"INCIDENCE OF RETINOPATHY OF PREMATURITY AMONG PREMATURE BABIES BORN AT A TERTIARY CARE HOSPITAL - A LONGITUDINAL STUDY WITH A SHORT FOLLOW UP".

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Endorsement by the HOD, Principal/Head of the Institution

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LIST OF ABBREVIATIONS

AOP	:	Apnoea of prematurity
APROP	:	Aggressive posterior retinopathy of prematurity
BT.WT.	:	Birth weight
CRYOROP	:	Cryotherapy for retinopathy of prematurity study
2, 3 DPG	:	2, 3- Diphosphoglycerate
ELBW	:	Extremely Low Birth Weight (<1000 gms)
ETROP	:	Early treatment for ROP trial
FIO2	:	Fraction of inspired concentration of oxygen
FFP	:	Fresh Frozen Plasma
gms	:	grams
Hb	:	Haemoglobin
IGF-1	:	Insulin-like growth factor-1
IVH	:	Intraventricular haemorrhage
ICROP	:	International Classification of ROP

LIST OF ABBREVIATIONS

Light-ROP	:	Light reduction on ROP study
NICU	:	Neonatal Intensive Cardiac Unit
O2	:	Oxygen
PHPV	:	Persistent Hyperplastic Primary Vitreous
PDA	:	Patent Ductus Arteriosus
PIH	:	Pregnancy Induced Hypertension
ROP	:	Retinopathy of Prematurity
RLF	:	Retrolental Fibroplasia
RDS	:	Respiratory Distress Syndrome
Transfusion	:	Fresh Frozen Plasma / Packedcell transfusion/Platelet
VLBW	:	Very Low Birth Weight (< 1500 gms)
VEGF	:	Vascular Endothelial Growth Factor
wks	:	weeks
YC	:	Yates Correction

ABSTRACT

Background: Retinopathy of prematurity (ROP) is a common blinding disease in children in the developed world despite current treatment, and is becoming increasingly prevalent in the developing world. Improved survival of preterm neonates has increased the incidence of retinopathy of prematurity (ROP) in India.

Objective: To know the incidence of ROP in preterm infants with birth weight ≤ 1500 grams and/or gestational age ≤32 weeks and to correlate between development of ROP and the risk factors.

Methods: A longitudinal study of 100 infants weighing ≤1,500 gms and/or GA ≤32 weeks at birth was conducted. The main clinical outcomes were the incidence of any stage of ROP and severe ROP. The variables considered for the study were: birth weight, gestational age, gender, oxygen, multiple gestations, occurrence of sepsis, respiratory distress syndrome, transfusion, apnoea, PIH, hypotension, fetal distress, anaemia and metabolic acidosis.

Results: The incidence of ROP in this study was found to be 16%. 18.75% babies were in stage 1, 68.75% were in stage 2 and 6.25% was in stage3 ROP. Only 6.25% developed APROP.

The mean birth weight of the ROP babies was 1272.81 ± 143.67 gm, while that of non-ROP babies was 1642.80 ± 216.60 gm. Lower birth weight was significantly associated with increased incidence (p = <0.001) of ROP. The incidence of ROP was 40% in babies weighing ≤ 1500gm at birth.

The mean gestational age of the ROP babies was 31.38 ±1.63 weeks, while that of non-ROP babies was 33.31 ± 1.74 weeks. The incidence of ROP was 26.32% in babies born ≤ 32 weeks of gestational age. Gestational age was found to be a significant risk factor for the development of ROP (p=0.004).

On Univariate analysis, risk factors associated with ROP were Oxygen, RDS &Transfusion (FFP/Platelet). On Multivariate analysis, RDS was found to be an important risk factor. Using the forward method, Oxygen and Transfusion were also found to be significant on Multivariate analysis.

Conclusion:

The present study reflects the problem of ROP in a tertiary care centre. Early examination was significantly associated with chances of early detection of ROP (p = 0.016) and hence all babies should have their first screening within the first four weeks after birth. In our opinion, the effective management of ROP requires a team effort of the neonatologist, ophthalmologist and the NICU staff. Regular screening programme with a criteria of birth weight <1500 gms and gestational age <32 weeks or both and babies more than 1500 gms and >32 weeks with other risk factors should be screened at the discretion of the neonatologist and ophthalmologist. Along with regular screening, an effective control of oxygen delivery, reduction of apneic spells and their early recognition and effective management of RDS are required.

Key Words: Retinopathy of prematurity, Birth weight, Gestational age, Risk factors

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INTRODUCTION:

Retinopathy of prematurity (ROP) is a vasoproliferative disorder affecting premature infants. It is one of the most common causes of visual loss in children and can lead to lifelong vision impairment and blindness.

There are approximately 45 million blind people in the world today out of which, 30% are in Asia. Of the total blindness, childhood blindness accounts for 4%. It is estimated that there are about 1.4 million blind children, 1 million of whom live in Asia. India shares 20% of the world's childhood blindness[1]. ROP afflicts over 3,00,000 infants worldwide[2] .In developing countries like India, the incidence of ROP has been reported at 24 – 47 % among high risk preterm infants [3]. It is important not only in terms of economic burden, but in its severe social implication, which is very long in terms of blind years.

In the context of our country, we are sitting at the summit of two volcanoes - one where all latest state of the art health care is available and the other, where even minimal basic health care is unavailable. ROP is known to grow in both these conditions. Among the preventable causes of blindness in children (57%), ROP figures very high in the agenda. Low birth weight and gestational age were found to be the most important risk factors for the development of ROP.

With neonatalogical units being equipped with the state-of-art technological background and highly qualified personnel providing optimum care of extremely immature newborns, ROP incidence is on a rise. By early detection and timely intervention, blindness due to ROP is preventable.

The purpose of this study is to know the incidence of ROP and to correlate it with maternal and neonatal risk factors.

AIMS AND OBJECTIVES

Primary objective - To know the incidence of Retinopathy of Prematurity in preterm infants, with birth weight ≤ 1500 grams and/or gestational age ≤32 weeks.

Secondary objective – To know the relationship between development of ROP and risk factors.

REVIEW OF LITERATURE

HISTORY

RETINOPATHY OF PREMATURITY (ROP) was originally designated Retrolental Fibroplasia (RLF) by Dr. Theodore L. Terry [4], who in 1942 first connected the condition with premature birth. It was based on his impression that the primary change involved a proliferation of the embryonic hyaloid system which incorporated the retina. He studied the unilateral pathological specimens (most likely to be PHPV) and provided details which he thought, may be identical with bilateral cases of retrolental fibroplasia. He postulated that "some new factor has arisen in extreme prematurity to produce such a condition" and he argued that this new factor was 'excess light'.

The term ROP was coined by Heath in 1951. In 1951, Dr Kate Campbell[5] observed that, in a smaller hospital where each infants family was charged for each tank of oxygen that was used, much less oxygen was administered, and there was a lower incidence of RLF. She concluded that, "normal oxygen environment of the newborn full-term infant is abnormal for the premature infant". More convincing evidence came within a year from Crosse and Evans[6] in England, and from a randomised trial by Patz et al[7] in the USA, followed by a cooperative trial by Kinsey et al[8] in 1956.

Two ROP epidemics occurred in industrial developed countries during the past 60 years. The first epidemic was diagnosed in 1940-1950. In spite of careful oxygen supply monitoring, in the course of 1970-80, a second ROP was

registered while it was noted that, a greater percentage of ELBW- infants survived in industrial developed countries.[9-11]

In 1981, Phelps[12] estimated the incidence of ROP associated with increase in survival rates of infants with birth weight less than 1000gms.

In 1970's Japanese physicians treated the ridge and adjacent avascular retina[13]. Hindle and Leyton, in Canada, treated the ridge, and the retina anterior and posterior to it. McPherson, Hittner, and Kretzner advocated cryo of the ridge and adjacent posterior avascular zone to destroy the spindle cells, the presumed source of angiogenic factor[14].

The International Classification of ROP was devised in 1983 under the leadership of John Flynn [15].

With the advent of telemedicine to aid in the screening and diagnosis of ROP and laser photocoagulation replacing cryotherapy as the standard mode of treatment, more and more babies are being diagnosed earlier and treated .

INCIDENCE

With progress in neonatology, the survival of ELBW-infants increased from 5-65% in the last 40 years, while in VLBW-infants, it increased from 35-90%. The latter increase in survival rates has provided a situation propitious to increasing the number of diagnosed ROP cases.

In the CRYO-ROP study, the incidence of the disease in a group of premature newborns, with a birth weight <1251gms was 65.8% and it was 81.6% for infants of less than 1000 g birth weight[16]. In the ETROP (multi centric) study done 15 years later, the overall incidence of ROP was found to be 68% in babies with birth weight <1251 gms. The overall incidence of more-severe ROP (prethreshold) was 36.9% among infants with ROP in the ETROP Study, whereas the incidence was 27.1% for patients in the CRYO-ROP Study who developed ROP[17].

A review of literature reveals that, the incidence and severity of ROP increases with decreasing gestational age and birth weight. The incidence of ROP in different studies done outside India was found to be 9.4%-38.9%[18-21].A study from the Indian subcontinent reveals the incidence to be 17.5%-46%[22-26]

Reports from developing countries indicate that, although the trend in ROP is not similar in all the units, there is an overall decrease in incidence of disease whenever there is an ongoing surveillance programme. The incidence of severe form of the disease (Threshold disease) is decreasing. Aggarwal and co-workers found a drop from 46 to 21% in their study over a period of 7 years[11]. A Danish study found a statistically significant decrease in incidence of ROP in

infants weighing more than 1250gm[20]. Similar observations were made in multicentre study in UK[27]. Nair[22] and colleagues, Gupta[28] and co-workers found no cases of ROP in babies weighing more than 1250gm.

Table 1: Incidence of ROP in Indian and International studies

INCLUSION CRITERIA			
INDIAN STUDIES INCIDENCE	G .AGE	BT. WT.	
1.Rekha[23],1996	≤ 34	<1500	46%
2.Maheshwari[24],1996	<35	<1500	20%
3.Patil[25],1997	<32	<1250	17.5%
4.Agarwal[26],2002	<35	<1500	20%
5.Gupta[28], 2003	≤35	≤1500	21.7%
6.Nair[22],2003	≤32	<1500	25.4%
INTERNATIONAL STUDIES			
1.Hussain[29],1999	<32	<1500	21.3%
2.Fledelius[20],2000	<32	<1500	9.4%
3.Blair[21], 2001	<30	<1250	38%
4.Conarth[30],2004	≤32	<1750	10%
5.Shah[18] 2005		<1500	29.2%
6.Fortes[31],2007		≤1000	48.9%
		≤1500	18.2%
7.Binkhathlan[32],2008	<36	<2000	56%

PATHOGENESIS

NORMAL RETINAL VASCULAR DEVELOPMENT:

Ocular blood supply involves the development of angiogenesis, arteriogenesis, and vascularization. Angiogenesis is the formation of endothelial lined blood vessels. Arteriogenesis is the addition of smooth muscle cells to endothelial cells, forming intact arterioles [33]. Vascularisation is the new arterialization of a tissue i.e.,the retina. The combination of these three elements in forming the embryonic vascular tree is termed vasculogenesis.

The posterior segment of the eye has a dual system of blood supply:choroidal and retinal. The choroidal blood supply nourishes the outer retina while the inner retina is supplied by the retinal circulation. Up to 16 weeks of gestation, choroidal vessels alone nourish both outer and inner retina, practically inner retina remains avascular. At about 16 weeks of gestational age, the first blood supply to inner retina appears in the form of mesenchymal "spindle cells". It arises from the adventitia of the hyaloid artery.The origins of the retinal circulation reside at the optic nerve head. The vasculogenic elements begin to spread out over the retina from there and vascularisation proceeds in a relatively concentric fashion out to the ora serrata [34]. Vessels reach the nasal ora first, because the fovea is the eye’s center, the optic nerve is therefore nasal to the retinal center and uninterrupted vascularisation must reach the closer point first i.e.,nasal ora. The rate of growth of the advancing spindle cells is 0.1nm/day and

reaches nasal ora by 36 weeks and then temporal ora serrata by 40 weeks of gestational age[35].

PATHOGENESIS OF ROP

At birth, fetal circulation is transformed by the switch from placental oxygenation to lung oxygenation. Oxygen saturation rises from mixed venous levels to arterial levels. However, these fetal lungs are immature and are not capable of fully mature oxygen transfer. Medical intervention provides inhaled supplemental oxygen, enhancing the oxygen transfer. So several factors lead to a potential initially hyperoxic state: mixed venous oxygenation to arterial oxygenation; supplemental inspired oxygen; immature but as yet undamaged lungs; and a low retinal metabolic rate of oxygen consumption. At some point after birth, this relative hyperoxia begins to change. The lungs become damaged, alveolar – blood oxygen exchange is compromised, and retinal metabolic demand for oxygen rises precipitously according to rigidly timed embryologic events. This gives rise to relative hypoxia. And this transition is not a smooth, linear one. There are undoubtedly dramatic swings during the gradual change over from hyperoxia to hypoxia.

Retinal vascularisation is modulated by VEGF. This process is acutely sensitive to relative states of hyperoxia and hypoxia. Hypoxia up-regulates VEGF and hyperoxia down-regulates its production. Along with VEGF, there are insulin like growth factor(IGF-1), basic fibroblast growth factor, and transforming growth factors associated with it.[33,36-39].

Typical ROP appears to occur in two phases, a vasocessation phase and a vasoproliferative stage. Phase I, the vasocessation phase, begins with the initial hyperoxia that occurs in the immediate neonatal period. Arterial oxygen, increased FIO2 (fraction of inspired oxygen) and low retinal oxygen utilisation secondary to a low metabolic rate, all combine to produce this relative hyperoxia. The relative nature of the hyperoxia is important. The tissue saturation may not be higher than the normotoxic levels of a mature retina, but retinal vascularisation should be normally occurring under the in utero hypoxic conditions characteristic of mixed venous blood supply. So, the tissue oxygen tension is high, VEGF production is diminished, and normal vascularisation ceases.

Phase II is the vasoproliferative stage. As one would expect, this phase is driven by hypoxia and increasing VEGF levels. The relative hypoxia is fuelled by decreasing alveolar oxygen exchange and increasing retinal oxygen utilization. It is important to recognize that transition between these two phases is not abrupt. It is undoubtedly a phased transition, albeit within a probably tight timeframe. During this possibly short transition period, the retina probably undergoes frequent and potentially wide-swings of hyperoxia /hypoxia. The sickest infants, undoubtedly have the most volatile transition phase of retinal oxygenation with medical intervention chasing the tissue oxygenation needs. It suggests that the ultimate cause of ROP is related to the mismatch of tissue oxygen need and tissue oxygen supply.

Two important hypothesis described are

a) *The Classical Theory*: Ashton and Patz[40] proposed the classical pathogenesis of ROP. Elevated arterial PO2 causes retinal vasoconstriction, leading to vascular closure and subsequent permanent vascular occlusion. Endothelial cell

proliferation occurs adjacent to closed capillaries when neonate returns to room air, leading to neovascularisation. Subsequent extension of this neovascularisation may reach vitreous, producing hemorrhage which leads to fibrosis and causes vitreous traction and retinal detachment.

b) *Spindle Cell Theory*: This theory proposed by Kretzer et al[41], postulates that the induction of retinal and vitreal neovascularisation occurs by spindle cell insult. After birth, the spindle cells are exposed to hyperoxic environment because of increased oxygen diffusion through the thin and avascular peripheral retina. Free radical formation occurs, which attacks compromised spindle cells, which have deficient anti-oxidative defense mechanism. These abnormal spindle cells stop migration and canalization, with neovascularisation occurring at the shunt site.

GROWTH FACTORS IN ROP

Retinal vascularisation is modulated by VEGF, which is constructed by highly regulated VEGF mRNA[42-45]. This process is acutely sensitive to relative states of hyperoxia and hypoxia. Hypoxia up regulates VEGF and hyperoxia down regulates its production. VEGF is not the only vasoactive molecule within the retina. There are insulin like growth factor (IGF-1), basic fibroblast growth factor, and transforming growth factors[33,36-39]. These cytokines create a complex milieu in which relative oxygen concentrations drive vascularisation and this vessel formation and tissue invasion create modifiable changes in the extracellular matrix[46-49]. Astrocyte development and migration parallel this vascularisation and may be the source of VEGF . If the avascular zone is larger and when this is exposed to the hyperoxic state, VEGF expression is decreased leading to vaso-

obliteration. This causes hypoxia and ischemia in non-perfused area, if insult is sustained. This again stimulates VEGF production and thus neovascularisation. Over the time VEGF production decreases, ROP will regress. If VEGF production increases or persists, ROP will progress. Manipulation of these factors could be beneficial therapeutically[50].

RISK FACTORS FOR DEVELOPMENT OF ROP[51]

ROP is a multifactorial disease and based on clinical and epidemiological studies, numerous risk factors for ROP have been proposed.

Definitive and well accepted factors

- Prematurity/ Gestational age/ Birth weight
- Oxygen supplementation

Associated factors

- Light
- Vitamin E deficiency
- Apnea with bag/ mask ventilation
- Methyl xanthine administration
- Respiratory distress syndrome
- Asphyxia / Hypoxia
- Shock
- Hypercarbia / Hypocarbia
- Acidosis / Alkalosis

- Sepsis
- PDA / Indomethacin
- Blood transfusions / Exchange transfusions
- Intraventricular hemorrhage
- Chronic in-utero-hypoxia
- Maternal factors – Anaemia

PREMATURITY AND BIRTH WEIGHT

In the CRYO-ROP study, the incidence of the disease in a group of premature newborns with a birth weight <1251gms was 65.8% and 81.6% for infants of less than 1000 g birth weight[16]. In the ETROP (multi centric) study done 15 years later, the overall incidence of ROP was found to be 68% in babies with birth weight <1251 grams.. The incidence of ROP in different studies done outside India was found to be 9.4%-38.9%[18-21].A study from the Indian subcontinent reveals the incidence to be 17.5%-46%[22-26]. A Danish study found a statistically significant decrease in incidence of ROP in infants weighing more than 1250gm[20]. Gupta[28] and co-workers found no cases of ROP in babies weighing more than 1250gm. A review of literature reveals that, the incidence and severity of ROP increases with decreasing gestational age and birth weight.

Contrary to these reports, in a retrospective study done by Anand Vinekar[52] et al they found that, severe ROP is often encountered in babies with birth weight more than 1250 grams in developing countries and suggested that, the western screening guidelines may require modifications before application in developing countries.

OXYGEN AND ROP

From the time ROP has been reported, excessive use of oxygen has been proved to be one of the main risk factor for the development of ROP. During this possibly short transition period, the retina probably undergoes frequent and potentially wide swings of hyperoxia /hypoxia. The sickest infants undoubtedly have the most volatile transition phase of retinal oxygenation. It suggests that the ultimate cause of ROP is related to the mismatch of tissue oxygen need and tissue oxygen supply.

Sudden discontinuation of oxygen and duration of oxygen therapy are also incriminated in the pathogenesis of ROP[53]. Gunn analyzed data from their low birth weight survivors and found a significant association between, the more severe grade of cicatricial disease and duration of oxygen therapy[54].Their finding concurred with the co-operative study of 1977, in which Kinsey emphasized that the strongest association with occurrence of ROP, apart from birth weight, was time in oxygen.

He also noted that, concentration of oxygen administered was significantly associated with ROP in infants under 1200gm. When comparing mean Pa02 levels in normal infants and in ROP infants, he found differences only in babies of low birth weight and only Pa02 levels greater than 150mmHg[53]. Hence, appropriate monitoring of actual Pa02 in infants at risk is essential. A definite safe range for arterial Pa02 is not known, nor do we know the critical duration of oxygen exposure. Until such guidelines are established, keeping Pao2 <100 mm Hg is recommended preferably between 50 and 70mm Hg and saturation between 90-95%[55].

LIGHT:

The LIGHT-ROP Study[56] was a multicenter, prospective, randomized, controlled, clinical trial designed to determine if a reduction in ambient light exposure to premature infants eyes would reduce the incidence of retinopathy of prematurity (ROP). Previous clinical reports were contradictory and had one or more methodological flaws. The investigators hypothesized that, reducing the amount of light that reaches the eyes of preterm infants may be effective in preventing ROP. The study showed that the reduction in the ambient-light exposure did not alter the incidence of ROP.

VITAMIN E DEFICIENCY:

Vitamin- E is a fat soluble antioxidant and as a result it is able to scavenge free radicals derived from oxygen. The premature infant and the retina are likely to be particularly vulnerable to the deleterious effects of these oxygen derived free radicals, as a result prophylactic vitamin E has been suggested for the management of retinopathy of prematurity (ROP). Three clinical trials[57-59] have documented the efficacy of vitamin E supplementation in suppressing the development of severe ROP. The spindle cells, mesenchymal precursors of the inner retinal capillaries, are the primary inducers of the neovascularisation associated with ROP. Exposure of spindle cells to elevated oxygen tension increases their gap junction area. This early morphologic event immediately halts the normal vasoformative process and eventually triggers the neovascularisation that is observed clinically 8–12 weeks later. Vitamin E supplementation above the

deficient plasma levels of these infants[60] suppresses gap junction formation and clinically reduces the severity, without altering the total incidence of ROP.

