A RETROSPECTIVE REVIEW OF THE RISK FACTORS ASSOCIATED WITH RETINOPATHY OF PREMATURITY IN THREE ACADEMIC HOSPITALS IN JOHANNESBURG, SOUTH AFRICA.

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Abstract

Purpose: Retinopathy of prematurity (ROP) is a leading cause of preventable blindness worldwide. Numerous studies have attempted to identify the many risk factors for this condition. While risk factors have been identified in previous studies, there remains a lack of information specific to the South African population. The purpose of this study was to try and identify possible risk factors in an African population that may differ from studies done in other areas of the world. Secondarily by identifying important risk factors in South African population, screening could be further tailored to try and decrease the load on a very strained health system as well as aiming education for smaller hospitals to try and manage babies correctly to decrease the prevalence of this devastating disease.

Methods: Medical information from an eight-year period for infants with proven ROP that required treatment and for their mothers was reviewed from three public sector hospitals in Gauteng. A matched control group of babies who did not present with ROP were compared to the ROP group.

Results: There were 64 babies in the treatment group and 63 babies in the control group (no ROP). Results showed that babies with ROP were significantly smaller (mean birthweight of 1064g compared to 1210g), had a shorter gestational age (average of 28.3 weeks compared to 29.9 weeks) and were significantly more likely to receive a blood transfusion.

Weaker evidence showed that the babies with ROP all received oxygen therapy, were more likely to undergo mechanical ventilation, receive nitric oxide and have neonatal sepsis. The treatment group also showed a trend towards having a higher likelihood of hypotension and hyperglycaemia (requiring insulin).

The ethnicity of the babies did not differ between groups with most of the babies being African (96% and 94% respectively, ROP and control groups). The prevalence of HIV between and within groups could not be reliably established due to lack of available data.

Conclusion: Well documented risk factors as well as some lesser established risk factors were shown to predict ROP in this population group. The lack of note keeping of important risk factors by attendant medical staff proved problematic and requires attention. The current ROP-screening guidelines in use in South Africa (screening babies of under 1500g) are acceptable and should not be reduced if economically possible, to the guidelines used in some first world countries (screening of babies under 1250g) as this would increase the chances of missing the diagnoses of ROP (7 out of 63 treated babies in this study's treatment group would have been missed) in premature infants.

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List of Abbreviations

Anti-VEGF	Anti-vascular endothelial growth factor
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
CPAP	Continuous positive airway pressure
DHA	Decosahexaenoic acid
HJ	Helen Joseph Hospital
ICU	Intensive care unit
IGF-1	Insulin-like growth factor 1
ROP	Retinopathy of prematurity
SA	South Africa
SJEH	St John Eye Hospital

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CHAPTER ONE - INTRODUCTION

The long term visual outcome for children with retinopathy of prematurity (ROP) include an increased risk for blindness and visual disability. Identifying the risk factors and understanding the pathogenesis are crucial for the treatment and ultimately the prevention of ROP.

Problem statement

As has clearly been documented, South Africa has become part of the so called "3rd epidemic of retinopathy of prematurity".[1,2,3] The increase in premature infant survival has been primarily responsible, with the associated complications of the condition, including blindness. In South Africa, we are still seeing numerous infants with severe ROP requiring treatment. Of concern is that often the condition is discovered too late with irreversible damage resulting.

Justification

Guidelines have been established for the management of ROP, such as those from the American Academy of Paediatrics and the Royal College of Ophthalmologists, however these are tailored to first world countries with well-resourced medical centres. South Africa currently adopts the Royal College recommendation of screening infants under 1500g birthweight. In a study undertaken at Chris Hani Baragwaneth Hospital it was found that no patients over a weight of 1250g at birth developed ROP. Thus, it is suggested that by following the Cryotherapy-ROP trial and only screening babies of a birth weight under 1250g there could be a significant saving of resources and screening time. [4] Of additional importance is the South African population demographic: disease profile and risk factors differ considerably from those first world countries. [5]

Additional barriers to overcome in the South African context include the relatively few trained ophthalmologists compared to the number of premature babies in the public health sector. According to the World Health Organization there are more than 1.2 million babies born annually in South Africa and around 14% are born prematurely. This equates to around 168 000 babies possibly needing ROP screening.[6] Within the public health setting there are additional difficulties in transporting ophthalmologists or the infants to larger and more specialized medical centres for screening and the relative lack of resources for the management of ROP country-wide.

As ROP is a preventable disease, identification of specific risk factors in the South African population demographic could facilitate the development of a very specific, more streamlined screening programme which would mean improved and timeous treatment of affected premature babies.

STUDY AIM

This study aimed to assess the risk factors in both premature babies and their mothers from within the South African population demographic.

Objectives

To identify the risk factors in already active (five contiguous or eight clock hours of extraretinal neovascularization) ROP infants requiring treatment.These include infant factors such as

birthweight, gestational age, oxygen therapy, ventilation, chronic lung disease, nitric oxide use, infant weight gain, hypotension, neonatal infections, blood transfusion and hyperglycaemia. Maternal characteristics under investigation include ethnicity, associated pre-eclampsia, chorio-amnionitis or other maternal infections and breastfeeding.

Secondarily by identifying important risk factors in the South African population, screening could be further tailored to try and decrease the load on a very strained health system as well as educating the staff involved in the management and care of these babies in smaller hospitals to try and decrease the prevalence of this devastating disease.

LITERATURE REVIEW

Retinopathy of prematurity is a vaso-proliferative disorder of the developing retina, that occurs mainly in preterm, very low birth weight (<1500g) new-borns, often subjected to hyperoxic conditions. [1]

Epidemiology

Worldwide about 10 percent of births are preterm (before 37 full weeks of gestational age). Estimates suggest that ROP is a cause of blindness in at least 50 000 children in countries with levels of development ranging from low to high.[1] ROP was first described as "retrolental fibroplasia" by Terry in 1942.[2] This was the first epidemic of the disease and seen with the use of unmonitored oxygen therapy for the treatment of respiratory problems in premature infants. At that point in time ROP became the single most common cause of childhood blindness in high income countries.[3] In 1952 Patz and colleagues demonstrated in a clinical study the association between oxygen and ROP.[2] This caused the use of oxygen to be dramatically restricted which led to a decrease in blindness from ROP, but also an increased rate of mortality and morbidity over the following few decades.

