



## **The study of Incidence and Risk Factors of Retinopathy of Prematurity In NICU.**

Dinesh Kumar Vuppu\*, Venugopal Reddy I<sup>1</sup>, Upendra Kumar DJ<sup>2</sup>

1. *Medical Director and Consultant Pediatrician, Ovum Hospital, Bangalore.*

2. *Pediatrician.*

**\*Correspondence to:** Dinesh Kumar Vuppu, Consultant Pediatrician and Neonatologist, Ovum Hospital, Bangalore.

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## Introduction

Retinopathy of prematurity (ROP), which was previously called as Retrolental Fibroplasia (RFL), is a vaso-proliferative disorder of the retina. Preterm infants are more prone for this disease especially low birth weight (LBW) neonates who are exposed to large amount of oxygen (O<sub>2</sub>). It is the major cause of preventable blindness in infants.<sup>1</sup>

The World Health Organisation (WHO) programme of Vision 2020 targeted against ROP mentioned that the incidence of ROP can be reduced by early screening and referral for treatment.<sup>2</sup> Spectrum of ROP is broad and ranges from a spontaneously recovering stage to a vision threatening sequelae. In infants with birth weight (BW) less than 1000grams, the risk of ROP is 82%, and 9.3% of them are potentially under the risk of blindness.<sup>3</sup>

Initially there was a low incidence of ROP in developing countries like India because there was no adequate screening and reporting and there was inadequate awareness regarding the grave consequences of the disease. But now there is an apparently increasing incidence with better screening protocols, more availability of assisted ventilation services and increased survival of preterms in newborn units.<sup>4</sup>

The pathogenic process involved in causation of ROP is multifactorial<sup>5</sup>. It is attributed to many possible risk factors like prematurity, hyperoxia, sepsis, necrotising enterocolitis, intraventricular hemorrhage (IVH), low birth weight (LBW), prolonged exposure to O<sub>2</sub>, severity of neonatal illnesses, severe respiratory distress requiring mechanical ventilation, shock, hypoxia, prolonged ventilatory support, need for blood transfusion, acidosis, anemia, high ambient light and vitamin E deficiency where as breast feeding was proposed to be having a protective effect.<sup>5,6</sup>

## Materials and Methods

Preterm babies with GA of less than 34wk or BW of less than 1750gm admitted to our NICU in Ruby hall clinic, Pune. The duration of the study was from 1st April 2014 to 30th September 2015.

A minimum sample size calculation of 80 preterm neonates was done.

**Method of collection of data:**

**Inclusion Criteria:** Preterm babies with GA of less than 34wk or BW of less than 1750gm delivered in (inborn babies) to the NICU of Ruby hall clinic,Pune

**Exclusion Criteria:** Children with suspected chromosomal anomalies.

**Place of Screening**

After taking informed consent from the parents, babies were assessed for the risk factors of ROP and recorded in a predesigned proforma. All the inborn neonates involved in the study were screened at the NICU in Ruby hall clinic,Pune.

The babies who developed any stage of ROP were taken as cases and the babies who did not have ROP were taken as controls. Laser treatment was performed based on the Early Treatment Guidelines for ROP (ETROP)

**Method of Study**

The study was a descriptive and observational study. A total of 80 preterm neonates satisfying the inclusion criteria were included in the study. As soon as the baby fulfilling inclusion criteria was admitted to NICU, the details were entered in a predesigned proforma which includes assessment of the risk factors. Informed consent was taken from the parents and baseline data were collected for each baby regarding date of birth, sex, single or multiple births, intrauterine growth retardation and other antenatal insults. During the stay, heart rate, blood pressure, apnea monitoring and O<sub>2</sub> saturation was done by continuous pulse oximetry. Clinical assessments and lab investigations for identifying the risk factors were carried out as follows.