APNOEA

In a study by Kim et al[61], they found that apnoea independently increased the incidence of ROP. Furthermore, frequent apnoeic attacks increased the progression of pre-threshold ROP to threshold ROP. In addition, apnoea may not only increase the risk of developing ROP, but may also worsen pre-existing ROP. A higher incidence of hypoxemia and apnoeic episodes requiring bagging was found among infants with severe ROP, than in a control group[26,54]. The babies with increased frequency of apnoea appeared to have longer duration of high pC02. Similarly in a study by Chen et al[62] they found that apnoea was one of the independent risk factors of ROP.

HYPOXIA

Szewczyk[63] was the first to suggest that, ROP was produced by too rapid a reduction of the level of oxygen after a child had been habituated to an enriched oxygen atmosphere. He found the condition could actually be treated by returning the infant to the high oxygen level, followed by a slow return to breathing air. He implied that ROP was a result of hypoxia and not due to oxygen toxicity.

An association between ROP and hypoxia was also noted by Cohen and co-workers, who found that 45% of 43 children with ROP had other abnormal neurological signs; 14% had a definite history of anoxia in the immediate postnatal period[64]. In reviewing the neonatal course of 50 infants who developed

ROP, Katzman and his colleagues found no significant difference in total duration of oxygen therapy, or exposure to different concentrations of oxygen, when those with more severe (stages III to V) disease were compared with those with less severe (Stages I to II) disease[65]. They did, however, find that the more severe stages were associated with significantly more hours spent with arterial PO2 levels below 35 mmHg.

Similar results were reported by Shoht and co-investigators among infants weighing < l,250gm[66].

HYPOCARBIA AND HYPERCARBIA

Hypercarbia prevents the retinal vasoconstriction that occurs as a normal and perhaps protective response to hyperoxia, as the retina would then more likely be exposed to the damaging effects of hyperoxic blood as suggested by Bauer and Widmayer[67].

Walbarsht and co-workers postulated that hyperoxia may be responsible for impaired removal of CO2 from the retina. This could result both from, arteriolar constriction with resultant decreased retinal perfusion and from the interference with haemoglobin binding of CO2, when haemoglobin in the retinal venous circulation is nearly 100% saturated with oxygen. For both these reasons, CO2 could accumulate and lead to retinal vasodilatation, which precedes the vasoproliferation of ROP[68].

It should be noted that the second cooperative study on ROP[10] did not find a clinical association between higher PCO2 and development of ROP, nor did the study of Shohat et al[66].

Similarly a study by Balazs Gellen et al.[69] showed that, neither variable blood carbon dioxide tension nor duration of hypercarbia or hypocarbia in the first 2 weeks of life was associated with the development or severity of ROP. A study by Liao SL et.al[70] found that hypercarbia or hypocarbia in the first 3 days of mechanically ventilated preterm neonates did not affect the subsequent development of ROP.

Shohat et al.[66] study found a highly significant association between hypocarbia and the development of severe ROP i.e., an increased frequency of episodes of hypocarbia (PaC02 <25) and alkalosis (pH>7.55) among infants developing ROP.

SEPSIS

Sepsis is an independent risk factor for the development of ROP. It may act through cytokines and endotoxins or by oxidative burst in the neutrophils consequent to infection. Its prevention and early control may reduce the incidence of ROP. Agarwal[26] reported positive blood cultures in 67 percent of infants who later developed ROP, and in only 31 percent of infants with normal eyes. Liu PM et al[71] in her study, found sepsis as the most significant factor contributing to ROP. In a study published in Paediatric Research[72] they found that, Candida sepsis is independently associated with increased severity of ROP and the need for laser surgery in ELBW infants .Similar findings were found in a study done by Parupia H, et al[73] where they found that, the risk of threshold ROP requiring laser treatment was higher in infants who developed fungal sepsis. Other studies also support these findings[54].

BLOOD TRANSFUSIONS

In recent years, the role of blood transfusions and iron intake as risk factors for ROP has been strongly emphasized[77,78]. Reports have provided conflicting views on the relative role that transfusions may play. Some studies suggest that anaemia per-se is a risk factor for ROP, whereas others contend that a high haematocrit ratio and frequent blood transfusions are important independent risk factors[79-81].

The usual explanation is that, tissue (including retinal) oxygen levels are increased by transfusion, owing to the reduced affinity of adult haemoglobin to oxygen, as compared to fetal haemoglobin. An alternative hypothesis is that, damaging effects of blood transfusion on the retina are mediated by, an increase

in free iron load which may react with various intermediates of oxygen generating highly reactive oxygen radical. Otherwise, protection against free iron is provided by ceruloplasmin and transferrin, but in preterm infants with gestational age lower than 34 weeks, the levels of these binding proteins are very low, and rapid saturation of transferrin occurs[77, 78].

A study done by Akter S et al[82] showed that, blood transfusion in first week of life and repeated blood transfusion resulting in large cumulative volume are very significantly associated with occurrence of ROP.

INTRAVENTRICULAR HAEMORRHAGE

Intraventricular haemorrhage has been significanty correlated with ROP. Hungerford et al[74] reported IVH in 79% of infants with any stage ROP. In a review of all infants weighing <l,500g, born between 1976 and 1978, and surviving for at least one month, Procianoy[75] and assistants found that IVH was the only condition significantly correlated with ROP. It was proposed that as cerebral and retinal circulations respond similarly to changes in PaO_2, $PaCO_2$ and blood pressure and the two conditions might be caused by a common mechanism.

Watts P et al[76] concluded that, with improvements in neonatal care and a reduction in the prevalence of severe IVH, there appears to be a weakening of the previously reported association between severe IVH and severe ROP. However, the presence of even a minor grade of IVH may be a significant risk factor for threshold ROP once stage 3 disease is encountered.

SURFACTANT

Repka[83] in his study concluded that, the widespread use of prophylactic surfactant therapy, will not change the incidence of retinopathy of prematurity in extremely low-birth-weight infants. However, the absolute number of affected patients will likely increase because of the decrease in mortality of extremely low-birth-weight patients, the patients most at risk for retinopathy of prematurity. However, surfactant replacement therapy may have a beneficial effect on the development of cicatricial, severe ROP. Several other studies revealed no significant effect of surfactant on the incidence or severity of ROP

Yet, others have shown that improving survival rates of very premature infants with synthetic surfactant, does not result in increased number of infants with impairments(e.g- neurodevelopmental defects, mental retardation, cerebral palsy and ROP), with detrimental effect on developmental outcome or late morbidity[83].

ROLE OF DEXAMETHASONE

The effects of early dexamethasone therapy on pulmonary mechanics and chronic lung disease and on the progression of ROP in very low birth weight infants have been investigated. In a prospective randomized controlled trial, Durand and colleagues[84] showed that, early dexamethasone therapy in very low birth weight infants markedly improves respiratory compliance and tidal volume, reduces fractional inspired oxygen concentration and mean airway pressure;

facilitates extubation; and reduces the duration of mechanical ventilation. It decreases chronic lung disease without causing additional risk for the development of infection, intraventricular hemorrhage and ROP. In a study done by Rosemary et al[85] in an urban population they found that antenatal dexamethasone administration appears to be associated with a decreased incidence of development of ROP of stage 2 or higher.

In a recent study, Tsukahara and associates[86] showed that, early dexamethasone therapy (at 4-7 days of age) for prevention of chronic lung disease in preterm infants did not influence the incidence of ROP.

OTHER RISK FACTORS ASSOCIATED WITH ROP

In recent years, additional factors have been implicated in the evolution of ROP. The task of defining any of these factors in the setting of other major factors, such as low birth weight and early gestational age, is again formidable. Because oxidative injury contributes to the development of ROP, bilirubin has been suggested as a physiologically important antioxidant. However, a recent study found no definite association between bilirubin levels and severe ROP.

The use of dopamine in the management of hypotension in high risk prematurely born infants (birth weight < 1,000 gm) has been associated with increased risk for the development of threshold ROP. Thus more vigilant screening of high-risk infants requiring dopamine therapy, for systemic hypotension may be warranted[87].

It is hypothesized that replacement of fetal blood by adult blood, would reduce the overall oxygen affinity of haemoglobin and consequently promote the unloading of oxygen, to the tissues at relatively lower arterial oxygen levels. Several studies have shown that, transfusion of adult blood either by exchange transfusion or top-up transfusion is associated with ROP[22, 88]. In an effort to elucidate further the oxidative influence in the development of ROP, Papp and co-workers examined the glutathione status of red blood cells in patients with ROP both in vivo and after an in vitro oxidative challenge. Infants with active ROP have the lowest levels of reduced glutathione (GSH), the highest levels of the oxidized form (GSSG), the highest GSSG/GSH ratios and the greatest fall in GSH after an in vitro oxidative challenge. After an in vitro oxidative stress, defective glutathione recycling was found in patients with preceding ROP (not active ROP) and was suggested as a factor predisposing to oxidative haemolysis. The glutathione redox ratio was warranted as a biochemical screen for active ROP in premature infants[89].

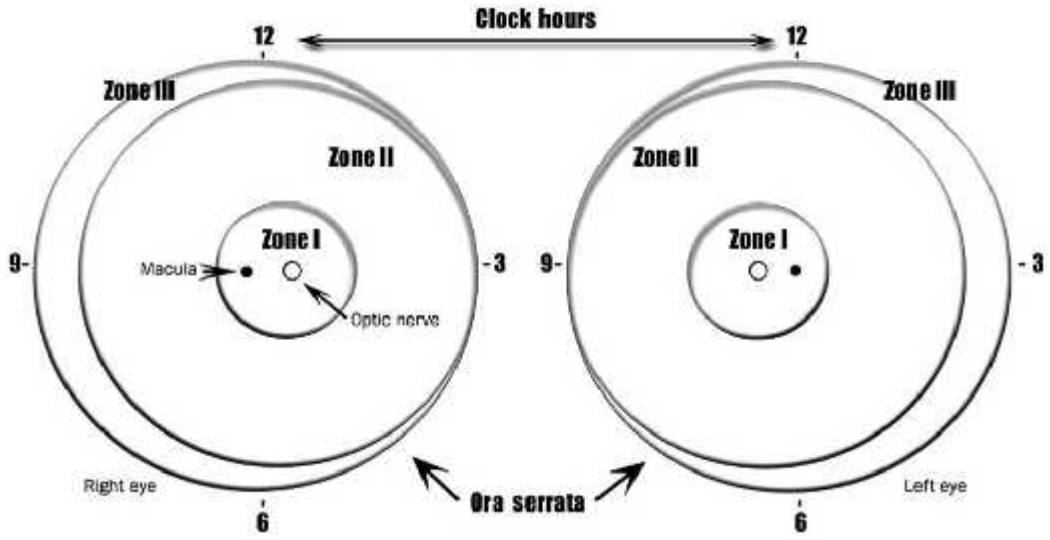
CLASSIFICATION OF ROP

INTERNATIONAL CLASSIFICATION OF ROP(ICROP)[15]:

A. Classification - Consists of five components.

1. Location refers to, how far the developing retinal blood vessels have progressed. The retina is divided into three concentric circles or zones.

Figure 1: International Classification of Retinopathy of Prematurity (ICROP) zones

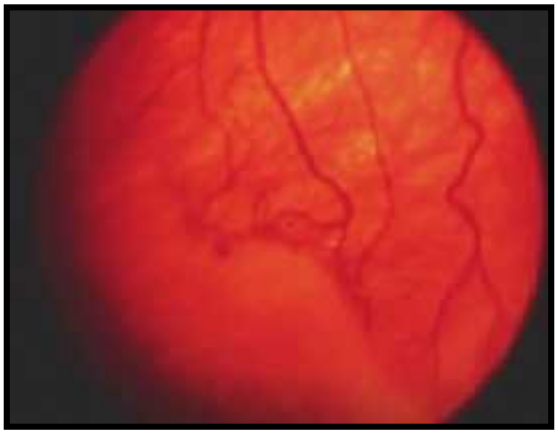


- Zone 1 consists of an imaginary circle with the optic nerve at the center and a radius of twice the distance from the optic nerve to the macula.

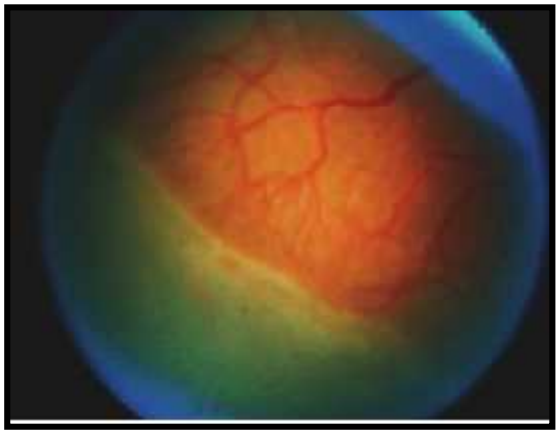
- Zone 2 extends from the edge of zone 1 to the equator on the nasal side of the eye and about half the distance to the ora-serrata on the temporal side.

- Zone 3 consists of the outer crescent - shaped area extending from zone 2 out to the ora serrata temporally.

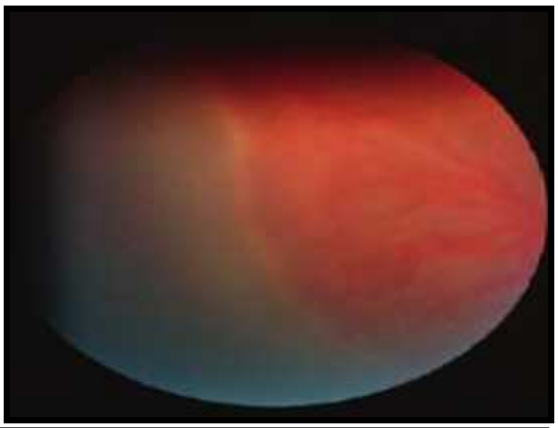
2. Stage of ROP:



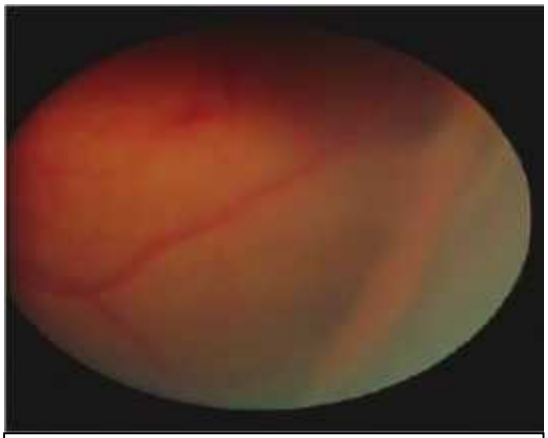
Photograph 1: Stage 1- Demarcation line



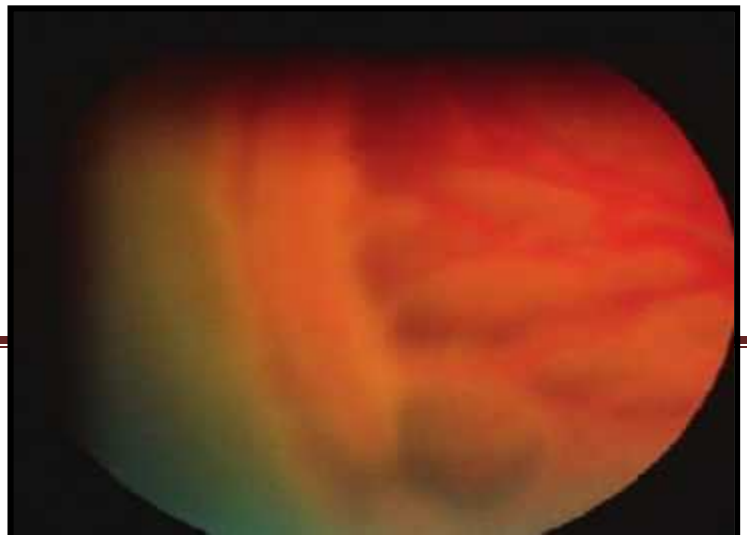
Photograph 2: Stage 2- Ridge



Photograph 3: Stage 3 ROP - Mild

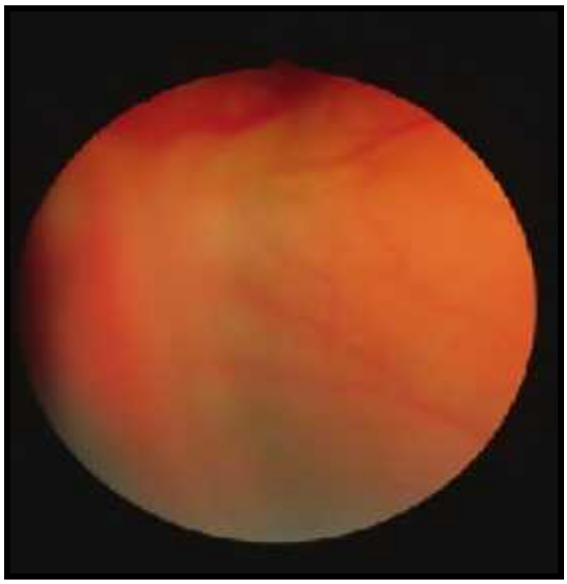


Photograph 4: Stage 3 ROP - moderate

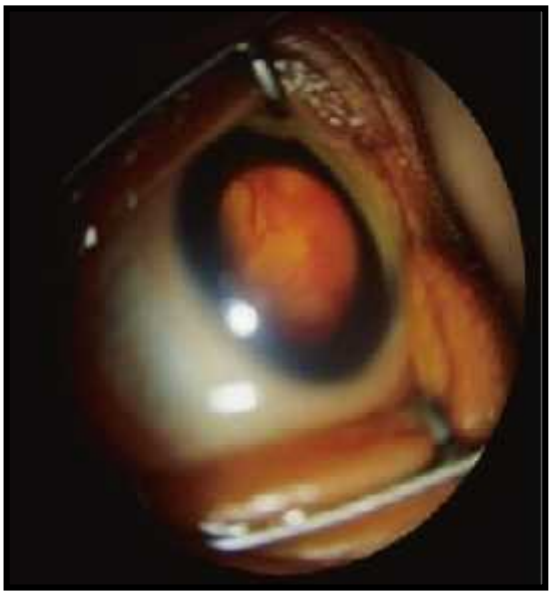


Photograph 5: Stage 3 ROP - severe

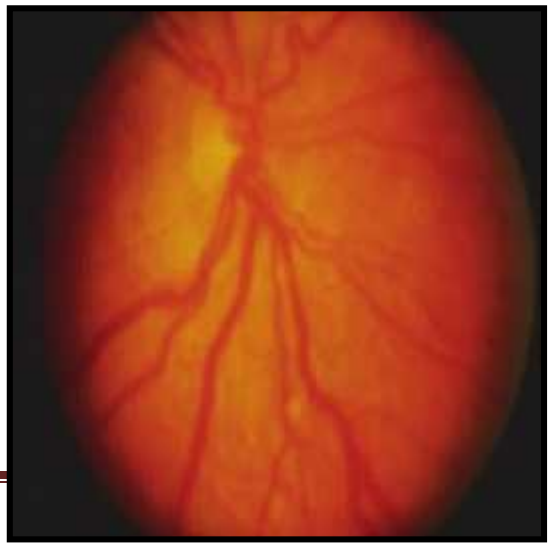
Stages of ROP (contd)



Photograph 6: Stage 4 ROP – Partial RD- Exudative and Tractional



Photograph 7: Stage 5 ROP – Open Funnel RD



Photograph 8: ROP – APROP

Photograph 9: Plus disease

Stage 1- A demarcation line appears as a thin white line that separates the normal retina from the undeveloped avascular retina .

Stage 2- A ridge of scar tissue with height and width replaces the line of stage 1. It extends inward from the plane of the retina.

Stage 3- The ridge has extra retinal fibro-vascular proliferation. Abnormal blood vessels and fibrous tissue develop on the edge of the ridge and extend into the vitreous.

Stage 4- Partial retinal detachment may result when scar tissue pulls on the retina.

• *Stage 4A* is partial detachment outside the macula, so that the chance for vision is good if the retina reattaches.

• *Stage 4B* is partial detachment that involves the macula, thus limiting the likelihood of usable vision in that eye.

Stage 5- Complete retinal detachment occurs. The retina assumes a funnel shaped appearance and is described as open or narrow in the anterior and posterior regions.

3. Plus disease:

It is an additional designation, which refers to the presence of vascular dilatation and tortuosity of the posterior retinal vessels. This indicates a more severe degree of ROP and may be associated with iris vascular engorgement, pupillary rigidity, and vitreous haze. Plus disease, that is associated with zone 1 ROP is termed rush disease; this type of ROP tends to progress extremely rapidly.

4. Extent - refers to the circumferential location of the disease and is reported as clock hours in the appropriate zone.