With the introduction of neonatal intensive care units in the early 1970's, a second epidemic of ROP in high income countries developed. This was later controlled by improvements in the neonatal ICU care as well as the introduction of retinal ablation therapy (in the form of cryotherapy).[3]

The so called "third epidemic of ROP" is now occurring with increasing incidence as more premature infants survive due to improved neonatal care.[3]

South Africa is forming part of this. Each year over a million babies are born in South Africa, 87 percent in public healthcare sector including almost half of this in district level facilities. Data suggest that 12.8 percent of babies born in the public sector have a birth weight of under 2500g.[5]

Approximately 168 000 babies are at risk of ROP and require screening each year.[1] This necessitates the implantation of accurate screening programmes, with as much information as possible about the risk factors to successfully minimise the consequences of ROP.[5]

Pathogenesis of ROP

In the human, the retina has no blood vessels until the fourth month of gestation at which time vascular complexes from the hyloid vessels at the optic disc grow toward the periphery of the retina. These vessels reach the nasal periphery after 8 months of gestation but only reach the temporal periphery about 1 month after normal term delivery.[7] Angiogenesis is stimulated by "physiologic hypoxia" of the developing retina, that is when the metabolic demands of the maturing neural retina outpace the oxygen supplied by the underlying choroid and the encroaching vascular network. Vasoactive factors (particularly vascular endothelial growth factor [VEGF]) are secreted by the avascular retina, which stimulate new vessel formation.[2]

Retinopathy of prematurity can be viewed as the arrest of the normal retinal neuronal and vascular development in the preterm infant, with ultimately pathological compensatory

mechanisms that result in aberrant vascularisation of the retina. The more profound the immaturity at birth and the persistence of developmental cessation due to exposure of the retina to harmful factors, as well as deficiency of factors usually provided in utero the more aggressive the later pathological response.[8]

Risk factors associated with ROP

ROP is a multifactorial disease with many risk factors and mediators involved. Ongoing research is done to determine the factors involved and to help us prevent blinding complications of this disease and to implement effective screening programmes.

Infant Risk Factors

Gestational age and birth weight

Low birth weight and prematurity are established risk factors for ROP. Both factors are related to the extent of the immaturity of the retina and its vascular development at birth. It has also been found the lower the gestational age and birth weight the greater the loss of factors normally provided to the foetus by the intrauterine environment.[1,2,3] The immature fetus is unable to take over this production at such an early stage. An added insult is that the low gestational age increases the duration of the infants' exposure to postnatal insults which also contributes to the infants' risk of ROP.

Studies have also shown in utero growth restriction to be a risk factor for ROP.[8]
Data from 10,481 babies, of which 643 developed sight threatening ROP have been analysed in preparation for the 2008 United Kingdom guidelines. All the babies fell into the existing criteria of gestation less than 32 weeks and/or birth weight of 1500g or less. Had the GA criteria been reduced by one week or birth weight by 250g, 9 babies requiring treatment would have been missed. Thus, the criteria have not been changed much, but recommendation was made to screen babies from between 31-32 weeks' gestation and 1251g-1501g.[9]

It is also important to keep social demographics in mind, as neonatal care in high income countries is relatively uniformly excellent, whereas middle-low income countries may range from excellent to poor neonatal ICU care and babies of greater maturity have been seen to develop ROP. Recent publications from India illustrate this issue with babies of less than 36weeks gestational age and greater than 2500g birth weight requiring treatment.[9]

Oxygen therapy

This is also a well-established risk factor of ROP since the 1950's. Since then several studies have been done to try and establish the best possible oxygen saturation to prevent ROP and yet still be effective enough to prevent infant mortality and morbidity.[1,2,3]

Tin and colleagues reported that in infants with a gestational age of less than 28 weeks at birth and an oxygen saturation (measured with pulse oximetry) of 88-98% for the first 8 weeks of

life, needed treatment for ROP four times as often than those with a saturation target of 70-90%. No difference in survival or cerebral palsy were seen.[8]

Oxygen saturation targets of 85-89% and 91-95% have been compared in two large, multicentre, double blind randomised studies. The SUPPORT trial consisted of 1316 infants born from 24-27 weeks and six days' gestational age. It showed that infants in the lower saturation group had slightly increased mortality (20% vs 16%). But a significantly smaller percentage developed retinopathy of prematurity (9% vs 18%).[8,9]

Another theory that is being studied is the possibility that oxygen in phase 2 of ROP could suppress high concentrations of oxygen regulated growth factors such as VEGF that cause proliferative disease. In a meta-analysis of ten studies, Chen and colleagues showed that the need for oxygen is different at different stages of ROP. Low saturation (70-96%) in the first few postnatal weeks and higher oxygen saturation (94-99%) at 32 weeks' postmenstrual age or older were both associated with decreased risk for progression of ROP.[5,8]

Also of importance is that fluctuations in oxygen saturation during the first 8 weeks of life are associated with higher risk of progression to severe ROP.[8]

Thus, it is of utmost importance to set out appropriate guidelines for neonatal staff to follow regarding the strict management of oxygen control to minimise fluctuations, as well as the avoidance of high saturation especially in phase one.

Mechanical ventilation

Duration of artificial ventilation has been found to be a significant risk factor for ROP. Seiberth and Linderkamp showed artificial ventilation for more than seven days to be a risk factor for ROP.[10] A study in 2009 by Nowadzky *et al* demonstrated the use of the bubble CPAP was associated with improved pulmonary outcomes in very low birthweight infants compared to conventional mechanical ventilation. Unfortunately, it was also shown to have double the incidence of ROP as compared to mechanical ventilation. CPAP has been shown to be a significant risk factor for ROP.[11]

Chronic lung disease

Chronic lung disease has been shown to be independently related to the presence of ROP. It is also of interest that low gestational age and chronic lung disease have been shown to have synergistic effects and that together they have a higher risk for the progression of ROP than each risk factor on its own.[12]

Inhaled nitric oxide

Treatment with nitric oxide for hypoxic pulmonary failure has been found to be a risk factor for ROP.[10] Inhaled nitric oxide vasodilates the pulmonary vasculature through relaxation of the smooth muscle cells thus promoting oxygenation. Due to its rapid effects, it is also associated with large fluctuations in arterial oxygen saturation and this is another known risk factor for

ROP.[10] There is however some doubt as to whether inhaled nitric oxide is correlated to use of supplemental oxygen, artificial ventilation and the length of stay in the neonatal ICU. However, in the study of the nationwide inventory of risk factors for ROP in the Netherlands, inhaled nitric oxide was still a significant risk factor after the correction of the variables.[10]

