines the extent of retinal vascular abnormalities, whereas zonal classification reflects the anatomical progression of the disease, with Zone I involvement being associated with more aggressive forms of ROP.[5] Studies have demonstrated that precise classification plays a crucial role in intervention timing and long-term prognosis, as Zone I ROP is linked to a higher risk of retinal detachment and poor visual outcomes compared to Zone II or III.[6] Emerging automated detection methods using artificial intelligence have improved ROP classification accuracy, but real-world applicability remains limited, particularly in resource-constrained settings.[7] Several well-documented risk factors contribute to ROP development, including low birth weight, gestational age, oxygen therapy, sepsis, and blood transfusions.[8] Studies from low- and middle-income countries have reported a significantly higher prevalence of severe ROP among preterm infants requiring prolonged mechanical ventilation and oxygen support.[9] In addition, neonatal comorbidities such as bronchopulmonary dysplasia, patent ductus arteriosus, and hyperglycemia have been linked to increased ROP severity.[10] The interplay of these risk factors highlights the importance of early screening and timely intervention, particularly in settings with high neonatal sepsis rates.[11] An emerging area of interest in ROP research is the impact of single versus multiple births on disease incidence and progression. Multiple pregnancies, particularly twins, triplets, and higher-order births, are associated with a heightened risk of prematurity and low birth weight due to intrauterine growth restriction.[12,13] Several studies have reported contradictory findings regarding whether multiple birth neonates exhibit higher or lower ROP rates compared to singletons. Conversely, research from India indicated that ROP progression was more aggressive in singletons, suggesting that intrauterine shared circulation in multiples may offer some protective effects.[14] However, these findings remain inconsistent, and no study has specifically analyzed staging and zone distribution differences between single and multiple birth preterm neonates. This represents a critical knowledge gap that requires further investigation. In Bangladesh, where neonatal care infrastructure is still evolving, ROP screening programs remain irregular and underutilized. A study from Dhaka Shishu Hospital found an ROP prevalence of 31.9%, with many infants presenting at advanced stages due to delayed diagnosis.[15] Limited access to trained ophthalmologists and inconsistent follow-up care further exacerbate the issue, emphasizing the need for region-specific screening guidelines.[16] Given that multiple birth rates have risen due to the increasing use of assisted reproductive technologies in Bangladesh, the impact of birth plurality on ROP severity and progression remains unexplored. Despite existing literature on ROP risk factors and prevalence, direct comparative data on staging and zone distribution in single versus multiple birth preterm neonates are lacking, particularly in low-resource settings such as Bangladesh.[17] This gap in knowledge prevents clinicians from optimizing screening protocols and tailoring interventions based on birth plurality. Addressing this gap is critical to refining ROP management strategies, improving resource allocation in neonatal intensive care units, and guiding policy changes to establish evidence-based neonatal care protocols. This study aims to conduct a comparative analysis of ROP staging and zone distribution between single and multiple birth preterm neonates in Bangladesh. By identifying whether multiples exhibit different disease progression patterns, this research seeks to contribute to the development of targeted screening recommendations and individualized follow-up strategies.

Materials and Methods

This cross-sectional analytical study was conducted at the Department of Ophthalmology, Dhaka Medical College Hospital, Dhaka, from January

2023 to December 2023, spanning a 12-month period. The study population included inborn preterm neonates admitted to the hospital. A total of 82 neonates meeting the inclusion criteria were enrolled using a consecutive sampling. The inclusion criteria consisted of neonates aged ≥30 days or more with a history of preterm birth (gestational age ≤35 weeks) and low birth weight (≤2000 g), categorized into Group A (single birth neonates) and Group B (multiple birth neonates, including twins, triplets, and quadruplets). Neonates with full-term birth (gestational age >35 weeks) or those who were severely ill were excluded. Ethical approval was obtained from the Ethical Review Committee (ERC) of Dhaka Medical College, Dhaka (Memo No. ERC-DMC/ECC/2022/438; Date: December 12, 2022), and informed written consent was collected from the legal guardians of all participants. Data collection involved history taking, physical examination, and recording baseline demographic and clinical variables (age, gender, gestational age, and birth weight) using a structured questionnaire. Birth weight was measured using a digital baby scale, ensuring neonates were without shoes or heavy clothing. Fundoscopic examination was performed after pupil dilation with tropicamide (0.5%) and phenylephrine (2.5%) drops, administered 60 min before the examination at 20-min intervals. A topical anesthetic was applied before indirect ophthalmoscopy with a 28D condensing lens and RetCam Shuttle to assess the presence and severity of ROP, Plus Disease (arterial tortuosity and venous dilation), and Aggressive Posterior ROP. ROP staging followed the standard five-stage classification, with zone determination based on proximity to the optic nerve. All collected data were stored securely in a separate data record form. Statistical analysis was conducted using the Statistical Package for the Social Sciences version 23 (IBM Corp, Armonk, NY). Categorical variables were summarized as frequencies and percentages, whereas continuous variables were described using means, medians, standard deviations, and percentiles. Student's *t*-test was applied for continuous data, whereas Chi-Square and Fisher's exact tests were used for categorical data. A $P < 0.05$ was considered statistically significant. Ethical considerations included ensuring patient privacy, data confidentiality, and voluntary participation, with participants given the right to withdraw at any time without consequences. No financial incentives were provided for participation.