**Defining risk factors:**

1. Oxygen exposure: Number of hours on oxygen, flow rate of oxygen and mode of oxygen delivery were noted for each child.
  2. Hypoxia and hyperoxemia: Arterial blood gas analysis was done as per unit protocols and episodes
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of hyperoxia and hypoxia was monitored. Continuous pulse oximetry was done in all babies who required any form of supplemental O<sub>2</sub>. Any values of SpO<sub>2</sub> less than 85% and more than 92% was noted. 85-92% of oxygen saturation is taken as the reference range.<sup>79</sup>

3. Apnea: Apnea was defined as a cessation of respiration for more than or equal to 20seconds or less than this duration accompanied by bradycardia (heart rate less than 100 beats per minute) or cyanosis.<sup>80</sup>

4. Hyperglycemia: Hyperglycemia was defined as whole blood glucose level of more than 125 mg%. Daily RBS monitoring was done thrice a day with a glucometer. Any glucometer reading more than 125 mg% was confirmed by checking the blood glucose levels by laboratory method.<sup>81</sup>

5. Thrombocytopenia: Thrombocytopenia was defined as platelet count less than 1.5 lakhs cells/cumm.<sup>82</sup>

6. Severe respiratory illness requiring mechanical ventilation: Duration of mechanical ventilation in hours, mode of ventilation, proximal inspiratory pressure. (PIP), peek end expiratory pressure (PEEP) and fraction of inspired O<sub>2</sub> (FiO<sub>2</sub>), was monitored for each case.

7. Severity of illness: RDS, surfactant administration, necrotizing enterocolitis, apnea of prematurity, pulmonary hemorrhage, patent ductus arteriosus, hypoxic ischaemic encephalopathy and IVH were considered as severe illnesses for the study.

8. Septicemia: Blood culture positivity for bacterial or fungal sepsis was noted. Clinical manifestations suggestive of pneumonia, meningitis, pyelonephritis, osteomyelitis, septic arthritis, shock, sclerema, necrotizing enterocolitis, disseminated intravascular coagulation were noted. Laboratory criteria like white blood cell count less than 5000cells/mm<sup>3</sup>, absolute neutrophil count less than 1000/mm<sup>3</sup> and band neutrophil ratio more than 0.2 were also considered.<sup>83</sup>

9. Anemia: Anemia was defined as hematocrit or hemoglobin level more than 2 standard deviations below the mean value for the age.<sup>84</sup> Since hematocrit is more reliable than haemoglobin for assessment of anemia, hematocrit was checked for all cases which were pale clinically or had a haemoglobin level below the mean.

10. Amount of blood transfusion: Number of units and ml/kg of whole blood transfused shall be noted. Amount of other blood products transfused like packed red blood cells, fresh frozen plasma and platelets were noted.

11. Duration of stay: Duration of stay in the hospital in days was calculated for each case.

### **Preparation And Precautions**

All the precautions were taken as per the AAP 2013 guidelines.<sup>69</sup> Since ROP screening examinations can have short-term effects on blood pressure, heart rate and respiratory function in the premature baby, examinations were kept as short as possible and precautions taken to ensure that emergency situations were dealt with promptly and effectively. Eye examination during screening lasts several minutes and may cause considerable pain to the neonate. Discomfort to the baby was minimized by pre treatment of the eyes with a topical Proparacaine and swaddling the baby. Babies were fed at least one hour before examination to avoid vomiting and aspiration. Hand washing was done and asepsis maintained. Neonatologist was available throughout the procedure in anticipation of any complications.

### **Time of 1st screening of Screening for ROP**

Every case was screened as per the AAP 2013 guidelines for ROP screening (table: 3)<sup>69</sup>

### **PROCEDURE**

Instruments used: The following instruments were used for screening:

1. Indirect ophthalmoscope
2. Pediatric wire speculum.
3. Scleral indentor.

Dilatation of the pupil: Pupils were dilated with Phenylephrine 2.5% and Tropicamide 0.5%. One drop of Tropicamide was instilled every 10-15 minutes for 4 times starting 1 hour before the scheduled time for examination. This was followed by Phenylephrine, one drop just before examination. Phenylephrine which is available in 10% concentration was diluted 4 times before use in neonates. Repeated instillation of Phenylephrine was avoided for the fear of hypertension.