Omega-3 long chain polyunsaturated fatty acids

The importance of omega-3 long chain polyunsaturated fatty acid and docosahexaenoic acid has been shown through mouse studies. In the third trimester of pregnancy there is a massive transfer of essential fatty acids from mother to fetus. This does not occur in preterm birth. [2] The principle omega 3 fatty acid is DHA and main retinal omega 6 fatty acid is arachidonic acid, the balance between these two fatty acids affects retina vasculature and neuronal survival. A deficit of omega 3 fatty acids has been shown to increase risk of ROP in animal models.
The effects of omega 3 DHA supplementation have been shown to be as strong as anti-VEGF therapy in the mouse model.[2]

Poor early weight gain and infant nutrition

It has been reported that there is an association between poor early weight gain and the risk of developing severe ROP. Inadequate nutritional status may affect normal retinal development due to protein/calorie deficiency as well as vitamin deficiency.[1]

Enteral protein intake and weight gain are directly associated with the concentration of IGF-1. The WINROP (weight IGF-1 Neonatal ROP) algorithm was developed in 2006 and uses the rate of postnatal gain as a reflection of IGF-1, or uses weight gain and IGF-1 levels together as highly predictive markers for infants at increased risk of development of proliferative ROP. It can be used to identify patients who will develop proliferative ROP up to 5 weeks before treatment is needed.[2]

Inositol is a carbohydrate found in high concentrations in breastmilk and lower serum levels are found in preterm infants who have received prolong parenteral feeding. This has been associated with ROP. Also of nutritional importance is Vitamin E. Vitamin E tends to increase ROP and blindness but increase risk of sepsis. This in turn causes a "catch 22" situation, as sepsis is associated with increased risk for ROP.[1]

Insulin-like growth factor

IGF-1 has been shown to be critical in both phase 1 and 2 of development of ROP. In premature infants, the sudden loss of maternal nutrients and factors contributes to the dramatic reduction in IGF-1. IGF-1 is very important in angiogenesis and so a minimal level of IGF-1 is required for vascular growth, thus explaining poor vascular growth in premature infants. This is supported by the increase growth and decreased ROP after early IGF-1 treatment seen in mouse pups.[2]

Inflammation and Infection in infants

Low birth weight premature infants are very susceptible to infection. It has been suggested that the exposure of the preterm infant to infectious and inflammatory mediators is associated with the increased risk of developing ROP. This has been proven in multiple studies such as ELGAN study.[3]
Multiple epidemiologic studies have shown that prenatal, perinatal and postnatal systemic inflammation is an additional risk factor.

Hyperglycaemia and insulin use

Raised neonatal glucose concentrations are a proven risk for ROP development. In a study of 372 infants born at a gestational age of less than 30 weeks, increased nutrition alone caused hyperglycaemia which required insulin use. These were both associated with an increase in ROP.[8]

Erythropoietin (EPO)

EPO is an oxygen related growth factor. Its main function is on erythrocyte production in the bone marrow but it also has a role in angiogenesis and retinopathy independent of VEGF. It has been suggested that early EPO supplementation in the first phase can promote vascular and neuronal survival, however if given late during phase two it can exacerbate proliferation. [13]

Blood transfusions

It has been shown that frequent blood transfusions are associated with ROP development. Newborn babies are transfused with adult packed red blood cells which have lower affinity for oxygen than fetal haemoglobin. This causes increased oxygen delivery to the retina. Also associated is the lifespan of the transfused red blood cells which is shorter than in adults and so causes an overload of free iron, with increased free radical production and resultant retinal damage. [14]

Hypotension

It was shown in a study to examine the relationship between the cause and severity of hypotension in premature infants and the development of severe ROP, that dopamine resistant hypotension is a significant risk factor for developing severe ROP. In this study 66% of 242 infants under 28 weeks' gestation developed hypotension and of these, 25% were dopamine resistant. The only cause related to the 19% who developed severe ROP was idiopathic dopamine resistant hypotension. Low cortisol levels were the link between the severe ROP and the idiopathic dopamine resistant hypotension.[15]

Maternal Risk Factors

Maternal pre-eclampsia

Pre-eclampsia has been associated with an increased risk of ROP. Although the exact pathogenesis is unclear it seems that maternal systemic inflammation plays an important role in developing pre-eclampsia as well as ROP. Many placental factors, cytokines and hormones cause an inflammatory response and placental ischaemia leads to further inflammation. Inflammation has been discussed previously in its role regarding ROP.[16]

Inflammation and Infection in mothers

Infants born to mothers with chorio-amnionitis and leucocytosis have been reported to be at increased risk for ROP.[16] Fungal infection has also been linked to chorio-retinitis and ROP. Candida sepsis has been independently associated with increased severity of ROP needing surgical treatment.[8] This is of massive importance in our population demographic as we see many cases of candida (also due to HIV) associations and screening with this risk factor in mind could help early prevention and treatment of at risk infants. This strongly suggests that the maternal factors need to be taken into account for screening guidelines.

Ethnicity

It has been shown that being born to a mother of black ethnicity is protective towards the development of ROP requiring treatment. [1] The reason for this association is unclear. In contrast to these findings a multi-ethnic population study in the midlands region of the UK, Asian and black infants were at greater risk for developing ROP requiring treatment compared to white infants. This is thought to be related due to the difference in screening criteria and possibly the ethnic ancestry of black infants in the UK.[1]

Very little is known about the incidence as well as the ethnicity risk in South Africa. A study done of children in blind schools in SA showed 10.6 percent were due to ROP and of this only 1.25 percent were of black population.[4] This may be due to the socioeconomic differences or limited access to medical or other care.

Delport *et al.* reported a frequency of 24.5 % of ROP in a black hospital with 6.4 % developing stage three disease and 4.2 % requiring treatment.[17] Retinopathy of prematurity in a South African tertiary hospital showed a slightly lower percentage of ROP-16.3 % and 1.6 % requiring treatment.[4] South Africa needs more information on our population demographics associated with ROP, as well as standardized screening programmes that suit our countries ethnicity, socio-economic circumstances and government health programmes.