5. Pre-plus disease - Vascular abnormalities of the posterior pole that are insufficient for the diagnosis of plus disease, but that demonstrate more arterial tortuosity and more venous dilatation than normal.

B. Definition of threshold and prethreshold ROP:

- **Threshold ROP** is present if five or more contiguous or eight cumulative clock hours (30-degree sectors) of stage 3 with plus disease in either zone 1 or 2 are present. This is the level of severity at which the risk of blindness is predicted to approach 50% and thus treatment is recommended.

- **Prethreshold ROP** is any of the following : zone 1 ROP of any stage less than threshold ; zone 2 ROP with stage 2 and plus disease; zone 2 ROP with stage 3 without plus disease; or zone 2 ROP at stage 3 with plus disease with fewer than the threshold number of sectors of stage 3. Infants with prethreshold ROP have a 1 in 3 chance of needing surgical treatment

and a 1 in 6 chance of extreme loss of vision if treatment is not done promptly when threshold is reached. With therapy, they have a 1 in 12 chance of extreme visual loss[35].

Aggressive posterior ROP (AP-ROP):

It is a rapidly progressing, severe form of ROP. If untreated, it usually progresses to stage 5 ROP. The characteristic features of this type of ROP are its posterior location, prominence of plus disease, and the ill-defined nature of the retinopathy. This may not have classical ridge or extraretinal fibrovascular proliferation. This rapidly progressing retinopathy has been referred previously as "type II ROP" and "Rush disease". Observed most commonly in Zone I, but may also occur in posterior Zone II.

DIAGNOSIS

Screening Window:

Progression of ROP follows a distinct time-table according to the post-menstrual age of the baby. Hardly any ROP is detected before 32 weeks of gestation. The median age for detection of stage 1 ROP is 34 weeks. Pre-threshold ROP appears at 36 weeks of post-menstrual age and threshold disease at 37 weeks. Vascularisation is complete by 40 weeks of gestation. Thus the crucial period for detection of ROP is from 32 weeks to 40 weeks of post-menstrual period. The critical phase is from; 34-35 weeks to 37-38 weeks age during which, the progression of the disease takes place and treatment may have to be instituted.

It may also be noted that, ROP usually does not develop before 2 weeks of postnatal age.

Babies to screen:

Selecting neonates for screening depends on incidence of ROP at different gestation ages. Based on current incidence and risk factors reported in Indian literature, following group of neonates should be screened.

- Babies with birth weight <1500 g
- Babies born at ≤32 weeks of gestation
- Selected preterm infants with a birth weight between, 1500 and 2000 g or gestational age of more than 32 weeks, with sickness requiring cardiorespiratory support, prolonged oxygen therapy, apnoea of prematurity, anaemia needing blood transfusion and neonatal sepsis or believed by their attending paediatrician or neonatologist to be at high risk. This 'third criterion' is important as it brings in many larger babies into the screening guidelines, without raising the screening parameters[90].

First screening - should be carried out at 31 weeks of gestation or 4 weeks of age, whichever is later.

Follow up:

Follow-up examinations are done based on the retinal findings.

- 1-week or less follow-up
    - Stage 1 or 2 ROP: Zone I
    - Stage 3 ROP: Zone II

- 1- to 2-week follow-up
    - Immature vascularisation: Zone I - no ROP
    - Stage 2 ROP: Zone II
    - Regressing ROP: Zone I
- 2-week follow-up
    - Stage 1 ROP: Zone II
    - Regressing ROP: Zone II
- 2- to 3-week follow-up
    - Immature vascularisation: Zone II—no ROP
    - Stage 1 or 2 ROP: Zone III
    - Regressing ROP: Zone III

Findings that suggest further examinations are not needed include:

- Zone III retinal vascularisation attained without previous Zone I or II ROP
- Full retinal vascularisation
- Postmenstrual age of 45 weeks and no prethreshold disease (defined as stage 3 ROP in Zone II, any ROP in Zone I) or worse ROP is present
- Regression of ROP

SCREENING[91]:

Neonates are best examined in the neonatal unit itself under supervision of attending pediatrician. Pupils are dilated with Phenylephrine 2.5% and Tropicamide 0.5%. One drop of Tropicamide is instilled every 10-15 minutes, for 4 times starting 1 hour before the scheduled time for examination. This is followed by Phenylephrine, one drop just before examination. Phenylephrine is available in 10% concentration; it should be diluted 4 times before use in neonates. Repeated instillation of phenylephrine is avoided for the fear of hypertension.

Screening of ROP involves, indirect ophthalmoscopy using 20 D or 28/30 D lens by an experienced ophthalmologist. After instilling a topical anesthetic drop like proparacaine, a wire speculum is inserted to keep the eye-lids apart. First, the anterior segment of the eye is examined to look for tunica vasculosa-lentis, pupillary dilation, and lens / media clarity; followed by the posterior pole, to look for plus disease; followed by, sequential examination of all clock hours of the peripheral retina. A scleral depressor is often used to indent the eye externally, to examine areas of interest, rotate and stabilize the eye.

Ophthalmological notes should be made after each ROP examination, detailing zone, stage and extent in terms of clock hours, of any ROP and the presence of any pre-plus or plus disease. These notes should include a recommendation for the timing of the next examination (if any) and be kept with the baby's medical record.

ROP screening examinations can have short-term effects on blood pressure, heart rate and respiratory function in the premature baby[92].The examinations should be kept as short as possible and precautions should be taken to ensure that emergency situations can be dealt with promptly and effectively.

Eye examination during screening lasts several minutes and may cause considerable pain to the neonate. A systematic review and meta-analysis comprising four studies have reported that, oral sucrose reduces pain during eye examination[92]. Of two studies reporting the role of topical proparacaine drops, one has observed significant pain reduction. Discomfort to the baby should be minimized by administering oral sucrose just before examination, pretreatment of the eyes with a topical proparacaine and swaddling the baby. Baby should not have been fed just before examination, to avoid vomiting and aspiration. Hand washing should be done and asepsis maintained.

RETCAM:

A wide-field digital camera (RetCam) capable of retinal imaging in preterm infants, has been evaluated as an alternative to indirect ophthalmoscopy for screening. Retinal images taken by camera can be stored, transmitted to expert, reviewed, analyzed and sequentially compared over time and are useful for telemedicine purposes. Studies comparing RetCam with the indirect ophthalmoscope, have reported variable sensitivity and good specificity[93].

However, due to high cost and due to limitations in diagnostic sensitivity, specificity, and accuracy when image quality is poor, it is not recommended to replace bedside ophthalmoscopic examination. Digital fundus images can be used as a useful adjunct to conventional bedside ROP screening by indirect ophthalmoscopy.

Treatment

Early Treatment of Retinopathy of Prematurity (ETROP) trial recruited neonates at 26 centres in the US and compared early treatment of high-risk prethreshold disease with conventional threshold treatment[94]. Four hundred and one babies meeting the criteria for 'high-risk' of an unfavourable outcome with prethreshold in at least one eye were randomized to receive either early or conventional treatment. The level of risk was determined by a risk analysis programme which used, among other factors, degree of ROP (stage, zone and presence of plus), rate of ROP progression, birth weight, gestational age and ethnicity to classify eyes as, either 'high-risk' (i.e. 15% chance) or ≥ 'low-risk' (<15% chance) of an unfavourable outcome without treatment. The results showed, an overall significant benefit for the early treatment of eyes with high-risk prethreshold disease. Based on results of ETROP, two new terminologies have been suggested:

Type 1 ROP:

- Zone I, any stage ROP with plus disease
- Zone I, stage 3 ROP with or without plus disease
- Zone II, stage 2 or 3 ROP with plus disease

Type 2 ROP:

- Zone I, stage 1 or 2 ROP without plus disease
- Zone II, stage 3 ROP without plus disease

Peripheral retinal ablation should be carried out for all cases with type 1 ROP and continued serial examinations are advised for type 2 ROP.

Treatment modalities :

Peripheral retinal ablation of avascular retina anterior to the ridge can be done by either cryotherapy or diode laser. Diode laser ablation has replaced cryotherapy ,due to lower rate of postoperative ocular and systemic complications and less damage to the adjacent tissues compared to cryotherapy. Other advantages are that, the laser spots are visible during treatment, minimizing the risk of missing areas requiring treatment and that, laser equipment is portable allowing use outside of the operating theatre. The procedure can be carried out under general anesthesia or under sedation, depending on the feasibility and expertise. Treatment for ROP should include, the entire avascular retina anterior to the ridge, with burn spacing between 0.5 to 1 burn-widths apart.

CRYOTHERAPY:

Cryotherapy is an ablative procedure used in severe active ROP. It is aimed at, destroying avascular peripheral retina in order to stop the rapidly growing vessels that are presumably being driven to grow by an angiogenic factor released by the peripheral avascular retina. The cryotherapy for Retinopathy of

Prematurity (Cryo-ROP) is a major landmark study in the battle against ROP. The Cryo-ROP study[53] recommended cryotherapy for threshold ROP, defined as 5 contiguous or 8 cumulative clock-hours of stage 3 plus ROP in zone I and zone II. The technique of cryotherapy involves using the cryo probe to create contiguous cryo marks on the avascular retina. Treatments are performed under continuous monitoring of heart rate, respiratory rate, blood pressure and oxygen saturation. General endotracheal tube anaesthesia is preferred. Cryotherapy is administered with a cryotherapy probe, such as a hammer head-shaped pediatric probe. Continuous cryotherapy is performed under direct observation of the fundus, avoiding over treatment and re treatment. After the cryo treatment, patients can be discharged home on a topical steroid, cycloplegic and antibiotic on the same day, if not anaesthetized[95].

Laser Photocoagulation:

The use of indirect laser via an ophthalmoscope, for retinal photocoagulation in the treatment of ROP has been established. McNamara and coworkers[95] conducted a prospective clinical trial, that randomly assigned infants with threshold ROP to cryotherapy versus argon laser photocoagulation and showed that infants treated with laser had less ocular inflammation, fewer systemic complications, and no significant difference in effectiveness as compared with those treated with cryotherapy. In addition, general anesthesia typically was not required for infants treated with laser (27% of infants in the Cryo-ROP study underwent general anesthesia). Some experts in ROP have also advocated earlier intervention with laser therapy in eyes with ROP, especially when there is plus disease in any zone (particularly in zone I) and vitreous haze.

Trans-scleral diode laser photocoagulation has been evaluated for the treatment of threshold ROP and results suggested that, it is as effective in the treatment of threshold ROP as is transpupillary diode laser photocoagulation. Trans - scleral diode laser photocoagulation seems to be an advantageous treatment method, if trans-pupillary treatment bears an increased risk of cataract formation[57].

Pre-anesthetic medication:

Oral feeds should be discontinued 3 hours prior to the procedure. Baby should be started on intravenous fluids, and put on cardio-respiratory monitor. Dilatation of pupil is done by using 0.5% Tropicamide and 2.5% Phenylephrine as described in the section on protocol for screening.

Anesthesia/ Sedation:

Topical anaesthesia alone provides insufficient analgesia for ROP treatment and should not be used. Babies may be treated under adequate sedation and analgesia in an operation theatre, if this can be arranged in a timely way. If shifting to operation theatre is not possible or is causing delay in treatment, babies may be treated more rapidly in the neonatal unit under adequate sedation and analgesia.

Procedure:

Both the eyes can be treated at the same sitting time, unless contraindicated by instability of the baby. If baby is not tolerating the procedure, consider abandoning the procedure for the time being. Vital signs and oxygen saturation should be monitored very closely.

Monitoring after laser therapy:

After laser therapy, first examination should take place 5-7 days after treatment and should be continued at least weekly for signs of decreasing activity and regression. Re-treatment should be performed usually 10-14 days after initial treatment, when there has been a failure of the ROP to regress.

Post-operative care:

The baby should be closely monitored. If condition permits, oral feeds can be started shortly after the procedure. Premature babies, especially those with chronic lung disease may have an increase or re-appearance of apneic episodes, or an increase in oxygen requirement. Therefore they should be carefully monitored for 48-72 hours after the procedure. Antibiotic drops (such as chloramphenicol) should be instilled 6-8 hourly for 2-3 days.

Future therapeutic targets[96]:

The discovery of the importance of VEGF and IGF-1 in the development of ROP is a step forward in our understanding of the pathogenesis of this disease. These studies suggest a number of ways to intervene medically in the disease process. The use of anti-VEGF therapy is the first medical treatment for neovascularisation in age-related macular degeneration and is likely to be useful for proliferative retinopathy. However, prevention of vessel loss will be even more important in the treatment of ROP, since the extent of the second destructive phase of ROP is determined by the amount of vessel loss in the first phase. The finding that, late development of ROP is associated with low levels of IGF-1 after

premature birth suggests that, physiological replacement of IGF-1 to levels found in-utero, might prevent the disease by allowing normal vascular development. In addition, the use of a specific agonist to VEGFR-1, PIGF-1, might be used early in the disease process to prevent vessel loss without promoting proliferative disease.

The current understanding of ROP pathogenesis also makes clear that, timing is critical in any medical intervention, since the two phases of ROP require very different approaches. Inhibition of either VEGF or IGF-1 early after birth can prevent normal blood vessel growth while, at the second phase, might prevent pathological neovascularisation. Similarly, providing VEGF or IGF-1 early on, might promote vessel growth, whereas late supplementation in the neovascular phase could exacerbate the disease. In the fragile neonate, any intervention must be made very carefully to promote normal physiological development of both blood vessels and other tissues.

PREVENTION

Prevention can be subdivided into the prevention of premature birth, eliminating ROP at the source; optimizing neonatal care, eliminating ROP by facilitating normal physiologic maturation; and preventing or minimizing ROP itself. The statistics on premature birth are not encouraging. Public health success would provide the greatest social, economic, and medical benefit but unfortunately is often not well funded. Maximizing neonatal care means mimicking the in-utero environment as much as possible. Unfortunately that eludes our technology. Neonatal care is still primarily reactive and focused in a piecemeal fashion on organ support.

Judicious oxygen therapy

Oxygen is a drug and it should be administered in a quantity that is absolutely necessary. Each neonatal care unit should have a written policy outlining appropriate use of oxygen therapy. If a preterm neonate born at < 32 weeks gestation needs resuscitation at birth, inhaled oxygen concentration (FiO2) should be titrated to prevent hyperoxia and achieve gradual increase in oxygen saturation (70% at 3 minute and 80% at 5 minute after birth)[97]. During acute care of a sick preterm neonate, ROP is more likely to develop if partial pressure of oxygen in arterial blood is more than 80 mm Hg[98]. Oxygen level in blood should be continuously monitored using pulse oximeter. It has been observed that, if oxygen saturation in a baby on oxygen therapy is kept between 85% and 93%, in about 90% samples partial pressure of oxygen is in desirable range (40 to 80 mm Hg)[99]. Various observational studies have reported that, incidence and severity of ROP is lowered if oxygen saturation targets are kept in desirable range and if units implement written policies regarding oxygen administration and monitoring[100,101].During recovery phase of respiratory illness in preterm neonates, targeting higher oxygen saturations has been associated with increased incidence/ severity of bronchopulmonary dysplasia without any benefit in stopping progression of ROP or improving growth and development[102,103].

Judicious use of blood transfusions

Transfusion of packed RBCs is another risk factor of ROP. Adult RBCs are rich in 2, 3 DPG and adult Hb binds less firmly to oxygen, thus releasing excess oxygen to the retinal tissue. Packed cell transfusions should be given when haematocrit falls below following ranges: ventilated babies 40%, babies with cardio-pulmonary disease but not on ventilators 35%, sick neonates but not having cardiopulmonary manifestations 30%, symptomatic anemia 25% and asymptomatic anemia 20%.

Vitamin E Supplementation

Very low birth weight neonates should receive 15-25 IU of vitamin E daily as supplement. However, higher doses given by intravenous route have been associated with, increased risk of neonatal sepsis[104].

Prenatal steroids

Use of prenatal steroids, is a well-known approach to prevent respiratory distress and intraventricular haemorrhage - the two important risk factors of ROP. Although there are some concerns that, prenatal steroids may induce ROP, this is not borne out by other studies. We believe prenatal steroids prevent acute illnesses in premature babies and should be administered to all mothers with preterm labor between 24-34 weeks of gestation. The preferred preparation of steroids for prenatal used is betamethasone in two doses of 12 mg each, given intramuscularly, 24 hours apart.

Bevacizumab

Intravitreal injection of bevacizumab, a neutralizing anti-VEGF molecule has been demonstrated to diminish the neovascular response significantly in animal models[105]. However, due to uncertainties with respect to the dosing, frequency, timing, and adjunct therapies to be used and potential to cause serious systemic adverse effects, use of bevacizumab is not recommended outside the scope of clinical trial.

METHODOLOGY

Source of Data: All preterm infants born with a birth weight of ≤1500 grams and/or ≤ 32 weeks of gestation at KLE'S Dr.Prabhakar Kore Hospital and Medical Research Centre, Belgaum .

Method of collection of Data:

A data collection instrument was used in which data was collected

1. by interview of parents
2. Hospital records
3. Examination of the infant

STUDY DESIGN:

Longitudinal study with a short follow-up

Study period:

One year and three months -1st January 2011 to 31st March2012.

Sample size: Sample size for incidence with specified confidence level and specified relative precision

$$n = [z\alpha / d] 2$$

z = specified confidence level (95% i.e. = 1.96)

d = relative precision (20%)

$$n = 97 \approx 100$$

Sample size = 100

Selection criteria:

Inclusion criteria

- Premature infants born ≤ 32 weeks and/or birth weight ≤1500 grams.
- Babies between 1501-2000grams and/or 33-35weeks who are at a higher risk of developing ROP like

a) Respiratory Distress Syndrome

b) Sepsis

c) Multiple blood transfusions

d) Multiple births

e) Apnoeic Episodes

f) Intraventricular haemorrhage

Exclusion criteria

- Babies from whom consent for the study could not be obtained.
- Babies who died before full vascularisation of retina.
- Babies who did not complete the follow up protocol for other reasons were excluded.

Consent and ethical clearance:

Informed/ written consent of parents was taken after explaining in detail about the methods and procedures involved in the study in their own vernacular language. Ethical clearance was obtained.

Definition of risk factors

Apnoea:

It is defined as a cessation of respiration for > 20 sec. which required resuscitation with bag and mask, with 100% O2.

Sepsis:

It was diagnosed by clinical picture, changes in leukocyte count, elevated CRP(C- reactive protein) and positive blood culture.

RDS (Respiratory Distress Syndrome):

Presence of at least two of the following criteria:

- Respiratory rate > 60/min in a quiet and resting baby.
- Sub costal/ intercostals space recessions.
- Expiratory grunt.

Place of screening:

All eligible babies were screened at Neonatal Intensive Care Unit.

Preparation of the child:

The pupils were dilated with a mixture of Phenylephrine 2.5% and Tropicamide 0.5% instilled 3 times at 10mins interval about 1 hour before the scheduled examination. Resistance to dilation was noted. Care was taken to wipe off any eye drops with sterile cotton that come out of eyes to cheeks and not to feed the baby immediately before examination as the child might vomit or aspirate.

Instruments used:

- Cordless Indirect ophthalmoscope with 20D lens.
- Pediatric wire speculum.
- Scleral indentor.

First examination: The following time table was used for screening

Table 2: Timing of First Screening Eye Examination Based on Gestational Age at Birth[106]

Gestation age at birth (weeks),	Age at initial examination (Postmenstrual age)	Chronological age
22	31	9
23	31	8
24	31	7
25	31	6
26	31	5
27	31	4
28	32	4
29	33	4
30	34	4
31	35	4
32	36	4

First screening examination was carried out at 31 weeks of gestation or 4 weeks of age, whichever was later. For this purpose, gestational age was calculated from the last menstrual period.

Follow up protocol:

If no ROP was detected at initial examination, the infants were re-evaluated once every two weeks until vascularisation was complete. If ROP was detected, the examinations were performed weekly for stage 1-2 disease and more frequently for stage 3 disease, till the disease started resolving or progressed to threshold stage. Babies showing evidence of regression were followed up till vascularisation was complete. Babies progressing to threshold stage were referred.

Procedure:

All preterm babies who satisfied any one of the inclusion criteria were taken up for the study.

The babies were enrolled into the study at birth. Parents were explained the nature of the examination and informed consent was taken. Demographic history and risk factors like respiratory distress syndrome, sepsis, multiple blood transfusions, multiple births, apneic episodes and oxygen given was documented using a data collection instrument.

First examination was done at 4 weeks post natal age (age in weeks after birth) by taking all aseptic precautions in a temperature controlled room in the presence of a neonatologist.

Indirect ophthalmoscopic examination was done .One drop of topical paracaine eyedrops was used to anaesthetise the cornea. A pediatric wire speculum was used to keep the eyelids apart .After decreasing the room

illumination the anterior segment was first visualized to look for tunica vasculosa lentis, pupillary dilatation and lens and media clarity. Then the posterior pole was examined for any Plus disease. A scleral indenter was used to visualize the periphery. The periphery was examined in all clock hours to look for the extent of changes from nasal to temporal retina. Care was taken not to put too much pressure on the globe. During examination, untoward neonatal complications were looked for and managed appropriately.