### **Screening**

Screening of ROP was done with Retcam Shuttle (Clarity MSI, USA) by an experienced ophthalmologist in our NICU. After instilling a topical anaesthetic drop like Proparacaine, a wire speculum was inserted to keep the eye-lids apart. First the anterior segment of the eye was 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 was used to indent the eye externally to examine areas of interest, rotate and stabilize the eye.

Notes were 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 included a recommendation for the timing of next examination and were kept with the medical record. After screening, the cases were classified as per ICROP on the basis of vascularization of the retina and characterized by its position (zone), severity (stage), and extent (clock hours).

### **Follow up.**

Follow up was done as per the recommended by the ICROP67.

### **Ethical Issues**

Informed consent of parents was taken after explaining in detail about the methods and procedures involved in the study in their own vernacular language. Institutional Ethical Committee Clearance was taken before the study was undertaken.



Figure 3: ROP screening being done at NICU Ruby hall clinic using a INDIRECT OPHTHALMOSCOPE



Figure 4: ROP LASER TREATMENT



## Results

### INCIDENCE OF ROP AMONG STUDY SUBJECTS (n = 80)

Overall incidence of ROP in the study group was 21.3% (17 babies). Out of them, 7 babies (41.2%) had stage-1 ROP and 10 babies (58.8%) had stage-2 ROP.

	Number of cases	% of cases
Controls	63	78.7
Cases	17	21.3
Stage 1 ROP	7	41.2
Stage 2 ROP	10	58.8

**Table 1: Incidence of ROP.**

### DISTRIBUTION OF SEX AMONG CASES AND CONTROLS

There was no statistically significant difference in sex distribution among cases and controls. Hence, cases and controls were approximately similar in terms of sex distribution.

SEX RATIO: The sex ratio in the study group was 1.29:1[Male: Female]

Sex	Cases		Control		P-value
	n	%	n	%	
Male	9	52.9	36	57.1	0.789 <sup>NS</sup>
Female	8	47.1	27	42.9	
Total	17	100.0	63	100.0	

**Table 2: Sex distribution among cases and controls**



**MODE OF DELIVERY AMONG CASES AND CONTROL**

11.8% of cases and 1.6% of controls were delivered vaginally. 88.2% of cases and 98.4% of controls were delivered by caesarean section (P value =0.113). The same is also depicted in Table 3.

**Table 3: Mode of Delivery among Cases and Control**

Mode of delivery	Cases		Control		P-value
	n	%	n	%	
Vaginal delivery	2	11.8	1	1.6	0.113 <sup>NS</sup>
LSCS	15	88.2	62	98.7	
Total	17	100.0	63	100.0	

**CLASSIFICATION OF CASES BY STAGE AND ZONE OF ROP AS PER ICROP.**

Of 7 babies who had stage 1 ROP, 2 babies had zone 2 and 5 babies had zone 3 disease. Of 10 babies who had stage 2 ROP, 5 had zone 2 and 5 had zone 3 disease. The same is depicted in table 4 and figure 4.

**Table 4: Stage and zone of ROP among the cases (n = 17).**

STAGE OF ROP	ROP IN ZONE 2	ROP IN ZONE 3
STAGE 1	2	5
STAGE 2	5	5
<b>Total</b>	<b>7</b>	<b>10</b>

**DISTRIBUTION OF BIRTH WEIGHT AMONG CASES AND CONTROLS**

The BW of the ROP babies ranged from 620gm-1700 gm while that of non- ROP babies ranged from 1100gm-1750gm. Maximum number of cases had BW ranging from 1000gm-1499gm where as maximum number of controls had BW ranging from 1500gm-1750gm. LBW was significantly associated with increased incidence of ROP (p<0.001). Table 5 and figure 5 depict the same.