Breast milk

Mother's milk and colostrum provide many unique nutrients including DHA (decosahexaenoic acid), hormones and growth factors including IGF-1. Most preterm babies require parenteral nutrition as they require more nutrients than what enteral feeding can give them. Breast feeding

and ROP risk have had many contradictory results with some studies showing protective results and others no association. Further studies are needed to clarify this association.[18]

Beneficial factors preventing development of ROP

Prenatal glucocorticoid steroids as well as female sex have shown to have a lower incidence of ROP. Higgens *et al.* have described the role of antenatal steroids given before birth, causing lung maturation.[10] Previous research has also shown that black maternal ethnicity is associated with decreased severity of ROP, however some studies have shown increased risk. More research is clearly needed to determine if there is any conclusive evidence. [10]

Contrary to this finding, Binet *et al.* found no difference between male and female sex and development of ROP. However, it was shown that male infants were more likely to die or have adverse outcomes. [10]

CHAPTER TWO - METHODS

Study Design

The study was designed as a retrospective investigation of all infants with established retinopathy of prematurity with adequate medical records who required treatment in St John Eye Hospital (SJEH), Charlotte Maxeke Johannesburg Academic hospital (CMJAH) and Helen Joseph Hospital (HJ).The patients were found by evaluating the theatre logbooks (as these patients would have received treatment in theatre for ROP) and getting the patient's hospital number, date of birth and name and surname. This information was used to trace files in both the ophthalmology department and the paediatric department.

Analysed records were sourced from the Ophthalmology and Paediatric departments, the following information was extracted: birth weight, birth age, gender, possible infant and maternal infection, HIV status if known, use of infant oxygen and ventilation, use of nitric oxide, use of insulin for hyperglycaemia, infant hypotension, infant blood transfusions, breastfeeding, maternal age, maternal ethnicity, maternal pre-eclampsia or eclampsia and whether the patient was booked or unbooked.

Once the information was collated it was tabulated in an Excel spreadsheet. Patients were assessed on the basis of ROP screening/diagnosis and were allocated to either the research sample or a control sample grouping. Control cases included babies who underwent screening in the CMJAH for ROP but were found to have no ROP on ocular examination. While research cases were defined as babies who had confirmed ROP and underwent treatment in either CMJAH or St Johns eye hospital for this disease.

Descriptive statistics were calculated using SPSS for variables where all data was available. Normal distribution was determined from Normal Q-Q Plots, ensuring that the assumption of normality was not violated. T-tests were conducted on both samples and Chi-Square tests were used to compare risk factors between groups.

Participants

Inclusion criteria

All infants with established retinopathy of prematurity who had undergone treatment. Treatment included laser ablation, cryotherapy and/or anti-Vascular endothelial growth factor intravitreal injections. Infants who were referred from peripheral hospitals but underwent treatment in the included centres will form part of the study.

A control group of patients undergoing screening but who do not fulfil the inclusion criteria were included into the study. These patients were found in the three above mentioned hospitals.

Exclusion criteria

ROP infants that have not undergone treatment or have not been treated in the abovementioned hospitals.

Sampling and intervention

This retrospective study included patients who had received treatment for retinopathy of prematurity within the three hospitals mentioned. The records were examined for both the fetal and maternal risk factors and these were analysed for significance in our population. These common risk factors both maternal and fetal, can aid us in early diagnosis and treatment, more effective screening or possibly even prevention of this disease in our country.

Data capture, collection and analysis

The names and hospital numbers of the patients who had undergone treatment for ROP were searched for and recorded. The patient data was used to look up files from the Paediatric Neonatal archives and the Ophthalmology archives in the relevant hospitals. (Note that Helen Joseph Hospital refers babies requiring treatment to CMJAH), and so no data was ever looked for and no information was ever retrieved from this hospital. This information was recorded under the data collected at CMJAH. The files were reviewed for any clinic, hospital and discharge notes for both the neonate and the mother and any relevant information was documented. The control group was analysed for the same maternal and infant risk factors as the retrospective group.

Ethics

This protocol was submitted to the Ethical Standards Committee for research involving human participants at the University of the Witwatersrand and received clearance (see Appendix 1).

Limitations

This was a retrospective study and depended on the availability of patient files and records from the relevant hospitals.

This study also involved not only primary academic hospitals but referrals from smaller, secondary referral hospitals with different standards and level of treatment as well as possible lack of equipment. This would be detrimental to proper risk factor analysis but would also offer insight into areas in which protocols and treatment could be improved.

This study is limited by its confines to only three treatment hospitals in a single city in South Africa.

CHAPTER THREE - RESULTS

Study sample

The number of infants included in the treatment study group were 39 babies from CMJAH and 25 babies from SJEH, with a total of 64 treated babies. The number of infants included in the control group were 63 babies from CMJAH (Table 1). The control group was only sourced from CMJAH as it has a proper screening form that is used by the Ophthalmologists with proper stats being kept for the last few months and so obtaining information on these babies was reliable and efficient.

The hospital where the infants in the control group were born are as follows: 35 in CMJAH, 7 in Edenvale hospital, 18 in Far east rand hospital and 3 in OR Tambo memorial hospital.

The hospital where the experimental group infants were born are as follows: 1 in Helen Joseph, 1 in Natalspruit hospital, 2 in OR Tambo memorial hospital, 1 in Pholosong hospital and 1 in Vosloorus hospital. The other 58 babies' data for this parameter was not found in the data collected.

Table 1: Summary of results

RISK FACTOR		CONTROL GROUP	TREATMENT GROUP
Maternal Ethnicity	African	94%	96%
	Caucasian	-	4%
	Coloured	6%	-
Sex of Baby	Male	62%	64%
	Female	38%	36%
Gestation (weeks)		29.9±2.4	**28.3±1.8***
Birthweight (g)		1209.62±378.44	**1063.58±192.86***
Oxygen Therapy Received		100%	100%
Mechanical Ventilation Required		20%	73%
Nitric Oxide Treatment		0%	0%
Neonatal Sepsis Present		43%	60%
Hypotension Present		0%	10%
Blood Transfusion Required		18%	50%
Hyperglycaemia needing Insulin		0%	11%
HIV Status: EXPOSED		0%	40%

Where: bold font and * denotes significant ($p<0.05$) difference. Results for risk factors denoted as "%" per sample should be interpreted with caution due to frequency of missing data.

Of Importance: Due to the missing data of patient information, especially in the primary sample, statistical analysis was not possible for reliable p value results.

Infant Risk factors

Gender

The male to female ratio within and between the experimental group and the control group was very similar, ensuring that any ROP-related findings were not associated with the sex of the babies. See Figure 1.

