UNIVERSITY OF CALGARY

Neurodevelopmental and Visual Outcomes of Preterm Infants with Retinopathy of Prematurity

by

Mohammed Yasir Abdulrahman Al-Hindi

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF COMMUNITY HEALTH SCIENCES

CALGARY, ALBERTA

APRIL, 2010

UNIVERSITY OF
CALGARY

Abstract

Objective: To examine the neurodevelopmental and visual outcomes of children who developed severe retinopathy of prematurity (ROP) at 36 months corrected age.

Methods: This was a retrospective cohort study with prospective neurodevelopmental and visual follow up carried out on eligible infants born preterm ≤ 28 weeks gestation and/or birth weight ≤1250 grams between 1996 and 2004.

Results: Of the 677 infants followed, 568 had no/mild ROP and 109 had severe ROP. The risk of developing severe Neurodevelopmental impairment among the severe ROP group was 3.4 times (95% CI 2.32 - 4.98) of the no/ mild ROP group. Gestational age, severe brain injury and severe ROP were independent risk factors for severe Neurodevelopmental impairment. All visual morbidities were higher in the severe ROP group.

Conclusion: Severe ROP is linked to increased risk of severe Neurodevelopmental impairment and visual morbidities.

Acknowledgement

I am so grateful to my supervisor, Dr. Reg Sauve who encouraged from the beginning, followed me carefully throughout my learning process, and supported me in my difficult times. Without his understanding and extraordinary efforts, this work could not have been done. I would also like to express my gratitude to my committee members, Dr. Stephen Wood and Dr. Wendy Yee for their kind help. I was honored that my consultant was Dr. Anna Ells, one of the distinguished authors in Retinopathy of Prematurity.

I am blessed with a good family. I am forever thankful to my mother and father for their spiritual support. I am also thankful to my wife, Israa and my children, Alaa, Bayan, Albatool, and Ibraheem for being patient with me during my extended absence, I promise to compensate for them. Other family members who suppurated me were Meraj, Sana', Ula, Olfat, Iman, Wajeeh, Al-Zahraa', Majid, and Salam.

I would like to acknowledge the support extended by the Department of Perinatal/Neonatal Medicine members at University of Calgary. I will never forget the support of Crystal, Florence, Heather, Selphee, and Everly.

My special thanks to Dr. Hasan, Dr. Majeedah, Dr. Yusuf, Dr. Lodha, Dr. Essa, and Dr. Belal for their social support. Last, but not least, Dr. Amochou and his lovely wife Anuradha Soraisham, thanks a lot.

Dedication

I dedicate this work to my wife, Israa Meraj Mirza who is recovering from her severe illness.

TABLE OF CONTENT

Contents **Page**

LIST OF TABLES

Table **Page**

LIST OF FIGURES

FIGURES **Page**

LIST OF ABBREVIATIONS

ANR	Antenatal Risk Score
ANS	Antenatal Steroid
ACH	Alberta Children Hospital
BC	British Colombia
BW	Birth Weight
BSID	Bayley Scales of Infant Development
BPD	Bronchopulmonary Dysplasia
CI	Confidence Interval
CHR	Calgary Health Region
CNN	Canadian Neonatal Network
CP	Cerebral Palsy
ELBW	Extremely low birth weights
ET	Early Treatment
FIM	Functional Independence Measure
GLM	Generalized Linear Model
GA	Gestational Age
HDI	Human Developmental Index
HI	Hollingshead Index
ICROP	International Classification of Retinopathy of Prematurity
IGF	Insulin-like Growth Factor
IVH	Intra Ventricular Hemorrhage
IQR	Inter Quartile Range
MRI	Magnetic Resonance Imaging
MR	Mental Retardation

M-H	Mantel Haenszel
MB	Multiple Birth
NICU	Neonatal Intensive Care Unit
NEC	Necrotizing Enterocolitis
NICHD	National Institute of Child health Human Development
NDI	Neurodevelopment Impairment
O2	Oxygen
PMA	Post Menstrual Age
PVL	Periventricular Leukomalacia
PDA	Patent Ductus Arteriosus
PNFU	Perinatal Follow Up
RLF	Retrolental Fibroplasia
ROP	Retinopathy of Prematurity
RR	Risk Ratio
SD	Standard Deviation
SGA	Small of gestational Age
TIPP	Trial of Indomethacin Prophylaxis in Preterm
VLBW	Very Low Birth Weight
WPPSI	Wechsler Preschool and Primary Scale of Intelligence

CHAPTER ONE

1.1 Introduction:

Retinopathy of prematurity (ROP) is a vasoproliferative disorder of the eye affecting premature, very low birth weight (VLBW < 1500 grams) infants. It is an important cause of visual impairment and blindness in childhood. In countries with infant mortality rates of less than 10 per 1000 live births, ROP accounts for 6% to 20% of childhood blindness (1;2).Variations in the incidence may occur between areas within the same country depending on the level of available postnatal care (1;3;4).

Retinopathy of prematurity, previously termed Retrolental Fibroplasia (RLF), was initially reported in 1942 by *Terry (5)*. In 1952, *Campbell* provided the first link between the use of oxygen and ROP (6). She suggested that the toxic effects of uncontrolled supplemental oxygen (O2) to newborns were responsible for the epidemic. She emphasized the importance of maintaining pregnancies beyond the 33rd week and avoiding the use of prophylactic oxygen therapy, advocating its use only in the treatment of cyanosis (6). It took another decade and more reports (7;8) for this association to be widely recognized and the practice of restricted O2 supplementation to be implemented. The decline in the use of greater than 40% supplemental O2 for premature infants in the 1950s was followed by a decline in the incidence of ROP (9;10), ending what is referred to as the "First Epidemic" of ROP in developed countries. However, decreased O2 use was feared to have resulted in cerebral hypoxic changes and an increase in brain damage and death among premature infants (7;9;11-13). For example, *Cross* (10) suggested that while the policy of restricting the amount of O2 in incubators diminished RLF rates in the U.K., it concurrently increased the

number of deaths in the first 24 hours of life. A rough estimate suggested that for each case of blindness prevented, there was an excess of 16 deaths (10). He also concluded that the similar findings of increased neonatal mortality were being observed in U.S. centers (10). These report led to more liberal, but careful use of O2 over the following two decades. At the same time there were enormous advances in the field of newborn medicine, this led to increased survival of smaller and less mature infants (14).

During the 1980s, reports emerged of a "Second Epidemic" of ROP (15), similar in size to the first epidemic. *Gibson et al* (16) studied data from a population-based register of handicapping conditions in British Colombia (B.C.), Canada, and a birth weight-specific census of live-born infants in B.C. These data were used to determine annual, population-level incidences of ROP-induced blindness during 1952 to 1983. Infants weighing <1000 g, i.e. extremely low birth weights (ELBW) at birth had a significantly increased standardized incidence ratio of 3.07 (95% confidence interval (CI) 1.26 - 11.06). No increases in risk were observed in heavier or lighter weight infants. This report, supported by subsequent reports from the U.S and Australia, concluded that this epidemic was due to increased survival rates of ELBW premature infants and not to new iatrogenic factors (16-18). Since the 1990s, in developed countries, the incidence of severe ROP decreased in some centers (19-23); on the other hand, other centers reported an increase in the incidence and severity of ROP (24;25).

Unfortunately, at least 50,000 children are blind from ROP globally (26). It is now becoming a significant cause of blindness in many middle income countries in Latin American and, Eastern Europe (26;27) while ROP is being reported more frequently in emerging countries like India (27) and China (28). The characteristics of babies developing severe ROP in these countries are different from the industrialized

countries, having a much wider range of birth weight (BW) and gestational age (GA). Rates of disease requiring treatment also tend to be higher suggesting that babies are being exposed to risk factors which are being better controlled in industrialized countries. This constitutes now "the third epidemic" of ROP (26).

The exact cause of ROP is not known. The occurrence of ROP is linked strongly to the degree of prematurity, BW, and exposure to supplemental O2 (24;29-32). ROP develops at 32-34 weeks post menstrual age (PMA), regardless of the GA at delivery (33). The current concept in its pathogenesis is the involvement of two major growth factors that regulate the neovascularization in the retina: Vascular endothelial growth factor (VEGF) and Insulin-like growth factor (IGF-1) (34). If they become deficient, normal vessels growth is inhibited; while in excess they stimulate new vessel formation (34). The pathogenesis of ROP is a two phase mechanism regulated by VEGF and IGF-1 (34). In Phase I, there is cessation of retinal vascular growth after premature birth. This is mediated by the cutoff of the placental supply of VEGF and IGF-1. Conversely, in phase II, the insufficient vascular growth of the developing retina creates hypoxia, which precipitates the release of both factors, stimulating new and abnormal blood vessel growth.

Genetics may play a role in the development of ROP. But the studies so far have found only associations rather than causal relationships (35;36). *Vannay et al* (35) conclude that, from a retrospective study, VEGF specific haplotype was more prevalent in the treated patients than in the untreated patients (13 of 86 versus 1 of 115; $p < 0.001$), and the association remained significant ($p < 0.01$) even after the adjustment for risk factors of ROP (GA, supplemental O2, and gender). While

Hutcheson et al (36) studied Norrie disease gene sequence variants in an ethnically diverse population with ROP in the U.S., and found a weak association (36).

In general, ROP occurs more in the white race than in blacks and more in males than in females (37-39). A number of other risk factors have been suggested to contribute to the incidence and severity of ROP. These factors include prolonged mechanical ventilation (40), early intubation, prolonged O2 supplementation, hypotension, patent ductus arteriosus (PDA), necrotizing enterocolitis (NEC) (41), chronic lung disease (37;42), intraventricular hemorrhage (IVH) (42), and Candida sepsis (38;40). It is controversial whether these are truly independent risk factors or simply indicators of the compromised health of the VLBW (40).

1.2 **Definition and Classification of Retinopathy of Prematurity:**

Coinciding with the history of ROP, the definition and classification of this disease also have evolved. The term ROP was introduced to replace RLF after the first expert meeting in 1984 (43). This definition was expanded to include retinal detachment as an end result of the progression of ROP in 1987(44). The former terminology described the disease in its late stage, while the latter (44) one extended the definition to describe the early phases of the disease (44).The updated consensus of "The International Classification of Retinopathy of Prematurity" (ICROP), see appendix (A) for related figures, (45) is now based on:

*(1) **Staging of the disease:***

<u>Stage I</u>: demarcation line between the vascular and avascular portions of the retina. It is the earliest sign of ROP.

<u>Stage II</u>: formation of a pink, ridge-like structure between the two regions.

Stage III: extra-retinal fibrovascular, fine vessels are seen growing from the ridge-like structure into the vitreous.

Stage IV: is partial detachment of the retina which could be extra foveal (IVa) or foveal (IVb).

Stage V: total retinal detachment.

(2) ***Extent of involvement:*** *This is recorded as hours of the clock or as 30º sectors along the junction of the avascular and vascular portions of the retina.*

(3) ***Location:***

Zone I: the posterior most regions around the optic disc and fovea, with a radius of twice the distance from the macula to the optic disc, and are the most critical region for development of visual acuity.

Zone II: extends from the periphery of zone I to the ora serrata on the nasal side and to approximately the equator on the temporal side.

Zone III: extends from the outer edge of zone II in a crescentic fashion to the ora serrata.

(4) ***Presence or absence of "plus disease":*** *presence of dilated and tortuous retinal blood vessels in the posterior pole of the eye. This can be seen at any stage. It is a hall mark of rapidly progressive disease.* ***Pre-plus disease*** *is any vascular abnormalities of the posterior pole that are insufficient to diagnose plus disease. (5)*

(5) ***Aggressive posterior ROP:*** *Rapidly progressive, does not pass through the stages, increased vascular dilatation and tortuosity that occur in all 4 quadrants and are out of proportion of the peripheral retinopathy.*

Extract with permission from: An International Committee for the Classification of Retinopathy of Prematurity. The International Classification of Retinopathy of Prematurity revisited. Arch Ophthalmology 2005, 123(7): 991-999. ©American Medical Association (45)

The natural history of mild ROP is usually favorable if there is regression by involution (45). With more severe forms, the final outcomes could include fibrosis and subsequent traction, detachment of the retina, and blindness (45). Laser photocoagulation has replaced cryotherapy and became the standard preventive measure for the progression of severe forms of ROP to blindness (46). The guidelines for the indication for treatment of ROP in Calgary Health Region (CHR), now part of Alberta Health Services, are in concordance with the Early Treatment of Retinopathy of Prematurity (ET-ROP) (46) defined as "*retinal ablation administered to the avascular retina to a selected group of higher-risk eyes among those that have reached prethreshold severity"(46).* **Prethreshold** is defined in this study as zone I location of disease with any ROP; or zone II, stage 2 with plus disease; or zone II ROP with any amount of stage 3; or zone II with less than 5 contiguous or 8 cumulative clock hours of stage 3 with plus disease (46). **Threshold** ROP is defined as Zone I or II location of the disease; five contiguous or eight composite hours of Stage 3 with plus disease (46). Once an infant is labeled as having a prethreshold ROP, risk factor criteria are applied that include: BW, GA, ethnicity, singleton versus multiple birth status, out born versus inborn status, severity of ROP, and rate of progression of ROP. Based on this, he/she is offered laser therapy (47).

1.3 Linking Retinopathy of Prematurity with Neurodevelopmental Outcome of Premature Infants

1.3.1 Predictors of Neurodevelopmental Outcomes

With the improvement of prenatal and perinatal management and the advancement of neonatal intensive care, more preterm infants, especially ELBW infants, have survived (48-50). These improvements included turning-key modalities of therapy like antenatal steroids, surfactant, advanced ventilation and parentral nutrition (51). Survival is directly proportional to GA and BW. From 1987/1988 to 1999/2000, survival of infants weighing 500 to 750 g improved from 44% to 65%; for infants weighing 751 to 1000 g survival improved from 66% to 88%; and for 1001- to 1500-g infants from 87% to 93%, in The National Institute of Child Health and Human Development (NICHD) -Neonatal Research Network (50). This had led to increased interest in their neurodevelopmental outcomes rather than only mortality. In the NICHD Neonatal Research Network, rates of neurodevelopmental impairment (NDI) (defined as the presence of any of the following: moderate to severe cerebral palsy, cognitive or motor scores that fall more than 2 standard deviations below the population mean on standardized testing, bilateral hearing impairment requiring amplification or bilateral blindness) in Network Centers in the 1990s ranged from 28% to 40% in infants born at 27 to 32 weeks and 45% to 50% in infants born at 22 to 26 weeks (52). Regional and local studies in the 1990s report similar, wide range major neurodevelopmental impairment rates from 20% to 48% (48;53-56). Center variability in outcomes is thought to be related to rates of neonatal morbidities such as sepsis, NEC, grade 3-4 IVH, and bronchopulmonary dysplasia (BPD) and differences in management style including rates of administration of antenatal steroids (ANS),

postnatal steroids, antibiotics, cesarean section rate, and use of ventilators (57). In CHR the prevalence of severe NDI at 36 months corrected age among children born at ≤28 weeks GA or ≤1250g BW between1996-2004 was 12% (58).

Although prematurity and ELBW are strongly associated with increased significant NDI (51), this does not mean that their rates were increasing. In fact a study by *Wilson-Costello et al (53)*, suggested the opposite. In their study neonatal therapies, survival and outcomes at 20 months' corrected age among all ELBW infants born in 2000-2002 (period III) were compared to 2 previous periods: 1982-1989 (period I) and 1990-1999 (period II). Across the three periods survival increased from 49% to 71%. Among those who had follow-up, the rate of cerebral palsy decreased from 13% to 5%, resulting in a decrease in NDI from 35% to 23%. As a result, during period III versus II, survival without impairment increased, whereas survival with impairment decreased (53). The author implicated a variety of perinatal and neonatal factors that could explain this, including increased ANS use and cesarean section delivery, as well as decreased sepsis, severe cranial ultrasound abnormalities, and postnatal steroid use (53). Of interest there was no change in the rate of chronic lung disease and ROP as a morbidity was not included in the analysis.

Unfortunately, as more survivors have longer follow-ups, those who had minimal or labeled as no disability could suffer from "low severity dysfunctions" (59;60). These include learning disabilities, borderline to low average IQ scores, attention-deficit hyperactivity disorder, specific neuropsychological deficits (e.g., visuomotor integration, executive function), and behavior problems. These dysfunctions might occur in up to 70% of VLBW infants depending on the degree of their BW (61-63). As a result, around 50% of VLBW children will require special

education services, 20% will need a self-contained learning disabilities placement, and 16% to 20% will repeat at least one grade (61).

There are many factors that could affect the neurodevelopmental outcomes of preterm infants. Socio-demographic factors like maternal age, race, level of education, socioeconomic status, social support, maternal physical and mental health, environmental exposure to positive and negative experiences has been implicated to affect neurodevelopmental outcomes (64). On the other hand, biological risks include GA, BW, perinatal events like chorioamnionitis and increased antenatal risk score (ANR) for hypoxic-ischemic encephalopathy (65). Events that could lead to brain insult in the postnatal period included chronic lung disease, recurrent apnea and bradycardia, transient hypothyroxemia of prematurity, hyperbilirubinemia, medications, and stress from hospitalization (66). Other additional considerations that have an impact on central nervous system integrity include abnormal neurological exam at discharge, home oxygen use and frequent hospitalization (65;66).

The mechanisms that lead to NDI are complex. They include multi-model pathogeneses that are intertwined together. Disruption of normal development of the brain occurs with premature birth. It involves the disruption of corticogenesis (66), synaptogenesis and developmental regulation of specific receptor populations (N-methyl-d-aspartate, AMPA, glutamate) (67). Injury to the brain could occur perinataly secondary to chorioamnionitis (68) or hypoxic- ischemic encephalopathy (69), early neonatal period e.g. IVH or hypoxia or late neonatal period e.g. hypothyroxinemia, post-natal steroid use. The above mechanisms might be evident early by early cranial ultrasound findings (IVH, periventircular leukomalacia, ventriculomegaly, echodensities) (69) or later by magnetic resonance imaging performed in adolescence

(70) even without apparent early brain injury, having smaller volumes than controls with respect to cortical gray matter, basal ganglia, corpus callosum, amygdala, and hippocampus (71). Either way, disruption or injury, the resultant abnormal neuronal connectivity or circuitry might lead to specific NDI (72). For example, an impact on cognitive functions were associated with reduced gray matter volume (71), decreased IQs were correlated with summed cortical volumes (73) and decreased complexity in cortical areas in children born preterm was associated with changes in visuospatial and semantic processing(74).

The above mentioned sequence of 1- the actual event, 2- probable neuroanatomic effect, 3- functional impact, and 4- observable outcome were simplified examples of four-staged model "*Conceptual model of neurobiologic impact*" that has been suggested in the work of *Taylor et al*(75) that explains variability of sequelae in VLBW. The biological factors influence mostly the neurologic and perceptual performance function.

Environmental factors play an important role in neurodevelopmental outcomes (76). Their role is more pronounced as early as 24 months of age and beyond (65). No single environmental factor appears to be responsible in neurodevelopmental impairment (77). Socioeconomic status is a common stable factor that could be measured objectively via a scoring system, keeping in mind that it is only one dimension of the diverse environmental influences. They influence mostly the verbal and general cognitive outcome (77).