**Table 5: Comparison of Birth Weight among cases and controls and its distribution among Stage 1 ROP and stage 2 ROP**

BIRTH WEIGHT (IN GRAMS)	NUMBER OF CASES		NUMBER OF CONTROLS
	STAGE 1 ROP	STAGE 2 ROP	
< 1000	0	2	0
1000-1299	4	1	2
1300-1499	1	5	22
1500-1750	2	2	39
Total	7	10	63

P value <0.001 (Significant).

### MEAN BIRTH WEIGHT AMONG CASES AND CONTROLS

The mean birth weight of the ROP babies was 1255.3gm  $\pm$  302.8 gm, while that of non-ROP babies the mean birth weight was mean 1480gm  $\pm$  170.4gm. Low birth weight was significantly associated with increased incidence of ROP on univariate analysis. (p value<0.001) The same is depicted in the form of table 6 and figure 6 below:

**Table 6: Comparison of Mean Birth Weight of Cases and Controls**

BIRTH WEIGHT (IN GRAMS)	CASE S (n=17)	CONTROL S (n=63)
Mean	1255.3	1480.0
Standard Deviation	302.8	170.4
P value <0.001 (Significant)		

### MEAN GESTATIONAL AGE AT BIRTH AMONG CASES AND CONTROLS

The mean GA of the cases was 30.12wk  $\pm$  2.29wk and the controls was 32.43wk  $\pm$  0.96wk. Cases had significantly lower mean GA when compared to controls which was statistically significant (P-value<0.001).

This is depicted in Table 7 below.

**Table 7: Mean gestational age among cases and controls.**

GESTATIONAL AGE (IN	CASE S	CONTROL S (N=63)
Mean (in weeks)	30.12	32.43
Standard Deviation	2.29	0.96
P value <0.001 (Significant)		

The same information is revealed from the figure 13 below.

The GA ranged from 25wks to 34wks among cases and 29wk to 34wk among controls. Of the total number of ROP cases, maximum number (82.4%) was amongst babies born with a GA of less than 32 wk, whereas among controls only one baby had a GA of less than 32wks, which was highly significant (p<0.001). The same is shown in table 8 and figure 8a below.

Stage 1 ROP was distributed amongst babies with GA at birth ranging between 29 wk-34 wk, whereas stage 2 ROP was found amongst babies with a lower mean GA at birth ranging from 25wk-32wk. This is shown in the Table 8 and figure8b which follows.

**Table 8: Comparison of Gestational Age at birth among cases and controls and its distribution in stages of ROP**

GA AT BIRTH	CASES		CONTROLS
	STAGE 1	STAGE 2	
25	0	1	0
28	0	3	0
29	1	2	1
30	1	2	0
31	1	1	0
32	1	1	47
33	2	0	0
34	1	0	15
Total	7	10	63

**GESTATIONAL AGE OF STUDY GROUP AT 1ST OPHTHALMOLOGICAL EVALUATION.**

GA of the cases at first ophthalmological examination ranged from 30 wk to 38 wks where as the controls had a GA ranging from 31wk to 37wk.

The GA at first ROP screening examination and distribution among cases and controls, and among stage 1 and stage 2 ROP is as shown in the table 9 and figure 9 that follows.

**Table 9: Gestational age of neonates at 1<sup>st</sup> ophthalmological evaluation.**

GA at first	Cases		Controls
	Stage 1 ROP	Stage 2 ROP	
30	0	1	0
31	0	1	1
32	1	2	6
33	0	1	16
34	1	2	10
35	0	1	14
36	5	2	8
37	0	0	8
Total	7	10	63

**Figure 9 : Comparison of Gestational age of neonates at 1<sup>st</sup> ophthalmological evaluation among cases and controls**

**MEAN GESTATIONAL AGE AT 1ST OPHTHALMOLOGICAL EXAMINATION FOR SCREENING OF ROP.**

The mean GA at first examination in cases was at 34.04wk ± 2.10wk and the mean GA at first examination among controls was at 34.3wk ± 1.53wk. There was no significant difference in the mean age at first screening examination of the cases and the controls. (P value= 0.512). The same is depicted in Table10 and Figure 10 below.