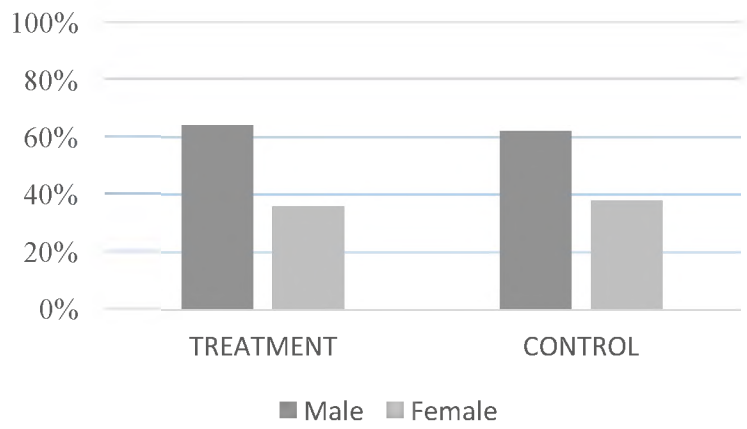

Figure 1: Gender of babies included in treatment and control samples

The following factors represent "good" evidence for risk factors for ROP. Of importance in this data is that the treatment group babies were significantly smaller at birth (measured in grams) and they were born after a shorter gestation period (more premature).

Birth weight

The treatment group had a significantly lower weight at birth compared to the control group ($p<0.05$, $t=2.607$, Figure 2).

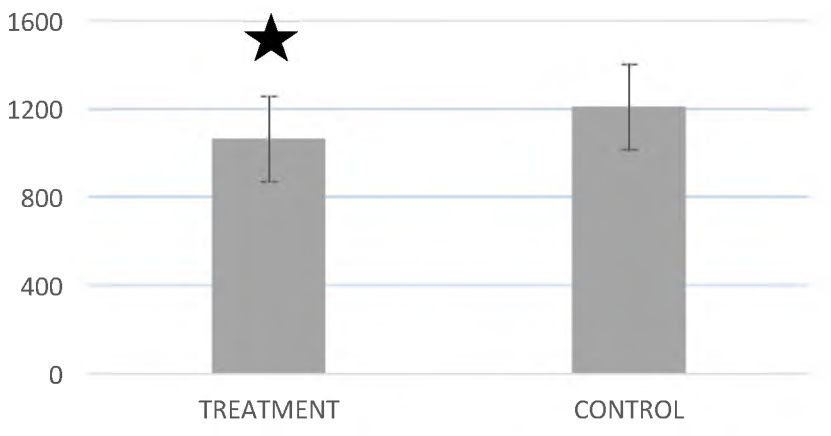

Figure 2: Birthweight (grams)

Within the treatment group there was valid data for n=43, and missing data for n=21. The mean weight for this group was 1063.58 g with a range in birth weight from 720 to 1480 g.

The control group had 63 valid data entries. The mean weight for this group was 1209.62g with a range from 710 g to 2730 g.

Gestation

The treatment group was shown to have a significantly ($p<0.05$, $t=3.586$) shorter gestation period compared to the control group (Figure 3).

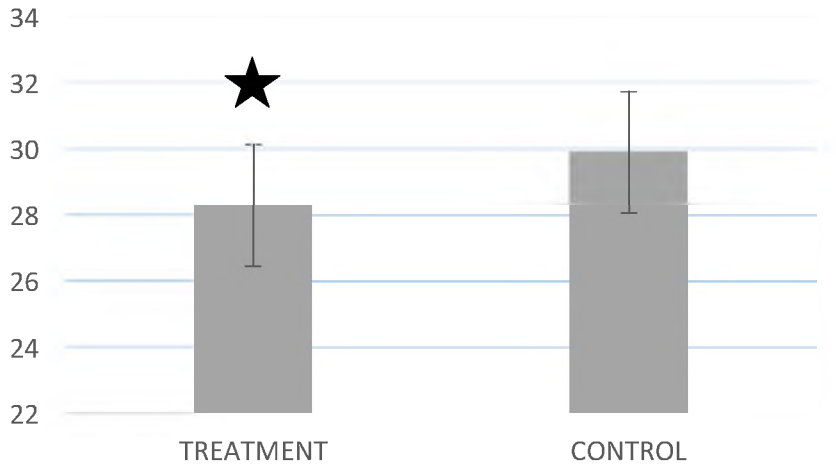

Figure 3: Gestation Period (weeks)

The treatment group had 43 babies with valid data and 21 babies with missing data. The mean age of gestation at birth was 28.3 weeks with a range from 24weeks to 32 weeks.

The control group had 63 babies with valid information and the mean age of gestation at birth was 29.87 weeks with a range from 24weeks to 37 weeks.

The following parameters represented risk factors with "weak" evidence in the current sample.

Blood transfusion

In the treatment group, half (50%) of the babies required blood transfusions compared to only 18% in the control group.

Oxygen therapy

Within the treatment group, 22% (n=14) of babies received oxygen therapy compared to 32% (n=20). However, this was calculated with the missing data included. (Treatment n=50, Control n=43). If the missing data is excluded, all in both samples then received oxygen therapy.

Mechanical ventilation

These results should be reviewed with care due to a large percentage of missing data from the files. Ventilation was required for 2% of the control group and 12.5% of the treatment group. Mechanical ventilation was not required for 6% of the control group and 5% of the treatment group.

If the data is considered with exclusion of the missing information the trend is that 80% of the control group did not need mechanical ventilation, where as 73% of the treatment group did require ventilation.

Nitric Oxide

79% of the control group did not receive NO, while 16% treatment group did not receive NO. By inference, excluding missing data, no participant in either group received NO treatment.

Neonatal Sepsis

There was no significant difference in occurrence of neonatal sepsis between the treatment and control groups. Excluding missing data, sepsis was reported in 43% of the control patients and in 60% of the treatment patients.

Hypotension

Excluding the missing data, 100% of control did not present with hypotension, while 10% of treatment did present with the condition.

Hyperglycaemia requiring Insulin

There were no cases in control group requiring insulin, whereas 11% of treatment group did require insulin to combat hyperglycaemia.

Records of HIV exposure and status were poor and thus HIV status as a risk factor in this instance represents "very weak" evidence for ROP development.

HIV Status

All the patients in the control group (n=63) were HIV negative, while 40% of the treatment group were exposed to HIV via an HIV positive mother. The treatment group sample size for whom HIV status had been recorded was very small (n=5). This data should be reviewed with caution.

Maternal Risk Factors

Maternal Ethnicity

Mothers of African origin were the majority in both samples: 94% control and 96% treatment groups. Coloured mothers made up the remainder of the control sample (6%) and Caucasian mothers comprised the remainder of treatment group (4%).