Results

The demographic profile of the study subjects ($n = 82$) revealed that the mean age of neonates in the single birth group was 31.70 ± 1.21 days, whereas it was 31.35 ± 1.26 days in the multiple birth group, with no statistically significant difference ($P = 0.379$). Regarding birth weight distribution, 36.6% of multiple birth neonates and 29.3% of singletons weighed between 1000 and <1500 g, whereas the majority in both groups weighed between 1500 and <2500 g (70.7% in singletons vs. 63.4% in multiples), with no significant difference in mean birth weight (1532 ± 276 g vs. 1482 ± 262 g, $P = 0.403$). Gestational age was also similar between groups, with 73.2% of singletons and 68.3% of multiples born at ≤33 weeks, and the mean gestational age showed no significant difference (32.12 ± 1.65 vs. 32.53 ± 1.48 weeks, $P = 0.363$). In terms of gender distribution, males were more predominant in the multiple birth group (68.3%) compared to 53.7% in the single birth group, whereas females were more common in the singleton group (46.3%) compared to 31.7% in the multiple birth group; however, this difference was not statistically significant ($P = 0.174$). Overall, no significant demographic differences were observed between single and multiple birth neonates in this study [Table 1].

The distribution of ROP stages among single and multiple birth neonates showed some variations, though none reached statistical significance. Stage 1 ROP was more common among single birth neonates (51.2%) compared to in multiple birth neonates (36.6%) ($P = 0.182$). Conversely, Stage 2 ROP was observed at a higher frequency in multiple birth neonates (63.4%) compared to singletons (43.9%), but the difference was not statistically

significant ($P = 0.076$). Stage 3 ROP was identified in 4.9% of single birth neonates, whereas no cases of Stage 3 ROP were reported in multiple birth neonates ($P = 0.494$). These findings indicate that while Stage 2 ROP appeared more frequently in multiple births, the overall distribution of ROP severity did not significantly differ between single and multiple birth neonates [Table 2].

The distribution of ROP zones showed significant differences between single and multiple birth neonates. Zone I ROP, the most severe form, was observed in 9.8% of single birth neonates and 4.9% of multiple birth neonates, with no statistically significant difference ($P = 0.675$). However, Zone II ROP was significantly more common in multiple birth neonates (65.9%) compared to 31.7% in singletons ($P = 0.002$). Conversely, Zone III ROP, which is associated with a better prognosis, was more frequent in single birth neonates (58.5%) compared to 29.3% in multiple birth neonates, showing a statistically significant difference ($P = 0.008$). These findings suggest that ROP in multiple birth neonates is more likely to occur in Zone II, while single birth neonates have a higher tendency for Zone III involvement, indicating possible differences in disease progression based on birth plurality [Table 3].

Table 1: Demographic profile of the study subjects (n=82)

Variable	Single	Multiple	*P*-value
Age (Days)	31.70±1.21	31.35±1.26	[a]0.379
Weight (g)			
1000–<1500	12 (29.3)	15 (36.6)	[b]0.639
1500–<2500	29 (70.7)	26 (63.4)	
Mean±SD	1532±276	1482±262	[b]0.403
Gestational age (weeks)			
≤33	30 (73.2)	28 (68.3)	[b]0.627
>33	11 (26.8)	13 (31.7)	
Mean±SD	32.12±1.65	32.53±1.48	[a]0.363
Gender			
Male	22 (53.7)	28 (68.3)	[b]0.174
Female	19 (46.3)	13 (31.7)	

[a]Unpaired t-test and [b]Chi-square test were done. SD: Standard deviation

Table 2: Stages of ROP of the single and multiple birth neonates (n=82)

Stages	Single *n* (%)	Multiple *n* (%)	*P*-value
Stage-1	21 (51.2)	15 (36.6)	[a]0.182
Stage-2	18 (43.9)	26 (63.4)	[a]0.076
Stage-3	2 (4.9)	0 (0.0)	[b]0.494

[a]Chi-square test and [b]Fisher's exact test were done.
ROP: Retinopathy of prematurity

Table 3: Zone where ROP is found in single and multiple birth neonates (n=82)

Zones	Single *n* (%)	Multiple *n* (%)	*P*-value
Zone-I	4 (9.8)	2 (4.9)	[b]0.675
Zone-II	13 (31.7)	27 (65.9)	[a]0.002
Zone-III	24 (58.5)	12 (29.3)	[a]0.008

[a]Chi-square test and [b]Fisher's exact test were done.
ROP: Retinopathy of prematurity