The changes in the retina were graded according to the International Committee for the Classification of Retinopathy of Prematurity (ICROP) guidelines-2005[15].

Method of Statistical Analysis:

The following methods of statistical analysis have been used in this study.

1. The results for each parameter (numbers and percentages) for discrete data and average (mean + standard deviation) for continuous data are presented in Tables and Figures. The proportions were compared using Chi-square test of significance.

Chi-Square ($\chi 2$) test for (2 x 2 tables)

GROUP	Attribute Characteristic finding		
	ABSENT	PRESENT	TOTAL
Group 1	A	B	a+b
Group 2	C	D	c+d
Total	a+c	b+d	N

a,b,c,d are the observed numbers.

N is the Grand Total

χ^2 with 1 DF $\frac{N(ad-bc)^2}{(a+b)(c+d)(a+c)(b+d)}$

DF=(r-1)*(c-1), where r=rows and c=columns

DF= Degrees of Freedom (Number of observation that are free to vary after certain restriction have been placed on the data)

2. Student "t' test.

The student't' test was used to determine whether there was a statistical difference between male and female subjects in the parameters measured.

Student's t test is as follows:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{s\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \quad \text{---- } t_{n1+n2-2} \quad \text{Where } s^2 = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{(n_1 + n_2 - 2)}$$

3. The association between potential related risk factors with ROP and without ROP were studied initially through an Univariate analysis. The categorical variables were assessed using Pearson chi-square and Yates correction applied where needed. Odds Ratio (OR) and 95% Confidence Interval (CI) was calculated. To estimate the independent effect of the factors that were significantly associated with ROP and without ROP the confounding effect they may have on each other, logistic regression analysis was done. The variables were included if their respective univariate analysis yielded P <0.10. A backward stepwise elimination procedure based on the likelihood statistics (using removal probability of 0.10 and considering the change in classification accuracy) was also performed to identify the best subset of variables.

In the entire test, the "*p*" value of less than 0.05 was accepted as indicating statistical significance. Data analysis was carried out using Statistical Package for Social Science (SPSS - 20) package.

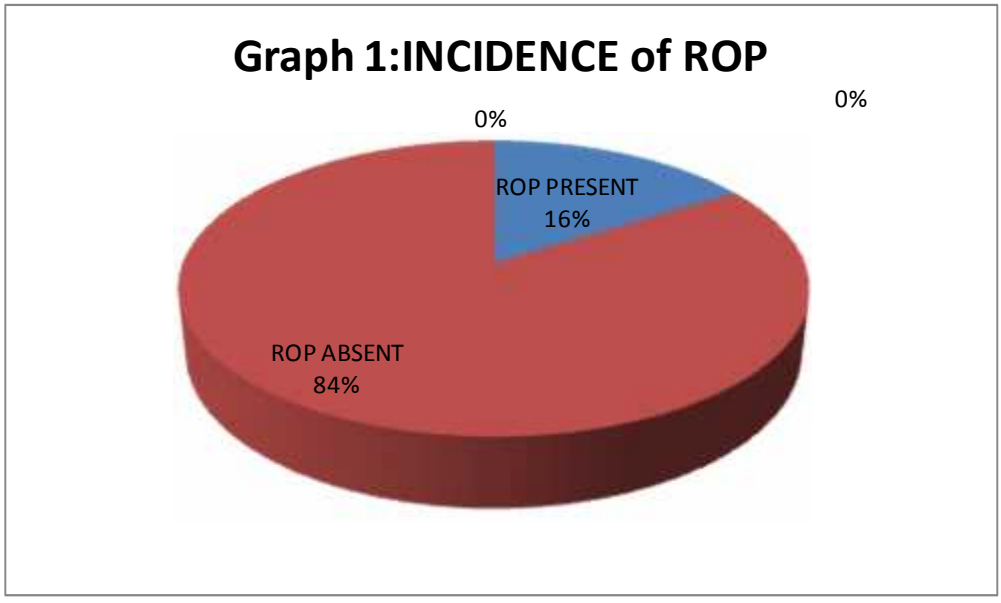
RESULTS

INCIDENCE

100 babies who fulfilled the inclusion criteria were screened and 16 babies were *found* to have ROP. The incidence of ROP in this study is 16%.

Table 3: Incidence of ROP

ROP	No.	%
PRESENT	16	16%
ABSENT	84	84%
TOTAL	100	100

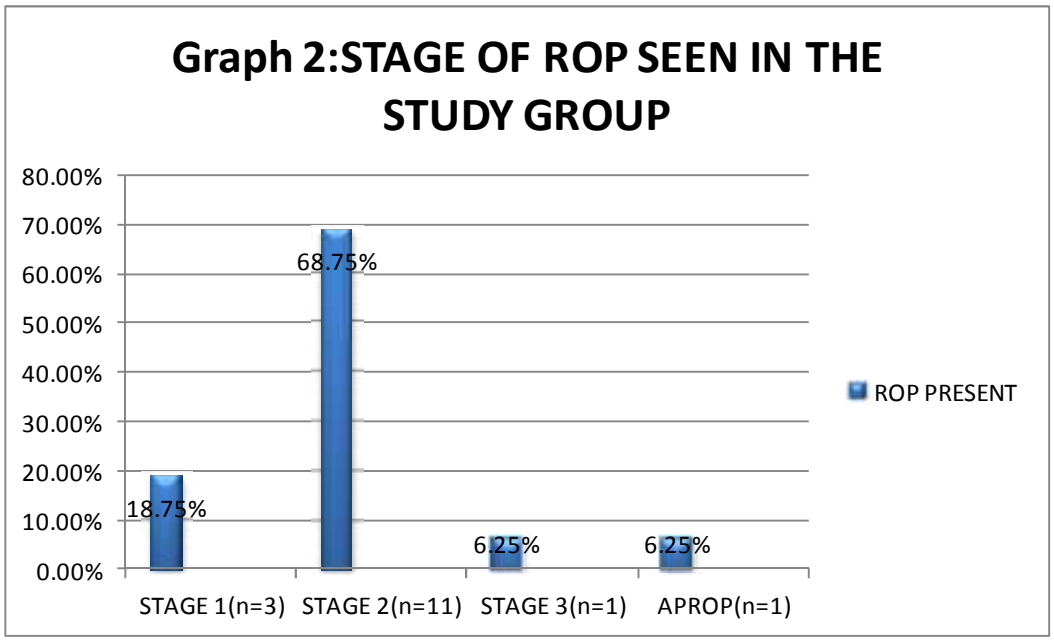


STAGES OF ROP:

Out of 16 babies with ROP, only 3 babies(18.75%) were in stage 1, 11 babies(68.75%) were in stage 2,1 baby(6.25%) was in stage3 and 1 baby(6.25%) developed APROP.

Table 4: Stages of ROP

	RETINOPATHY OF PREMATURITY STAGES				
	1	2	3	APROP	TOTAL
ROP PRESENT	3	11	1	1	16
%	18.75%	68.75%	6.25%	6.25%	100%



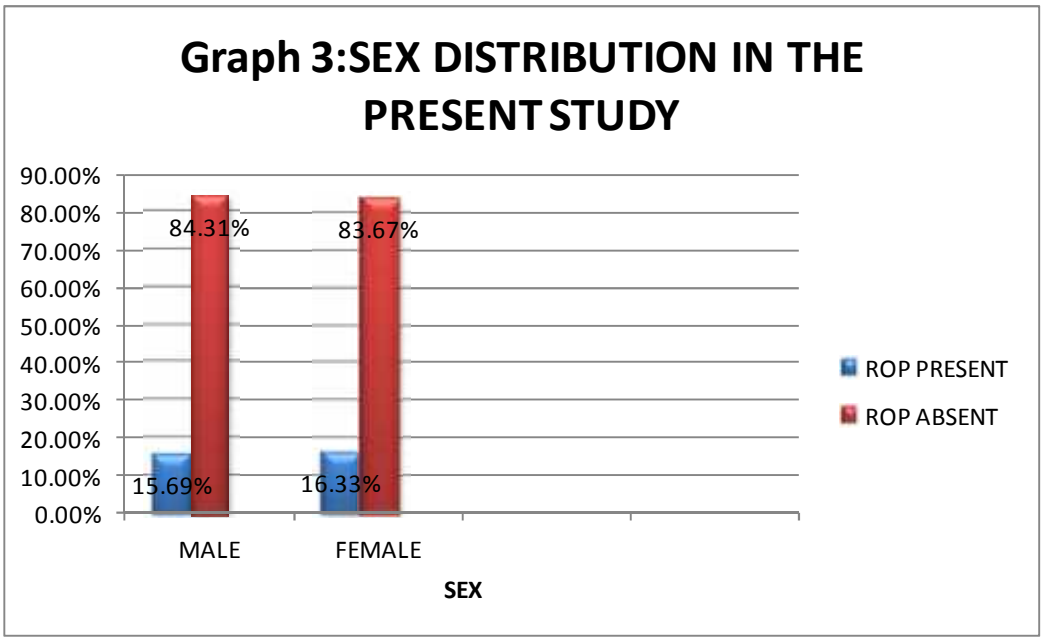
SEX DISTRIBUTION:

Out of 100 babies screened, 51 were male and 49 were female. Among 51 male babies 8 (15.69%) developed ROP and out of 49 female babies 8 (16.33%) had ROP. In this study gender *did not* significantly influence the incidence (p=0.928) of ROP.

Table 5: Sex distribution of ROP babies

	SEX and ROP			
		ROP		
		PRESENT	ABSENT	TOTAL
SEX	MALE	8(15.69%)	43(84.31%)	51
	FEMALE	8(16.33%)	41(83.67%)	49
	TOTAL	16	84	100

P value =0.928



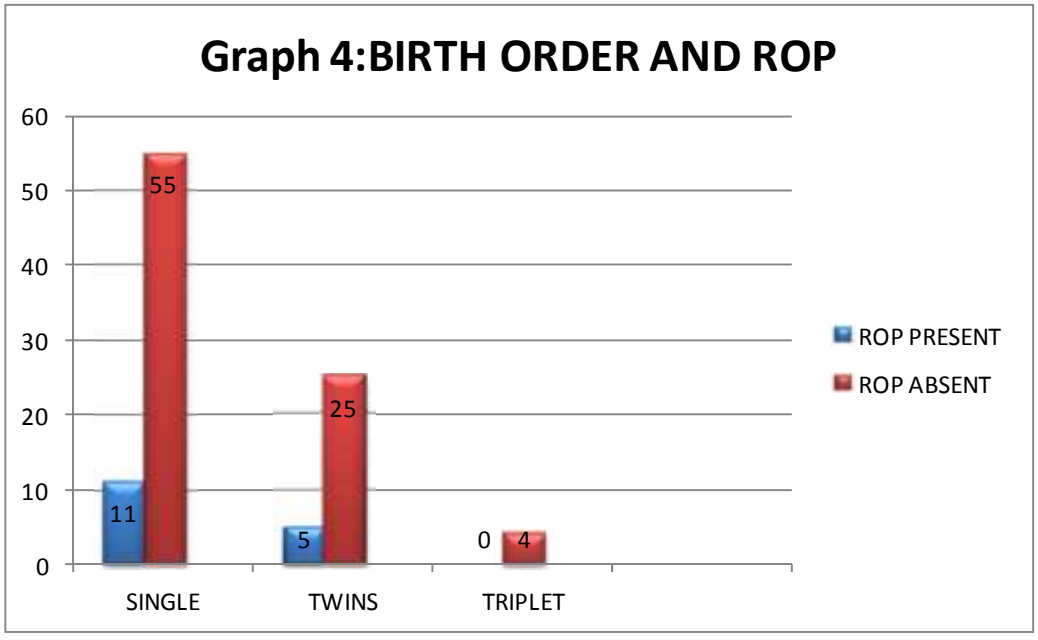
BIRTH ORDER:

Out of 100 babies, 66 babies were singletons, 30 babies were twins and 4 babies were triplets. Out of 66 singletons, 11 babies developed ROP. Only 5 of the 30 twins developed ROP and no triplet developed ROP. Birth order was *not found* to be significantly associated (p = 0.971) with ROP in the present study.

Table 6: Birth order and ROP

	BIRTH ORDER AND ROP			
		ROP		
		PRESENT	ABSENT	TOTAL
BIRTH ORDER	SINGLE	11	55	66
	TWIN	5	25	30
	TRIPLET	0	4	4
	TOTAL	16	84	100

P value = 0.971



BIRTH WEIGHT AND ROP:

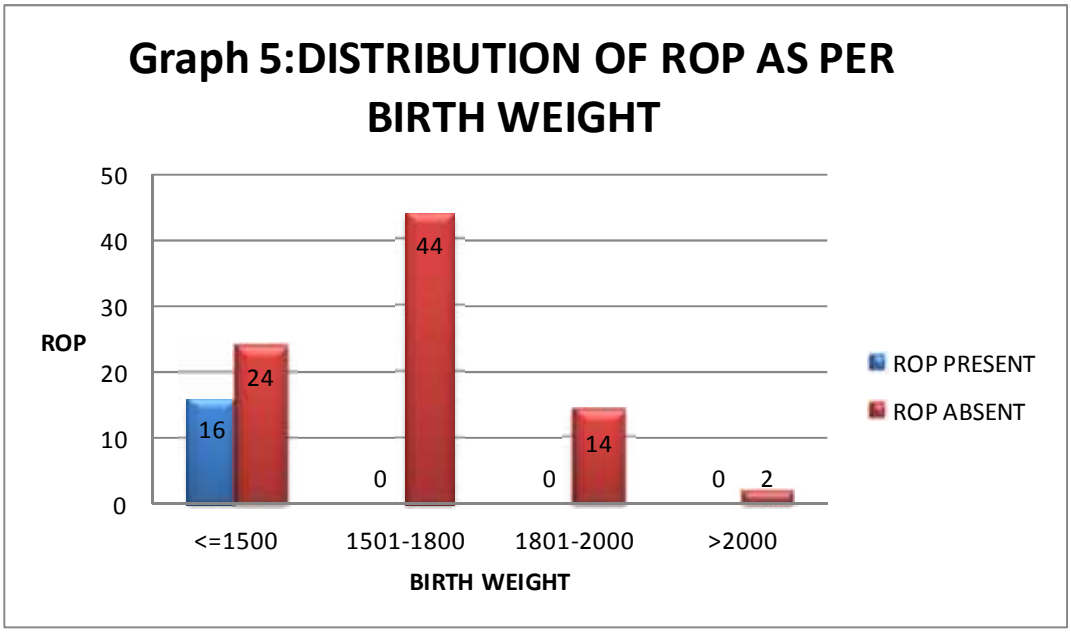
The birth weight of the ROP babies ranged from 1000-1500 gm (mean 1272.81 ± 143.67 gm), while that of non-ROP babies ranged from 1100-2100 gm (mean 1642.80 ± 216.60 gm). Lower birth weight was *significantly associated* with increased incidence (p = <0.001) of ROP. The incidence of ROP was 40% in babies weighing ≤ 1500gm at birth.

Table 7: Distribution of ROP as per birth weight-1

	DISTRIBUTION AS PER BIRTH WEIGHT			
		ROP		
		PRESENT	ABSENT	TOTAL
BIRTH WEIGHT	<=1500	16	24	40
	1501-1800	0	44	44
	1801-2000	0	14	14
	>2000	0	2	2
	TOTAL	16	84	100

Table 8: Distribution of ROP as per birth weight-2

ROP	N	MEAN	STD DEVIATION	BT.WT. RANGE		T VALUE	P VALUE
Present	16	1272.81	143.67	1000	1500	6.328	<0.001
Absent	83	1642.80	216.60	1100	2100		


Graph 5:DISTRIBUTION OF ROP AS PER BIRTH WEIGHT
50
40
30
20
10
0
ROP
16
24
0
44
0
14
0
2
<=1500
1501-1800
1801-2000
>2000
BIRTH WEIGHT
ROP PRESENT
ROP ABSENT

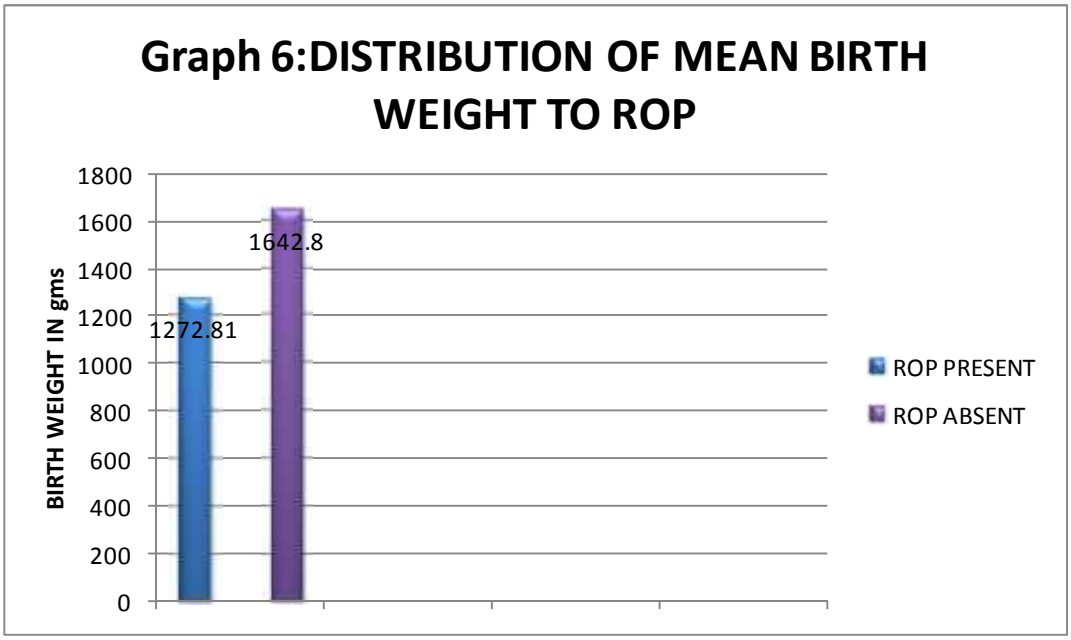
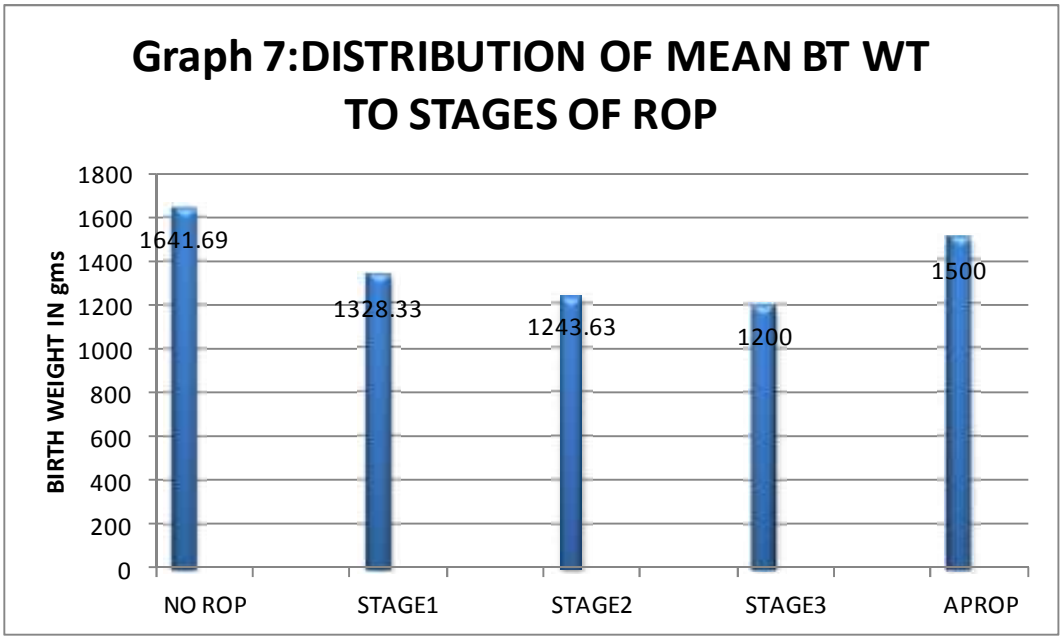

Graph 6:DISTRIBUTION OF MEAN BIRTH WEIGHT TO ROP
1800
1600
1400
1200
1000
800
600
400
200
0
BIRTH WEIGHT IN gms
1642.8
1272.81
ROP PRESENT
ROP ABSENT

Table 9: Distribution of birth weight and stage of ROP

STAGE OF ROP	BIRTH WEIGHT				
	NO	MEAN	S.D	MIN	MAX
NO ROP	84	1641.69	215.53	1100	2100
STAGE 1	3	1328.33	106.80	1250	1450
STAGE2	11	1243.63	145.34	1000	1500
STAGE3	1	1200	--------	1200	1200
APROP	1	1500	--------	1500	1500



GESTATIONAL AGE AND ROP:

The gestational age of the ROP babies ranged from 28 -34 weeks (mean 31.38 ±1.63 weeks), while that of non-ROP babies ranged from 30-38 weeks (mean 33.31 ± 1.74 weeks). The incidence of ROP was 26.32% in babies born ≤ 32 weeks of gestational age. Gestational age was *found to be a significant* risk factor for the development of ROP (*p*=0.004).