CHAPTER FOUR - DISCUSSION

This study aimed to evaluate the infant and maternal risk factors associated with ROP in a specifically South African sample. The importance of this would be to develop an effective and applicable screening tool in order to detect ROP timeously and facilitate prompt treatment.

Worldwide around 10% of births are preterm (before 37 weeks' gestation) and according to the World Health Organization this number is higher in South Africa, at around 14%.[6,8] This equates to approximately 168 000 babies in South Africa alone.[1] It is estimated that 87% of all the babies born in South Africa are born within the public health sector.[5] This represents an extreme burden on the health care system, specifically for the Ophthalmologists, Paediatricians and of course infants and family involved. Babies undergoing screening require multiple examinations and possibly visits to different facilities, resulting in stressful situations for both the parents and the infant.[1]

A new and more specific guideline for screening for ROP in SA would include multiple risk factors that would be documented. This would be vital information for further research and detecting ROP in our specific population. This would differ from the standardised guidelines such as those from the Royal College of Ophthalmologists which currently include only weight and gestation.[5]

The total number of babies treated within this time frame were 38 babies from St Johns eye hospital (of which 25 were included in the study) and 40 from CMJAH (of which 39 were included in the study).

A weakness of the study was due to no data being found in records or incomplete information in medical records. This study included information from 2008 and many risk factors now identified as important were not common knowledge at that stage and so were possibly not deemed important to be included in the patients' medical notes. As seen in a study done in 2006 at Chris Hani Baragwanath, a large number of babies with ROP were lost to follow-up and so affected the results of the study. Ten years later lack of data, lost files and poor follow-up is still a major problem.[4] Maternal information and risk factors were also not routinely documented for the infant cases included in this study.

Infant Risk Factors

Gender

The current study showed that both the control and the treatment group had a very similar ratio of male versus female babies and this is important as it cannot cause any confounders in the findings. In a study done in the USA nearly two million babies were analysed after preterm labour and it was shown that 50% of male infants were more likely to born prematurely than female babies. However, this study concentration on white woman and so this could be different in our population group.[19] The ratio of male to female babies who had ROP was almost double (20 male babies versus 11 female babies). There are no studies in South Africa with data relating to any sex prevalence of ROP. Worldwide only a few studies have mentioned the sex ratio in babies with ROP. Most of these studies do not present sex as a risk factor. [20] Only a few show otherwise, with an increased male preponderance such as seen in this study. In a study done in Lithuania it was shown that male babies developed ROP twice as often as female babies (71 versus 37 babies) .[21] Female sex was considered a possible protective factor in one study done in Australia and New Zealand. [22]

Birthweight

The current findings indicate that babies who develop ROP are of significantly lower birthweight than babies who are pre-term yet do not develop ROP. In this study babies with ROP had a mean birthweight

of 1063 grams. The maximum weight of an infant treated with ROP in this study was 1480 grams, which differs from a study done at Chris Hani Baragwanath by Mayet *et al.* where the maximum weight was 1250 grams.[4,5] It was suggested that screening guidelines be changed to only babies under 1250 grams to reduce stress on resources, however in this study it has been shown that by doing this ROP could be missed. In this study 7 of the 63 babies in the treatment group weighed between 1250g and 1500g and so 11% would have been missed if babies only under 1250g were screened.

This correlates with the 2008 United Kingdom guidelines in which 10,481 premature babies were screened and 643 developed sight threatening ROP. The babies screened were according to the guidelines of less than 1500 grams in weight or under 32 weeks' gestation. Had the birthweight criteria been reduced by 250g then nines babies needing treatment would have been missed.[9] In a study done in Greece of 189 premature infants, 24 developed ROP and the mean birthweight was 939 grams, which is lower than found in our study.[12] However, in contrast, high income countries with better neonatal care and much stricter oxygen use guidelines often screen babies of less than 31 weeks' gestation and birthweight under 1250 grams. This is done with what is called a 'sickness" criteria. Should any babies over this guideline have any other possible additional risk factors such as sepsis or blood transfusions then a recommendation is made to screen them. [9] This would not be advisable in a third world country such as South Africa as the premature births often occur in smaller hospitals and clinics and the monitoring of oxygen use and any other risk factors is often sub-optimal. By decreasing our screening criteria, babies with ROP might be missed leading to irreparable damage. Currently, in India which is classed as a low to middle income country, babies have been shown to have ROP if they are less than 2500 gram birthweight or less than 36 weeks' gestational age.[9] However, increasing our guidelines to such parameters would put extreme stress on an already overburdened system. A possible recommendation would be to put a "sickness criteria" into our guidelines.

Gestation

The current findings indicate that neonates with ROP had a significantly shorter gestation period than those who did not develop ROP. The average gestation in the babies with ROP was 28 weeks in this study compared to 29.8 weeks in the control group. Premature gestational age is the most commonly documented risk factor for the development of ROP.[1,7,8,9,10,13,14,15,] Similar gestational age findings have been reported by Giapros *et al.* with a mean age of 27.2 weeks in the group with ROP and a mean age of 30.3 weeks in the group with no ROP.[12]

Worldwide, multiple studies have been done with regard to gestational age and the development of ROP. Although they all show that prematurity is a risk factor, the ages differ slightly based on whether the country is a first or third world country (thought to be due to the level of neonatal facilities and care).[7] Studies have been done to try and determine whether various risk factors act synergistically to increase the risk of ROP. It was shown that low gestational age and chronic lung disease have a higher risk of ROP then the sum of each risk factor alone.[12]

If more of these relationships could be established it would be easier to try and pinpoint patients at increased risk for ROP. In contrast to the previous finding low gestational age along with sepsis and oxygen usage were all found to be independent risk factors of each other.[12] Of importance is that the risk of ROP is higher with lower gestational age as was shown in the CRYO-ROP trial. Babies under 750 grams were reported to have ROP of any stage in 90 % of patients.[3] The earlier a baby is born the more extreme the loss of essential factors that are usually supplied by the mother and the placenta. The infant is not capable of taking over the production of these factors in its premature state and this leads to poor weight gain, increased risk for sepsis, ventilation and transfusions. This is a vicious cycle with an increased risk for the development of ROP.[7] The findings of this study are the first to confirm this risk factor in a South African sample.

Blood Transfusions

The current findings suggest that neonates at risk for ROP will require blood transfusions. Fifty percent (50%) of the babies in the treatment group required transfusions compared to only 18% in the control group. While this information certainly indicates a trend in support of this risk factor, further research is required to ratify these findings.