Discussion

ROP remains a significant cause of preventable childhood blindness, with birth plurality emerging as a potential factor influencing its progression and severity. The present study sought to compare the staging and zone distribution of ROP in single versus multiple birth preterm neonates and revealed notable findings that align with and contrast previous literature. While no significant differences were observed in ROP staging, the zone distribution of ROP demonstrated statistically significant differences, particularly with respect to Zone II and Zone III involvement. The demographic profile of single and multiple birth neonates in the current study was largely comparable, with no significant differences in birth weight ($P = 0.403$), gestational age ($P = 0.363$), or gender distribution ($P = 0.174$). This aligns with findings by Fraser *et al*. and Anand *et al*., who similarly reported no independent association between multiple pregnancy and adverse neonatal outcomes after adjusting for gestational age and birth weight.[18,19] However, multiple birth neonates have been noted in some studies to experience greater neonatal complications, potentially impacting ROP progression.[20] In terms of ROP

staging, our study found that Stage 1 ROP was more frequent in single birth neonates (51.2%) compared to multiple birth neonates (36.6%), whereas Stage 2 ROP was more frequent in multiple birth neonates (63.4%) than singletons (43.9%). However, these differences were not statistically significant ($P = 0.182$ and $P = 0.076$, respectively). These results are consistent with findings by Petriçli *et al*., which demonstrated that ROP staging did not significantly differ between single and multiple birth neonates.[21] Similarly, Riazi-Esfahani *et al*. observed that threshold ROP rates were comparable between the two groups (6.1% in multiples vs. 7.1% in singletons, $P = 0.62$), reinforcing the notion that birth plurality itself does not significantly alter ROP staging patterns.[22] Conversely, ROP zone distribution demonstrated statistically significant differences between single and multiple birth neonates. The prevalence of Zone II ROP was significantly higher in multiple birth neonates (65.9%) compared to singletons (31.7%) ($P = 0.002$), whereas Zone III ROP was significantly more frequent in single birth neonates (58.5%) compared to multiple birth neonates (29.3%) ($P = 0.008$). These findings align with those of Hua *et al*. (2015), who similarly reported that multiple birth neonates exhibited higher rates of Zone II ROP ($P = 0.024$) (Hua *et al*., 2015). In addition, Di Pietro *et al*. identified multiple birth as a significant risk factor for Zone II ROP, reinforcing the greater predisposition of multiple birth neonates to more central retinal involvement.[17] The higher incidence of Zone III ROP in singletons, as observed in the present study, may reflect differences in disease regression patterns between the two groups. Ju *et al*. reported that Zone III ROP exhibited the highest rates of spontaneous regression (100%), supporting the notion that Zone III ROP is associated with better prognosis.[23] Similarly, Dos Santos Motta *et al*. (2011) found that Zone III ROP was significantly more common in singletons, reinforcing that single birth neonates may experience more peripheral ROP involvement with a lower risk of severe disease progression.[24] Notably, Stage 3 ROP was observed in 4.9% of single birth neonates, whereas no cases were recorded in multiple birth neonates ($P = 0.494$). Although this difference was not statistically significant, it aligns with previous studies suggesting lower Stage 3 ROP prevalence in multiple births. Patel *et al*. (2023) reported no cases of Stage 3 ROP among multiple birth neonates.[25] Similarly, Riazi-Esfahani *et al*. (2008) found that Stage 3 ROP was more frequent in singletons but did not reach statistical significance.[22] This observation may indicate differences in disease progression patterns or screening intervals between the two groups. Overall, the findings of this study highlight the importance of considering birth plurality when assessing ROP risk, particularly in terms of zone distribution. While staging patterns did not significantly differ, multiple birth neonates demonstrated a higher prevalence of Zone II involvement, which is associated with a greater need for intervention. Conversely, single birth neonates had a higher prevalence of Zone III ROP, which has better prognostic implications. These differences underscore the need for tailored screening and follow-up strategies based on birth plurality to optimize early detection and management. Future studies with larger sample sizes and longitudinal follow-up are warranted to further elucidate the clinical implications of these findings.

Limitations of the study

The study was conducted in a single hospital with a small sample size. Hence, the results may not represent the whole community.

Conclusion

The current study highlights important differences in ROP zone distribution between single and multiple birth preterm neonates, underscoring the clinical relevance of birth plurality in ROP management. Although no statistically significant differences were found regarding ROP staging, multiple birth neonates showed a significantly higher prevalence of Zone II ROP, whereas single birth neonates had significantly more cases of Zone III involvement. These findings suggest differences in disease progression and prognosis between the

two groups, emphasizing the need for tailored screening and follow-up strategies based on birth plurality. Further large-scale, longitudinal studies are warranted to validate these findings and to inform the development of refined guidelines for effective ROP screening and intervention strategies, ultimately aiming to reduce the risk of severe visual impairment among preterm infants.

Funding

No funding sources.

Conflict of Interest

None declared.

Ethical Approval

The study was approved by the Institutional Ethics Committee.

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