The relation between biological factors and environmental factors are thought to be transactional (77). This assumed an inherent plasticity in the child (biologic) and the

environment. The child is thought to be constantly reorganizing and self-righting. A poorly stimulating environment would interfere with self-righting and vice versa (77).

1.3.2 Retinopathy of Prematurity and Neurodevelopmental Outcomes:

Vascular endothelial growth factor is important in the development of ROP (34). Although there are no studies that look at the effects of VEGF deficiency on neurodevelopmental outcome in neonates, it might have a negative effect in the development of the brain. This is evident in a study done by *Kim et al* (78) in which they showed the neurogenic effects of VEGF during germ layer formation of human embryonic stem cells (78). On the other hand, IGF-1 is important for the early development of the brain (79). It was suggested that regulation of the IGF-1 level plus other factors might improve the neurodevelopmental outcomes of ELBW (80). As IGF-1 is implicated in the development of ROP in phase I (34), it could be postulated that infants who had severe ROP are also at increase risk of impaired neurodevelopment outcome. Finally, some associated morbidities like BPD and NEC have been linked with impaired neurodevelopment (81;82), hence, the inclusion of these morbidities as modifier or confounders may help in delineating the causal pathway of ROP.

1.3.3 Literature Review

There are few studies that assessed directly the association of ROP with neurodevelopmental outcome but the results are variable (83;84). *Sugimoto et al* (83), did a retrospective study to examine the relationship between ROP and neurologic morbidities in 1081 Japanese VLBW infants. The main outcome was cerebral palsy

(CP) or mental retardation (MR). Adjusting for BW subgroups, they found no significant association between ROP and CP or MR. On the other hand, *Msall et al* (84) conducted a longitudinal follow up of children who participated in the Multicenter Cryotherapy for ROP Study (CRYO-ROP) (85). The outcome was neurodevelopmental function at 5.5 years determined by the Functional Independence Measure for Children (WeeFIM) (85). This study included 255 infants with BW <1251 grams at birth from 23 centers who developed threshold ROP and received cryotherapy to not more than one eye. They found that with the increase in ROP severity from no ROP to threshold ROP the disability increased from 3.7% to 19.7%, respectively. They also reported high rates of functional limitation with unfavorable visual acuity (84).

Schmidt et al (86) examined 910 infants with BW of 500 to 999 g who were enrolled into Trial of Indomethacin Prophylaxis in Preterms (TIPP) and survived to a PMA age of 36 weeks for poor long-term outcome. This was defined as the combined end point of death or survival to 18 months with 1 or more of CP, cognitive delay, severe hearing loss ,and bilateral blindness. Each of the neonatal morbidities (BPD, brain injury and severe ROP) was similarly and independently correlated with a poor 18-month outcome. Odds ratios (OR) were 2.4 (95% CI 1.8-3.2) for BPD, 3.7 (95% CI 2.6-5.3) for brain injury and 3.1 (95%CI 1.9-5.0) for severe ROP. In children who were free any of the 3 morbidities and with any of the 1, any 2, and all 3 neonatal morbidities, the rate of poor long-term outcomes was 18%, 42%, 62% and 88% , respectively (86).

1.4 Visual Outcomes of Retinopathy of Prematurity

The published literature includes many reports of visual outcomes of ROP. Blindness secondary to ROP is a still a major complication accounting for 3% to 14% in ountries with high Human Developmental Index (HDI)(26), while it reaches as high as 60% in moderate HDI countries. In low HDI countries, it is either not reported or under reported (26). Apart from blindness, if ROP was untreated complications might include myopia, early development of cataracts, iris neovascularization, glaucoma, retinal pigmentation, retinal folds, dragging of the retina, lattice-like degeneration, retinal tears, and rhegmatogenous and exudative retinal detachments (87). *Palmer et al* (88) examined 254 survivors, at age 15 years, from 291 preterm children with BW< 1251 g and threshold ROP in one or both eyes, who participated in the CRYO-ROP trial. Thirty percent of treated eyes and 51.9% of control eyes (P<.001) had unfavourable structural outcomes (defined as posterior retinal fold or worse). Between 10 and 15 years of age, new retinal folds, detachments, or obscuring of the view of the posterior pole occurred in %4.5 of treated and 7.7% of control eyes. Unfavourable visual acuity (distance visual acuity 20/200 or worse) outcomes were found in 44.7% of treated and 64.3% of control eyes (P<.001). They concluded that the benefit of cryotherapy for treatment of threshold ROP was maintained across 15 years of follow-up. They also suggested long-term, regular follow-up (88). After implementation of laser therapy as a standard treatment for ROP, a long term follow up assessment of the refractive and biometric outcomes was conducted at mean of 11 years on 16 laser-treated eyes with threshold ROP and compared to 9 eyes with subthreshold untreated ROP (89). Although the trend toward increased myopia in treated eyes did not achieve statistical significance (p=0.08), the myopia in this group appeared to be slowly progressive in nature. The laser-treated eyes had reduced

anterior chamber depth compared with the subthreshold eyes (p=0.02). Otherwise, the two groups did not differ significantly in terms of eye structure or physiologic accommodation (89). Studies that followed up patients who received early treatment of ROP as per ET-ROP criteria showed that around 70% of high-risk prethreshold ROP (pre-ROP) eyes were myopic in early childhood, and the proportion with high myopia increased steadily between ages 6 months and 3 years. Timing of treatment of high-risk pre-ROP did not influence refractive error development. The prevalence of myopia and high myopia was higher in eyes with retinal residua of ROP than in eyes with normal-appearing posterior poles (90). The high-risk pre-ROP group developed astigmatism in nearly 43% of treated eyes which was not influenced by timing of treatment or by characteristics ROP (91). The above findings reinforce the need for long term follow-up eye examinations in infants with high risk pre-ROP.

1.5 Rationale and Significance

Sugimoto's (83) study included a retrospective cohort of VLBW infants. Birth weights were grouped to explore effects on neurodevelopmental outcome. They only reported CP and MR rates. There were no further data that assess cognitive function or level of CP, MR or visual disability. Lastly, they used the older ICROP classification.

In the *Msall* (84) study, the major drawback was that they used the infants who had cryotherapy treatment to only one eye. The other eye was left as a control. If the other eye would have had severe ROP or even retinal detachment, it was still untreated. In fact this study was terminated 9 months prior to the closing date as treatment showed significant benefits (92). This could have great impact on their outcomes. Furthermore laser therapy and earlier treatment is now proven to be more

beneficial with better visual outcomes (93), this could affect the neurodevelopmental outcomes. Finally, they used the WeeFIM tool which has restricted study in Canada.

In *Schmidt's* (86) study, the latest ICROP had not been established and not all centers used ET-ROP criteria. Also, centers differed in management using either cryotherapy or laser. Patients were followed up to 18 months corrected age.

Our study, though retrospective, will include results of prospectively collected data from all eligible infants. The ROP was classified as per the latest ICROP and treated by laser photocoagulation as per ET-ROP criteria (personal communication with Dr. Anna Ells) (47). Assessment will include elaborate details, at 36 months corrected age, of the component of neurodevelopment of these infants which is internationally standardized and most developmental programs follow in Canada. The visual outcomes and their relation to neurodevelopmental outcomes will also be discussed.

In the Calgary Health Region, the incidence of severe ROP requiring laser photo coagulation was reported to be 4.8% from 1991-2000 (47). After this report, an observational study showed that the incidence of overall ROP has increased over the last decade (1996-2004) from 37.2% to 58.8%, the severe form from 11.5% to 21.3 %, zone I disease from 0.75 to 4.8 %, and plus disease from 4.8% to 11.2%. Laser therapy rate was also increased from 6.8 to 14.9 % (94). These results were alarming and there is a need for further follow up studies to address outcomes of affected infants. This study would add more information in understanding the association of ROP to neurodevelopmental outcome. Moreover, it will form building blocks for further follow up studies of regional or national level. Finally, it is hoped to help in

allocating appropriate resources and give clearer information for prognosis for the parents.

1.6 Research question/ hypothesis/ objectives:

1.6.1 Research Question: Do preterm infants weighing ≤1250 g at birth and/or born at ≤28 weeks gestation that developed severe ROP, have different neurodevelopmental and visual outcomes as compared to those infants with mild or no ROP at 36 months corrected age**.**

1.6.2 Hypothesis: Preterm infants weighing ≤1250 g at birth and/or born at ≤28 weeks gestation that developed severe ROP, have different neurodevelopmental and visual outcomes than those who have mild or no ROP 36 months corrected age**.**

1.6.3 Primary objective: To examine the neurodevelopmental and visual outcomes of infants born weighing ≤1250 grams (g) and/or at ≤28 weeks, who developed severe forms of ROP, at 36 months corrected age**.**

1.6.4 Secondary objectives: To examine other potential perinatal and postnatal morbidities that could affect ROP and neurodevelopmental/ visual outcomes.

CHAPTER TWO: METHODOLOGY

2.1 Research Design: This study was a retrospective cohort study with prospective neurodevelopmental and visual follow up at 36 months corrected age.

2.2 Participants:

2.2.1 Major Inclusion Criteria: All infants with a birth weight of ≤1250 g and/or ≤28 weeks gestation admitted to NICU in the CHR between January 1, 1996 and December 31, 2004 were eligible for the study. Surviving infants who had neurodevelopmental and visual assessments at 36 months adjusted age were included in the study.

2.2.2 Major Exclusion Criteria: Infants who had documented congenital or chromosomal abnormalities and those who died before the final assessment were excluded from the study.

2.3 Interventions: These included prospective data collection of the following:

2.3.1 Ophthalmologic Screening: All eligible infants had ophthalmic screening examinations carried out according to the Canadian Screening Guidelines for ROP by Pediatric Ophthalmologists (47). The first examinations were performed between 4-6 weeks of chronological age. Subsequent examinations were scheduled at 2-week intervals if no ROP was present and weekly if ROP was detected (55). Clinical examinations were performed by indirect ophthalmoscopy and/or direct ophthalmoscopy through an infant gonioscopy lens of the entire retina. The quality and quantity of ROP were recorded according to the IC-ROP (45;47). Stage I & II ROP were considered as **Mild ROP**, otherwise stage III and above ROP, plus disease, posterior disease, or zone I disease were considered as **Severe ROP**.

2.3.2 Neurodevelopmental Follow Up Examinations: Once infants were discharged from NICU, they were registered into Perinatal Follow Up (PNFU) clinics. Multidisciplinary comprehensive examinations were carried out independently by Pediatricians, Psychologists, Audiologists, Physiotherapists, and Ophthalmologists on every child. Clinical dietitians and social services were incorporated if necessary. The examinations were carried out around 4, 8, 12, 18, and 36 months corrected age. In this study, the enrolled patient must have completed the 36 months assessment.

The neurological assessment of tone, strength, reflexes and posture were done according to the technique described by *Amiel-Tison* (95). Infants were scored as normal if no abnormalities were observed in the neurological examination. **Cerebral palsy** was diagnosed if the child had non-progressive motor impairment characterized by abnormal muscle tone in at least one extremity and decreased range or control of movements (96). It was considered **severe** if CP was severe enough to render the patient immobile, or **mild** if the patient was ambulatory.

The Bayley Scales of Infant Development-II (BSID-II) (97) or Wechsler Preschool and Primary Scale of Intelligence-III (WPPSI-III) (98) were administered by experienced testers. If the **Mental Developmental Index** (MDI) score was below 70 (2 standard deviations below the mean of 100), for those tested with BSID-II or WPPSI-III, the child was considered to have **severe cognitive delay**. **Mild cognitive delay** was considered if the MDI score was <84 (1-2 SD below the mean of 100). The score of 49 were assigned, by convention, to infants who could not be tested due to severe neurodevelopmental impairment.

Deafness was defined as sensorineural hearing loss requiring hearing aids. However, **mild hearing loss** was considered in case where patients were not requiring hearing aids.

Visual impairment was defined as acuity in the best seeing eye of < 20/60 and **blindness** as visual acuity < 20/200 following refractive correction.

Severe neurodevelopmental impairment (NDI) was defined as presence of one or more of the following: severe cerebral palsy, deafness, blindness or cognitive delay> 2 SD. **Mild NDI** was defined as cognitive delay within 1-2 SD, hearing loss, not requiring amplification, visual impairment remediated with corrective lenses and ambulatory CP.

2.3.3 Ophthalmological Follow Up: Visual morbidities were assessed as part of PNFU clinics. All infants in the geographic study area who had serious ROP, and any who developed visual disability or blindness from ROP, became known to the pediatric ophthalmologists at the Eye clinic (47). Furthermore, details of other visual morbidities were assessed and recorded.

2.4 Data Collection: Participants' demographic, clinical characteristics, and daily interventions, during NICU stay are collected prospectively for the PNFU program by a certified, full time nurse who completes a standardized data set about each infant. Once discharged from the neonatal unit, participants are examined in the PNFU clinics and data is obtained during the same visit. All data is entered into a database program (Microsoft® Access 2003) at the PNFU center and into (Microsoft ® Excel 2003) spreadsheet for ACH eye clinic. The databases will be merged and imported into Stata IC 10.0™ (TX, USA.) for analysis.

2.5 Major Outcomes:

2.5.1 Primary Outcome: The primary outcome of the study is the rate of severe neurodevelopmental impairment (NDI).

2.5.2 Secondary Outcome: Visual morbidities include: visual acuity, refraction, ocular structure, and ocular motility and mild NDI.

2.6 Sample Size Calculation: *Msall et al* (84) reported severe disability in infants with severe ROP to be 20%, whilst 3.7% in infants with no ROP. Although this was based on Functional Independence Measure for Children (WeeFIM) tool (84), we believe our assessment for NDI would be similar. Based on an expected incidence of NDI of 20% in infants with severe ROP, and 3.7% in infants without ROP, a sample size of 73 subjects per group will have 80% power ($1-\beta=0.8$) to detect a statistically significant difference($\alpha=0.05$). From a previous study, the prevalence of severe ROP was 16.5% from 774 infants survived until ophthalmological screening, i.e. 126 patients, from the period 1996 to 2003. Based on PNFU loss to follow up estimated to be 33.4%, we believe our sample size is achievable. Including infants from 2004 would give us even extra numbers to account for loss to follow up.

2.7 Data Analysis: Eligible infants with severe ROP will be compared with those without severe ROP. Univariate descriptive statistics will be used to identify potential data entry errors and characterize participants. Continuous variables will be described in terms of mean, median, range, standard deviation, skewness, and quartiles. Histograms and box plots will be generated to evaluate assumptions of normality and to detect outlying values. *Missing data* will be treated as follows: For ophthalmological and neurodevelopmental outcomes, data will be deleted list wise method. For other clinical characteristics, data will be deleted pair wise manner.

Bivariate analysis will be used to examine characteristics of participants as well as for the primary neurodevelopmental and visual outcomes. Statistical analyses will be performed by Student's t-test for continuous variables, and chi square test for categorical variables. Regression methods will be used to study the effect modification/ confounding of possible co-morbidities.

2.8 Limitations: Selection bias could result from loss to follow up. It is estimated that we might lose around 33% of infants who entered the PNFU by 36 months corrected age. This is either due to death before final assessment (20%) or actual loss to follow up (14%) for which assessments at younger and older ages can be analyzed separately.

CHAPTER THREE: RESULTS

3.1 Flow of Patient's Selection:

Patients that composed our sample were selected as shown in figure 1. The PNFU database retrieved 1065 infants who met the eligibility criteria, while the Eye database included 102 infants. Matching of the two databases was based on Infant's name, identification numbers, year of birth, BW, and gestation at birth. A total of 98 infants were found to be congruent, while 4 infants from PNFU data could not be matched. These infants were not included in analysis. Out of 1065 infants included, the prevalence of ROP was (35%). The exposure status was examined and infants were categorized as follow:

I- Missing ROP status: 191 (18%) infants had missing ROP status, 82 of them died before ROP screening. Although 65 children had documented final assessment at 36 months corrected age, they were excluded from the final analysis as their data were deleted *list wise*.

II- No ROP: 500 (47%) infants were not exposed i.e. had no ROP, out of which 371 (74%) infants had documented final assessment at 36 months corrected age. The reasons for the 129 dropouts were as follows:

- Five infants had missing data*.
- Sixty four infants died before initial PNFU visit**.
- One had congenital abnormality.
- Fifty nine had assessment less than 36 months:

- Eight died before final assessment.
- Fifty one were lost to follow up*.

* The actual loss to follow up was 56 infants (11%).

** Infant mortality was 64 (13%).

III- Mild ROP: 239 (22%) infants were exposed to (had suffered from) mild ROP, out of which 197 (82%) infants had a documented final assessment at 36 months corrected age. The reasons for the 42 dropouts were as follows:

- Three infants had missing data*.
- Five had congenital abnormality.
- Thirty four had assessment less than 36 months:
    - Five died before final assessment.
    - Twenty nine were lost to follow up*.

* The actual loss to follow up was 32 infants (13%).

IV. Severe ROP: 135 (13%) infants were exposed (had suffered from) severe ROP, out of which 109 (81%) children had documented final assessment at 36 months corrected age. The reasons for the 26 dropouts were as follows:

- Three infants had missing data*.
- Seven had congenital abnormality.
- Sixteen had assessment less than 36 months:
    - Four died before final assessment.
    - Twelve were lost to follow up*.

*The actual loss to follow up was 15 infants (11%).