**Table 10: Mean Gestational Age at 1<sup>st</sup> ophthalmological evaluation among cases and controls.**

GA AT 1 <sup>ST</sup>	CASES (n=17)	CONTROLS (n=63)
Mean	34.06	34.37
SD	2.05	1.59
<b>P-value= 0.512 (non-Significant).</b>		

### STAGE OF ROP AND THE GESTATIONAL AGE AT COMPLETE VASCULARIZATION OF RETINA AMONG THE STAGES OF ROP AND CONTROLS

All the controls attained full vascularization by 45wk of GA. Among cases, all stage 1 ROP cases attained full vascularization by GA of 46wk where as stage 2

ROP cases took up to GA of 49wk for full vascularization. The same is depicted in Table 11 and Figure 11 below.

**Table 11: Comparison of age at complete vascularization among cases and controls and its distribution among the stages of ROP.**

GESTATIONAL AGE AT COMPLETE VASCULARIZATION (IN	CASES		CONTROL S
	STAGE 1	STAGE 2	
36	0	0	8
38	0	0	9
40	1	2	1
41	1	0	0
42	3	3	6
43	0	2	21
45	1	0	1
46	1	1	0
47	0	1	0
49	0	1	0
Total	7	10	63

### MEAN GESTATIONAL AGE AT COMPLETE VASCULARIZATION AMONG CASES AND CONTROLS

Mean GA at which complete vascularization of retina was evident was 43.06wk  $\pm$ 2.63wk for cases where as the controls attained complete vascularization at a mean GA of 40.48wk $\pm$ 2.50wk which was significantly earlier than the ROP cases (P value<0.001). The same is shown in table 12 and figure 12.

**Table 12: Mean Gestational Age at Complete Vascularization among Cases and Controls**

<b>GESTATIONAL AGE AT COMPLETE VASCULARIZATION (IN WEEKS)</b>	<b>CASES (N=17)</b>	<b>CONTROLS (N=63)</b>
Me	43.0	40.48
S	2.6	2.5
P-value <0.001 (Significant)		

**NEED FOR OXYGEN ADMINISTRATION**

Ten babies among the cases (58.8%) and 15 babies among the controls (23.8%) needed oxygen therapy by any of the modes like through oxygen hood, oxygen prongs, bubble CPAP (continuous positive airway pressure) or mechanical ventilation. The requirement for oxygen was significantly more among the cases when compared to the controls (P value <0.001) as shown in table 18.

**Table 13: Stage of ROP among study subjects and their need for oxygen**

<b>NEED FOR OXYGEN</b>	<b>CASES</b>		<b>CONTROLS</b>
	<b>STAGE 1</b>	<b>STAGE 2</b>	
Yes	2	8	15
No	5	2	48
P-value = 0.002 (Significant)			

**COMPARISON OF VARIOUS PARAMETERS OF OXYGEN ADMINISTRATION AMONG CASES AND CONTROLS**

For all the babies in the study group who required oxygen, the duration of oxygen requirement, the minimum and the maximum duration of saturation of arterial oxygen (SpO<sub>2</sub>) attained and the duration of the maximum and minimum SpO<sub>2</sub> were continuously recorded using transcutaneous pulse oximetry technique by using the trend setter mode and significance assessed between the documented values among cases and



controls.

The mean duration of oxygen administered was  $70.00 \pm 48.96$  hours among the cases and  $49.60 \pm 23.39$  hours among controls. (P-value<0.05) the same is shown in table14 and figure 14a.