The increased risk for the development of ROP and its association with blood transfusion requirement has been noted elsewhere in various studies.[14] In a study done by Romagnoli *et al.* it was shown that babies with ROP received almost double the number of blood transfusions.[14] The pathogenesis of this risk factor is not well understood, however, infants are transfused with adult blood cells and these have a lower affinity for oxygen than fetal cells and are most probably leading to an increased delivery of oxygen to the preterm baby which directly affects the vascularization of the Retina and the development of ROP.[14] Future recommendations would include transfusion guidelines and possibly using allogenic cord red blood cells which may decrease side effects of using adult blood.[14]

Oxygen Therapy

The current findings show that only 22% of the treatment group received oxygen, compared to 32% in the control group. This abnormal statistic compared to worldwide data, is due to most of the data in this category being absent from notes collected. In the treatment group 50 babies of the 64 had no information regarding oxygen administration in the notes collected. If the missing data was removed from both groups and new calculations done, then all the babies in both groups received oxygen. Oxygen therapy should be precisely documented in all premature babies and this should be an essential recommendation that needs to be made country wide.

Although oxygen saturation levels are better controlled than in previous years we are still far from obtaining the optimum level for an exact amount of time that prevents infant mortality but still decreases the risk of ROP.[8] This level of care and monitoring is part of protocols in most first world countries, however in developing countries (especially in the smaller clinic and hospitals), babies are still being treated with unmonitored 100% oxygen. [8]

Multiple large studies have been done worldwide to try and determine the exact oxygen saturation level for the age of the babies, weight and duration. These included the SUPPORT, COT, BOOST and BOOST-II trials.[8] A combination of the results of these studies was reported in a study protocol, NeOProM (Neonatal Oxygenation Prospective Meta-analysis Collaboration study protocol).[23] This was a large study in which 4,911 infants were initially entered and they were divided into a group receiving low oxygen saturation (2,456 babies) and a group receiving high oxygen saturation (2,455 babies).[23] This study showed that ROP was decreased if the oxygen saturation level was low (around 85-89%), but that mortality was significantly increased in this group.[23] From the outcomes of this major study it is currently recommended that oxygen saturation levels are maintained between 90-95%.[23]

This upper limit needs to be monitored very carefully to try and prevent increasing risk of ROP. Babies that undergo fluctuations in oxygen saturation are also shown to have an increased risk of developing ROP and all efforts should be made to try and maintain a stable level of saturation that is not over 95%.[8] In South Africa more education and strict guidelines need to be applied to all centres that deliver babies to try and decrease the prevalence of ROP.

Mechanical Ventilation

The outcomes regarding mechanical ventilation in this study should be reviewed with caution as most of the data in both the treatment and the control group was missing. Once again, this emphasises the importance of strict and complete note-keeping that needs to become part of a nationwide programme. However, a trend was noted with the treatment group having almost a 10 times higher rate of babies receiving mechanical ventilation. Studies have shown that mechanical ventilation is a risk factor for the development of ROP [1], with 100% of babies having ROP receiving mechanical ventilation compared to 70% of babies without ROP shown by Giapros *et al.*[12] The longer the duration of mechanical ventilation received the higher the risk for the development of ROP.[1,10,15]

Nitric Oxide

The current findings were interpreted with minimal information due to a very high percentage of missing data. By calculating the statistics with missing data excluded, neither the treatment group nor the control group received nitric oxide. Nitric oxide is used to treat pulmonary failure and it was found by Van Sorge *et al.* that the use of nitric oxide was associated with an increased risk of ROP. It was also noted that it was independently associated with the risk of ROP after variables such as concurrent oxygen use and mechanical ventilation were corrected for.[10] Conversely, Donahue *et al.* showed that although the use of nitric oxide reduced mortality and chronic lung disease, it had no effect on the risk of developing ROP.[10] More information regarding the use of nitric oxide, the exact dose and the duration and its effect on ROP is needed.

Neonatal Sepsis

The data in this study shows that there was no significant difference in the occurrence of sepsis in the treatment group compared to the control group. There was a 60% occurrence of sepsis in the treatment group and 43% in the control group. Studies have shown that infants who developed sepsis early on had an increased risk of developing ROP.[16] Of importance, fungal infection has also been associated with an increased risk of severe ROP.[7] According to Lee *et al.* it was shown that both prenatal and postnatal sepsis was an independent risk factor for the development of ROP.[16] The exact mechanism is currently unknown however the effect of sepsis on increasing free radical molecules and a lower level of antioxidants due to immaturity may play a role [13,16]

Hypotension

The current data shows that if the missing data is excluded then 10% of the treatment group were diagnosed with hypotension and none of the control group had any hypotension. In some studies, hypotension has been shown to be a risk factor for the development of ROP, however this has not been a consistent finding.[15] Of importance, hypotension commonly occurs with neonatal sepsis and this could possibly be the cause of increased risk of developing ROP. In a study done by Catenacci *et al.* Hypotension was shown to be a risk factor for the development of ROP.[15]

Hyperglycaemia

Current data shows that 11% of the treatment group needed insulin to treat hyperglycaemia, compared to no babies in the control group. Studies have previously shown that high glucose levels in preterm infants needing insulin increase the risk for the development of ROP.[8]

HIV status

The current data shows that 40% of the treatment group were HIV exposed and all the patients in the control group were HIV negative. The sample size of patients with data was very small and so these results should be reviewed with caution. In our population group this information would have been very important due to the high prevalence of HIV. Note keeping for ROP babies should definitely include this information in the future and further studies would be beneficial to see if this has any effect on the risk of developing ROP. In a study done at Tygerberg children's hospital it was shown that ROP was not an associated risk factor.[24]

Maternal factors

Ethnicity

In this study, the majority of mothers were of African origin (over 90% in the treatment and control group). This is probably due to the population profile that is treated in these hospitals (mainly of African origin). This makes the comparison of different ethnicities impossible. The possible effect of ethnicity and genetics on the development of ROP is of importance and more research needs to be done in the South African population.

It was shown that being of black ethnicity is associated with a decreased risk of developing severe ROP in study done by Husain *et al.*[1] In contrast a study examining a multi-ethnic population showed that black infants were at higher risk for developing ROP.[12] Currently in South Africa more data needs to be collected comparing Caucasian, Coloured and African babies to determine who is most at risk in our population.