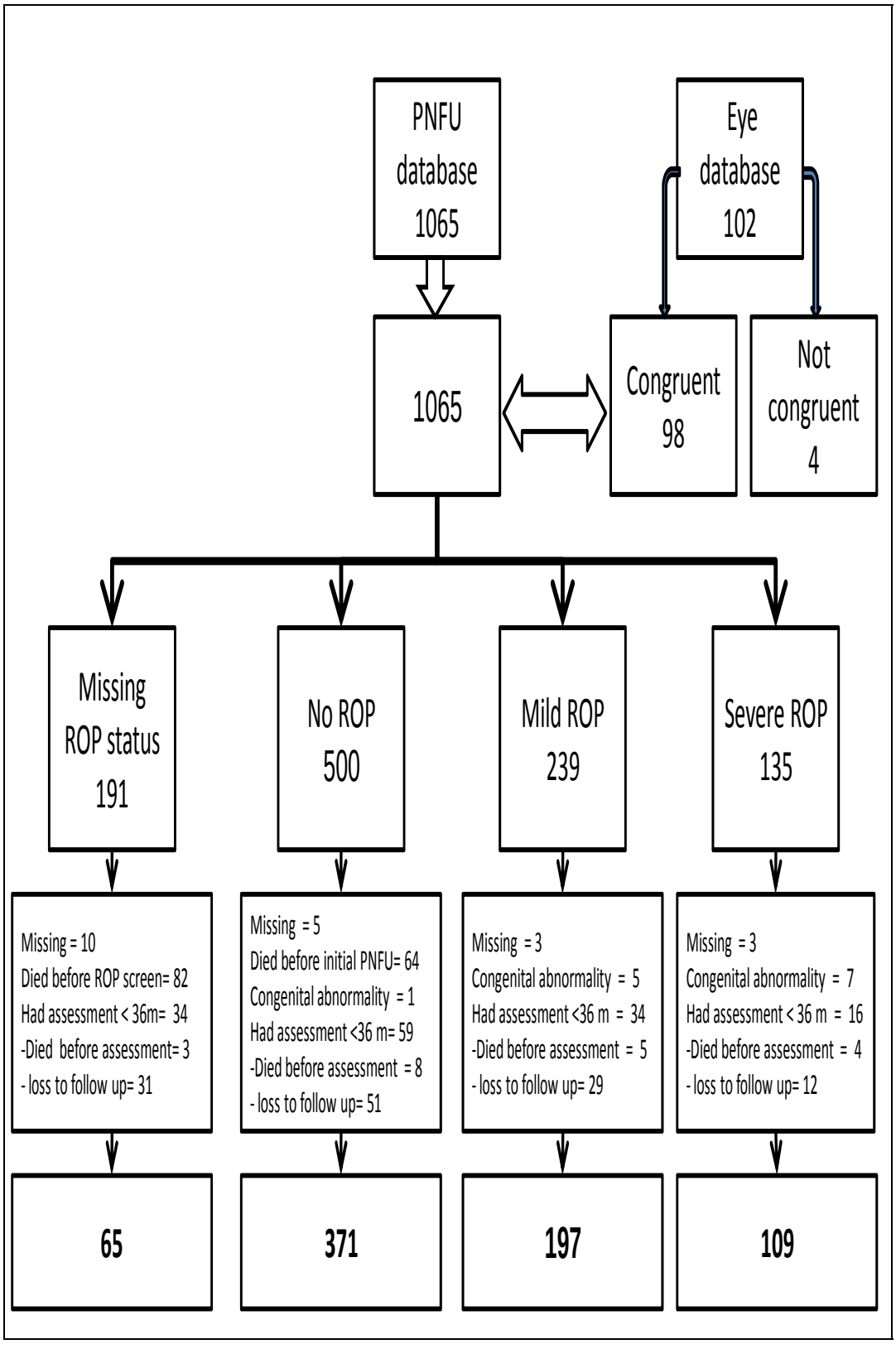

Figure 1: Flow of patients' selection

3.2 Maternal Characteristics:

This section elaborates on the maternal variables of infants included in the study. These infants were stratified as per their exposure status i.e. no ROP, mild ROP and severe ROP. The variables were carefully selected, based on previous literature, to examine the maternal demographics and specific potential risk factors that could affect the occurrence of ROP or could have an impact on NDI. The details of variables studied were as follows:

3.2.1 Maternal Age at the Time of Birth (years): the distribution of maternal age at the time of birth among infants with different ROP status is shown in figure 2. Since maternal age distributions were not skewed, one-way analysis of variance (ANOVA) was performed. There were no difference between the means of maternal age between the ROP groups (F=2.25, p=0.106).

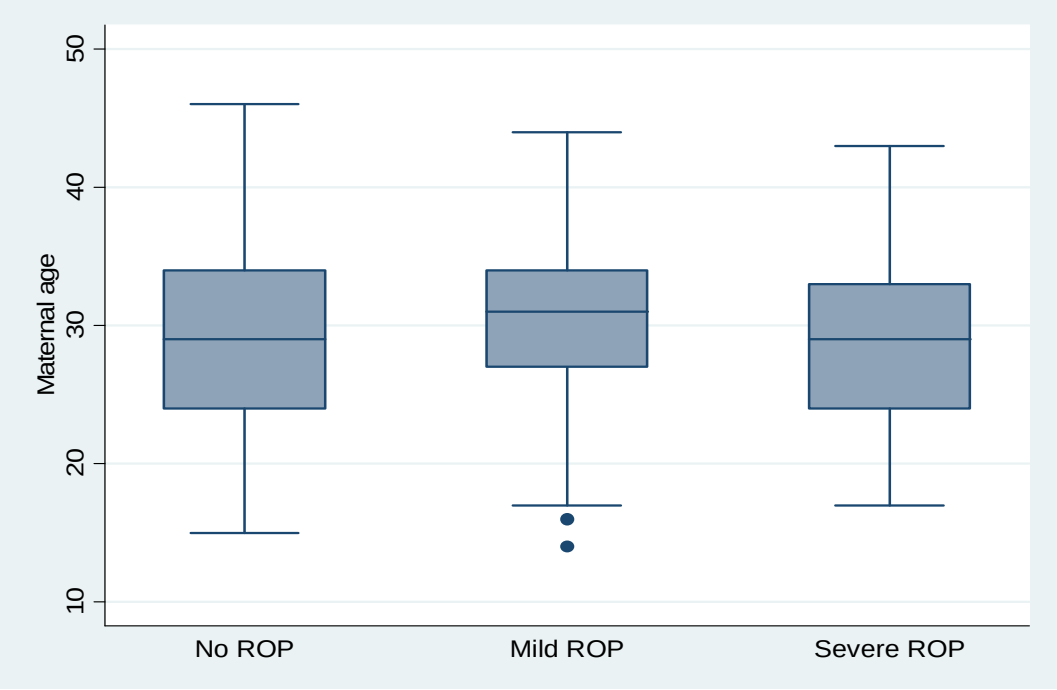

Figure 2: Box plots of maternal age among the different ROP groups. The line in the middle of the box represents the median. The lower and the upper boarders of the boxes represent

the 50% IQR. The lower and the upper whiskers represent the 95%CI. The dots represent the outliers. These will apply to later box plots.

3.2.2 Ethnicity: Maternal ethnicity were recorded as Caucasian, native, others and unknown. The data were coded as 0 for not Caucasian, 1 for Caucasian. The distribution of maternal ethnicity among the infants with different ROP severity is shown on table 1. Excluding the missing, the proportion of Caucasian was lower in the severe ROP group. A Chi-squared test was performed. The results showed that the proportions of Caucasian were different among the ROP groups (χ^2=9.47, p=0.009). This result has to be interpreted with caution since there were significant missing records in the 3 groups.

Maternal Race	No ROP	Mild ROP	Severe ROP	Total
Not Caucasian	72	27	28	127
(%)	(19.41)	(13.71)	(25.69)	(18.76)
Caucasian	233	123	49	405
(%)	(62.8)	(62.44)	(44.95)	(59.82)
Missing	66	47	32	145
(%)	(17.79)	(23.86)	(29.36)	(21.42)
Total	371	197	109	677
(%)	(100)	(100)	(100)	(100)

Table 1: Maternal ethnicity of infants with the different ROP groups.

3.2.3 Hollingshead Index (HI)(30): This index was used to represent the social status of the mother. The score is calculated by multiplying the scale value of occupation by a weight of five and adding the scale value for education multiplied by a weight of three. The higher the score, the better social status. The distribution of maternal HI among the ROP groups is shown in figure 3. Since HI distributions were

not skewed, ANOVA was performed. There was a difference between the means of HI between the ROP groups (F=3.98, p=0.019). From the table we notice that the mean HI of severe ROP and was lower than the mild ROP group. This result has to be interpreted with caution since there were significant missing records in the 3 groups.

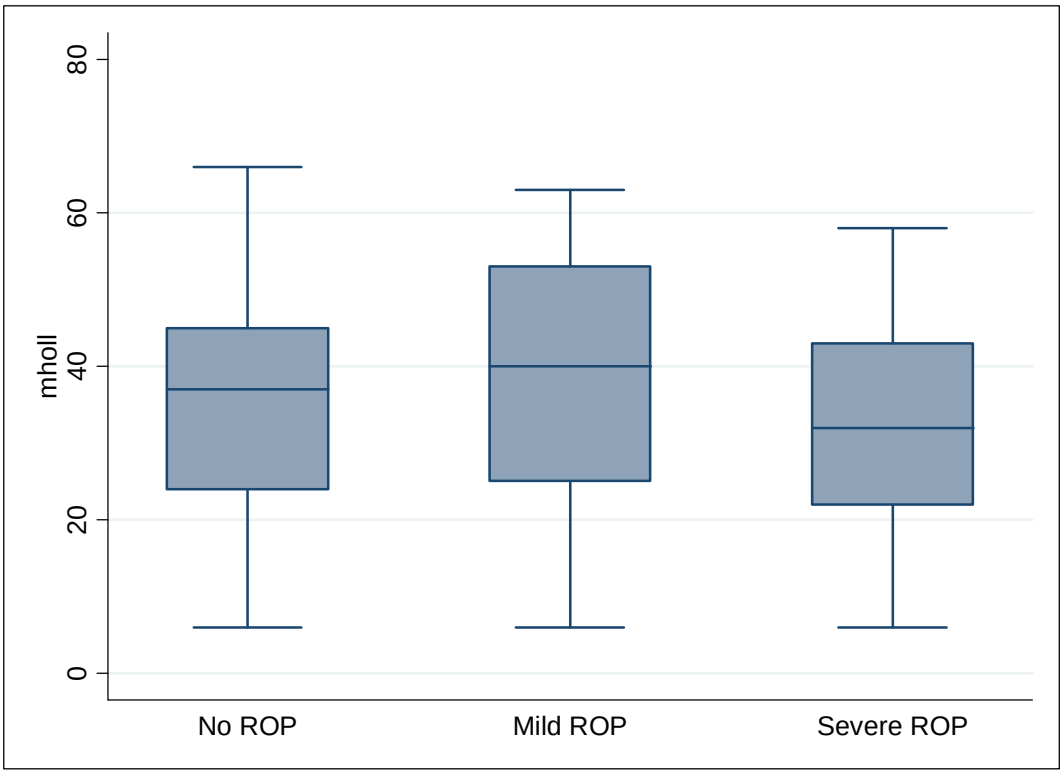

Figure 3: Box plots of maternal Hollingshead index among the different ROP groups.

3.2.4 Antenatal Risk Score: This score is a composite of various risk factors that affects the pregnancy outcomes. It includes pre-pregnancy, past obstetric, antenatal and other risk factors. See the complete scoring system on appendix (B). The sum of scores would provide the antenatal risk assessment as follows:

a. Low risk: 0 to 2.

b. Moderate risk: 3 to 6.

c. High risk: ≥7.

The distribution of ARS is shown on figure 4. Repeated median tests were performed. The results showed that there was no difference between the medians of ANR scores between the ROP groups (No ROP & Mild ROP: Z=0.9 & p=0.35. No ROP & Severe ROP: Z=1.4 & p=0.15. Mild ROP & Severe ROP: Z=0.6 & p=0.56).

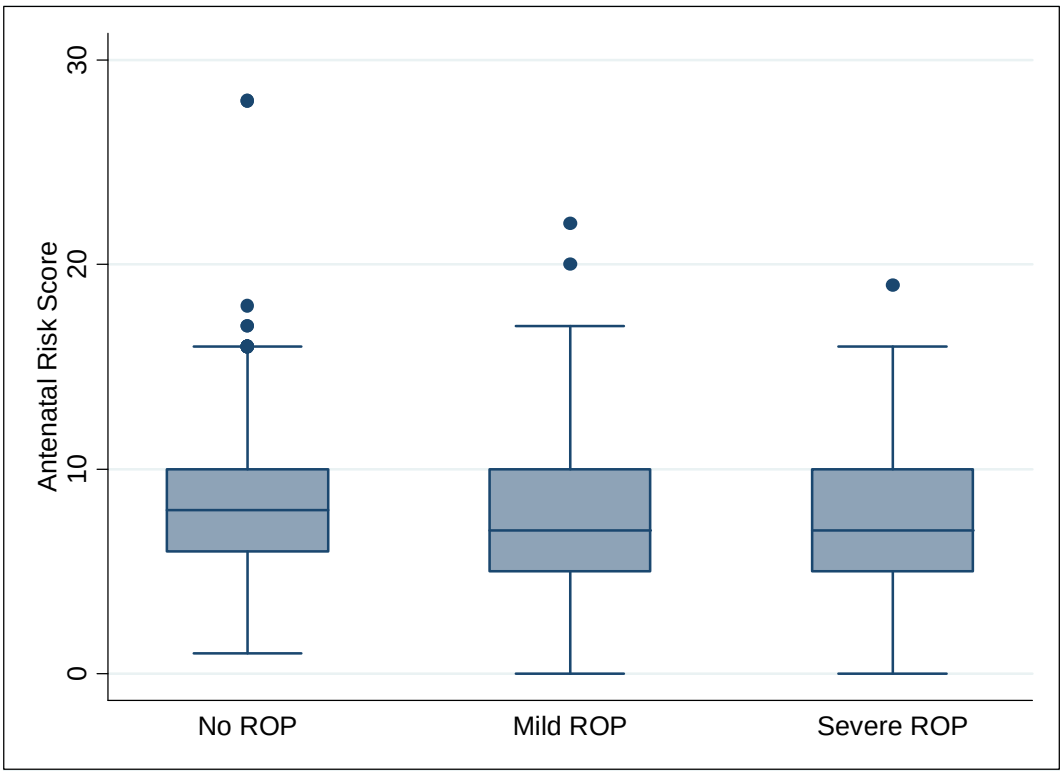

Figure 4: Box plots of antenatal risk score among the different ROP groups.

3.2.5 Antenatal Steroid: Mothers at risk of premature delivery are given ANS (betamethasone) to accelerate fetal lung maturation. This was coded as 0 for those who did not receive ANS or 1 for those who received ANS. The results are shown in table 2. Excluding the missing, the proportion of mothers who received ANS was lower in the severe ROP group. A Chi-squared test was performed. The results showed that the proportions of mothers who received ANS were different among the ROP groups (χ^2=7.15, p=0.03).

Antenatal steroids	No ROP	Mild ROP	Severe ROP	Total
Not given	54	27	26	107
(%)	(14.88)	(13.92)	(25)	(16.19)
Given	309	167	78	554
(%)	(85.12)	(86.08)	(75)	(83.81)
Total	363	194	104	661
(%)	(100)	(100)	(100)	(100)

Table 2: Antenatal steroid among the different ROP groups.

3.2.6 Multiple Births: This was coded as 0 for singleton or 1 for multiple pregnancies. The results are shown in table 3. A Chi-squared test was performed. The proportions of multiple pregnancies were not statistically different among the 3 groups (χ^2=4.37, p= 0.112).

Birth	No ROP	Mild ROP	Severe ROP	Total
Singleton	263	126	81	470
(%)	(70.9)	(64)	(74.3)	(69.4)
Multiple	108	71	28	176
(%)	(29.1)	(36)	(25.7)	(26)
Total	371	197	109	677
(%)	(100)	(100)	(100)	(100)

Table 3: Multiple birth among the different ROP groups.

3.2.7 Summary of Maternal Characteristics: Summary of maternal characteristic are listed in table 4:

Maternal characteristics	No ROP	Mild ROP	Severe ROP	P value
Age (±SD)	29.2 (6)	30.3 (5.8)	29.2 (6.3)	0.106
Ethnicity (% Caucasian)	233/305 (76)	123/150 (82)	49/77 (64)	0.009
Hollingshead index (±SD)	36 (15)	38 (16)	33 (13)	0.02
Antenatal Risk score (50%IQR)	8 (6-10)	7 (5-10)	7 (5-10)	
Antenatal steroid (%)	309/363 (85)	167/194 (86)	78/104 (75)	0.03
Multiple births (%)	106 (29)	71 (36)	28 (26)	0.1

Table 4: Summary of maternal characteristics. Data analyzed excluding missing.

3.3 Infants' Characteristics:

This section elaborates on the infant's variables during their NICU stay. The infants were stratified as per their exposure status i.e. no ROP, mild ROP and severe ROP. The variables were carefully selected, based on previous literature, to examine infant’s demographics and specific potential risk factors that could affect the

occurrence of ROP or could have an impact on NDI. The variables studied were the following:

3.3.1 Gestation Age at Delivery by Weeks: Gestational age distribution among ROP groups is shown in figure 5. It was noticed that there were unequal variances, mostly because of sekewed distribution. To correct this, the small for gestation (<10% birth weights) were omitted. Applying the ANOVA test, the gestation at birth decreases as the severity of ROP increases (F=116, p<0.001).

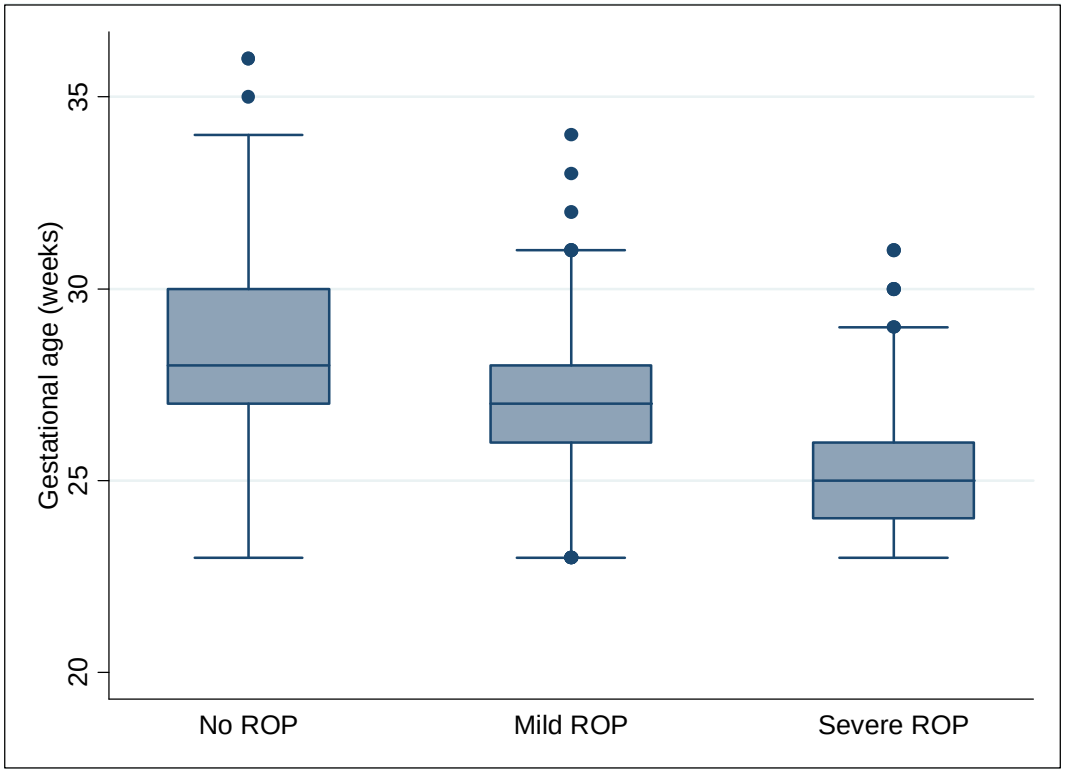

Figure 5: Box plots of gestational age among the different ROP groups.

3.3.2 Birth Weight (grams): birth weight distribution among ROP groups is shown in figure 6. Applying ANOVA test, the mean birth weight decreases as the severity of ROP increases (F=84, p<0.001).