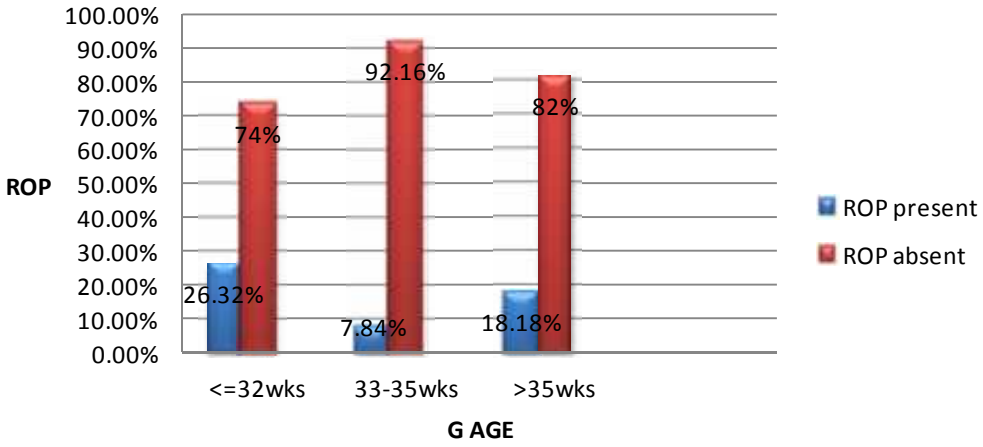
Table 10: Distribution of gestational age and ROP-1

	DISTRIBUTION OF GESTATIONAL AGE			
		ROP		
		PRESENT	ABSENT	TOTAL
GESTATIONAL AGE	<=32	10(26.32%)	28(73.68%)	38
	33-35	4(7.84%)	47(92.16%)	51
	>35	2(18.18%)	9(81.82%)	11
	TOTAL	16	84	100

Table 11: Distribution of gestational age and ROP-2

	ROP	NO	MEAN	S.D	GEST.AGE		T VALUE	P VALUE
					MIN	MAX		
	PRESENT	16	31.38	1.628	28.00	34.00	3.351	0.004
	ABSENT	84	33.31	1.742	30.00	38.00		

Graph 8:FREQUENCY OF ROP TO GESTATIONAL AGE



Graph 9:DISTRIBUTION OF MEAN GESTATIONAL AGE TO ROP

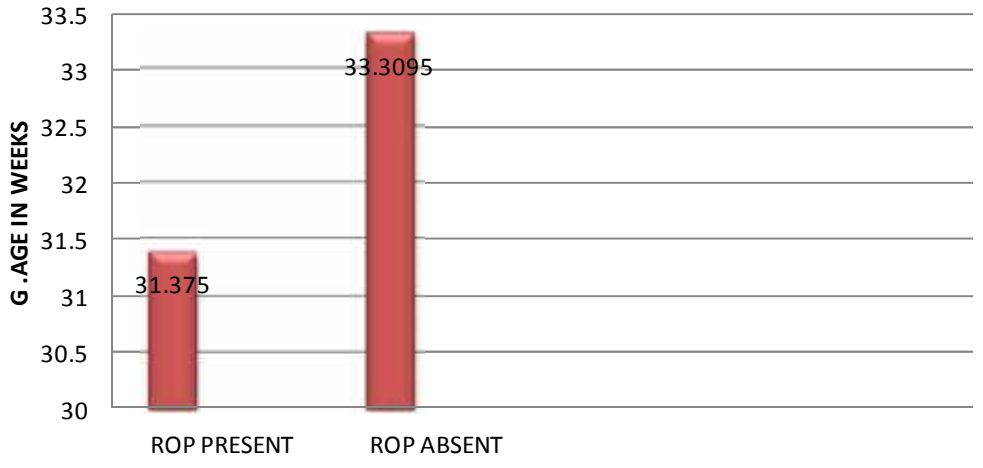
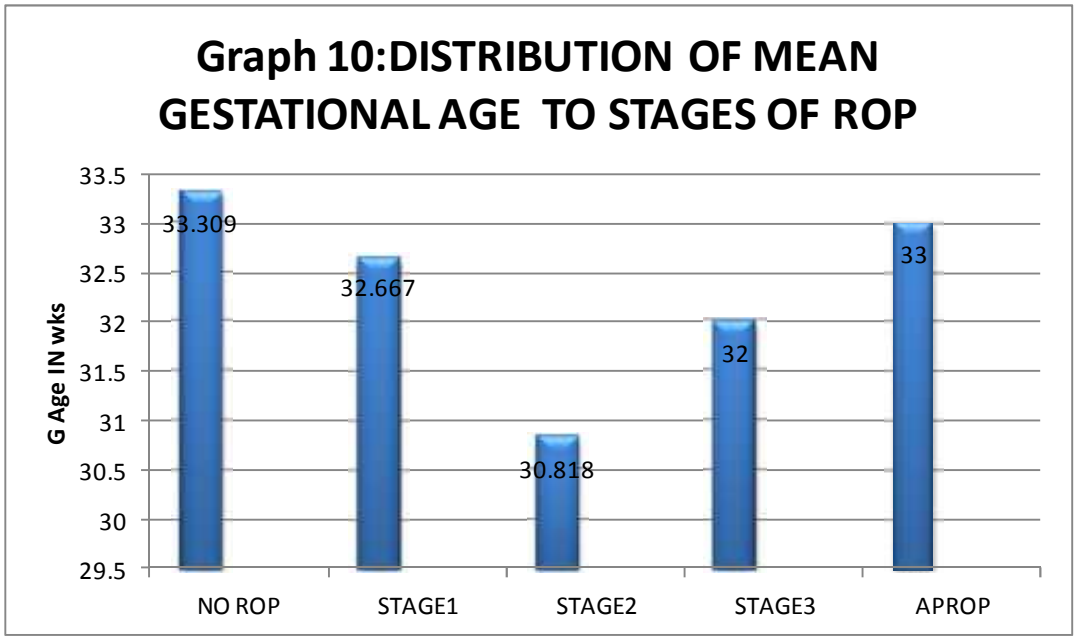


Table 12: Distribution of gestational age to stage of ROP

Stage of ROP	GESTATIONAL AGE				
	No.	Mean	S.D.	Min	Max
NO ROP	84	33.31	1.742	30.00	38.00
STAGE1	3	32.67	0.577	32.00	33.00
STAGE2	11	30.818	1.662	28.00	34.00
STAGE3	1	32.00	-	32.00	32.00
APROP	1	33.00	-	33.00	33.00



POST CONCEPTIONAL AGE AT FIRST EXAMINATION AND ROP:

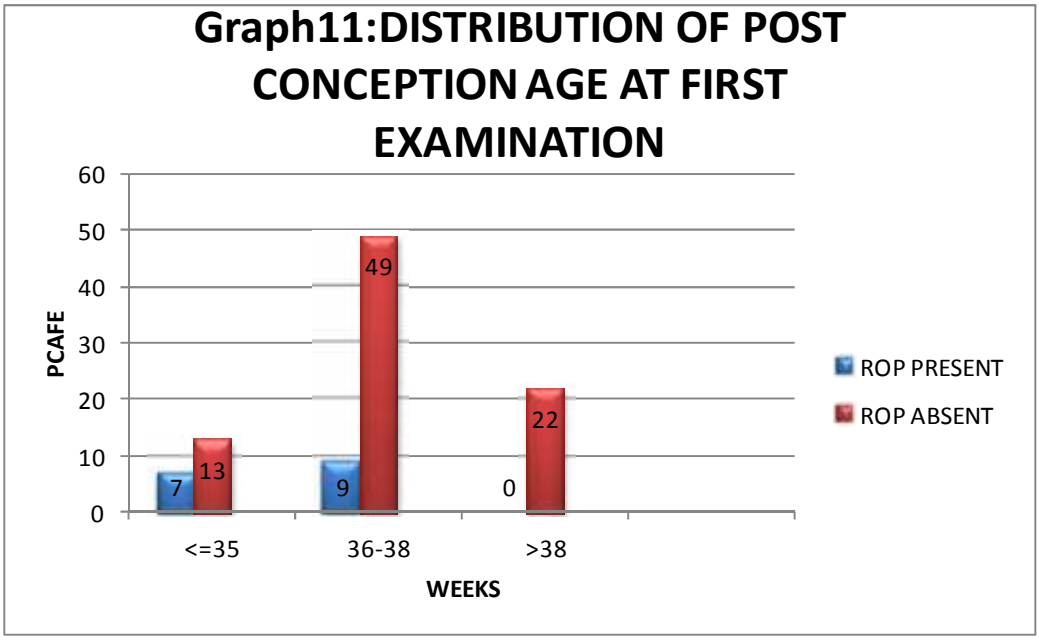
Post Conceptional Age at First Examination among ROP babies ranged from 33 – 38 weeks (mean 35.69 ± 1.58 weeks), while that of non-ROP babies ranged from 34 - 41weeks (mean 37.38 ± 1.78 weeks). Early examination *was significantly* associated with chances of early detection of ROP ($p = 0.016$).

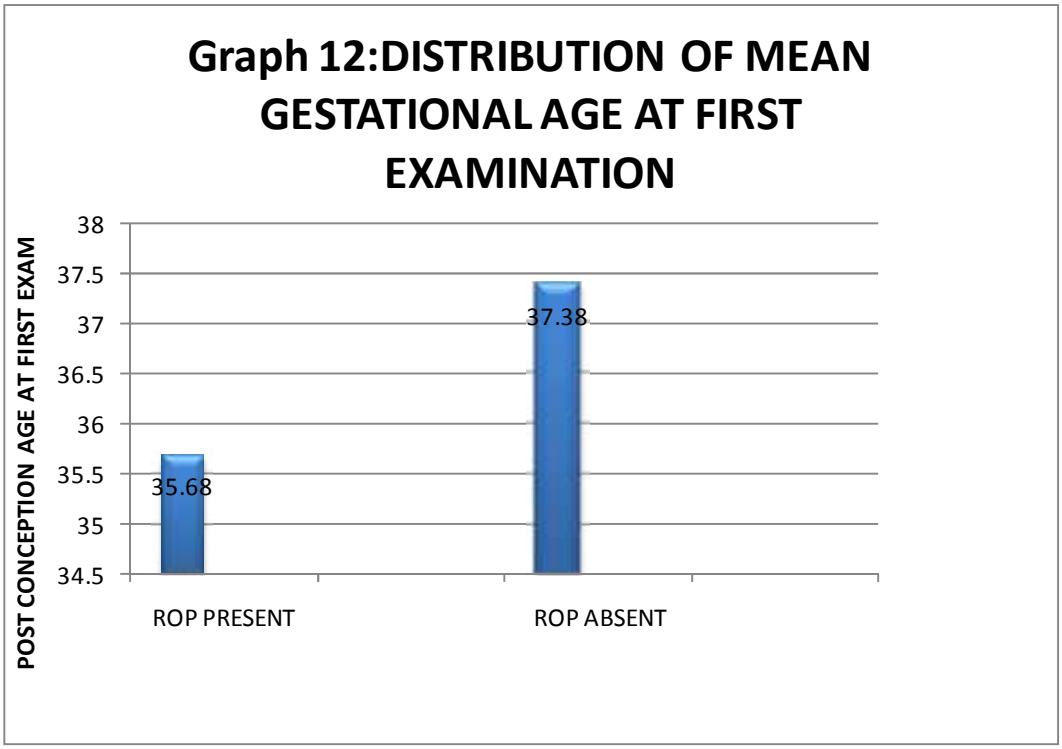
Table 13: Post conceptional age at first examination and ROP-1

		ROP		
	wks	PRESENT	ABSENT	TOTAL
POST CONCEPTIONAL AGE	<=35	7	13	20
	36 - 38	9	49	58
	>38	0	22	22
	TOTAL	16	84	100

Table 14: Post conceptional age at first examination and ROP-2

ROP	No	Mean	S.D	POST CONC. AGE		t value	p value
				MIN	MAX		
Present	16	35.687	1.579	33	38	2.724	0.016
Absent	84	37.381	1.782	34	41		


Graph11:DISTRIBUTION OF POST CONCEPTION AGE AT FIRST EXAMINATION
60
50
40
30
20
10
0
PCAFE
7
13
9
49
0
22
<=35
36-38
>38
WEEKS
ROP PRESENT
ROP ABSENT


Graph 12:DISTRIBUTION OF MEAN GESTATIONAL AGE AT FIRST EXAMINATION
38
37.5
37
36.5
36
35.5
35
34.5
POST CONCEPTION AGE AT FIRST EXAM
35.68
37.38
ROP PRESENT
ROP ABSENT

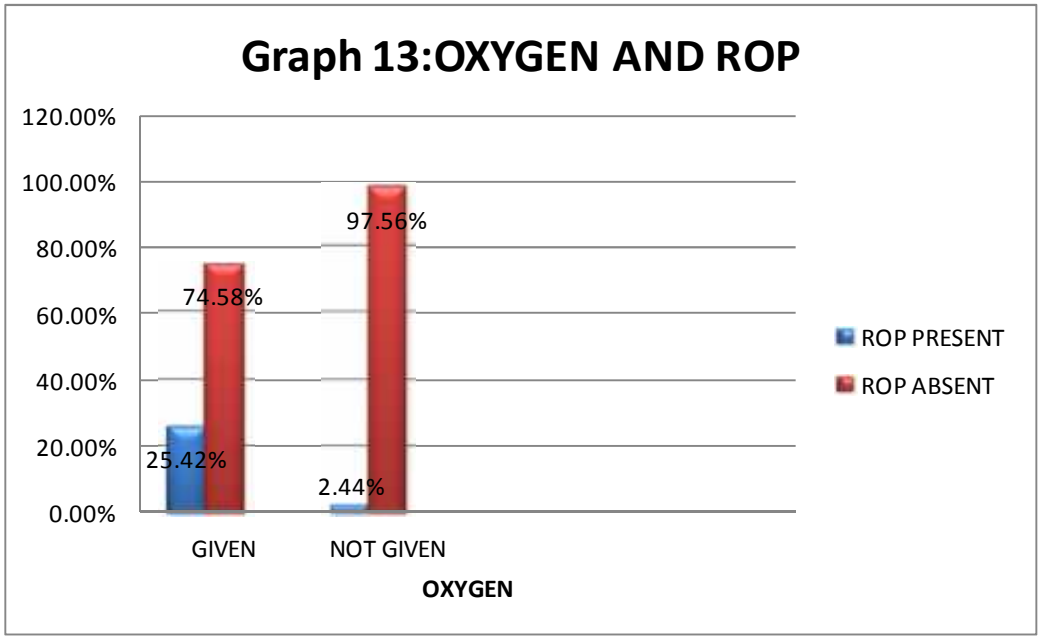
OXYGEN AND ROP:

Out of 100 babies screened 59 were given O2 and 15 (25.42%) babies developed ROP. Oxygen administration *was a significant risk factor* for the development of ROP (p = 0.005).

Table 15: OXYGEN AND ROP

	OXYGEN AND ROP		
		ROP	
OXYGEN		Present	Absent	Total
	Given	15(25.42%)	44(74.58%)	59
	Not Given	1(2.44%)	40(97.56%)	41
	Total	16	84	100

Yates chi- square = 7.785 Yates p value = 0.005 df=1



RESPIRATORY DISTRESS SYNDROME AND ROP:

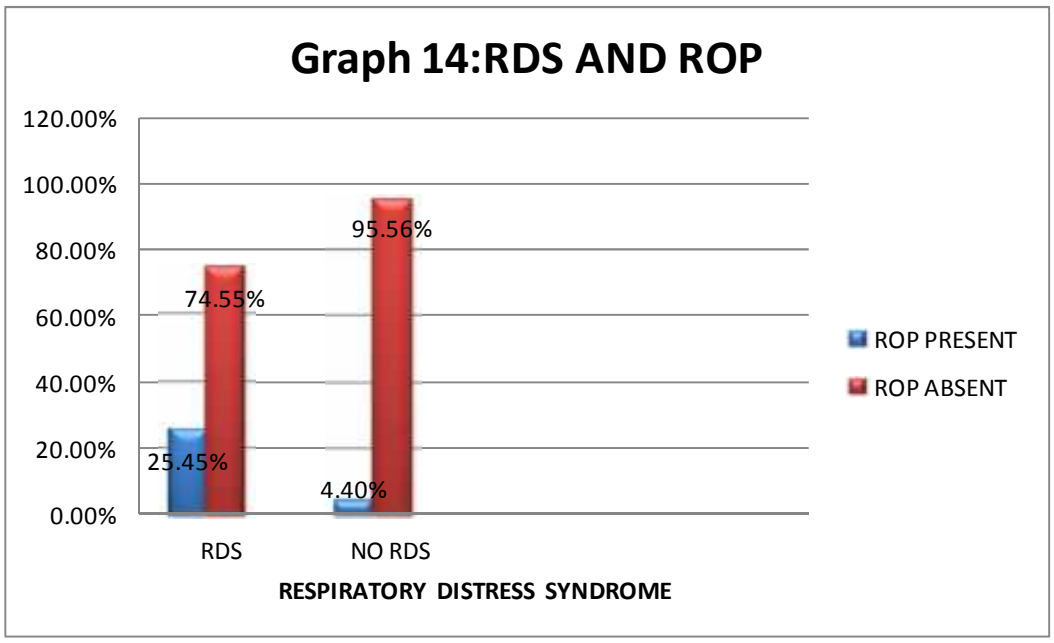
Out of 100 babies screened 55 had RDS and 14 (25.45%) babies developed ROP.

RDS *was a significant risk factor* for the development of ROP (p = 0.009).

Table 16: RESPIRATORY DISTRESS SYNDROME AND ROP

	RESPIRATORY DISTRESS SYNDROME AND ROP			
		ROP		
		Present	Absent	Total
RDS	Present	14 (25.45%)	41 (74.55%)	55
	Absent	2 (4.44%)	43 (95.56%)	45
	Total	16	84	100

Yates chi square = 6.641 Yates p value = 0.009



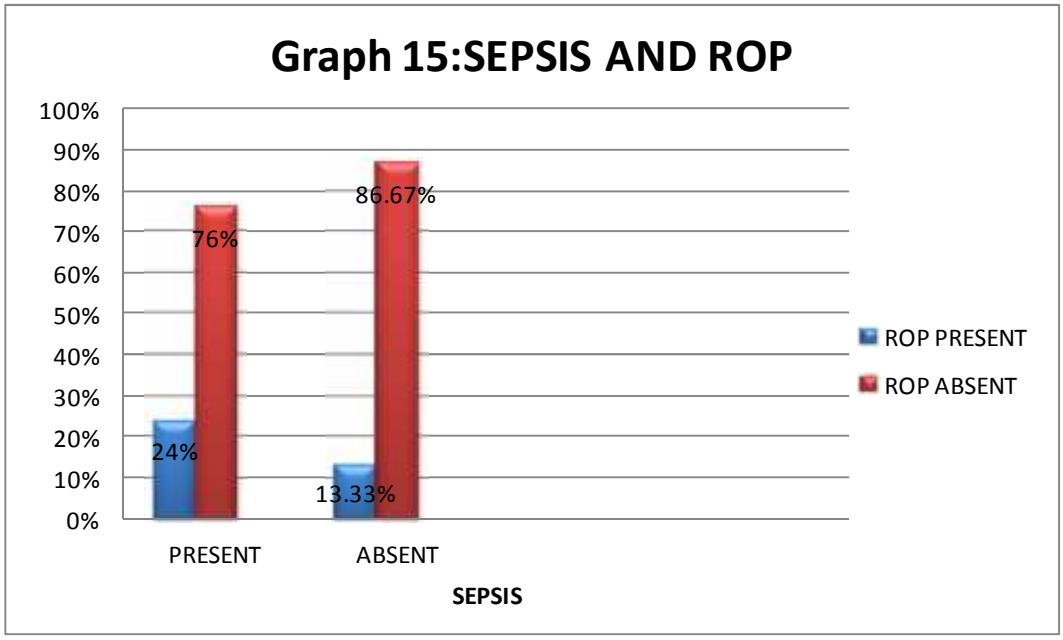
SEPSIS AND ROP:

Out of 100 babies screened, 25 had sepsis and 6(24%) developed ROP. Sepsis was *not a significant risk factor* for the development of ROP in this study (*p*=0.345).