The other maternal factors that were to be collected in this study were age, maternal infection and maternal preeclampsia. Due to lack of data in the notes collected his information was almost never documented or missing and so no analysis of these parameters could be carried out. This is an important recommendation to the department of paediatrics and ophthalmology to always be vigilant about writing down all the parameters that may influence the baby developing ROP. Although these factors were previously deemed irrelevant it is becoming more apparent through research that multiple factors affect the development of ROP and of note this includes maternal infection and pre-eclampsia. In a previous study, it was shown that chorio-amnionitis adds to the risk of developing ROP in a premature baby.[16]

The effect of pre-eclampsia on the development of ROP has had mixed results in studies with some showing an increased risk and others showing no risk.[16] What would be very important in our population is determine if it has a risk in our population profile. South Africa is a third world country with a high rate of HIV and this could cause increased rates of maternal immunosuppression, thus leading to increased maternal infection and inflammation. It would be vital to know if this causes an increased risk of ROP. Improving maternal care and health would have a widespread effect on both Mother, baby and health resources.

CHAPTER FIVE - CONCLUSIONS AND RECOMMENDATIONS

The data reflects important positive and negative findings relating to babies with ROP in the public health sector in South Africa. It shows that this population group is more likely to develop ROP if they are are of lower birth weight, smaller gestational age and have received blood transfusions. It shows a trend towards an increased risk for the development of ROP if they have undergone mechanical ventilation, had neonatal sepsis, received nitric oxide, had hypotension and hyperglycaemia. Maternal ethnicity was mainly African in origin due to the population subtype and lack of comparison against other population groups. HIV as a possible risk factor was not able to be calculated due to lack of missing data in these files.

Maternal factors are seemingly becoming more prominent in the possible development of ROP in the infant. This is thought to be due to risks causing preterm birth as well as possible inflammatory and circulating factors from the mother that could affect the infant. More information regarding mother's health and HIV status is vital to determine if this is true.

A weakness of this study was not the lack of babies that have been treated for ROP in this study, but rather a lack of relevant information in the notes collected at the various hospitals. Many new risk factors have emerged in the last few years other than just gestational age and oxygen use. These important possible risk factors need to be documented in a proper fashion by both the paediatrician and the ophthalmologist if we are to try and prevent this devastating disease. By proper note keeping and implementation of proper guidelines not only in larger hospitals but in all clinics that have the facilities for childbirth, we could clamp down on babies at risk and implement better screening and faster treatment.

Of importance, in this study 7 babies over the weight of 1250g (but under 1500g) were noted to have developed ROP (equates to 11% of the treatment group). This is in contrast to previous research done in South Africa. In keeping with these findings, our screening criteria should not be reduced to screening babies only under the weight of 1250g as being done in some first world countries if at all possible. Although our screening system is overburdened and screening less babies would make a positive impact on all the medical staff involved as well as the families and infant being screened, we stand a risk of missing possible blinding ROP. However, we do not need to go to such extremes such as in countries like India who screen babies from 2500grams and under. Based on the results of this study screening babies under 1500g and including a "sickness criteria" would suffice.

An important recommendation from this study is to implement a standardised neonatal ROP screening form that can be used country wide for the documentation of important risk factors both in the infant and the mother. I have included a template in appendix 3 that I have designed from this study. If such a form was used, we would be able to perform large scale studies with the data and hopefully be able to determine exactly which other risk factors are specific to this population. We would be able to determine if HIV is a risk factor and whether maternal risk factors are important.

It would show us important information regarding the level of care in smaller hospitals versus larger hospitals and where we need to work on healthcare education relating to ROP.

By ensuring properly educated healthcare workers from the nursing staff in primary level hospitals to the specialists in the tertiary hospitals we could firstly prevent, secondly screen efficiently with the correct risk factors known and if need be, treat this disease in the best possible manner in our wonderful country of South Africa.

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APPENDIX 1

Ethics approval

R14/49 Dr Suzanne Smith

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M160218

NAME: (Principal Investigator)	Dr Suzanne Smith
DEPARTMENT:	Neurosciences Division of Ophthalmology Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	A Retrospective Review of the Risk Factors Asscociated with Retinopathy of Prematurity, in Three Academic Hospitals in Johannesburg
DATE CONSIDERED:	26/02/2016
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof Trevor Carmichael
APPROVED BY:	Professor P. Cleaton-Jones,Chairperson, HREC (Medical)
DATE OF APPROVAL:	01/03/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004,10th floor, Senate House/2nd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated,from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially review in February and will therefore be due in the month of February each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

Date Feb 2017

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX 2

Data capture sheet

Patient serial number	
Infant data:	
Birth weight	
Birth Gestation	
Sex of infant	
Hospital where infant was born	
Was the patient booked or unbooked	
Age in weeks when ROP was discovered	
Oxygen therapy received	Yes or No
If yes- What was the duration	
Was the infant mechanically ventilated	Yes or No
Did the infant have chronic lung disease	Yes or No
Did the infant receive nitric oxide	Yes or No
Did the infant have neonatal sepsis	Yes or No
Did the infant have hypotension	Yes or No
Did the infant receive blood transfusions	Yes or No
Did the infant have hyperglycaemia requiring insulin use	Yes or No
Did the infant receive breastmilk	Yes or No
Infant HIV Status	
Maternal data:	
Maternal age	
Maternal ethnicity	
Maternal infection	Yes or No
If yes- What type of infection	
Did the mother have pre-eclampsia or eclampsia	Yes or No
Mother HIV status	

APPENDIX 3

Proposed ROP Screening Form as designed by myself (Dr Suzanne Mari Smith)

Date Screened:

First screen: **Y / N** , If No : Current number of follow up:

Patient Name:

Hospital Number:

Birth Hospital:

Booked: **Y / N**

Gender: **M / F**

Race: **B / W / C / I**

Infant Data

Oxygen Received: **Y / N**	Duration: Saturation level:
Mechanical Ventilation: **Y / N**	Duration:
CPAP Received: **Y / N**	Duration:
Nitric Oxide Received: **Y / N**	Duration: Dosage:
Chronic Lung Disease: **Y / N**	Type of Lung Disease:
Neonatal Sepsis: **Y / N**	Type of Sepsis:
Blood Transfusions: **Y / N**	Number Received:
Hypotension: **Y / N**	Dopamine Received: **Y / N**
Hyperglycaemia: **Y / N**	Insulin Received: **Y / N**

HIV: Exposed / Positive / Negative	
Other:	

Maternal Data

Pre-eclampsia / Eclampsia: **Y / N**	
Maternal Sepsis: **Y / N**	Type:
Maternal Age:	

Ophthalmologist Review:

RE: LE:

Zone: Zone:

Stage: Stage:

Treatment Received:

Date of Next Follow-Up:

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