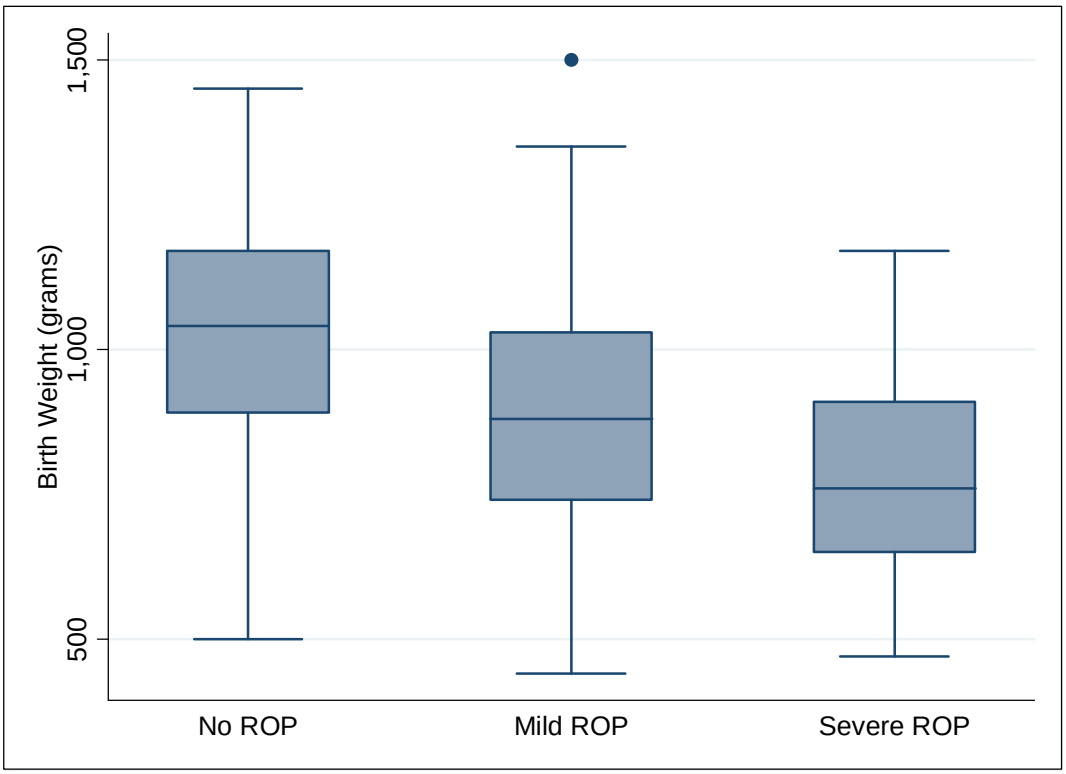

Figure 6: Box plots of birth weight among the different ROP groups

3.3.3 Sex: Gender distribution among infants with different ROP status revealed a lower proportion of females in the severe ROP group, table 5. A Chi-squared test was performed. The results showed that the proportions of females were different among the ROP groups (χ^2=6.9, p<0.03).

Sex	No ROP	Mild ROP	Severe ROP	Total
Male	187	100	70	357
(%)	(50.4)	(50.76)	(64.22)	(52.73)
Female	184	97	39	320
(%)	(49.6)	(49.24)	(35.78)	(47.27)
Total	371	197	109	677
(%)	(100)	(100)	(100)	(100)

Table 5: sex distribution among the different ROP groups

3.3.4 Small for Gestational Age (SGA): The proportion of SGA infants was significantly different (χ^2=16.1, p<0.001). The trend was the more severe the ROP, the less the proportion of SGA, table 6.

Small for gestation	No ROP	Mild ROP	Severe ROP	Total
Not SGA	283	170	96	549
(%)	(76.28)	(86.29)	(88.07)	(81.09)
SGA	88	26	10	124
(%)	(23.72)	(13.2)	(9.17)	(18.32)
Missing	0	1	3	4
(%)	(0)	(0.51)	(2.75)	(0.59)
Total	371	197	109	677
(%)	(100)	(100)	(100)	(100)

Table 6: Small for gestational age among the different ROP groups

3.3.5 Apgar's Score: Apgar's score is a measure of the newborn's well being (99). It is assigned at 1 and 5 minutes of life. Our score of interest is the 5 minutes Apgar's score, see appendix B.2. The distribution of Apgar's score among ROP groups is shown in figure 7. Repeated rank-sum (median) test showed no difference between the median Apgar's between no and mild ROP groups (Z=1.5, p=0.13) but statistical significant difference between severe ROP group and either no or mild ROP (Z=4.4 and p<0.001, Z=2.7 and p=0.006, respectively). This difference is thought not to be clinically significant.

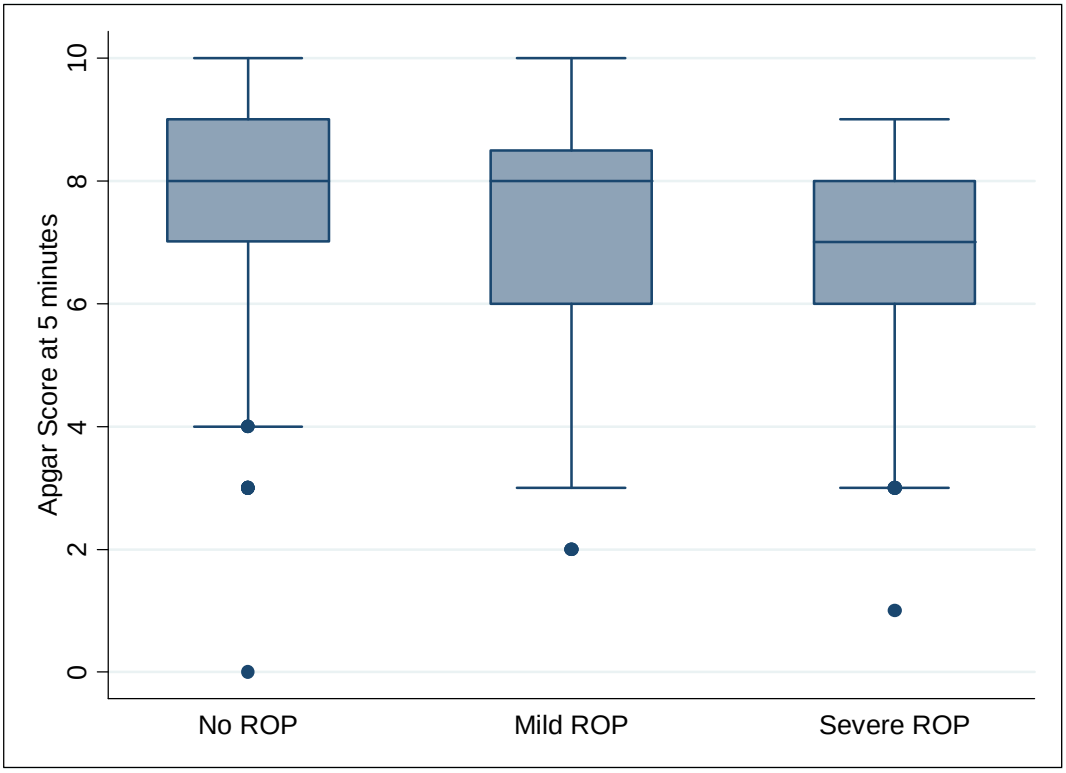

Figure 7: Box plots of Apgar's score among the different ROP groups

3.3.6 Severe Brain Injury: Defined as the presence of any of the following abnormal findings on brain imaging: grade III/IV IVH, periventricular leukomalacia (PVL), cortical atrophy, ventricular dilatation or echodenscities. The proportion of infants who had severe brain injury among the ROP groups was higher in the severe ROP group, table7. A Chi-squared test was performed. The results showed that the proportions of severe brain injury were different among the ROP groups (χ^2=13.8, p=0.001).

Severe brain injury	No ROP	Mild ROP	Severe ROP	Total
None	338	173	85	596
(%)	(91.11)	(87.82)	(77.98)	(88.04)
Present	33	24	24	81
(%)	(8.89)	(12.18)	(22.02)	(11.96)
Total	371	197	109	677
(%)	(100)	(100)	(100)	(100)

Table 7: Severe brain injury among the different ROP groups

3.3.7 Patent Ductus Arteriosus: This morbidity was categorized into 8 conditions depending on treatment options and availability of data. Surgical ligation was considered a major morbidity and was categorized as 0 (none or PDA not needing surgery) or as 1 (surgically ligated PDA). The proportion of PDA which needed surgical ligation among the ROP groups was higher in the severe ROP group, table 8. A Chi-squared test was performed. The results showed that the proportions of PDA were different among the ROP groups (χ^2=103, p<0.001).

PDA	No ROP	Mild ROP	Severe ROP	Total
None or not needing surgery	340	139	53	532
(%)	(91.64)	(70.56)	(48.62)	(78.58)
surgically ligated PDA	31	58	56	145
(%)	(8.36)	(29.44)	(51.38)	(21.42)
Total	371	197	109	677
(%)	(100)	(100)	(100)	(100)

Table 8: Patent ductus arteriosus among the different ROP groups

3.3.8 Necrotizing Enterocolitis: This condition was categorized into several stages, as per Bell's criteria (100), depending on the severity and treatment. The presence of stage II (confirmed NEC) was considered significant morbidity. Therefore, NEC was categorized as 0 (< stage II) or 1 (≥ stage II). The proportion of infants who had NEC (≥ stage II) among ROP groups was higher in the severe ROP group, table 9. Excluding the missing, a Chi-squared test was performed. The results showed that the proportions of NEC were just different among the ROP groups (χ^2=6, p<0.048).

NEC	No ROP	Mild ROP	Severe ROP	Total
<Stage II	315	159	86	560
(%)	(89.24)	(85.03)	(80.37)	(86.55)
≥Stage II	38	28	21	87
(%)	(10.76)	(14.97)	(19.63)	(13.45)
Total	353	187	107	647
(%)	(100)	(100)	(100)	(100)

Table 9: Necrotizing enterocolitis among the different ROP groups

3.3.9 Bronchopulmonary Dysplasia: Defined as oxygen requirement at 36 week post-menstrual age. It was categorized as 0 for no BPD or as 1 for BPD. The proportion of BPD was higher in the severe ROP group, table 10. Excluding the missing, a Chi-squared test was performed. The results showed that the proportions of BPD were different among the ROP groups (χ^2=126, p<0.001).

BPD	No ROP	Mild ROP	Severe ROP	Total
No BPD	188	35	5	228
(%)	(56.8)	(18.9)	(4.9)	(36.9)
BPD	143	150	97	390
(%)	(43.2)	(81.1)	(95.1)	(63.1)
Total	331	185	102	618
(%)	(100)	(100)	(100)	(100)

Table 10: Bronchopulmonary dysplasia among the different ROP groups

3.3.10 Sepsis: Is a systemic infection which was categorized into various conditions, depending on diagnostic criteria and availability of the data. Confirmation by blood/cerebrospinal fluid culture was considered significant sepsis. Therefore, it was re-coded as 0 for no sepsis or as 1 for confirmed sepsis. The proportion of sepsis was

higher in the severe ROP group, table 11. A Chi-squared test was performed. The results showed that the proportions of sepsis were different among the ROP groups (χ^2=38.3, p<0.001).

Sepsis	No ROP	Mild ROP	Severe ROP	Total
None or suspected	331	146	72	549
(%)	(89.2)	(74.1)	(66.1)	(81.1)
Confirmed	40	51	37	128
(%)	(10.8)	(25.9)	(33.9)	(18.9)
Total	371	197	109	677
(%)	(100)	(100)	(100)	(100)

Table 11: Sepsis among the different ROP groups

3.3.11 Infant's Characteristics Summary, table 12:

Infant's characteristics	No ROP	Mild ROP	Severe ROP	p value
Gestation by weeks(±SD)	28.7 (2.2)	26.7 (1.9)	25.5 (1.8)	<0.001
Birth weight grams(±SD)	1019 (181)	884 (203)	772 (182)	<0.001
Sex (%male)	187 (50.4)	100 (50.76)	70 (64.22)	0.03
SGA (%)	88 (24)	26 (13)	10 (9.4)	<0.001
Median Apgar (50% IQR)	8 (7-9)	8 (6-8)	7 (6-8)	0.04
Severe brain injury (%)	33 (9)	24 (12)	24 (22)	0.001
PDA (%)	31 (8)	58 (29)	56 (51)	<0.001
NEC (%)	38/353 (11)	28/187 (15)	21/107 (29)	0.048
BPD (%)	143/331 (43)	150/185 (81)	97/102 (95)	<0.001
Sepsis (%)	40/379 (11)	51 (26)	37 (34)	<0.001

Table 12: Infant's characteristics. SGA: Small for gestational age. Severe brain injury: any IVH G III/IV, PVL, cortical atrophy, ventricular dilatation or echodenscities. PDA: surgically ligated patent ductus arteriosus. NEC: ≥ stage II necrotizing enterocollitis. BPD: bronchopulmonary dysplasia i.e. O2 at 36 week PMA. Sepsis: culture proven.

3.4 Primary outcomes

3.4.1 Neurodevelopmental Outcomes:

The proportion of severe NDI in children who had no ROP was 7.6% (28/371), while 12.2% (24/197) in infants who had mild ROP. Children who had severe ROP, 31.2% (34/109) developed severe NDI.

Primary Outcome	No ROP	Mild ROP	Severe ROP
Severe NDI (%)	28 (7.6)	58 (19)	
Severe NDI (%)	28 (7.6)	24 (12.2)	34 (31.2)
Severe NDI (%)	52 (9.2)		34 (31.2)

Table13. Primary outcome analysis:

3.4.1.1. Combining the No/Mild Retinopathy of Prematurity Groups:

We elected not to pursue further comparison between no ROP and mild ROP groups for the following reasons:

- Not our primary research question.
- Not large enough sample size (poor power): it would need 761 infants in each group.

- The statistical analyses comparing between no ROP and mild ROP group were as follows:
- Estimated the risk difference was only 4.6
- Risk ratio (RR) was 1.6, 95% confidence interval (CI) between 0.96 and 2.7. The Fisher's exact test had p= 0.09, i.e. not significant.
- Maternal and infants' characteristics were, in general not significantly different, with exception of gestation and birth weight.

The no ROP and mild ROP groups were combined into one group (no/mild ROP). The results of maternal and infants' variables after combination are shown in table 20. There were no changes in interpretation of the results. Maternal characteristics were different in the two groups, except in the antenatal risk score and multiple birth. Infants' characteristics indicate that infants with severe ROP had more morbidity in all parameters.

Maternal characteristics	No/Mild ROP	Severe ROP	p-value
Age by years (±SD)	28 (2.2)	25.5 (1.8)	<0.001
Ethnicity (% Caucasian)	356/455 (78.2)	49/77 (64)	0.005
Hollingshead index (±SD)	36 (15)	32 (13)	0.037
Antenatal Risk score (±SD)	8 (3.6)	7.4 (3.5)	0.17
Antenatal steroid (%)	476/557 (85.5)	78/104 (75)	0.008
Multiple births (%)	179 (31.5)	28 (26)	0.227
Infant's characteristics			
Gestation by weeks(±SD)	28 (2.2)	25.5 (1.8)	<0.001
Birth weight grams(±SD)	972 (199)	772 (182)	<0.001
Sex (%male)	287 (50.5)	70 (64.2)	0.009
SGA (%)	114 (20)	10 (9.4)	0.009
Median Apgar (50% IQR)	8 (7-9)	7 (6-8)	<0.001
Severe brain injury (%)	57 (10)	24 (22)	<0.001
PDA (%)	89 (15.7)	56 (51)	<0.001
NEC	66/540	21/107	

(%)	(12.2)	(20)	0.04
BPD (%)	293/516 (57)	97/102 (95)	<0.001
Sepsis (%)	91 (16)	37 (34)	<0.001

Table 14: Maternal and infants characteristics among no/mild ROP & severe ROP groups.

3.4.1.2 Association between Severe Retinopathy of Prematurity and Severe Neurodevelopmental Impairment:

The following are explanations of the crude assessment produced for the primary outcome, table 15:

- **The risk** of developing severe NDI among the exposed (severe ROP) is 31%, while the risk is 9.2% among the unexposed (no/mild ROP).

- **The risk difference** among the exposed – the unexposed is (31%-9%) = 22% (95% CI 0.13 - 0.31). The 95%CI does not contain the null; hence, this difference is statistically significant. It suggests that out of risk (31%) of severe NDI in children who had severe ROP, 22% was attributed to severe ROP only.

- **The risk ratio (RR)** is the estimated ratio of the risk of developing severe NDI among the exposed (severe ROP) over the risk of developing severe NDI among the unexposed (no/mild ROP) = 3.4 (95% CI 2.32 - 4.98), i.e, the estimated risk of developing severe NDI in children who had severe ROP is 3.4 times the risk of developing severe NDI in children who had no/mild ROP. This point estimate is statistically significant ($X2=40$, $p<0.001$). Also 95% CI do not contain the null.

- **The attribution fraction of exposure** was the risk difference/the risk of developing severe NDI if exposed= 22/31=70% (95% CI 57% to 80%). This means

that, in the crude assessment, the risk of having severe NDI attributed to severe ROP only, was 70% in children who had severe ROP.

- **The attribution fraction of population** was the risk of developing the outcome in exposed(31%)-[risk of developing the outcome in population(number of those who have the outcome/total number of population 86/677=12%) – risk of developing disease in non-exposed (9%)]=28%; this means that the incidence of severe NDI, attributed to developing severe ROP only, was 28%. In another words, by crude assessment, 28% of severe NDI could be prevented in our study group if they did not develop severe ROP.

Of note, the risk of developing severe NDI risk was unchanged if we stratify the exposure to make comparison between no ROP vs. severe ROP (RR=4.4, 95%CI=2.6 to 6.5, p<0.001) and mild ROP vs. severe ROP (RR=2.6, 95%CI=1.6 to 4, p<0.001). This association was also true if we compare severe NDI in children with no ROP vs. any ROP (RR=2.5, 95%CI=1.6 to 3.8, p<0.001).

	Severe ROP	No/mild ROP	Total
Severe NDI	34	52	86
None	75	516	591
Total	109	568	677
Risk	0.31	0.09	
	Point Estimate	95%	CI
Risk Difference	0.22	0.13	0.31
Risk Ratio	3.4	2.33	5
Attributable fraction of exposure	0.7	0.57	0.8
Attributable fraction of population	0.28		
Odds ratio	4.5	2.75	7.37
ch2(1)	40	Pr>ch2	<0.001

Table 15: Association between severe ROP and Neurodevelopmental impairment

3.4.1.3 Role of Blindness: Further examining the role of blindness and its effect on severe NDI, we stratified the outcome (severe NDI) to: Severe NDI attributed to blindness alone, severe NDI attributed to blindness plus other components (severe cognitive delay, deafness and severe cerebral palsy), table 16.

	Overall	No or Mild ROP	Severe ROP	p value
Prevalence of blindness	12/677 (1.8)	4/568 (0.7)	8/109 (7.3)	<0.001
Proportion of blindness in children with severe NDI	12/86 (14)	4/52 (7.7)	8/34 (23.5)	0.04
Proportion of blindness alone in severe NDI	2/86 (2.3)	0/52 (0)	2/34 (5.9)	0.07
Proportion of blindness associated with other components of severe NDI	10/86 (11.6)	4/52 (7.7)	6/34 (17.6)	0.16

Table 16: examining the contribution of blindness to the outcome.*t test.