Table 17: SEPSIS AND ROP

	SEPSIS AND ROP			
		ROP		
		Present	Absent	Total
SEPSIS	Present	6(24%)	19(76%)	25
	Absent	10(13.33%)	65(86.67%)	75
	Total	16	84	100

Yates chi square = 0.893 Yates *p* value = 0.345



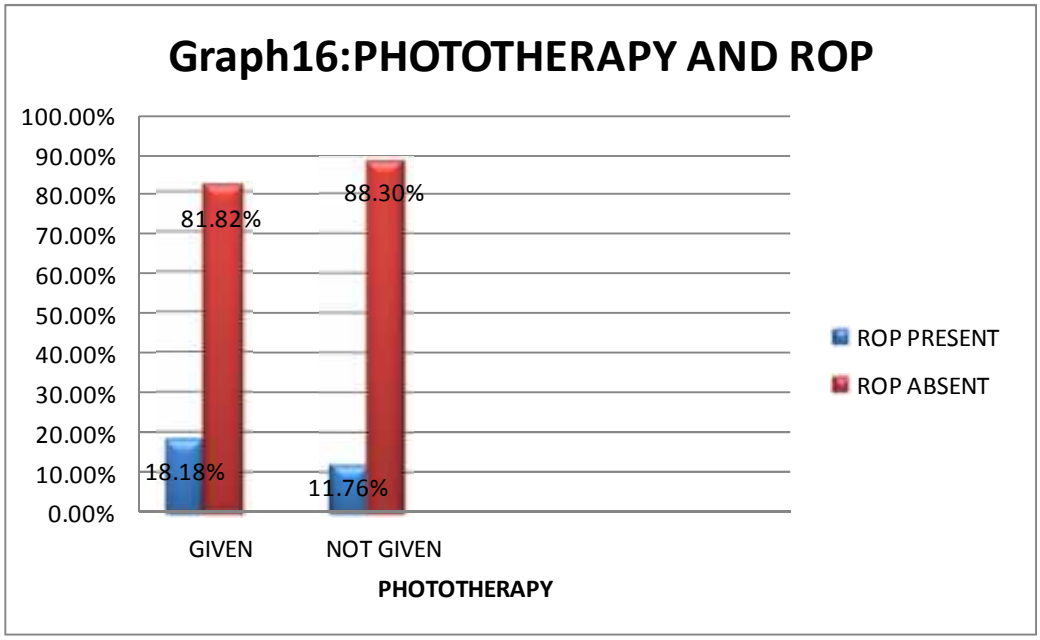
PHOTOTHERAPY AND ROP:

Out of 100 babies screened, 66 had been given phototherapy and 12(18.18%) developed ROP. Phototherapy was *not a significant factor* for the development of ROP (p = 0.588).

Table 18: PHOTOTHERAPY AND ROP

	PHOTOTHERAPY AND ROP			
		ROP		
		PRESENT	ABSENT	TOTAL
PHOTOTHERAPY	GIVEN	12(18.18%)	54(81.82%)	66
	NOT GIVEN	4(11.76%)	30(88.30%)	34
	TOTAL	16	84	100

Yates chi square = 0.293 Yates p value = 0.588



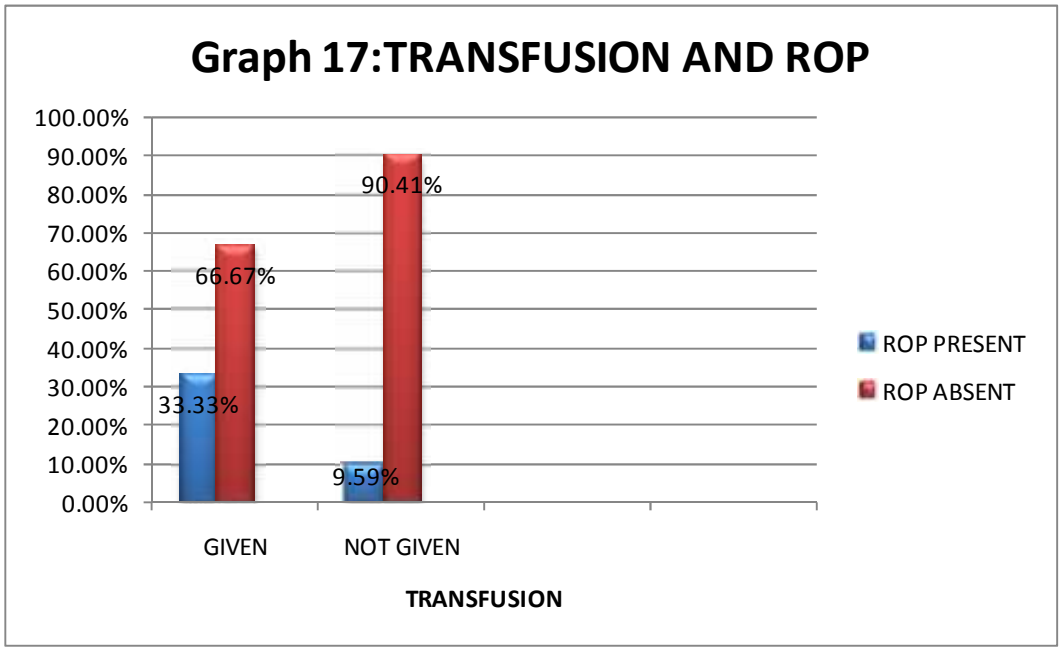
TRANSFUSION (FFP/ Packed cell/ Platelet):

Out of 100 babies screened, 27 were given transfusion (either FFP / Packed cell) and 9(33.33%) developed ROP. Transfusion *was found* to be a significant risk factor for the development of ROP in this study (p=0.004)

Table 19: TRANSFUSION AND ROP

	TRANSFUSION AND ROP			
		ROP		
		PRESENT	ABSENT	TOTAL
TRANSFUSION	GIVEN	9(33.33%)	18(66.67%)	27
	NOT GIVEN	7(9.59%)	66(90.41%)	73
	TOTAL	16	84	100

Chi square = 8.268 p value = 0.004



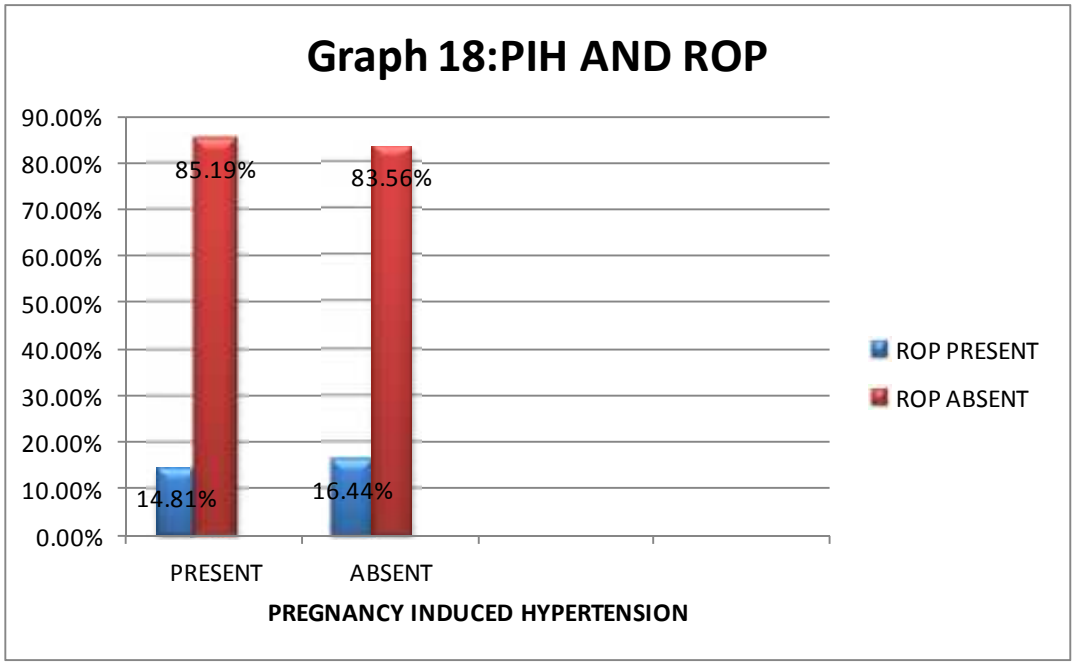
PREGNANCY INDUCED HYPERTENSION AND ROP:

Out of 100 babies screened, mother's of 27 babies had PIH and 4(14.81%) developed ROP. PIH was *not found* to be a significant risk factor for the development of ROP in this study (p=0.913).

Table 20: PREGNANCY INDUCED HYPERTENSION AND ROP

	PREGNANCY INDUCED HYPERTENSION AND ROP			
		ROP		
		PRESENT	ABSENT	TOTAL
PIH	PRESENT	4(14.81%)	23(85.19%)	27
	ABSENT	12(16.44%)	61(83.56%)	73
		16	84	100

Yates Chi Square = 0.012 Yates p value = 0.913



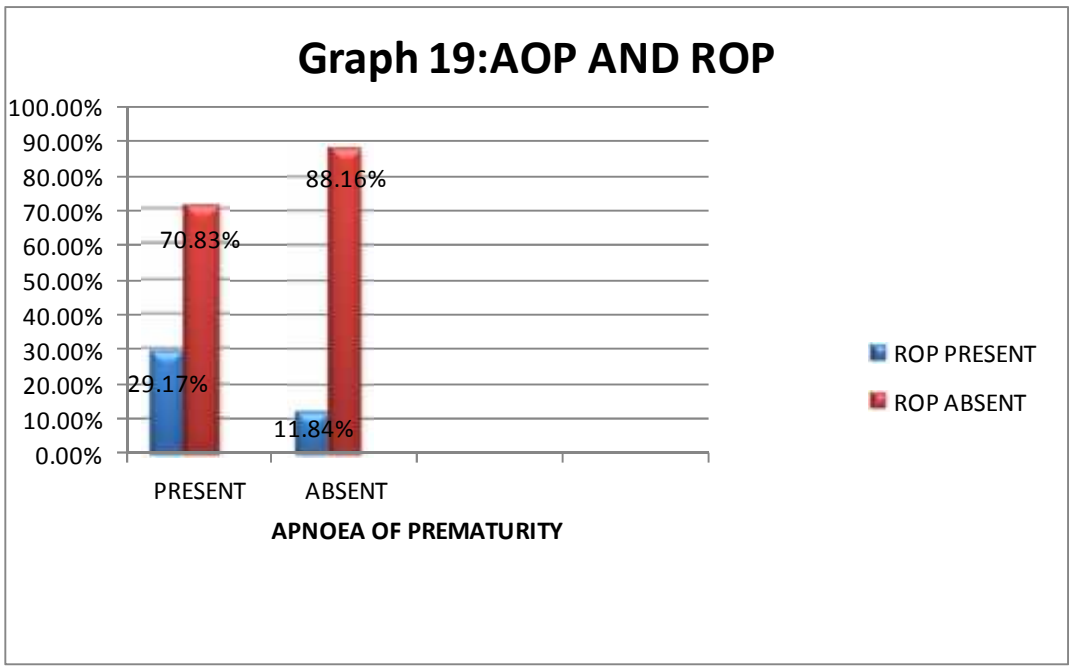
APNOEA OF PREMATURITY AND ROP:

Out of 100 babies screened, 24 babies had AOP and 7(29.17%) developed ROP. AOP *was found* to be a significant factor for the development of ROP in this study (*p*=0.044).

Table 21: APNOEA OF PREMATURITY AND ROP

	APNOEA OF PREMATURITY AND ROP			
		ROP		
		PRESENT	ABSENT	TOTAL
AOP	PRESENT	7(29.17)	17(70.83%)	24
	ABSENT	9(11.84%)	67(88.16%)	76
	TOTAL	16	84	100

Chi Square = 4.073 *p* = 0.044



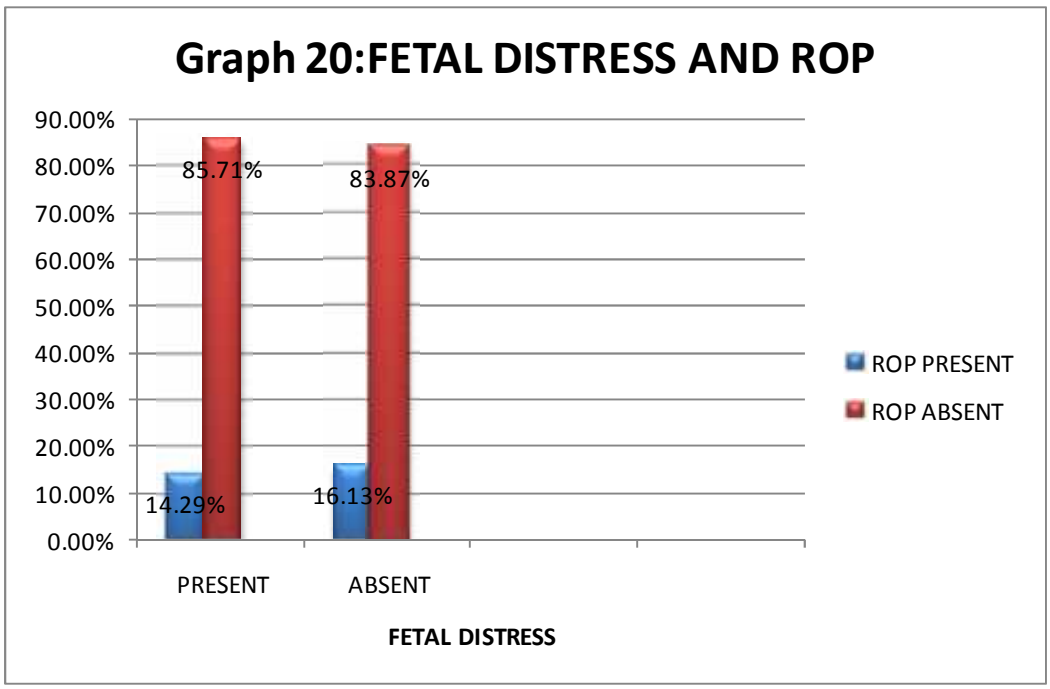
FETAL DISTRESS AND ROP:

Out of 100 babies screened, 7 had Fetal distress and only 1(14.29%) baby developed ROP. Fetal distress was *not found* to be a significant risk factor in the study (p=0.684).

Table 22: FETAL DISTRESS AND ROP

	FETAL DISTRESS AND ROP			
		ROP		
		PRESENT	ABSENT	TOTAL
FETAL DISTRESS	PRESENT	1(14.29%)	6(85.71%)	7
	ABSENT	15(16.13%)	78(83.87%)	93
	TOTAL	16	84	100

Yates Chi square = 0.165 Yates p value = 0.684



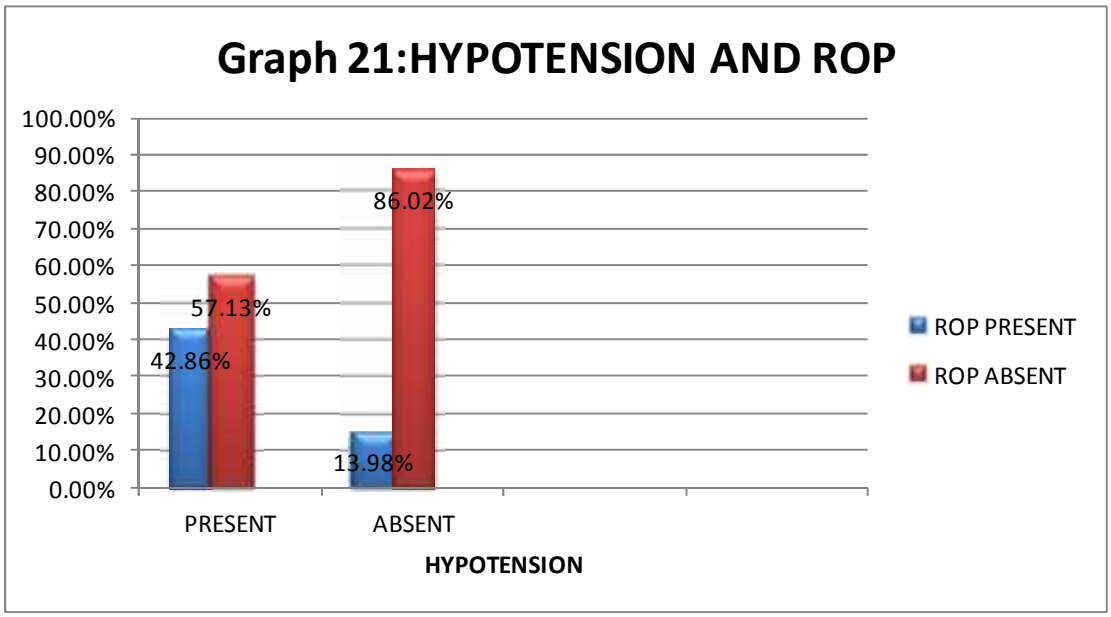
HYPOTENSION AND ROP:

Out of 100 babies screened, 7 babies had hypotension and 3 (42.86%) developed ROP. Hypotension was *not found* to be a significant risk factor for the development of ROP (p=0.140)

Table 23: HYPOTENSION AND ROP

	HYPOTENSION AND ROP			
		ROP		
		PRESENT	ABSENT	TOTAL
HYPOTENSION	PRESENT	3(42.86%)	4(57.13%)	7
	ABSENT	13(13.98%)	80(86.02%)	93
	TOTAL	16	84	

Yates Chi Square = 2.177 Yates p value = 0.140



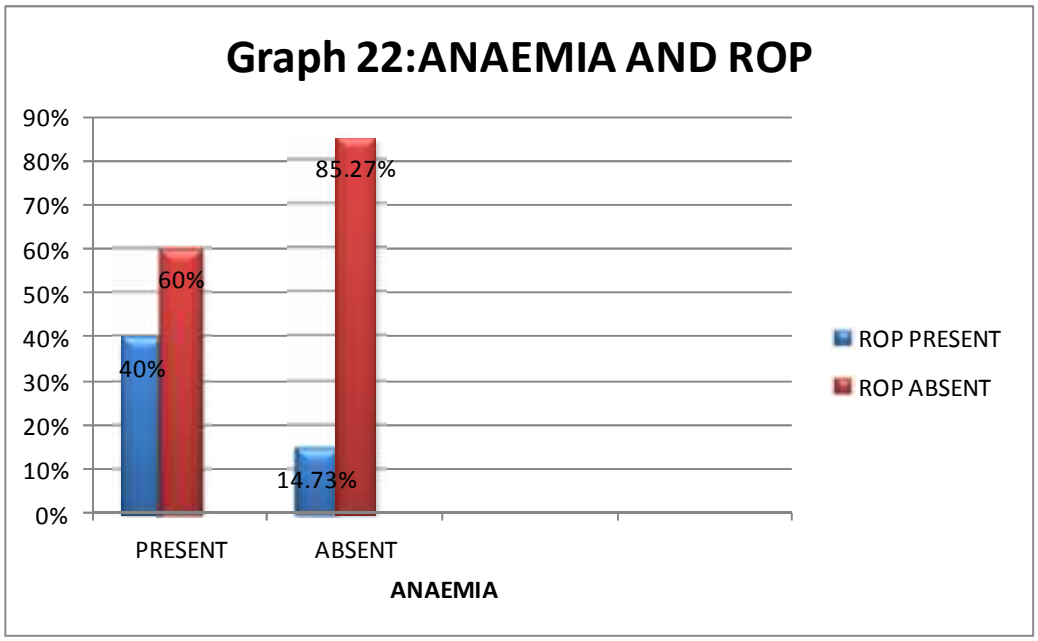
ANAEMIA AND ROP:

Out of 100 babies screened, 5 babies were found to be anaemic and 2(40%) developed ROP. Anaemia was *not found* to be a significant risk factor for the development of ROP (p=0.381).

Table 24: ANAEMIA AND ROP

	ANAEMIA VS ROP			
		ROP		
		PRESENT	ABSENT	TOTAL
ANAEMIA	PRESENT	2(40%)	3(60%)	5
	ABSENT	14(14.73%)	81(85.27%)	95
	TOTAL	16	84	100

Yates Chi square = 0.768 Yates p value = 0.381



METABOLIC ACIDOSIS AND ROP:

Out of 100 babies screened, 2 had metabolic acidosis and only 1(50%) developed ROP. Metabolic acidosis was *not found* to be a significant risk factor for the development of ROP (p = 0.726).