The above table showed that the prevalence of blindness was higher in the severe ROP group compared to no/mild ROP (7.3 vs. 0.7, p<0.001). The proportion of blindness was higher in children with severe NDI who had severe ROP compared to no/mild ROP (23.5 vs. 7.7, p=0.04).

In this study, blindness alone was a not major contributor to severe NDI as it only occurred in 2.3% in children with severe NDI, however, all these cases occurred in those who had severe ROP. In spite of this trend toward an increase, there was no

statistically significant difference whether blindness alone occurred in the no/mild ROP or severe ROP groups (0 vs. 5.9%, p=0.07).

Blindness occurred more commonly associated with other components of severe NDI (11.3%) and although there was a trend toward an increase in children who had no/mild ROP compared to those who had severe ROP (7.7 vs. 17.6%), but this was not statistically significant (p=0.16).

3.4.1.4 Stratified Analysis to Each Component of Severe Neurodevelopmental Impairment: Further sub-analysis of prevalence of each component of severe NDI in the no/mild ROP and severe ROP groups showed that the prevalence of severe CP, severe cognitive delay and severe hearing impairment (deafness) were significantly higher in the severe ROP group (p<0.001). See table 17, 18, 19.

Cerebral palsy	No/Mild ROP	Severe ROP	Total
None	533	86	619
(%)	(93.84)	(78.9)	(91.43)
Mild	13	11	24
(%)	(2.29)	(10.09)	(3.55)
Severe	22	12	34
(%)	(3.87)	(11.01)	(5.02)
Total	568	109	677
(%)	(100)	(100)	(100)

Table 17: Prevalence of cerebral palsy among ROP groups

Cognitive delay	No/Mild ROP	Severe ROP	Total
None	451	60	511
(%)	(81.56)	(56.6)	(77.54)
Mild	64	23	87
(%)	(11.57)	(21.7)	(13.2)
Severe	38	23	61
(%)	(6.87)	(21.7)	(9.26)
Total	553	106	659
(%)	(100)	(100)	(100)

Table 18: prevalence of cognitive delay among ROP groups

Hearing impairment	No/Mild ROP	Severe ROP	Total
None	544	100	644
(%)	(95.77)	(91.74)	(95.13)
Mild	19	0	19
(%)	(3.35)	(0)	(2.81)
Severe	5	9	14
(%)	(0.88)	(8.26)	(2.07)
Total	568	109	677
(%)	(100)	(100)	(100)

Table 19: Prevalence of hearing impairment among ROP groups

3.4.1.5. Classical Analysis:

Effect modification or confounding caused by various maternal and infant variables on the association between severe NDI and severe ROP were investigated by "classical" stratified analysis, the relative risks between severe NDI and exposure to severe ROP were calculated in strata of the third variable. The hypothesis of underlying equal relative risks in strata through a test of homogeneity. The significant results (p-value<0.05, Wald statistics) support that the third variable as an effect modifier. If the test was not significant, then crude and Mantel-Haenszel (M-H) relative risks were calculated, assuming that the stratum specific relative risks were uniform. A comparison of the crude and M-H combined relative risks provided information on the confounding by the third variable.

A quantitative criterion was suggested to assess whether adjustment for the confounding variable is required or not (101). Mostly, it is calculated as:

Unadjusted relative risk (Crude- Adjusted relative risk (M-H combined) X 100
Adjusted relative risk (M-H combined).

For the current study, a variable was considered as confounder if it caused a change in the adjusted risk ratio by 15%.

For the measured data, visual assessment was performed on box plots of the variable plotted against severe NDI as a major category and severe ROP as a subcategory. If box plots are similar among groups then it is assumed that there are no effect modifications. Any differences in the box plots among the groups then presence of either effect modification or confounding is entertained.

Table 20 illustrates the effect of various maternal and neonatal characteristics on the association between severe NDI and severe ROP were investigated by the "classical" stratified analysis. None of the included variables demonstrate significant effect modification. Most variables showed observed changes in the RR , for example, sex as a modifier decreased the risk of developing severe NDI in the severe ROP from 4 (95% CI 2.5-6.3) to 2.3 (95% CI 1.1-4.7), however, this reduction is of not much clinical or practical significance, also the 95% CI were overlapping and test of homogeneity was not significant. This applies for the rest of the variables.

Variable	Absent RR (95%CI)	Present RR (95%CI)	Test of Homogeneity (M-H) p value
Maternal Characteristics			
% Caucasian	4.1(2.1-8.2)	2.4(1.3-4.4)	0.3
Antenatal steroids	4.2(1.9-9.5)	3.3(2.1-5.2)	0.6
Multiple birth	3.8(2.4-5.9)	2.7(1.3-5.5)	0.4
Infant's Characteristics			
Sex(m/f)	4(2.5-6.3)	2.3(1.1-4.7)	0.2
SGA	3.3(2.2-5)	2.1(0.5-8)	0.5
Severe brain injury	3.1(1.9-5.2)	2.2(1.3-3.9)	0.3
PDA	3(1.7-5.6)	2(1.2-3.5)	0.3
NEC	3.7(2.4-5.9)	2(0.9-4.5)	0.17
BPD	4(0.6-25)	2.5(1.7-3.8)	0.6
Sepsis	3.1(1.8-5.2)	2.6(1.5-4.6)	0.6

Table 20: assessment of modification effect using stratified analysis for discrete variables

Table 21; illustrates the comparison between the crude RR and the M-H combined (adjusted) RR. Severe brain injury, PDA, BPD and Sepsis were 26%, 42%, 36% and

17% away from the crude, respectively. Hence, they were considered possible confounder and were included into further analysis.

Variable	Crude RR(95%CI)	M-H Combined (95%CI)	Difference between crude and adjusted risk(%)
Maternal Characteristics			
% Caucasian	3.4(2.3-4.9)	3.1(2-4.9)	9.7
Antenatal steroids	3.5(2.4-5.1)	3.6(2.4-5.2)	2.8
Multiple birth	3.4(2.3-4.9)	3.4(2.3-5)	0.0
Infant's Characteristics			
Sex(m/f)	3.4(2.3-4.9)	3.3(2.3-4.9)	3
SGA	3.4(2.3-4.9)	3.1(2.1-4.8)	9.7
Severe brain injury	3.4(2.3-4.9)	2.7(1.9-3.9)	25.9
PDA	3.4(2.3-4.9)	2.4(1.6-3.6)	41.7
NEC	3.4(2.3-4.9)	3.2(2.2-4.7)	6.2
BPD	3.4(2.3-4.9)	2.5(1.7-3.8)	36
Sepsis	3.4(2.3-4.9)	2.9(1.9-4.2)	17.2

Table 21: Assessment of confounding using stratified analysis for discrete variables

For the measured maternal variables, a series of box plots were generated followed by explanation for each graph.

Figure 8, showes a comparison of distribution of maternal age across the severe NDI and ROP status. Examining the box plots, maternal age distributions seemed similar across the groups, hence were not included into further analysis. The same observation is applied to HI, see figure 9. Although we see that the median tend to be lower in the severe ROP groups, but the 50% IQR and the 95%CI overlap. Hence, HI was not included into further analysis. In figure 10, the ANR score was

examined across the same groups. After removing the outlier results, the ANR distribution was considered similar across the groups.

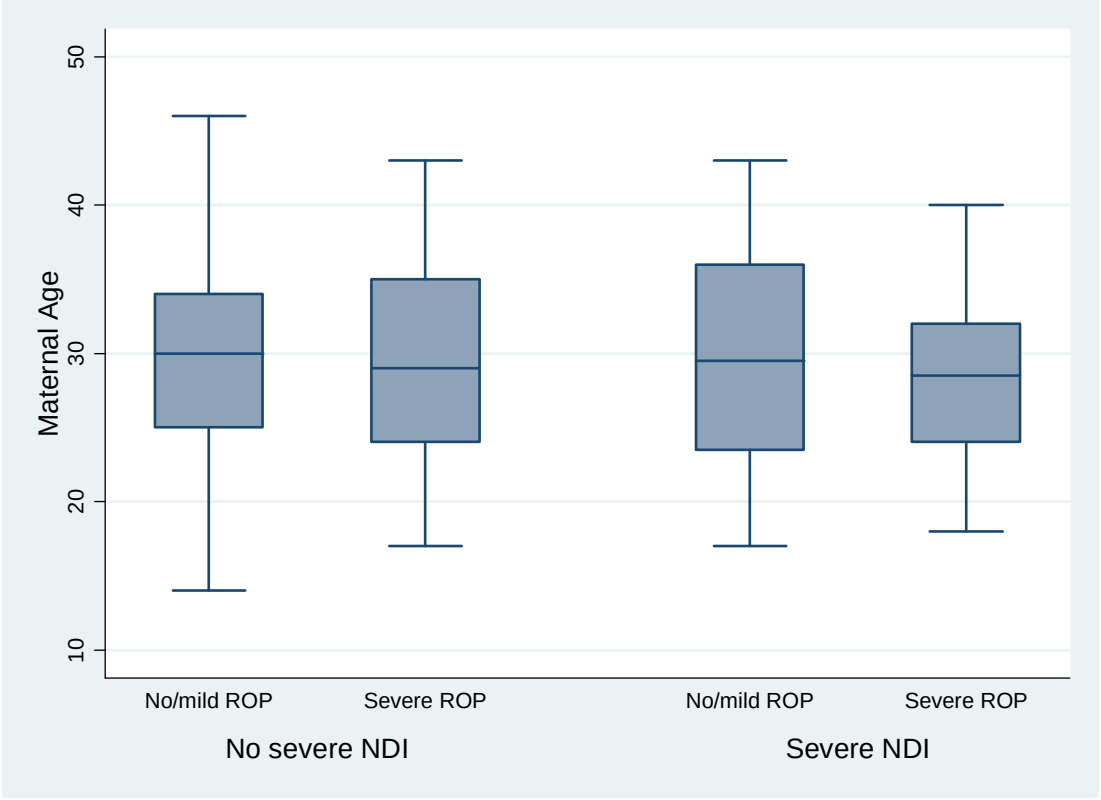

Figure 8: Comparison of box plots of maternal age in ROP groups among Neurodevelopmental status.

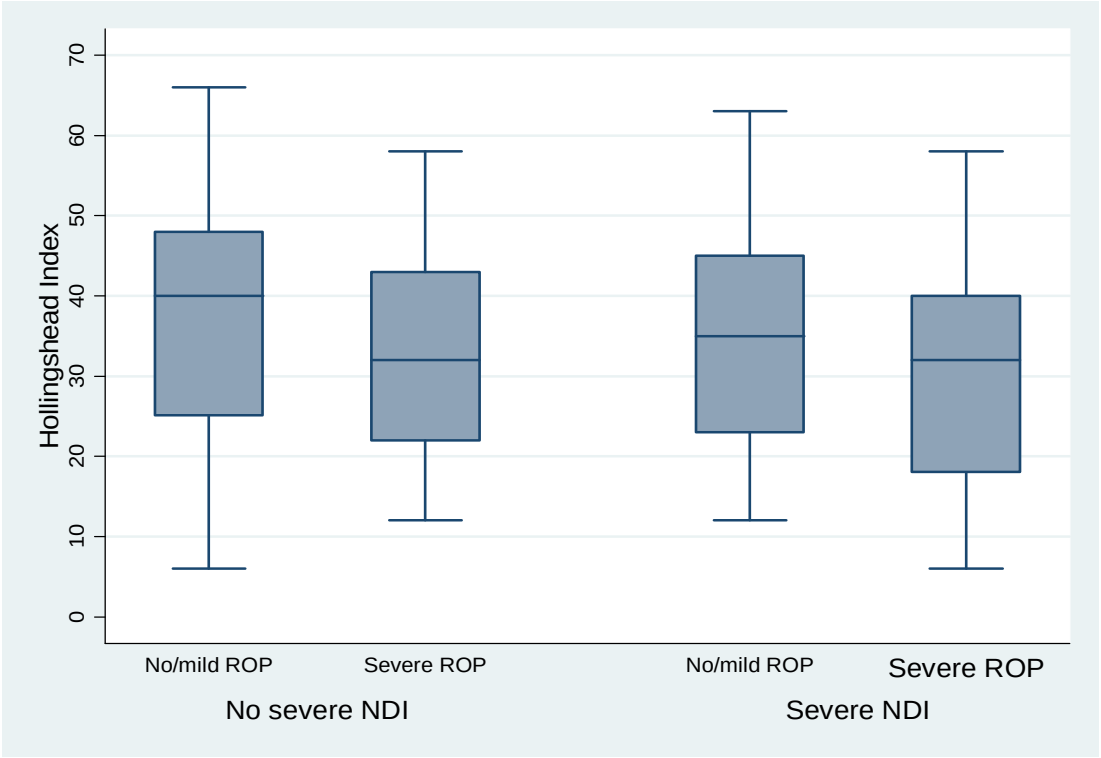

Figure 9: Comparison of box plots of Hollingshead Index in ROP groups among Neurodevelopmenta status

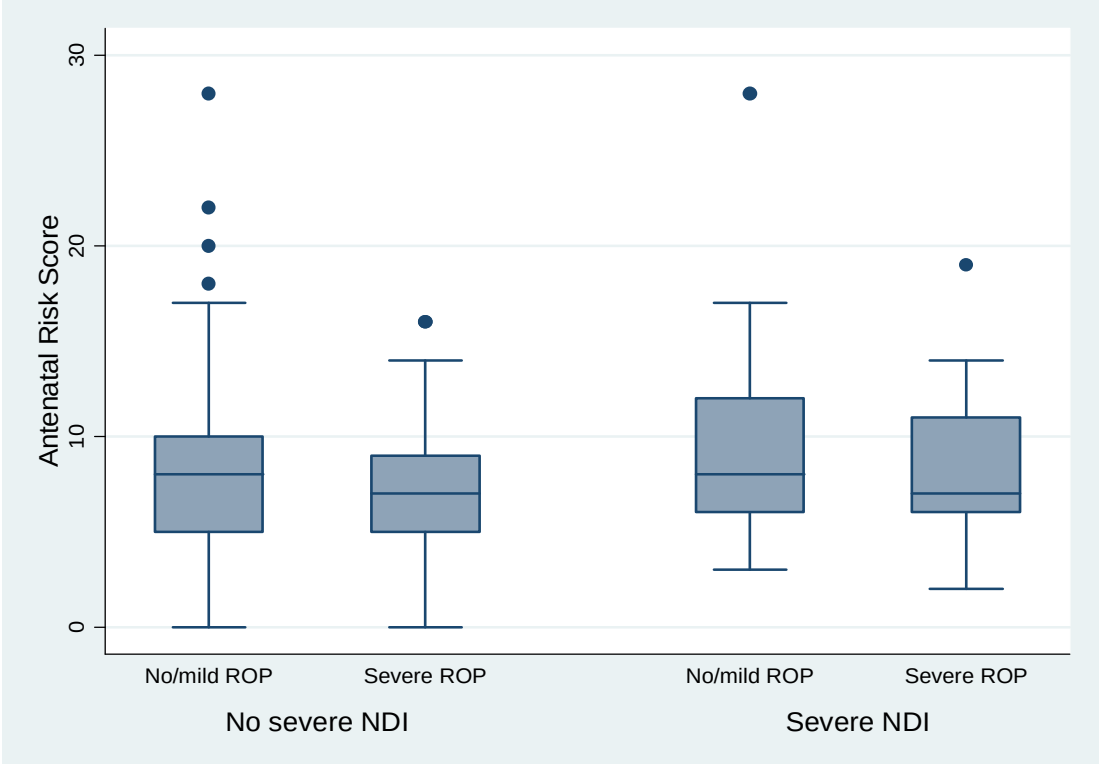

Figure 10: Comparison of box plots of antenatal risk scores in ROP groups among NDI status

For the assessment of possible effect modification/confounding of the measured neonatal variables on the severe NDI and severe ROP association, the following variables were dichotomized: gestation to <28 weeks and above or equal 28 weeks, birth weight into <1000g and ≥1000g. Table 22, illustrates thtat there wer no effect modification, while table 23 illustrates a possibility of confounding for both BW and GA since the M-H adjusted RR was 21.4% and 25.9% away from the crude, respectively. Hence, should be adjusted for in further analysis.

Variable	Absent RR (95%CI)	Present RR (95%CI)	Test of Homogeneity (M-H) p value
Birth weight	2.9(1.9-4.5)	2.2(0.6-8.5)	0.7
Gestation	2.7(1.8-4.2)	2.9(0.9-4.1)	0.9

Table 22: Assessment of modification effect using stratified analysis for birth weight and gestational age

Variable	Crude RR(95%CI)	M-H Combined (95%CI)	Difference between crude and adjusted risk(%)
Birth weight	3.4(2.3-4.9)	2.8(1.9-4.2)	21.4
Gestation	3.4(2.3-4.9)	2.7(1.8-4.1)	25.9

Table 23: Assessment of confounding using stratified analysis for birth weight and gestational age

3.4.1.6 Logistic Regression

The goal of logistic regression is to find the best fitting, yet biologically reasonable, model to describe the relationship between the dichotomous characteristic of interest (dependent variable = response or outcome variable) and a set of independent (predictor or explanatory) variables. Logistic regression models were

developed with the underlying assumptions that the model was specified correctly, cases were independent and none of the X variables were linear function of the others. The assumptions were checked; no important variables were omitted and no extraneous variables were included.

The matrix of correlations among x-variables was examined to assess the collinearity among x-variables.

For discrete variables the tetrachoric correlation method was used (102). Tetrachoric computes estimates of the tetrachoric correlation coefficients of the binary variables. All these variables should be 0, 1, or missing values. Tetrachoric correlations assume a latent bivariate normal distribution (X1, X2) for each pair of variables (v1, v2), with a threshold model for the manifest variables (vi = 1 if and only if Xi > 0). The means and variances of the latent variables are not identified, but the correlation, r, of X1 and X2 can be estimated from the joint distribution of v1 and v2 and is called the tetrachoric correlation coefficient (102).

In our study we considered "tetrachoric correlation coefficient (rho)" of≥0.75 as significant correlation. None of the discrete variables reached this level. Therefore all were considered in further analysis.

For measured variables, correlations were examined and a "correlation coefficient of (r)>0.5 was considered significant. Gestational age and BW were found highly correlated, (r)=0.6. In this study, BW was chosen to be analysed. This was based on literature that recommended using a more accurate, measurable BW rather than an estimated GA that at best measurement has ± 1 week variation, except in in-vitro fertilization (65).

Since we had 34 infants who had severe NDI in the severe ROP group, we were limited to study only 3 variables under the rule of thumb that we can assess only 1 variable per 10 events (103). These variables have also to be biologically plausible. Birth weight was the first variable chosen as it is one of the most common and strongest predictor reported in literature to affect neurological outcome. It is also associated with severe ROP as concluded in our study. Since BW is an event that precedes severe ROP, it would be of importance to assess for confounding separately. Severe brain injury is another important predictor of severe disability. It is also strongly associated with severe ROP as it was demonstrated in this study and it is not thought to be an intermediate factor in the causal pathway. Finally, BPD is one of the strong predictors of disability and well associated with severe ROP.

Other factors were not included as we were limited with the events. They are also thought to be in line of the causal pathway e.g. PDA, sepsis, that leads to increased brain injury. Others were not chosen as missing values were considered large e.g. HI.

In a logistic regression approach, a model 1, table 24, included:

- Outcome variable: Severe NDI.

- Exposure variable: Severe ROP.

- Birth weight, severe brain injury, and BPD to adjust as possible confounders.

Severe NDI	Coef.	Std. Err.	z	P>z	[95% Conf. Interval]	
No/mild vs severe ROP	0.91	0.30	3.05	0.00	0.32	1.49
Birth weight	0.00	0.00	-2.46	0.01	0.00	0.00
Brain injury	1.71	0.29	5.84	0.00	1.14	2.28
BPD	0.66	0.37	1.80	0.07	-0.06	1.37
_cons	-1.42	0.77	-1.84	0.07	-2.92	0.09

Table 24: Model 1:Number of obs. 618 Log likelihood = -201.7 LR chi2(5)=81 Prob>chi2 <0.001

For model assessment to check how BW behave prior to any interpretation, Model 1 was used to predict log odds of severe NDI (pr(yh)) as BW advances. Figure11 shows the fractional polynomial fit of pr(yh) in the y axis against BW. It was noticed that as BW advances, the pr(yh) decreases, however this prediction reaches its nadir and levels by 1250g. This suggests that the probability of developing severe NDI reaches its usual population prevalence by 1250g regardless of the morbidity. It understandable from the inclusion criteria (≤1250 and/or 28 weeks) that some more mature preterm infants will be included up to 1500g while their GA are 28 weeks, these constitute either the LGA preterm or "most probably" wrong estimation of GA. It seems that this group of infants, though small in numbers could confound our severe NDI and severe ROP association. One of the solutions of this problem is to restrict the analysis to only those who are < 1251g.

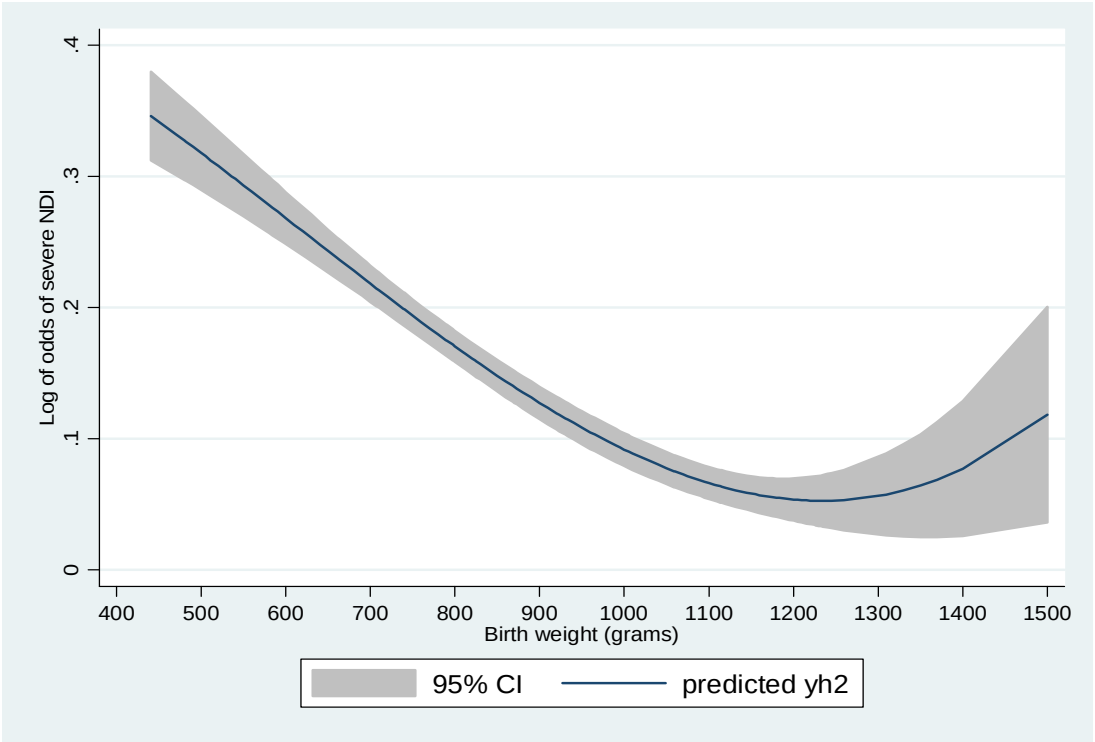

Figure 11: Prediction of log odd of severe NDI (pr(yh)) as BW advances using the fractional polynomial fit of pr(yh) in the y axis against BW

On further assessment post estimation of model 1, it correctly classified, at predicted probability cut off=0.5, the severe NDI in 88% of the cases. Also area under the curve (AUC), interpreted as the percent of all possible pairs of cases in which the model 1 assigns a higher probability to a correct case than to an incorrect case, was 0.78.

After restricting the analysis to those infants born <1251g, model 2 was produced see table 25.

severe NDI	Coef.	Std. Err.	z	P>z	[95% Conf. Interval]	
No/mild vs severe ROP	0.91	0.30	3.06	0.00	0.33	1.49
Birth weight	0.00	0.00	-2.54	0.01	0.00	0.00
Brain injury	1.68	0.30	5.66	0.00	1.09	2.26
BPD	0.61	0.37	1.66	0.10	-0.11	1.33
_cons	-1.29	0.79	-1.64	0.10	-2.83	0.25

Table 25: Model 2: Number of obs = 611 Log likelihood = -199 LR chi2(5)=69 Prob > chi2<0.001

Further, the post estimation assessment of model 2, it correctly classified, at predicted probability cut off=0.5, the severe NDI in 88 % of the cases and AUC was 0.78. Finally, the predicted log odds of severe NDI as BW advances showed more "linear" relation, without plateau, if we restricted our analysis to < 1251 g, Figure12.

Of note, the sensitivity and strength of the model was unchanged by restricting the BW to <1251g. Nevertheless, for correct modeling we will continue to use model 2.

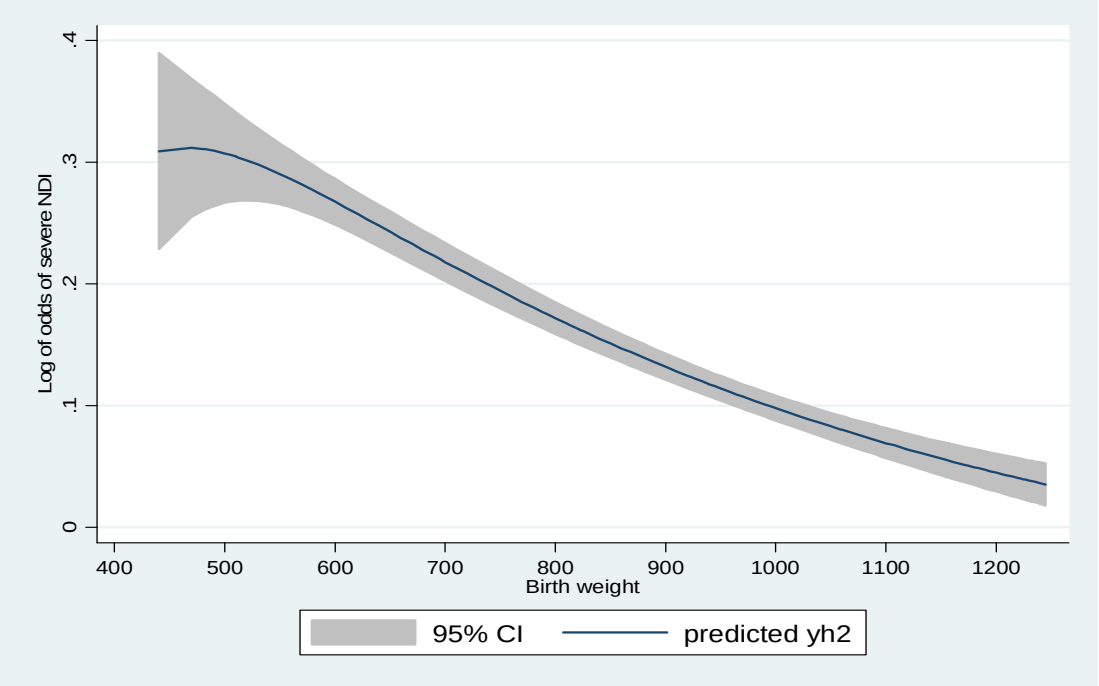

Figure 12: Prediction of log odd of severe NDI (pr(yh)) as BW advances using the fractional polynomial fit of pr(yh) in the y axis against BW (restricting to< 1251g)

It would be more convenient to interpret the results of the model if we report the OR which the antilogarithm of odds coefficient.

severe NDI	Odds Ratio	Std. Err.	z	P>z	[95% Conf. Interval]	
No/mild vs severe ROP	2.49	0.74	3.06	0.00	1.39	4.46
Birth weight	0.9982	0.0007	-2.5400	0.0110	0.9968	0.9996
Brain injury	5.34	1.58	5.66	0.00	2.99	9.54
BPD	1.84	0.68	1.66	0.10	0.89	3.78

Table 26: Model 2, reporting with odds ratio

As we previously mentioned logistic regression could provide evidence of confounding for measured variable by assessing the coefficients or the OR of the interested variable.

First we must assess for effect modification. The interaction term represents effect modification. The risk coefficient on the interaction term, ß3, provides an estimate as to which log of odds depend on the strata of the third variable. A

significant p-value on the interaction term rejects the null hypothesis, ß3 = 0 and confirms the third variables as an effect modifier.

If the p-value is insignificant, a second model without the interaction term and a third model without the interaction term and the third variable are developed. A comparison of OR or odds coefficients on the independent variable between the second and third models provided information on confounding by the third variable. The OR on independent variables between the second and third models is compared in order to assess the third variable as a confounder.

severe NDI	Odds Ratio	Std. Err.	z	P>z	[95% Conf. Interval]	
Model 3a						
No/mild vs severe ROP	1.74	1.99	0.48	0.63	0.18	16.42
Birth weight	0.9977	0.0007	-3.1500	0.0020	0.9962	0.9991
Interaction term	1.00	0.00	0.52	0.60	1.00	1.00
Model 3b						
No/mild vs severe ROP	3.10	0.85	4.13	0.00	1.81	5.32
Birth weight	0.9979	0.0006	-3.3800	0.0010	0.9966	0.9991
Model 3c						
No/mild vs severe ROP	4.50	1.14	5.93	0.00	2.74	7.39

Table 27: Model 3 a,b,c: Assessing modification effect and confounding of BW

As seen from model 3a in table 27, the p-value of the interaction term was not significant, also the 95%CI includes zero. Hence, there was evidence for the hypothesis that β3=0, as a result we conclude no effect modification of BW on severe ROP.

Therefore we proceed for model 3b and model 3c in the same table. The assumed common “adjusted” OR of severe ROP in model 3b was quite different from the “crude” OR in model 3c. Hence, there evidence of confounding by BW.

To report the RR instead of OR, we used the “bireg” command which carries out the Generalized Linear Model (GLM) for binomial family with log link for RR Model 2, table 28.

severe NDI	Risk Ratio	Std. Err.	z	P>z	[95% Conf.	Interval]
No/mild vs severe ROP	1.81	0.39	2.75	0.01	1.19	2.77
Birth weight	0.9988	0.0006	-2.2100	0.03	0.9977	0.9999
Brain injury	2.98	0.58	5.67	0.00	2.04	4.36
BPD	1.77	0.58	1.75	0.08	0.93	3.36

Table 28: Model 2, reporting RR

If we restrict our analysis for those infants born <1251g, interpreting the RR produced from model 2 were as follows:

- The estimated risk of developing severe NDI in children who suffered from severe ROP was 1.8 the risk in children who were did not suffer from severe ROP, adjusting for BW, brain injury and BPD. This RR was significant since the p-value was 0.01 and 95% CI did not include 0.

- With each unit increase in BW, the estimated risk of developing severe NDI was 0.99 i.e decreases adjusting for severe ROP, brain injury and BPD. This RR was significant since the p-value was <0.03 and 95% CI did not include 0.

- The estimated risk of developing severe NDI in children who had severe brain injury was 2.98 times the risk in children who did not have severe brain injury, adjusting for severe ROP, BW and BPD. This RR was significant since the p-value was <0.00 and 95% CI did not include 0.

- The estimated risk of developing severe NDI in children exposed to BPD was 1.75 times compared with children who were not exposed adjusting for severe ROP,

BW and brain injury. This RR was not significant since the p-value was 0.08 and 95% CI included 0.

3.4.2 Visual Outcomes:

3.4.2.1 Laser Therapy: As expected, those who had no ROP did not have laser therapy. Laser therapy was done on 2% of infants who had mild ROP, while it was done on 78% of infants who had severe ROP. Median chronological age at which laser was done was 82 days (50% IQR 67-91), while the median corrected GA was 36.7 week (34.5-38.6), table 29.

	No ROP n=371	Mild ROP n=197	Severe ROP n=109
Laser therapy (%)	0	4(2)	85(78)
Median Age (IQR - days)		82(67-91)	
Median CGA (IQR)		36.7(34.5-38.6)	

Table 29: Laser therapy

3.4.2.2 Location of Retinopathy of Prematurity: We can find that there are 4 missing report, 378 no-ROP and 7 cases had mild ROP in the right eye, 12 cases had mild ROP in the left eye, 182 mild ROP in the both eyes, 5 cases had severe ROP in 1 eye which is the right eye and left had mild ROP 9 cases had left ROP 80 cases severe ROP in the both side. Table 30 indicates that mild or severe ROP are mostly bilateral. There is no severe ROP with single eye alone.

Right eye	Left eye	Number	Percentage
missing	Missing	4	0.6
No ROP	No ROP	378	55.8
No ROP	Mild	7	1
Mild	No ROP	12	1.8
Mild	Mild	182	26.9
Mild	Severe	5	0.7
Severe	Mild	9	1.3
Severe	Severe	80	11.8
Total		677	100

Table 30: Analysis of ROP location.

3.4.2.3 Visual Impairment: Normal vision was achieved in 93.8% of no-ROP, 88.8% in mild ROP and 84.4 in severe ROP groups. Visual impairment was diagnosed in 5.7% of children who had no ROP, 10.2% mild ROP and 8.25% in severe ROP group.

Percentage of Blindness in those who had no ROP was 0.5%, 1% in mild ROP and 7.34% in the severe ROP group ($p<0.001$), table 31.

Visual outcomes	No ROP	Mild ROP	Severe ROP	p value
Normal (%)	348(93.8)	174(88.8)	92(84.4)	<0.001
Impairment (%)	21(5.7)	20(10.2)	9(8.26)	
Blindness (%)	2(0.5)	2(1)	8(7.34)	

Table 31: Visual outcomes. Impairment: acuity in the best Seeing Eye of < 20/60. Blindness: acuity < 20/200 following refractive correction.

3.4.2.4 Visual Morbidities:

- **Over all** visual morbidity not including severe impaired vision or blindness was 7% for no-ROP group, 11.2% in the mild ROP group and it was 41.3% in severe ROP group with p value of <0.001
- **Impaired visual acuity,** not to the level of impaired vision nor blindness, was 2.2% for no-ROP, 3.1% in the mild ROP, 15.6% in the severe ROP (p value of <0.001).
- **Impaired refraction**, defined as any of the following: astigmatism, hyperopia, myopia and anisometropia, was 4.9% in the no ROP group, 6.6% in the mild ROP and 33% in the severe ROP group(p value <0.001)
- **Impaired ocular motility** defined as esotropia, exotropia, hypertropia, nystagmus or restrictive syndrome**,** was 1% in the no-ROP group, 6.1% in mild ROP group and 12.8% in severe ROP group (p value <0.001).
- Infants who received **ophthalmological surgical treatment** other than laser therapy was 4% in the no-ROP group, 4.6% the mild ROP and 29.4% in the severe ROP group (p value of <0.001).
- **Ocular structural abnormality:** defined as any of the following: abnormal external structure, abnormal anterior segment, abnormal vitreous, abnormal retina "not ROP"/choroids, abnormal optic nerve or glaucoma, was 0.5% in the no-ROP group, 1% in the mild ROP and 5.5% in the severe ROP (p value of 0.02).

Table 32 summarizes the visual morbidities.

Visual morbidity	No ROP	Mild ROP	Severe ROP	p value
Total Visual morbidity* (%)	36/371 (7)	23/197 (11.2)	45/109 (41.3)	<0.001
Impaired visual acuity (%)**	8/371 (2.2)	6/197 (3.1)	17/109 (15.6)	<0.001
Impaired refraction (%)	18/371 (4.9)	13/197 (6.6)	36/109 (33)	<0.001
Impaired ocular motility (%)	4/371 (1)	12/197 (6.1)	14/109 (12.8)	<0.001
Ocular structural abnormality, not retinal (%)	2/371 (0.5)	2/197 (1)	6/109 (5.5)	0.001
Treatment other than laser (%)	15/371 (4)	9/197 (4.6)	32/109 (29.4)	<0.001

Table 32: Visual Morbidity * overall visual morbidity combining all PNFU data (not including severe visual impairment or blindness) ** Not to the level of impaired vision or blindness.

3.5.2.5 Combining the no/mild Retinopathy of Prematurity Groups: We combined the no and mild ROP into one group and compared them with the severe ROP group in regards to visual morbidity for further analysis, table 33.

Visual morbidity	**No/mild ROP**	**Severe ROP**	**Total**
None (%)	520 (91.5)	64 (58.7)	584 (86.3)
Present (%)	48 (8.5)	45 (41.3)	93 (13.7)
Total (%)	568 (100)	109 (100)	677 (100)

Table33: Visual morbidity combining no/mild ROP

The total visual morbidity not including severe impaired vision or blindness was 8.5% for no/mild ROP group, while it was 41.3% in severe ROP group (χ^2=83.2, p<0.001).

Association between severe ROP and visual morbidity was examined, see table 34.

	Severe ROP	No/mild ROP	Total
Visual Morbidity	45	48	93
None	64	530	548
Total	109	568	677
Risk	0.41	0.085	
	Point Estimate	95% CI	
Risk Difference	0.33	0.23 - 0.42	
Risk Ratio	4.88	3.4 - 6.9	
ch2(1)= 83.2		Pr>ch2 <0.001	

Table 34: Association between severe ROP and visual morbidity

The risk of developing visual morbidity among the exposed (severe ROP) is 41%, while the risk is 8.5% among the unexposed (no/mild ROP).

The risk ratio is the estimated ratio of the risk of developing visual morbidity among the exposed (severe ROP) over the risk of developing visual morbidity among the unexposed (no/mild ROP) = 4.9 (95% CI 3.4 - 6.9). In other words, the estimated risk of developing severe NDI in children who had severe ROP is 4.9 times the risk of developing severe NDI in children who had no/mild ROP. This point estimate is statistically significant (X2=83.2, p<0.001). Also 95% CI do not contain the null.

In a logistic regression model followed by GLM with RR link function to produce RR (binreg command), the outcome was visual morbidity, exposure was severe ROP and variable to be adjusted were BW and laser treatment. The risk of

developing visual morbidity in infants who had severe ROP remained high (RR=3.0, 95%CI 1.6-5,8, p<0.001) even after adjusting for BW and laser treatment, table 35.