Table 25: METABOLIC ACIDOSIS AND ROP

	METABOLIC ACIDOSIS AND ROP			
		ROP		
		PRESENT	ABSENT	TOTAL
METABOLIC ACIDOSIS	PRESENT	1(50%)	1(50%)	2
	ABSENT	15(15.31%)	83(84.69%)	98
	TOTAL	16	84	100

Yates Chi Square = 0.123 Yates p value = 0.726

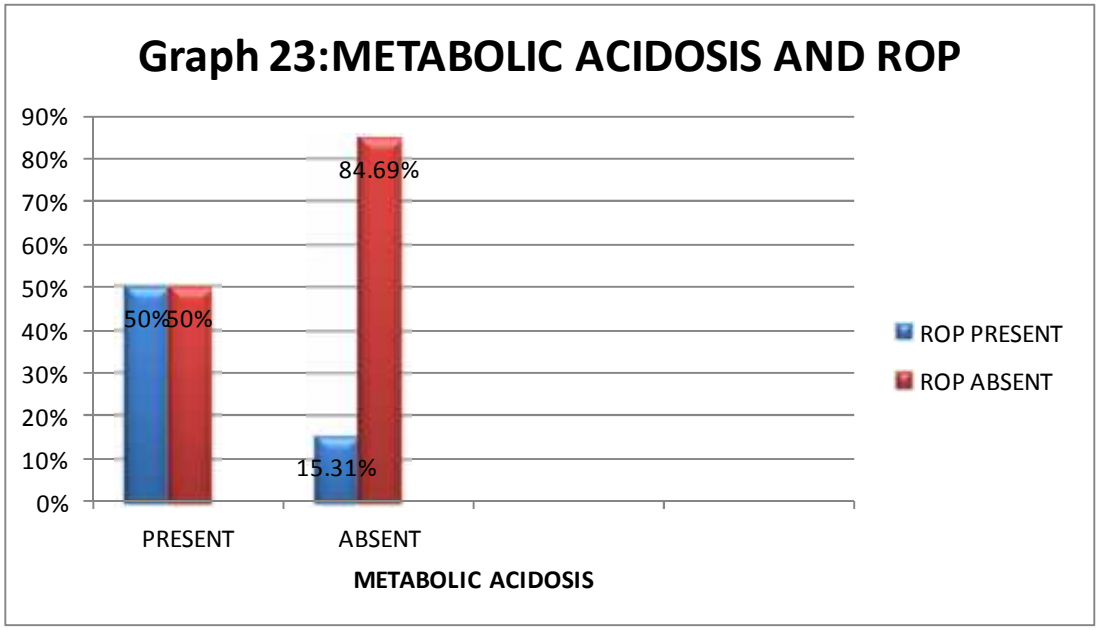


Table 26:Univariate analysis of risk factors

Gestational age	$X^2_2 = 5.572$	$p = 0.062$
Birth weight	$X^2_2 = 5.572$	$p = 0.062$
Oxygen	$X^2_1 = 9.509$	$p = 0.002$
Respiratory-distress syndrome	$X^2_1 = 8.129$	$p = 0.004$
Sepsis	$X^2_{YC} = 0.893$	$p = 0.345$
Phototherapy	$X^2_1 = 0.688$	$p = 0.407$
Sex	$X^2_1 = 0.008$	$p = 0.930$
Birth order	$X^2_2 = 0.294$	$p = 0.672$
Transfusion	$X^2_{YC} = 6.596$	$p = 0.010$
PIH	$X^2_{YC} = 0$	$p = 1$
Apnoea	$X^2_{YC} = 2.886$	$p = 0.089$
Foetal Distress	$X^2_{YC} = 0$	$p = 1$
Hypotension	$X^2_{YC} = 2.177$	$p = 0.140$
Hypertension	Fisher Exact	$p = 1$
Anaemia	Fisher Exact	$p = 0.180$
Metabolic acidosis	Fisher Exact	$p = 0.296$

By univariate analysis, risk factors associated with ROP are oxygen, RDS & transfusion (FFP/Platelet/Packed cell).

Gestational age category, birth weight category and apnoea can also be included in multivariate analysis as $p < 0.1$.

Table 27: Multivariate analysis of risk factors

Risk factors	Adjusted Odds ratio	*p* value	95% Confidence Interval
OXYGEN	11.342	0.070	0.82 – 156.8
RDS	2.117	0.487	0.25 -17.50
TRANSFUSION	6.005	0.015	1.42 – 25.36
GESTATIONAL AGE-CATEGORY			
≤ 32 WEEKS	1.20	0.864	0.15 – 9.8
32-35 WEEKS	0.41	0.424	0.04 – 3.69
>35 WEEKS	-	-	
BIRTH WEIGHT			
<1500	1.20	0.864	0.15-9.8
1501-1800	0.41	0.424	0.04 – 3.69
1801-2000	-	-	
APNOEA	1.380	0.664	0.32 – 5.89

RDS is found to be an important risk factor for ROP in the present study on multivariate analysis.

Using forward method two risk factors were identified

Table 28: Forward method of risk factors

OXYGEN	OR = 19.046	*p*= 0.007	95% CI	2.22 – 162.71
FFP	OR = 6.812	*p*= 0.003	95%CI	1.94 – 23.885

DISCUSSION

Retinopathy of prematurity (ROP) is a vasoproliferative disorder affecting premature infants. It is one of the most common causes of visual loss in children and can lead to lifelong vision impairment and blindness.

Out of 45 million blind people in the world today, there are about 1.4 million blind children. ROP afflicts over 3,00,000 infants worldwide[2] .In developing countries like India, the incidence of ROP has been reported at 24 – 47 % among high risk preterm infants[3]. It is important not only in terms of economic burden, but in its severe social implication, which is very long in terms of blind years. Among the preventable causes of blindness in children (57%), ROP figures very high in the agenda. Low birth weight and gestational age were found to be the most important risk factors for the development of ROP.

We screened babies admitted to our NICU with birth weight ≤ 1500g and gestation ≤ 32 weeks. Infants with birth weight >1500g and gestation more than 32 weeks were screened only if they had additional risk factors. In an article, Chawla et al[91], have suggested the same screening criteria.

INCIDENCE:

With neonatalogical units equipped with the state of art technological background and highly qualified personnel providing optimum care of extremely immature newborns, ROP incidence is on a rise.

The overall incidence in the present study was found to be 16%, with only one case of severe ROP (APROP). It is of current knowledge that, aggressive posterior ROP seems to occur especially among smaller and more immature neonates .In our study the baby which developed APROP, the birth weight was 1500 grams and gestational age was 33 weeks. In the CRYO-ROP study, the incidence of the disease in a group of premature newborns with a birth weight <1251gms was 65.8% and 81.6% for infants of less than 1000 g birth weight[16]. In the ETROP (multi centric) study done 15 years, later the overall incidence of ROP was found to be 68% in babies with birth weight <1251 grams. The overall incidence of more-severe ROP (prethreshold) was 36.9% among infants with ROP in the ETROP Study, whereas the incidence was 27.1% for patients in the CRYO-ROP Study who developed ROP[17].

A review of literature reveals that, the incidence and severity of ROP increases with decreasing gestational age and birth weight. The incidence of ROP in different studies done outside India was found to be 9.4%-38.9%[18-21].A study from the Indian subcontinent reveals the incidence to be 17.5% - 46%[22-26] comparable to the present study. Patil et al[25] in 1997 reported overall incidence as 17.5% and no severe ROP. They studied 40 babies with <32wk or < 1250gms.

Maheshwari et al[24] in 1996 reported overall incidence as 20% and severe ROP as 7%. They studied 66 babies with <35wk or < 1500gms.Gupta et al[28] in 2003 reported overall incidence as 21.7% and severe ROP as 5%. They studied 60 babies with ≤ 35wk or ≤1500gms. Dutta et al[88] screened 108 babies of ≤32 wk or ≤1700gms and reported overall incidence as 21%.

The incidence of severe form of the disease (Threshold disease) is decreasing. Agarwal and co-workers[11] found a drop from 46 to 21% in their study over a period of 7 years. A Danish study[20] found a statistically significant decrease in incidence of ROP in infants weighing more than 1250gm. Similar observations were also made in a multicentre study in UK[27]. Nair[22] and colleagues, Gupta[28] and co-workers found no cases of ROP in babies weighing more than 1250gm.

TABLE 29: Comparison of incidence of present study with other national and international studies

INCLUSION CRITERIA			
INDIAN STUDIES	G AGE	BT WT	INCIDENCE
1.Rekha[23],1996	≤ 34	<1500	46%
2.Maheshwari[24],1996	<35	<1500	20%
3.Patil[25],1997	<32	<1250	17.5%
4.Agarwal[26],2002	<35	<1500	20%
5.Gupta[28], 2003	≤35	≤1500	21.7%
6.Nair[22],2003	≤32	<1500	25.4%
7.Present study	≤32	≤1500	16%
INTERNATIONAL STUDIES			
1.Hussain[29],1999	<32	<1500	21.3%
2.Fledelius[20],2000	<32	<1500	9.4%
3.Blair[21], 2001	<30	<1250	38%
4.Conarth[30],2004	≤32	<1750	10%
5.Shah[18] ,2005		<1500	29.2%
6.Fortes[31],2007		≤1000	48.9%
		≤1500	18.2%
7.Binkhathlan[32],2008	<36	<2000	56%

Post Conception Age at First Examination:

Post Conception Age at First Examination among ROP babies ranged from 33 – 38 weeks (mean 35.69 ± 1.58 weeks), while that of non-ROP babies ranged from 34 – 41 weeks (mean 37.38 ± 1.78 weeks). Early examination was significantly associated with chances of early detection of ROP (p = 0.016) in the present study.

RISK FACTORS: In our study birth weight, gestational age, oxygen, Respiratory Distress Syndrome, transfusion and apnoea of prematurity were found to be significant risk factors on Chi Square analysis. On univariate analysis, risk factors associated with ROP were oxygen, RDS and transfusion (FFP/Platelet) whereas on multivariate analysis, RDS was found to be an important risk factor. Using the forward method oxygen and transfusion were also found to be significant on multivariate analysis.

Birth Weight and Gestational age:

In our study both low birth weight (p=<0.001) and prematurity (p= 0.004) were found to be significant risk factors for the development of ROP.

The birth weight of the ROP babies ranged from 1000-1500 gm (mean 1272.81 ± 143.67 gm), while that of non-ROP babies ranged from 1100-2100 gm (mean 1642.80 ± 216.60 gm). Lower birth weight was significantly associated with increased incidence (p = <0.001) of ROP. The incidence of ROP was 40% in babies weighing ≤ 1500gm at birth.

The gestational age of the ROP babies ranged from 28 -34 weeks (mean 31.38 ±1.63 weeks), while that of non-ROP babies ranged from 30-38 weeks (mean 33.31 ± 1.74 weeks). The incidence of ROP was 26.32% in babies born ≤ 32 weeks of gestational age. Gestational age was found to be a significant risk factor for the development of ROP (p=0.004) in this study.

Oxygen:

In our study oxygen was found to be significant risk factor for the development of ROP on Chi square analysis (p=0.005), on univariate analysis and also on multivariate analysis. Out of 100 babies screened 59 were given O2 and 15 (25.42%) babies developed ROP. The causal link between ROP and supplemental oxygen has been confirmed by controlled trials and clinical studies[24,107]. Gunn analyzed data from their low birth weight survivors and found a significant association between, the more severe grade of cicatrical disease and duration of oxygen therapy[54].Kinsey noted that, concentration of oxygen administered was significantly associated with ROP in infants under 1200gm. When comparing mean Pa02 levels in normal infants and in ROP infants, he found differences only in babies of low birth weight and only Pa02 levels greater than 150mmHg[53]. However, a safe level of oxygen usage has not been defined, keeping Pao2 <100 mm Hg is recommended, preferably between 50 and 70mm Hg and saturation between 90-95%[55] . Preliminary work has suggested that, continuous oxygen monitoring may reduce the incidence of ROP[108].

Table 30: Comparison of oxygen as a risk factor of ROP in different studies

Study	p-value
Gupta[28] et al	0.002
Rekha23et al	0.005
Present study	0.005

RESPIRATORY DISTRESS SYNDROME AND ROP:

Out of 100 babies screened 55 had RDS and 14 (25.45%) babies developed ROP. RDS was found to be a significant risk factor for the development of ROP (p = 0.009). RDS was also found to be significant on univariate and multivariate analysis. Gupta[28] et.al reported ROP in 33% of babies with RDS. Gupta [28]et.al, Akkoyun [109] et.al also found RDS to be a significant risk factor for the development of ROP.

Table 31: Comparison of respiratory distress syndrome as a risk factor in different studies

STUDY	P VALUE
Gupta et al[28]	0.02
Vinekar et al[52]	0.007
Akkoyunn et al[109]	0.029
Present study	0.009

TRANSFUSION: Out of 100 babies screened, 27 were given transfusion (FFP / Packed cell/ Platelet) and 9(33.33%) developed ROP. Transfusion was found to be a significant risk factor for the development of ROP in this study (p=0.004).Transfusion was also found to be significant on univariate and multivariate analysis in our study. A potential role for blood transfusions and /or anaemia in the pathogenesis of retinopathy of prematurity (ROP) has been suggested[110, 111]. However a number of prospective, randomized, trials have failed to show a link between ROP and anaemia or transfusions[112, 113]. Dutta[88] et.al reported that packed cell and double volume exchange transfusion in the neonatal period as a major risk factor for the development of ROP.

Apnoea of prematurity (AOP):

Out of 100 babies screened, 24 babies had AOP and 7(29.17%) developed ROP. AOP was found to be a significant factor for the development of ROP in this study (p=0.044). Agarwal[26] et. al(54.1%), Gupta[28] et.al(54.5%) found apnea to be a significant risk factor for the development of ROP. In a study by Kim et al[61], they found that apnoea independently increased the incidence of ROP. Furthermore, frequent apnoeic attacks increased the progression of pre-threshold ROP to threshold ROP. A higher incidence of hypoxemia and apnoeic episodes requiring bagging was found among infants with severe ROP than in a control group[26, 54]. Similarly in a study by Chen et al[62] they found that apnoea was one of the independent risk factors of ROP. Its appropriate management might reduce the incidence of ROP.

Other risk factors: In our study we did not find sepsis, phototherapy, PIH, fetal distress, hypotension, anaemia and metabolic acidosis to be associated with ROP as found in other studies.

LIMITATIONS OF THE STUDY: The sample size is small and may not represent all premature babies. To know the true incidence and risk factors involved, it is advisable to undertake larger study over a period of years.

CONCLUSION

1. The present study reflects the problem of ROP in a tertiary care centre.

2. The incidence of ROP in the present study is 16%.

3. Out of 16 babies with ROP, only 3 babies(18.75%) were in stage 1, 11 babies(68.75%) were in stage 2,1 baby(6.25%) was in stage3 and 1 baby(6.25%) developed APROP.

4. The birth weight of the ROP babies ranged from 1000-1500 gm (mean 1272.81 ± 143.67 gm), while that of non-ROP babies ranged from 1100-2100 gm (mean 1642.80 ± 216.60 gm). Lower birth weight was significantly associated with increased incidence (p = <0.001) of ROP. The incidence of ROP was 40% in babies weighing ≤ 1500gm at birth.

5. The gestational age of the ROP babies ranged from 28 -34 weeks (mean 31.38 ±1.63 weeks), while that of non-ROP babies ranged from 30-38 weeks (mean 33.31 ± 1.74 weeks). The incidence of ROP was 26.32% in babies born ≤ 32 weeks of gestational age. Gestational age was found to be a significant risk factor for the development of ROP (p=0.004).

6. Oxygen, Respiratory distress Syndrome, Transfusion and Apnoea of Prematurity were found to be significant risk factors on Chi Square analysis.

7. On Univariate analysis, risk factors associated with ROP were Oxygen, RDS and Transfusion (FFP/Platelet/Packed cell) .

8. On Multivariate analysis, RDS was found to be an important risk factor. Using the forward method, Oxygen and Transfusion were also found to be significant on Multivariate analysis.

9. Early examination was significantly associated with chances of early detection of ROP (p = 0.016) and hence all babies should have their first screening within the first four weeks after birth.

10. In our opinion, the effective management of retinopathy of prematurity requires a team effort of the neonatologist, ophthalmologist and the NICU staff.

11. Regular screening programme with a criteria of birth weight <1500 gms and Gestational age <32 weeks or both and babies more than 1500 gms and>32 weeks with other risk factors should be screened at the discretion of the neonatologist and ophthalmologist.

12. Along with regular screening, an effective control of oxygen delivery, reduction of apnoeic spells and their early recognition and effective management of RDS are required.

SUMMARY

A one year longitudinal study with a short follow up of three months was done to know the incidence of ROP and to correlate with the risk factors causing it at KLE'S Dr Prabhakar Kore Hospital and Medical Research Centre, Belgaum.

100 babies satisfied the inclusion criteria and were enrolled in the study. The incidence of ROP in the study was 16%. Out of 16 babies with ROP, only 3 babies(18.75%) were in stage 1, 11 babies(68.75%) were in stage 2,1 baby(6.25%) was in stage3 and 1 baby(6.25%) developed APROP.

The mean birth weight of the ROP babies was 1272.81 ± 143.67 gm, while that of non-ROP babies was 1642.80 ± 216.60 gm. Lower birth weight was significantly associated with increased incidence (p = <0.001) of ROP. The incidence of ROP was 40% in babies weighing ≤ 1500gm at birth. The mean gestational age of the ROP babies was 31.38 ±1.63 weeks, while that of non-ROP babies was 33.31 ± 1.74 weeks. The incidence of ROP was 26.32% in babies born ≤ 32 weeks of gestational age. Gestational age was found to be a significant risk factor for the development of ROP (p=0.004).

Oxygen, RDS and transfusion were found to be important risk factors on univariate analysis for the development of ROP. RDS was found to be an important variable on multivariate analysis. By using the forward method oxygen and transfusion were also identified to be important.

Our study concluded that early examination was significantly associated with chances of early detection of ROP (p = 0.016) and hence all babies should

have their first screening within the first four weeks after birth. In our opinion, the effective management of ROP requires a team effort of the neonatologist, ophthalmologist and the NICU staff. Regular screening programme with a criteria of birth weight <1500 grams and gestational age <32 weeks or both and babies more than 1500 grams and >32 weeks with other risk factors should be screened at the discretion of the neonatologist and ophthalmologist. Screening should be intensified in the presence of risk factors like administration of oxygen, RDS, transfusion and apnoea.

Along with regular screening, an effective control of oxygen delivery, reduction of apnoeic spells and their early recognition and effective management of RDS are required.

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DATA COLLECTION INSTRUMENT

" INCIDENCE AND RISK FACTORS OF RETINOPATHY OF PREMATURITY - A ONE YEAR LONGITUDINAL STUDY"

PATIENT OPD NO- PATIENT ID NO-

NAME:_____

BIRTH WEIGHT(gms): INFORMED CONSENT:

GESTATIONAL AGE(wks): DATE OF BIRTH:

ADDRESS:_____
___

Ph no:_____

SEX: (1-MALE,2-FEMALE)

CATEGORY: (1-AFD/2-SFD/3-LFD)

BIRTH ORDER: (1-SINGLE/2-TWIN/3-TRIPLET)

DELIVERY: (1-SVD/2-FORCEPS/3-VACUUM/4-LSCS)

MATERNAL RISK FACTORS: (1-YES/2-NO)

a) PIH: f)IDDM:

b) Placenta previa: g)GDM:

c)Abruptio placenta: h)Fetal bradycardia:

d)Fetal distress: i)Fetal tachycardia:

e) Meconium stained liquor: J)Antenatal steroids:

Neonatal Risk factors 0=no/1=yes

a.1 minute APGAR	☐	b.5 minute APGAR	☐
c.Respiratory Distress	☐	d.Apnoea of prematurity	☐
e.PDA	☐	f.CCF	☐
g.Sepsis(proved/suspected)	☐	h.NEC	☐
i.Pneumonia	☐	j.Meningitis	☐
k.Intracranial Haemorrhage	☐	l.Hypotension	☐
m.HIE	☐	n.Anaemia	☐
o.Respiratory acidosis	☐	p.Metabolic acidosis	☐
q.Hyperoxia(paO_2>100mmHg)	☐	r.Hypoxia(paO_2<50mmHg)	☐
s.Hypercapnia($paCO_2$>50mmHg)	☐		

Treatment 0=no/1=yes

a.Oxygen supplementation	☐	b.Surfactant	☐
c.Aminophylline	☐	d.Dexamethsone	☐
e.Phototherapy	☐	f.Transfusion (FFP/Platelet/Packed cell)	☐

ROP SCREENING PROFORMA

NAME:_____

DATE OF SCREENING:

CHRONOLOGICAL AGE(wks):

POST CONCEPTIONAL AGE(wks):

ANTERIOR SEGMENT EXAMINATION OF THE EYE:

	RE	LE
TUNICA VASCULOSA LENTIS		
PUPILLARY DILATATION		
LENS		
MEDIA CLARITY		

FUNDUS EXAMINATION:

RIGHT EYE

IMMATURE

ZONE

STAGE OF ROP

CLOCK HOURS INVOLVED (1-12)

PLUS DISEASE

AP-ROP

LEFT EYE

IMMATURE

ZONE

STAGE OF ROP

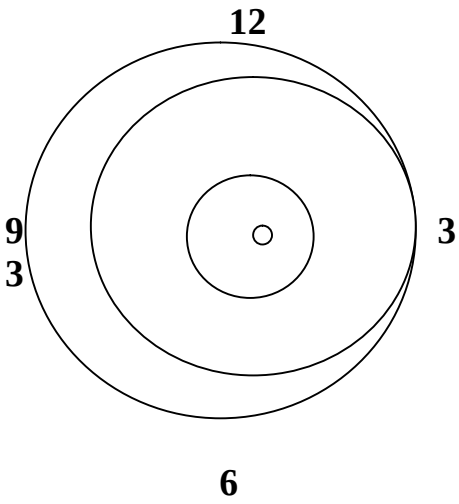
CLOCK HOURS INVOLVED(1-12)

PLUS DISEASE

AP-ROP

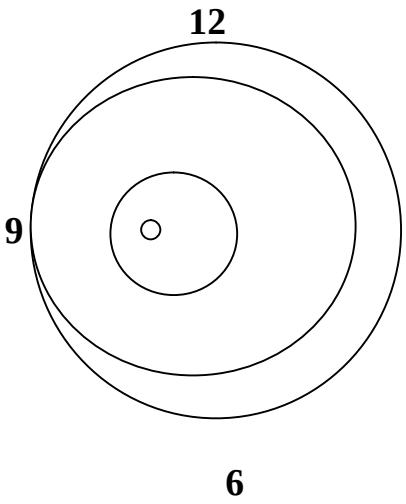
RIGHT EYE

MATURE ☐



LEFT EYE

MATURE ☐



IMPRESSION:_____

_____

FOLLOW UP EXAMINATION:

Follow up	Gestational age	Post natal age	Stage of ROP	Next follow – up date
1				
2				
3				

CONSENT FOR PARTICIPATION IN RESEARCH STUDY

Parent/Guardian of :_____

You are invited to participate in our research study titled "INCIDENCE AND RISK FACTORS OF RETINOPATHY OF PREMATURITY - A ONE YEAR LONGITUDINAL STUDY IN KLES Dr. PRABHAKAR KORE HOSPITAL AND MEDICAL RESEARCH CENTRE, BELGAUM" conducted by Dr***** , Post Graduate in M.S.OPHTHALMOLOGY under the guidance of Dr. ****** ,Professor, Department of Ophthalmology, J.N.Medical college , Belgaum.