Visual morbidity	Risk Ratio	Std. Err.	z	P>z	[95% Conf. Interval]	
Severe ROP	3.01	1.02	3.25	0.00	1.55	5.84
Birth weight	0.9993	0.0005	-1.3800	0.20	0.9983	1.0003
Laser Rx	1.56	0.52	1.31	0.18	0.81	2.98

Table 35: Model 4 produced by GLM with RR link function

3.4.3 Other Secondary Outcomes:

- The prevalence of mild NDI was 20% in the no-ROP group, 28% in mild ROP group while it was 37% in severe ROP group (p= 0.002).
- The prevalence of any NDI was 26% in the no-ROP group, 37% in the mild ROP group while it was 57% in the severe ROP group (p<0.001).

Secondary outcome	No ROP n=371	Mild ROP n=197	Severe ROP n=109	p value
Mild NDI (%)	67/343(20)	49/173(28)	28/75(37)	0.002
Any NDI (%)	95(26)	73(37)	62(57)	<0.001

Table 36: Secondary outcomes

CHAPTER FOUR: DISCUSSION

4.1 Summary of Results

The prevalence of ROP was 35% in the 1065 infants included over the study period. In 239 (22%) infants there was mild ROP and 135 (13%) infants had severe ROP, out of which 197 (82%) and 109 (81%) of them had a final assessment at 36 months corrected age, respectively. Infants who had no ROP were 500 (47%), out of which 371 (74%) had final assessment at 36 months corrected age.

The no ROP and mild ROP groups were combined into one group (no/mild ROP). Maternal characteristics were different in the two groups except in the antenatal risk score and multiple birth. Infants' characteristics indicate that infants with severe ROP had more morbidity in all parameters. **The risk** of developing severe NDI in the severe ROP group was 3.4 times the risk in the no/mild ROP group**,** RR 3.4 (95% CI 2.32 - 4.98) ($X2=40$, $p<0.001$). The risk of developing severe NDI risk was still significant if we stratify the exposure to make comparison between no ROP vs. severe ROP (RR=4.4, 95%CI=2.6 to 6.5, $p<0.001$) and mild ROP vs. severe ROP (RR=2.6, 95%CI=1.6 to 4, $p<0.001$). This association was also true if we compare severe NDI in children with no ROP vs. any ROP (RR=2.5, 95%CI=1.6 to 3.8, $p<0.001$).

The role of blindness was examined by stratifying the outcome (severe NDI) to: severe NDI attributed to blindness alone or severe NDI attributed to blindness plus other components (severe cognitive delay, deafness and severe CP). The prevalence of blindness was higher in severe ROP group compared to no/mild ROP (7.3 vs. 0.7, $p<0.001$). The proportion of blindness was higher in children with severe NDI who had severe ROP compared to no/mild ROP (23.5 vs. 7.7, $p=0.04$). In this study, blindness alone was a not major contributor to severe NDI, as it only occurred in

2.3% in children with severe NDI, however, all these cases occurred in those who had severe ROP. In spite of this trend toward an increase, there was no statistically significant difference whether blindness alone occurred in the no/mild ROP or severe ROP groups (0 vs. 5.9%, p=0.07). Blindness occurred more commonly associated with other components of severe NDI (11.3%) and although there was a trend towards an increase in children who had no/mild ROP compared to those who had severe ROP (7.7 vs. 17.6%) but this was not statistically significant (p=0.16).

Further sub-analysis of prevalence of each component of severe NDI in the no/mild ROP and severe ROP groups showed that the prevalence of severe CP, severe cognitive delay and severe hearing impairment (deafness) were significantly higher in the severe ROP group (p<0.001).

In logistic regression analysis, severe ROP, BW and severe brain injury were independent risk factors for severe NDI.

Laser therapy was done in 2% of infants who had mild ROP, while it was done in 78% of infants who had severe ROP at a median chronological age of 82 days (50% IQR 67-91), while the median corrected GA was 36.7 week (50% IQR34.5-38.6).

Mild or severe ROP are mostly bilateral. There was no severe ROP with single eye alone.

Visual impairment was diagnosed in 5.7% of children who had no ROP, 10.2% mild ROP, and 8.25% in severe ROP group. Percentage of Blindness in those who had no ROP was 0.5%, 1% in mild ROP and 7.34% in the severe ROP group (p<0.001).

Over all visual morbidity not including severe impaired vision or blindness was 7% for no-ROP group, 11.2% in the mild ROP group and it was 41.3% in severe ROP group, p <0.001**.** Impaired visual acuity not to the level of impaired vision or blindness was 2.2% for no-ROP, 3.1% in the mild ROP, 15.6% in the severe ROP (p <0.001). Impaired refraction was 4.9% in the no ROP group, 6.6% in the mild ROP and 33% in the severe ROP group (p<0.001)**.** Impaired ocular motility was 1% in the no-ROP group, 6.1% in mild ROP group and 12.8% in severe ROP group (p<0.001). Infants who received ophthalmological surgical treatment other than laser therapy was 4% in the no-ROP group, 4.6% the mild ROP and 29.4% in the severe ROP group (p<0.001). Ocular structural abnormality was 0.5% in the no-ROP group, 1% in the mild ROP and 5.5% in the severe ROP (p=0.02).

We combined the no and mild ROP into one group (no/mild ROP) and compared them with the severe ROP group in regards to visual morbidity. The total visual morbidity not including severe impaired vision or blindness was 8.5% for no/mild ROP group, while it was 41.3% in severe ROP group (χ^2=83.2, p<0.001). The risk of developing visual morbidity among the severe ROP group was 4.9 times the risk among the no/mild ROP group, RR 4.9 (95% CI 3.4 - 6.9; p<0.001). The risk of developing visual morbidity in infants who had severe ROP remained high (RR=3, 95%CI 1.6-5.8; p<0.001) even after adjusting for BW and laser treatment.

The prevalence of mild NDI was 20% in the no-ROP group, 28% in mild ROP group while it was 37% in severe ROP group (p= 0.002). The prevalence of any NDI was 26% in the no-ROP group, 37% in the mild ROP group while it was 57% in the severe ROP group (p<0.001).

4.2 Discussion of Results

The prevalence of ROP in CHR was 35% among infants born ≤1250 g BW or ≤28 GA between 1996 and 2004, the severe form accounting for 13%. This prevalence covers the catchment area of CHR that included Southern Alberta and parts of Eastern British Colombia (BC). *Schiariti et al (104)* reported at 10 year analysis of incidence of ROP in BC, the incidence of ROP between the second period of analysis 1997-2001that coincides to our selection period was 44% with severe form accounting for 14% (104). The Canadian Neonatal Network reported overall ROP prevalence over 23 centres in Canada at 35% with severe forms around 13% (105). Therefore our study reported similar prevalence that concurs with regional and national prevalence's.

The study of epidemiology and associated risk factors started as early as the discovery of the disease (previously RLF) (5) in preterm infants, hence marking the first and the most constant risk factor, prematurity. Links to oxygen exposure came a decade later (6). As more premature infants survived, more studies were published describing the potential risk factors for developing ROP. These studies sprouted in the 1980s and 1990s, and were either centre or population based (41;106-122). After the year 2000, these studies decreased (29;40;123-128) as more of the pathogenesis of ROP was revealed and the reporting of these potential risk factors became repeatedly similar. This study supports the previously referenced literature with the following risk factors: GA, BW, ANS coverage, PDA, NEC, sepsis, BPD, and severe brain injury. The Univariate analysis of theses morbidities confirming the fact that infants who had severe ROP are younger gestation at birth and lower birth weight, as well as suffered more morbidity. A previous thesis by *Ding* (129) elaborating the associated risk factors with the development of severe ROP that included more than 36 variables

with similar conclusions. Of note, the risks factors for developing ROP did not differ with time at which the study was conducted, modification of classification of ROP or from centre to centre.

It was documented that ROP occurred more in Caucasians (39). This study confirms the finding. The lower numbers of Caucasians in the severe ROP groups could be explained by the high missing records. Of interest, the reporting of aboriginal infants with ROP is rare in published literature. There were 33 aboriginal infants, of which 6 had mild ROP and 7 had severe ROP in this cohort. Their BW and GA were comparable to the Caucasian and they had similar prevalence of severe ROP. Nevertheless, all aboriginal children who developed ROP had severe NDI regardless of ROP severity. The incidence of severe ROP in aboriginals was higher in another study that reported threshold ROP in 11 of 34 Alaskan natives compared with 10 of 93 non-natives (130). This was not explained by differences in prenatal or NICU morbidity except that the intervals from birth to extubation and birth to cryotherapy were shorter for natives (130). These alarming results should warrant further studies describing ROP in this ethnicity.

Small for gestational age infants is a special group of newborns, who were in stress in-utero to the extent that it affected their growth potential. We excluded chromosomal abnormalities that could cause some of them. The prevalence of ROP in SGA was much less than in the appropriate for gestational age (AGA) infants. It is thought that this could be due to earlier maturation of the retina mostly secondary to higher levels of circulating IGF-1 at birth (131). Also SGA infants suffer less morbidity due to their earlier organ maturation (132). Nevertheless, a previous study that compared risk factors associated with the occurrence of ROP in AGA vs. SGA

preterm infants, in similar regional cohort was conducted. It included similar morbidities to this study and concluded that the risk factors associated with the development of ROP were similar to those in AGA infants (132).

Infants who had severe ROP had 3.4 times the risk of developing severe NDI in comparison to infants without severe ROP. This finding agrees with the study of *Msall et al* (84) which was a longitudinal follow up of children who participated in the CRYO-ROP trial (85). The outcome studied was neurodevelopmental function at 5.5 years determined by the WeeFIM (84). This study included 255 infants with birth weight <1251 grams at birth from 23 centers who developed threshold ROP and received cryotherapy to not more than one eye. They found that with the increase in ROP severity from no ROP to threshold ROP the disability increased from 3.7% to 19.7%, respectively. In the *Msall* (84) study, infants who had threshold ROP received cryotherapy treatment to only one eye. The other eye was left as a control. If the other eye would have had severe ROP or even retinal detachment, it was still untreated. In fact this study was terminated 9 months prior to the closing date, as treatment showed significant benefits (92). Although they used WeeFIM tool at 5.6 years, which has not been utilized as frequently in Canadian studies, their results were similar to our study in spite of using laser therapy at earlier stages of the disease (prethreshold) and obviously treatment of both eyes if needed. This could be explained by lower BW (772 vs. 831 g) and earlier GA (25.5 vs. 26.5) in our study vs. CRYO-ROP study, respectively. In addition, our assessment was at around 36 months corrected age, while *Msall* (84) study was at 5.6 years. Our tools of assessment were different than *Msall* (84) study. Hence, the similarity between the two studies should be interpreted with caution.

Our study results were opposite those of *Sugimoto et al* (83). They did a retrospective study to examine the relationship between ROP and neurologic morbidities in 1081 Japanese VLBW infants. Adjusting for BW subgroups, they found no significant association between ROP and CP or MR. *Sugimoto's* (83) study included a retrospective cohort of infants with higher BW <1500 grams at birth. They reported only CP and MR rates. There were no further measures assessing cognitive function or level of CP, MR or visual disability. Lastly, they used the older ICROP classification. All this could have a great impact on reported outcomes.

The TIPP study by *Schmidt et al* (86) had similar mean GA and mean BW to our study. They used a cohort of infants who underwent a multicentre trial of indomethacin prophylaxis and followed them up longitudinally up to 18 months corrected age. Their tools of assessment and main outcomes were similar to our study. On the other hand, our study was done in a regional cohort of infants with no specific intervention, but with a prospective collection of data of both neurodevelopmental and visual outcomes at 36 months corrected age. Our results support the *Schmidt*'s (86) study, but without using death as a component of poor outcome. If we take each component of severe NDI from our study and compare them with the components of poor neurodevelopmental outcomes of the TIPPS trial, we find that CP, impaired MDI, and blindness were increased significantly in infants with severe ROP. Rates of deafness were not similar.

The role of blindness was explored in details in this study. The prevalence of blindness was 1.8% or 18 in every 1000 living children born ≤1250g or ≤28 weeks by 3 years of age over a 9 year period, giving an average incidence rate ratio of 2 per 1000 every year. Of this 1.1% or 12 in 1000 caused by severe ROP over 10 years,

given an average of 1.3 per 1000 every year . To consider this at a regional level with estimated 20,000 live births in years 2004/2005 (133), as a result it is estimated that blindness due to severe ROP could occur in 2.6 out of 10,000 live births each year. Fortunately this low prevalence is accompanied by strict ophthalmological screening and neonatal follow up, so the risk of missing a blind child due to ROP seems minimal. In comparison to the retrospective analysis in BC between 1992 and 2002, which reported only 2 infants with blindness due to severe ROP in this period (104), this difference in number of cases of blindness (8 cases vs. 2 cases in Southern AB vs. BC, respectively) needs to be investigated in future studies.

Severe ROP was associated with an increase in all components of severe NDI (severe CP, severe cognitive delay or deafness). Our study reports these outcomes beyond 18th month of age for the first time in literature, to the best of our knowledge. These associations have to be explored elaborately in a larger scale study or database. It would be interesting to investigate whether severe ROP increases any of the severe morbidities, independently or not.

This study found that BW, severe brain injury and severe ROP independently increase the risk of severe NDI. These were interesting findings and could fit the causal pathway in terms of biological plausibility, temporality, strength of association, consistency with previous literature. The mechanisms that could have caused the severe NDI were discussed in details in section 1.3.1 for BW and brain injury; section 1.3.2 for ROP.

Laser photocoagulation therapy was consistent with the ET-ROP study in regards to its indication and timing. The prevalence of its use was 23.7%, which is higher than the CNN 2004 report of 13% in 13 centres (105). These centres had

variable laser/cryo therapy rates ranging from 0% to 30% depending on their unit specific ROP incidences (105). Newer therapy has been introduced, anti-vascular endothelial growth factor (anti-VEGF). Intravitreal bevacizumab (avastin) is one of the anti-VEGF that had been used, particularly in treatment of severe ROP with excellent regression and no ocular or systemic side effects (134;135). Nevertheless, this promising treatment still requires rigorous randomized controlled trials to evaluate its effectiveness against well studied laser therapy. It should include observation of ocular, systemic and neurological shorter and long term outcomes (136).

Literature rarely reports the location of ROP in terms of being unilateral or bilateral. This could be due to the way authors look at the disease. Ophthalmologists usually report the disease or its treatment "per eye", while others report them as "worst severity of the disease or received treatment" regardless of its location. This study is coded ROP like the later group. Reporting that mild or severe ROP is mostly present bilaterally and severe ROP rarely affects a single eye alone, supports the current concept of pathogenesis of ROP, see section 1.1, any changes in the levels of IGF-1 or VEGF, exposure to periods of hyperoxia and hypoxia should affect both eyes rather than the single eye.

Blindness, as discussed above, was more likely in the severe ROP group; on the other hand visual impairment, not to the level of blindness, was almost the same prevalence among all 3 groups. This could be explained that effective screening and early treatment of severe ROP had led to control the prevalence of visual impairment, otherwise if this measure failed due to aggressive disease, the final trajectory expected is blindness. Nevertheless, severe ROP was an independent risk factor for visual

morbidity after adjusting for BW and laser treatment. This result has a great implication on follow up of patients with severe ROP.

Since this study used ET-ROP criteria and laser treatment, similar centres were compared. *Schiariti et al (104)* did a retrospective review on 1348, infants born <1250 g, over 10 years period (1992-2001) in BC. Their objectives were to describe the incidence trend and long-term (4-6 years) visual outcomes of infants diagnosed with stages 3 to 4 ROP or laser-treated ROP. Although they divided the 10 years into 2 periods to compare the trend, we will compare the overall provenances. Of the 887 included, stages 3 to 4 ROP or laser-treated ROP were present in 94 (10.6%). They had similar mean BW and GA to our study. Visual outcomes were available for 78 children (82% compared to 81% in our study). Binocular visual impairment was present in 13 (16%) children (visual acuity≤20/60) plus 2 children were blind. Refractive errors, including myopia and astigmatism, were documented in 59 (75%) while impaired ocular motility, including nystagmus, exotropia and strabismus, was reported in 12 (15%) children. Ocular structural abnormality was reported in 2 patients. It was difficult to assess total visual morbidity, as per our definition, as some patients might have 2 (2.5%) morbidities at the same time. Compared to our results they had detected higher refractive errors (75% vs. 33%) while other parameters were similar. In spite of the similarities of the two studies, *Schiariti (104)* study had visual follow up 4-6 years as compared to 3 years. On the other hand, they had no comparison group since their centre did not follow up those who did not have severe ROP or who did not need laser therapy.

"Early treatment ROP" compared to treatment at" threshold ROP" had more favorable retinal structural outcomes evaluated at 2 years corrected age for children

involved in multicentre trial of ET-ROP (93). Their structural outcomes were defined differently than our study. Nevertheless, by 3 years of age, nearly 43% children developed astigmatism (91), 70% developed myopia in either group, and 27% developed higher degree of myopia (90). The latter figure was similar to our centre which reported refractive errors prevalence of 33%.

The above studies that reported visual outcomes stressed the importance of long term ophthalmological follow up. The duration of follow up could even extend up to adolescent age. *Palmer et al (88)* followed 254 survivors from 291 preterm children with BW <1251 g and severe threshold ROP in one or both eyes, who participated in the CRYO-ROP trial up to age 15 years. Of those 30% of treated eyes and 60% of control eyes had unfavorable structural outcomes. Between 10 and 15 years of age, new retinal folds, detachments, or obscuring of the view of the posterior pole occurred in 4.5% of treated and 7.7% of control eyes. Unfavorable visual acuity outcomes were found in 44.7% of treated and 64.3% of control eyes. The long term 6 year follow up of ET-ROP is still underway (93).

4.3 Strengths

Though this study had a retrospective component, it had had a prospective collection of outcome data. This included pre-defined comprehensive maternal and neonatal characteristics, as well as multidisciplinary, updated and widely accepted tools of neurodevelopmental and visual outcomes. This was supported by a well established PNFU with accumulated experience of more than 30 years. Data was collected by full time certified personnel to assure quality and accuracy. As a result,

out of 1065 infants included, only 44 (4%) and 11 (1%) were excluded from the analysis because of missing (not filled) data of ROP status and neurodevelopmental assessment, respectively.

The CHR was blessed by excellent ophthalmologists who introduced and adopted the latest definitions of IC-ROP as well as implementing the ET-ROP criteria early. They used laser therapy consistently before and during the study period.

The unified use of IC-ROP and ET-ROP, as well as the regularly updated definitions of morbidities as mentioned above, is a great tool to reduce information bias.

The sample size was achieved to allow analyses to detect significant differences in the main outcomes.

This is the first report, to our knowledge, that assessed the neurodevelopmental outcomes with elaboration on its component, and visual outcomes of preterm infants with severe ROP at 36 months corrected age in Canada.

This study can now form a building block to study longer term outcomes at school age and adolescence and probably adulthood.

4.4 Limitations

This study still has a retrospective component that has its own problems. Selection bias could result from loss to follow up. Of the 1065 infants screened for ROP, 168 had actual loss to follow up, compromising 15.7%. However, of 874 infants

that had documented ROP status, 103 had actual loss to follow up compromising 12% of the cases. These percentages were anticipated prior to data retrieval and appropriate sample size was achieved.

Confounding could not be adjusted for some variables as we were limited in the number of the events and some variables had large numbers of missing data e.g. HI. It would be interesting to assess these variables in future studies.

4.5 Recommendations

Screening for ROP must continue, as per published guidelines, with a goal to follow those infants with ROP for prolonged periods up to the adulthood.

Special attention should be paid to infants with severe ROP, as they may suffer more neurodevelopmental disability. This necessitates special awareness programs for the caregivers as well as allied health personnel.

Prolong neurodevelopmental assessment up to adolescence should be carried out in order to detect learning, social and adaptive problems, even in those with mild ROP**.**

Further studies should be conducted to evaluate the prevalence and morbidities of ROP in the aboriginals, since rare literature focus on this group.

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APPENDIX A

Stages of Retinopathy of Prematurity

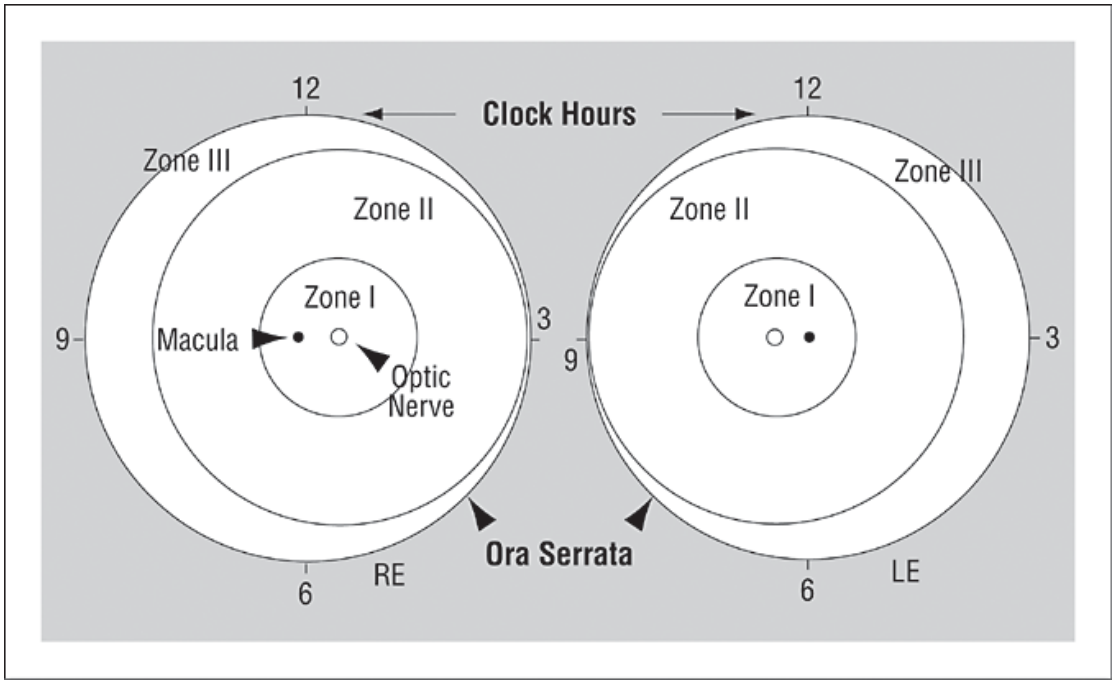

Figure 13: Scheme of the retina showing zone boarders and clock hours used to describe location and extent of ROP

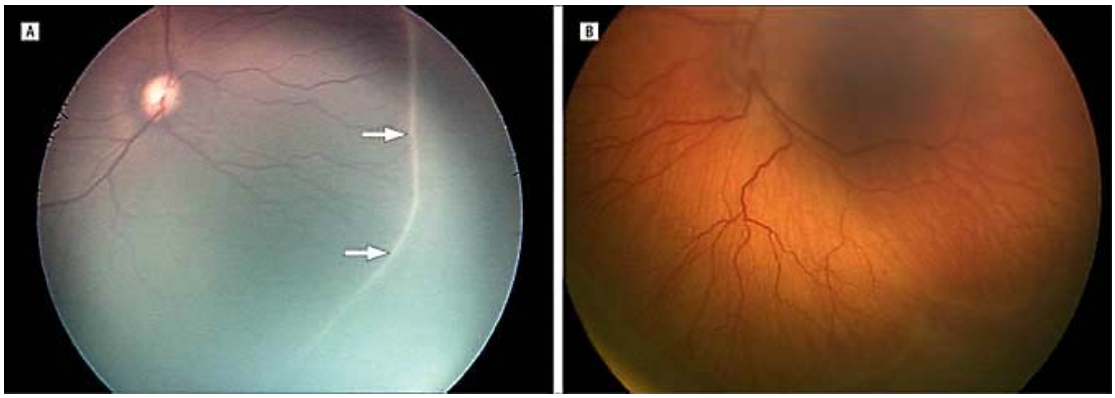

Figure 14: Stage I ROP showing demarcation line. A, View of the demarcation line in stage I ROP. B, Another example of the demarcation line seen in stage I ROP

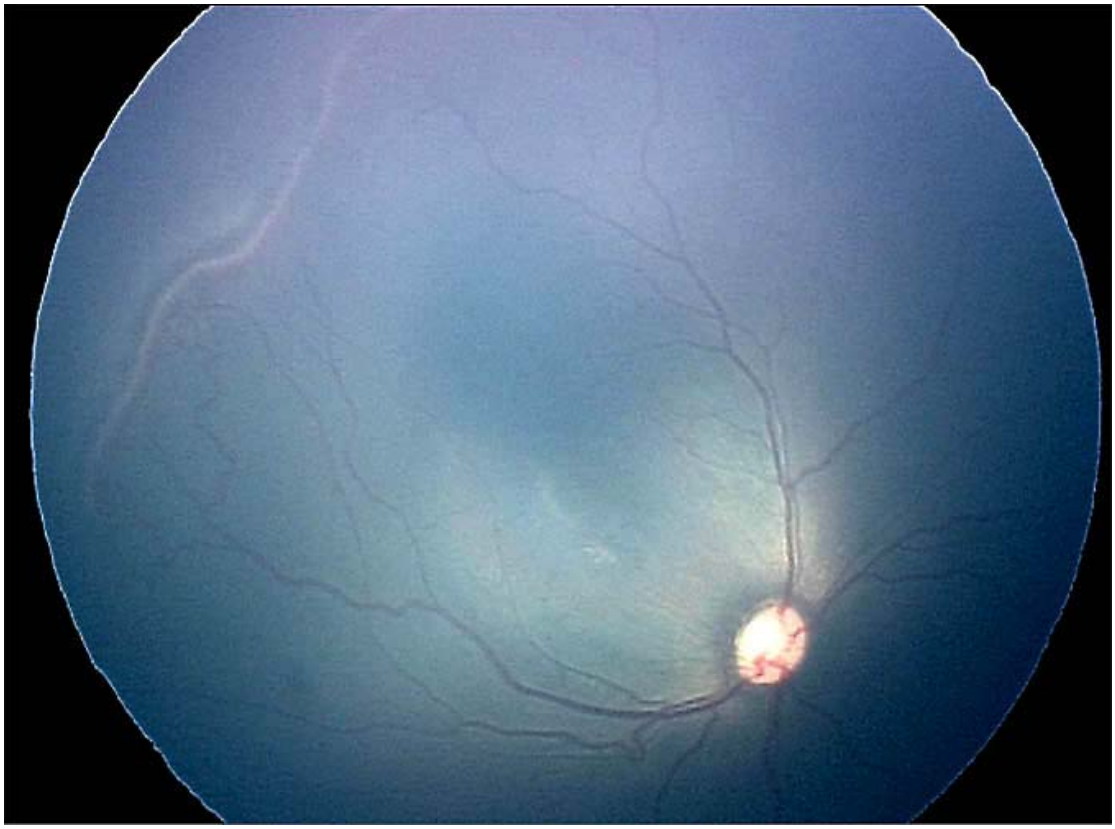

Figure 15: Stage II ROP showing ridge formation

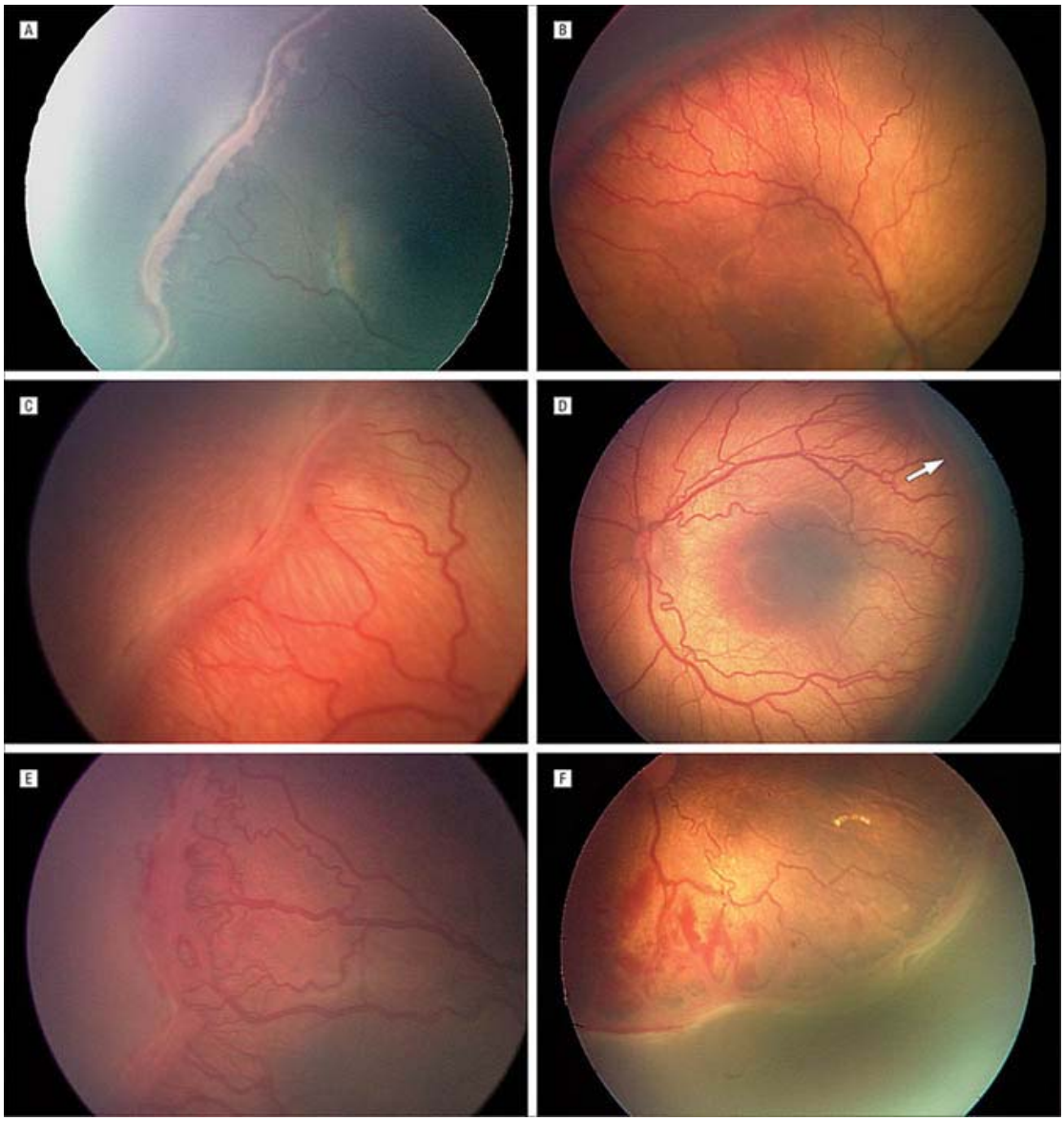

Figure 16: Stage III ROP showing mild to severe neovascularization. A, View of mild stage III ROP. B, View of stage III moderate ROP with fingerlike extensions posterior to the ridge. Note that the posterior pole vessels show increased tortuosity and dilatation. C, View of moderate stage III ROP. D, View of moderate stage III ROP. Substantial amounts of extraretinal fibrovascular proliferation are seen infiltrating the vitreous posterior to the ridge. Note the tortuosity and dilatation of posterior pole vessels that are insufficient for plus disease. E, View of severe stage III ROP with massive infiltration of neovascular tissue surrounding the ridge. F, View of severe stage III ROP with infiltration of the vitreous with a dominantly fibrotic proliferation

Figure 17: Stages IVa and b ROP. A, Example of stage IVb, extrafoveal partial retinal detachment. Note also the straightening of temporal vascular arcade. B, Fundus photograph of stage IVb, partial retinal detachment involving macula. C, Fundus photograph showing stage IVb retinal detachment with extensive temporal dragging of vessels and macula

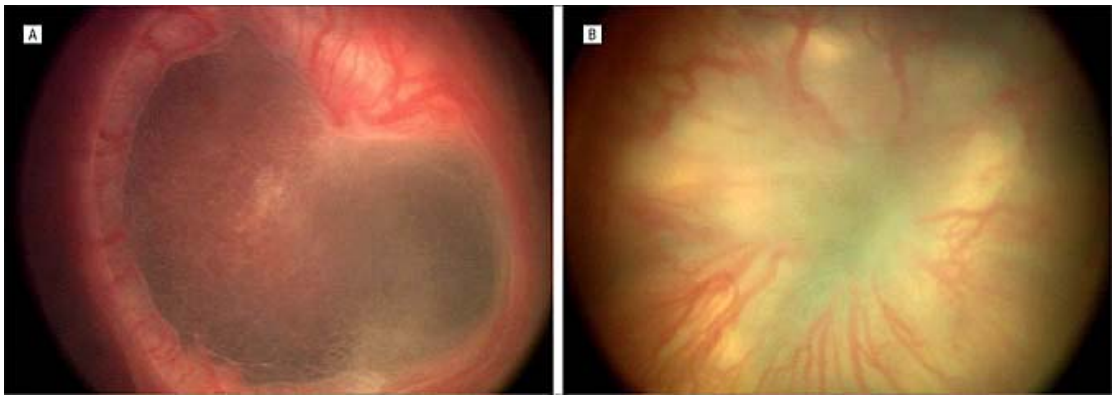

Figure 18: Stage V ROP. A, View of stage V ROP, total retinal detachment with open funnel configuration. B, View of stage V funnel retinal detachment that is open anteriorly but narrowed posteriorly

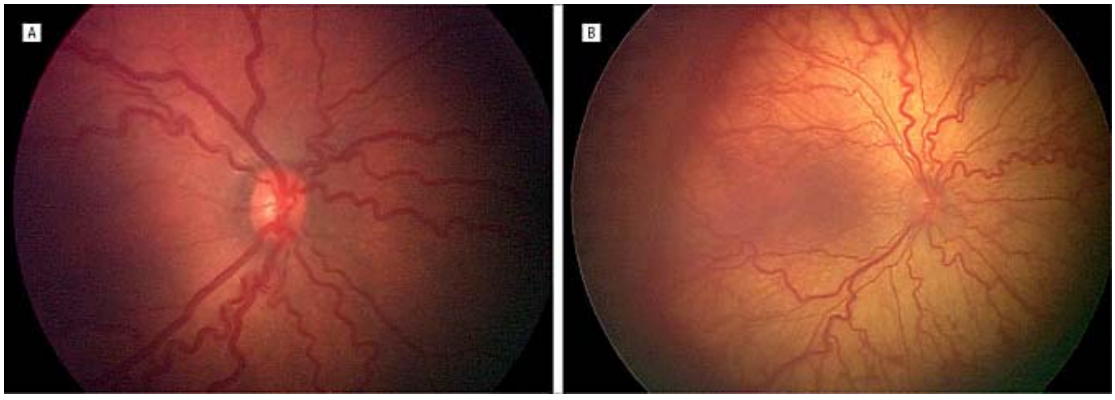

Figure 19: Plus disease. A, View of ROP with plus disease. B, Another example

Appendix B

Risk score

1.Calculation of antepartum risk score

Part A- Pre-Pregnancy	**Score**	**Part C- Problem in Current Pregnancy**	**Score**
Age ≤ 17 at delivery	1	Diagnosis of large for dates	2
Age ≥ 35 at delivery	2	Diagnosis of small for dates	3
Weigh ≥ 91kg	1	Polyhydramnios / Oligohydramnios	2
Weigh ≤ 45kg	1	Multiple Pregnancy	3
Height < 152 cm	1	Malpresentation (s)	3
		Membranes ruptured before 37 weeks	2
Diabetes		Bleeding < 20 weeks	1
Controlled by diet only	1	Bleeding ≥ 20 weeks	3
Insulin used	3	Pregnancy induced hypertension	2
Retinopathy documented	3	Proteinuria ≥ 1+	1
		Gestational diabetes documented	1
Heart disease		Blood antibodies (Rh, Anti C, Anti K,	3
Asymptomatic	1	etc.)	1
Symptomatic	3	Anemia (Hb < 100 grams/L)	1
		Pregnancy ≥ 41 weeks	1
Hypertension		Poor weight gain (28-36 weeks < 0.5 kg/	
140/90 or greater	2	week or weight loss)	1
Antihypertensive drugs	3	Smoker – anytime during pregnancy	
Ch. renal disease documented	2		
Other medical disorders (Epilepsy, severe asthma, lupus, Crohn's disease)	1		
Part B – Post Obstetrics History		**Part D – Other Risk Factors**	
Neonatal Death (s)	3	Major fetal anomaly.	3
Stillbirth (s)	3	Acute Medical Disorder (Acute asthma,	3
Abortion between 12 – 20 weeks and Under 500 grams birth weight.	1	Thyrotoxicosis, UTI, etc.).	
Delivery at 20 – 37 weeks	1	**Substance use**	
Cesarean section	2	Alcohol - ≥ 3 drinks on any one occasion	3
Small for date	1	during pregnancy.	
Large for date	1	Alcohol - ≥ 1 drink per day throughout	3
RH isoimmunization – affected infant.	1	pregnancy.	
		Drug dependent.	3
RH isoimmunization – affected infant.	3		
Major congenital anomaly (Downs, Heart, CNS defects).	1		
TOTAL ANTEPARTUM RISK SCORES			

Cont. Calculation of intrapartum risk scores

	Score
≤ 34 weeks	**2**
35 – 36 weeks	**1**
Meconium in labour	**1**
Pregnancy induced hypertension	**1**
Anemia	**1**
Fever	**1**
Fetal heart rate abnormalities	**1**
Bleeding	**1**
Ruptured membranes > 24 hours	**1**
Seizures	**1**
Coagulopathy	**1**
TOTAL INTRAPARTUM RISK SCORES	

2.Evaluation of Apgar Scores

Sign	**SCORE**		
	0	**1**	**2**
Heart rate	Absent	< 100 beats per minute	> 100 beats per minute
Respiratory effort	Absent	Weak, irregular	Strong, regular
Muscle tone	Flaccid	Some flexion	Well flexed
Reflex irritability	No response	Grimace	Cry
Skin color	Blue, pale	Body pink , extremities blue	Entire body pink