Respected Parent/Guardian we request you to enroll your ward to participate in our study as he/she is eligible to do so.

Your wards participation in this study is voluntary and your decision to/not to participate in the study will not affect your relationship with J.N.MEDICAL COLLEGE. If you decide to participate (ward) you are free to withdraw at any time.

Purpose of the study: The purpose of this study is to know the prevalence of Retinopathy of Prematurity(ROP) and to correlate it with maternal and neonatal risk factors.

Procedure involved: If you agree(ward) to enroll yourself in this study I will enquire about your baby's mode of delivery, any problems encountered during/after delivery, any other risk factor concerning the mother or baby for development of Retinopathy of Prematurity from you and the hospital records . Then I will be dilating the baby's eyes by instilling dilating drops into the eyes.

Indirect ophthalmoscopic examination (used to see inside of the eye) will be done and findings noted.

Risks and Benefits :The screening examination can have short term effect on blood pressure, heart rate (bradycardia) and respiratory function in the premature baby. The baby could vomit/aspirate and hence care is taken not to feed the baby atleast one hour before examination. The examination will be kept as short as possible and precautions taken to ensure that emergency situations can be dealt promptly and effectively.

Your baby's participation in this study will help in early diagnosis of this condition and thus early referral for treatment .

It will help us know the prevalence of Retinopathy of Prematurity and also the risk factors causing it and thus is beneficial for a better management of at risk babies.

Cost for participation in this study: There will not be any extra cost incurred by the participant. We will not be paying for /or treating the child as that is not part of our study.

Privacy and confidentiality: The only people to know that you(baby) are part of the study are the members of the research team. No information about you or information provided by you during the research will be disclosed to others without your written permission.

Compensation: In the event of injury related to the study, treatment will be made available through KLES Dr.Prabhakar Kore Charitable Hospital & MRC, Belgaum. There is no compensation or payment for such medical treatment by law. The doctors and the staff will provide facilities and medical attention to you.

Authorization to publish results :When the results of the study are published or discussed in a conference no information would be displayed that would disclose your identity. Any information that is obtained in connection with this study and that can be identified with you will remain confidential.

Questions: If you have any questions about the study you may please contact

1. Chief Investigator- Dr***** P.G Department of Ophthalmology JNMC Belgaum
2. Dr******, Professor ,Guide, Department of Ophthalmology,JNMC, Belgaum
3. Dr ******, Professor & HOD, Department of Pediatrics, JNMC, Belgaum
4. Dr***** ,Principal, JNMC, Belgaum and Chairman of institutional ethics committee

CONSENT FOR PARTICIPATION IN RESEARCH

TRIAL

I, Parent /Guardian of _____ voluntarily agree for the participation of my ward as a subject of this study. By signing this consent form I am not giving up any of my legal rights . I may withdraw from the study anytime .I am signing the consent form after having read or been read for me in my vernacular language, including the risks and benefits and having all my questions answered.

Subject

Name:_____

Parent / guardians Name:_____

Signature or Left thumb

impression:_____

Witness

name:_____

Signature or Left thumb impression:_____

Investigators name : Dr Alok B S

Signature of the investigator:_____

Date: Place:

Name of Guide : Dr Mahesh I Magdum Co-Guide : Dr S M Dhaded

Signature of Guide:_____ Co-Guide :_____

KEY TO MASTER CHART

A	Anaemia
AOP	Apnoea of Prematurity
BO	Birth Order
BW	Birth Weight
CBW	Code for Birth Weight
CGA	Code for Gestational Age
FD	Fetal Distress
GA	Gestational Age
Hypt	Hypertension
Hyt	Hypotension
MA	Metabolic Acidosis
N	Name
O	Oxygen
PCAFE	Post Conception Age at First Examination
P	Phototherapy
PIH	Pregnancy Induced Hypertension
ROP	Retinopathy of Prematurity
RDS	Respiratory Distress Syndrome
S	Sepsis
St	Stage of ROP
Sex	Gender of the baby
T	Transfusion(FFP/Packed cell/Platelet)
Z	Zone of ROP

KEY TO MASTER CHART

CGA :	1 :≤ 32 2: 33-35 3:>35
CBW:	1:≤1500 2:1501-1800 3:1801-2000
O:	1: Given 2: Not given
RDS:	1: Present 2: Absent
S:	1: Present 2: Absent
P:	1: Given 2: Not given
ROP:	1: Present 2: Absent
Z:	1: Zone 1 2: Zone 2 3: Zone 3 4: Mature retina
St:	1: Stage 1 ROP 2: Stage 2ROP 3: Stage 3ROP 4: APROP
S:	1: Male 2: Female
BO:	1: Single 2: Twin
T:	1: Given 2: Not given
PIH:	1: Present in mother 2: Absent in mother
AOP:	1: Present 2: Absent
FD:	1: Present 2: Absent
Hyt:	1: Present 2: Absent
Hypt:	1: Present 2: Absent
Anaemia:	1: Present 2: Absent
MA:	1:Present 2:Absent

S NO	IPNO	N	GA	CGA	BW	CBW	O	RDS	S	P	PCAFE	ROP	Z	St	Sex	BO	T	PIH	AOP	FD	Hyt	Hypt	A	MA
1	1459056	B/O NAG1	32	1	1550	2	1	1	2	1	36	2	4	5	1	2	2	2	2	2	2	2	2	2
2	1459056	B/O NAG2	32	1	1650	2	1	1	2	1	36	2	4	5	1	2	2	2	2	2	2	2	2	2
3	1464069	B/O SUN	34	2	1350	1	1	1	1	2	38	2	4	5	1	1	1	1	1	2	2	2	2	2
4	1464416	B/O MAL	33	2	1750	2	1	2	2	1	37	2	4	5	2	1	2	2	1	2	2	2	2	2
5	396451	B/O RES	36	3	2000	3	2	2	1	2	40	2	4	5	1	1	2	2	2	2	2	2	2	2
6	396848	B/O SNE	36	3	1400	1	1	1	2	1	40	2	4	5	1	1	2	2	2	1	2	2	2	2
7	1469056	B/O ROO1	34	2	1200	1	1	1	2	1	38	2	4	5	2	2	2	1	2	2	2	2	2	2
8	397120	B/O ROO2	34	2	1700	2	1	1	2	1	38	2	4	5	1	2	2	1	2	2	2	2	2	2
9	397185	B/O LAX	30	1	1100	1	1	1	1	1	34	1	2	2	1	1	2	1	2	2	2	2	2	2
10	397708	B/O MAN	37	3	1750	2	1	1	2	1	41	2	4	5	2	1	2	2	2	2	2	2	2	2
11	1472368	B/O PRA	35	2	1800	2	1	1	2	1	39	2	4	5	1	1	2	2	2	2	2	2	2	2
12	1472611	B/O MAD1	30	1	1600	2	1	1	2	1	35	2	4	5	2	2	2	2	1	2	2	2	2	2
13	1472612	B/O MAD2	30	1	1750	2	1	1	2	1	35	2	4	5	2	2	2	2	2	2	2	2	2	2
14	1476203	B/O MAN	33	2	2100	4	2	1	2	1	37	2	4	5	1	1	2	2	2	2	2	2	2	2
15	1478498	B/O SAV	32	1	1750	2	2	2	2	1	36	2	4	5	1	1	2	2	2	2	2	2	2	2
16	399439	B/O NEE	32	1	1830	3	1	1	1	2	36	2	4	5	1	1	1	2	2	2	2	2	2	2
17	399477	B/O GIR	35	2	2000	3	2	1	2	2	39	2	4	5	1	2	1	2	2	2	2	2	2	2
18	399481	B/O GIR	35	2	1800	2	1	1	2	2	39	2	4	5	2	2	2	2	2	2	2	2	2	2
19	1494372	B/O GEE1	33	2	1500	1	1	1	1	1	37	1	1	4	1	2	2	1	2	2	2	2	2	2
20	1497346	B/O GEE	33	2	1700	2	1	2	2	1	37	2	4	5	1	2	2	1	2	2	2	2	2	2
21	1498462	B/O ASH1	33	2	1750	2	1	1	2	1	37	2	4	5	1	2	2	2	2	2	2	2	2	2
22	1498463	B/O ASH2	33	2	1350	1	1	1	2	1	37	2	4	5	2	2	2	2	2	2	2	2	2	2
23	1498675	B/O GAY	31	1	1200	1	1	1	2	1	34	1	2	2	1	1	2	2	2	2	2	2	2	2
24	1500016	B/O ROO	32	1	1520	2	1	1	1	1	36	2	4	5	2	1	2	1	2	2	2	2	2	2
25	1503914	B/O DEE	34	2	1430	1	2	2	1	1	38	2	4	5	2	1	2	1	2	2	2	2	2	2

26	1518442	B/O JAY	30	1	1210	1	1	2	1	1	34	1	2	2	1	1	1	2	1	2	1	2	2	2
27	1520283	B/O REN	35	2	1850	3	1	1	2	1	39	2	4	5	1	2	2	2	2	2	2	2	2	2
28	403952	B/O NAN	32	1	1100	1	1	1	2	1	36	1	2	2	2	1	2	2	2	2	2	2	2	2
29	404260	B/O SHO	36	3	1500	1	2	2	2	2	40	2	4	5	1	1	1	2	2	2	2	1	1	1
30	404636	B/O SUN	34	2	1700	2	2	2	2	2	38	2	4	5	1	1	2	2	2	2	2	2	2	2
31	1544815	B/O REV	34	2	1930	3	1	1	2	1	38	2	4	5	2	1	2	2	2	2	2	2	2	2
32	1546498	B/O MAH	34	2	1400	1	1	1	2	2	38	2	4	5	1	1	2	2	2	2	2	2	2	2
33	1553313	B/O MAN	33	2	1750	2	1	1	2	2	38	2	4	5	2	2	2	2	2	2	2	2	2	2
34	407583	B/O DEE	35	2	1900	3	2	2	2	2	38	2	4	5	1	1	2	1	2	2	2	2	2	2
35	1560753	B/O ASH	31	1	1270	1	1	1	1	1	35	1	2	2	2	2	1	2	1	2	1	2	1	2
36	409348	B/O PAR	33	2	1410	1	1	1	1	1	37	2	4	5	2	1	2	1	2	2	2	2	2	2
37	408455	B/O SAN	34	2	1440	1	1	1	1	1	38	2	4	5	2	1	2	2	2	2	2	2	2	2
38	409783	B/O SUN	34	2	1850	3	2	2	2	2	38	2	4	5	1	1	2	2	2	2	1	2	2	2
39	413169	B/O RAJ	33	2	1800	2	1	1	2	2	37	2	4	5	1	1	2	2	2	1	2	2	2	2
40	411557	B/O MEE	38	3	1800	2	2	2	1	1	41	2	4	5	2	1	2	2	2	2	2	2	2	2
41	411626	B/O SAV	34	2	1000	1	1	1	2	1	38	1	2	2	1	1	1	1	1	2	2	2	2	2
42	412910	B/O AKS	34	2	1600	2	2	2	2	2	38	2	4	5	2	1	2	2	2	2	2	2	2	2
43	411029	B/O SUM	32	1	1300	1	1	1	1	1	36	2	4	5	1	1	1	1	2	1	2	2	2	2
44	411290	B/O TRU	33	2	1200	1	1	1	1	1	37	2	4	5	2	1	2	1	2	2	2	2	2	2
45	413118	B/O MAN	33	2	1900	3	2	2	2	2	37	2	4	5	1	1	2	2	2	2	2	2	2	2
46	413165	B/O NIK	33	2	1500	1	2	2	2	2	37	2	4	5	1	1	2	1	2	2	2	2	2	2
47	413213	B/O VIN 2	32	1	1800	2	2	2	2	2	36	2	4	5	2	2	2	2	2	2	2	2	2	2
48	1618138	B/O NAN	32	1	1200	1	1	1	2	1	36	1	2	3	2	1	2	2	1	2	2	2	2	2
49	1606773	B/O KAV	32	1	1600	2	2	2	2	2	36	2	4	5	1	1	2	2	2	2	2	2	2	2
50	416528	B/O GEE1	35	2	1543	2	2	2	2	2	39	2	4	5	2	2	1	1	2	2	2	2	2	2
51	416529	B/O GEE2	35	2	1440	1	2	2	2	2	39	2	4	5	2	2	1	1	1	2	2	2	2	2
52	416535	B/O ROO	33	2	1900	3	2	2	2	2	37	2	4	5	2	1	2	2	2	2	2	2	2	2
53	417637	B/O PAV	33	2	1560	2	2	2	2	2	37	2	4	5	2	1	2	2	2	1	2	2	2	2
54	417760	B/O NIR	31	1	1250	1	1	1	1	1	35	2	4	5	2	1	1	1	1	2	1	2	2	2

55	418223	B/O VID	31	1	1500	1	1	1	1	1	35	2	4	5	2	1	2	1	2	2	1	2	2	2
56	1641802	B/O SAV1	33	2	1640	2	1	1	2	1	37	2	4	5	1	3	2	1	2	2	2	2	2	2
57	1641803	B/O SAV2	33	2	1810	3	2	2	2	2	37	2	4	5	1	3	2	1	2	2	2	2	2	2
58	1641804	B/O SAV3	33	2	1580	2	1	2	2	1	37	2	4	5	1	3	2	1	2	2	2	2	2	2
59	1641823	B/O AFR1	32	1	1780	2	1	1	2	1	36	2	4	5	2	2	2	2	2	2	2	2	2	2
60	1641824	B/O AFR2	32	1	1600	2	2	2	2	2	36	2	4	5	2	2	2	2	2	2	2	2	2	2
61	1654017	B/O GAN	32	1	1350	1	1	1	1	1	38	2	4	5	1	1	2	2	1	2	2	2	2	2
62	1659608	B/O SHI	33	2	1500	1	1	1	1	1	36	2	4	5	2	1	1	1	1	2	2	2	1	2
63	1689081	B/O AFR1	33	2	1450	1	1	2	2	2	37	1	3	1	2	2	1	2	2	2	2	2	2	2
64	1689082	B/O AFR2	33	2	1800	2	2	2	2	2	37	2	4	5	2	2	1	2	2	2	2	2	2	2
65	1694280	B/O SAN	31	1	1350	1	1	1	1	1	35	2	4	5	2	1	2	2	1	2	2	2	2	2
66	1697324	B/O YAL	35	2	1100	1	1	1	1	1	37	2	4	5	1	1	2	2	1	2	2	2	2	2
67	1698535	B/O JIG	28	1	1500	1	1	1	2	2	33	1	2	2	2	1	1	2	1	2	2	2	2	2
68	1707270	B/O ARC2	33	2	1600	2	2	2	2	2	39	2	4	5	1	3	1	2	1	2	2	2	2	2
69	1701184	B/O DEV	34	2	1530	2	1	1	2	1	38	2	4	5	1	1	2	2	2	2	2	2	2	2
70	1701234	B/O SHA	32	1	1700	2	2	2	2	1	37	2	4	5	2	2	1	2	1	1	2	2	2	2
71	1716056	B/O SUJ	34	2	1450	1	1	1	1	1	38	2	4	5	2	1	2	2	1	2	2	2	2	2
72	1718093	B/O SOU	33	2	1660	2	2	2	2	1	36	2	4	5	1	1	2	2	2	1	2	2	2	2
73	1719900	B/O SAR	30	1	1240	1	1	1	1	1	38	1	2	2	2	1	2	2	1	2	2	2	2	2
74	1721231	B/O SHA	30	1	1300	1	1	1	2	1	37	1	2	2	2	2	1	2	2	2	2	2	2	2
75	432119	B/O TAN	36	3	1800	2	2	2	2	1	34	2	4	5	2	1	1	2	1	2	2	2	2	2
76	1757851	B/OMAN1	36	3	1860	3	2	2	2	1	34	2	4	5	1	2	2	2	2	2	2	2	2	2
77	1759019	B/O VEN1	32	1	1559	2	2	2	2	1	40	2	4	5	2	2	2	2	2	2	2	2	2	2
78	1759033	B/O VEN2	35	2	1660	2	2	2	2	1	40	2	4	5	2	2	2	2	2	2	2	2	2	2
79	1767993	B/O DEE	30	1	1770	2	1	1	2	1	36	2	4	5	1	1	2	2	2	2	2	2	2	2
80	1768802	B/O PRA	35	2	1990	3	2	2	2	1	39	2	4	5	2	1	2	2	2	2	2	2	2	2
81	1778632	B/O VRA	31	1	1790	2	2	2	2	1	34	2	4	5	1	1	2	2	2	2	2	2	2	2
82	1816932	B/O ANN	34	2	2100	4	2	2	2	2	39	2	4	5	2	1	2	2	2	2	2	2	2	2
83	1826032	B/O REN	30	1	1240	1	1	1	1	1	35	2	4	5	1	1	1	2	1	2	2	2	2	2

84	1787867	B/O FAR	36	2	1800	2	1	2	2	1	38	2	4	5	1	1	1	1	2	2	2	2	2	2
85	437629	B/O ANJ	33	1	1900	3	2	2	2	2	34	2	4	5	2	1	1	2	1	2	2	2	1	2
86	1794182	B/O BHA	30	1	1700	2	2	2	2	1	40	2	4	5	1	1	2	2	1	2	2	2	2	2
87	1793618	B/OMAN1	36	3	1500	1	1	1	2	1	37	2	4	5	1	1	2	2	2	2	2	2	2	2
88	1757851	B/OMAN2	31	1	1750	2	2	2	2	1	34	2	4	5	2	2	2	2	2	2	2	2	2	2
89	1794185	B/O SUK	31	1	1560	2	1	1	2	1	40	2	4	5	1	1	2	2	2	2	2	2	2	2
90	1826175	B/OGEE1	32	1	1450	1	1	1	1	1	35	2	4	5	2	2	2	2	2	2	1	2	2	2
91	1826178	B/OGET2	33	2	1250	1	1	1	2	2	35	1	3	1	2	2	2	2	2	2	2	2	2	2
92	1798419	B/O RAJ	32	3	1285	1	1	1	1	1	36	1	3	1	1	1	1	2	2	2	2	2	2	2
93	438949	B/O SUR	33	3	1360	1	1	1	2	2	37	1	2	2	1	1	1	1	2	2	2	2	2	2
94	1802789	B/O PAD	36	3	1420	1	2	2	2	1	38	2	4	5	1	1	1	1	1	2	2	2	2	2
95	1814245	B/O ANU	35	2	1920	3	2	2	2	2	35	2	4	5	2	1	2	2	2	2	2	2	2	2
96	1839761	B/O BHA	34	2	1600	2	2	2	2	1	40	2	4	5	2	1	2	1	2	2	2	2	2	2
97	1839868	B/O REN	34	2	1750	2	2	2	2	2	41	2	4	5	2	1	2	1	2	2	2	2	2	2
98	444393	B/O MAL	32	1	1560	2	2	2	2	2	40	2	4	5	1	1	1	2	2	2	2	2	2	2
99	444475	B/O SHA	31	1	1620	2	1	2	2	1	39	2	4	5	1	1	2	2	2	2	2	2	2	2
100	1817402	B/O SHA	30	1	1400	1	2	1	2	1	34	1	2	2	1	1	1	2	1	1	1	2	1	1