The mean of maximum SpO<sub>2</sub> attained in the cases was  $98.76\% \pm 1.20\%$  and that in the control was  $97.53\% \pm 1.41\%$  (P-value=0.012). The mean of minimum SpO<sub>2</sub> was significantly lower in the cases ( $81.88\% \pm 7.36\%$ ) when compared to the levels in the controls ( $87.87\% \pm 4.05\%$ ) (P Value =0.009). These 2 data showed that the cases were exposed to significantly wider levels of fluctuation in the arterial oxygen saturation than the controls. The same is shown in table 14 and figure 14b.

**Table 14: Comparison of O<sub>2</sub> Duration, Mean of maximum SpO<sub>2</sub> and mean of minimum SpO<sub>2</sub> among cases and controls**

		<b>Cases (n=17)</b>	<b>Controls (n=63)</b>
O <sub>2</sub> Duration in hours	Mean	70.00	49.60
	SD	48.96	23.39
	P Value =0.045 (Significant)		
SpO <sub>2</sub> Max in%	Mean	98.76	97.53
	SD	1.20	1.41
	P Value =0.012 (Significant)		
SpO <sub>2</sub> min in%	Mean	81.88	87.87
	SD	7.36	4.05
	P Value =0.009 (Significant)		

**UNIVARIATE ANALYSIS OF VARIOUS RISK FACTORS**

**COMPARISON OF THE DISTRIBUTION OF APGAR SCORES AMONG CASES AND CONTROLS**

In the present study groups, no case or control had one minute APGAR score less than 3, but cases had a lower APGAR at both 1 and 5 minutes when compared to the controls. There was a statistically significant difference in the distribution of APGAR score between cases and controls recorded at first and fifth minute of life (P value <0.01 for both).

The mean APGAR score at 1 minute of the cases was  $7.06 \pm 0.83$  and the controls was  $6.32 \pm 0.95$ .( p<0.01)

The mean APGAR score at 5 minutes of the cases was  $8.88 \pm 0.33$  and the controls was  $8.46 \pm 0.62$  (p<0.01).

Mean APGAR scores at 1 minute and 5 minutes were significantly lower among case when compared to the controls, signifying that neonates with perinatal asphyxia are at risk of ROP. The same shown in table 15 and figure 15.

**Table 15: Comparison of distribution of APGAR score among cases and controls.**

PARAMETER	CASES		CONTROLS		P VALUE
	Mean	SD	Mean	SD	
APGAR at 1-Min	7.06	0.83	6.32	0.95	0.004 (Significant)
APGAR at 5 min	8.88	0.33	8.46	0.62	0.008 (Significant)

P-values by unpaired 't' test.

#### EVALUATION OF MATERNAL AND FETAL RISK FACTORS AMONGST CASES AND CONTROLS

17.6% of the cases had maternal pregnancy induced hypertension compared to

22.2% of the controls, which was not statistically significant.

7.4% of cases had maternal antepartum hemorrhage compared to 15% in the controls which was not significant.

Non from the cases had antenatal steroid exposure compared to 20.6% in the controls which was not statistically significant.

None from. The cases had meconium stained amniotic fluid indicating intrauterine asphyxia compared to 33.3% of the controls, which was statistically significant.

**Table 16: Evaluation of maternal and fetal risk factors of ROP amongst cases and controls**

Parameter	Cases	Controls	P value	Significance
<b>Pregnancy Induced Hypertension</b>				
Yes	3	14	0.999	Not significant
No	14	49		
<b>Ante Partum Hemorrhage</b>				
Yes	0	13	0.060	Not significant
No	17	50		
<b>Maternal Steroid administration (Dexamethasone)</b>				
Yes	0	21	0.004	Significant

**COMPARISON OF DISTRIBUTION OF BIRTH ORDER AMONG CASES AND CONTROLS**

Birth order of 1, 2 and 3 was equally distributed among cases and controls, which signify that the groups were comparable. This is shown in table 17.

**Table 17: Birth order and its distribution among cases and controls**

PARAMETER (BIRTH)	CASES	CONTROLS	P VALUE	SIGNIFICANCE
1	6	42	0.059	Non Significant
2	8	14		
3	3	7		

## COMPARISON OF VARIOUS NEONATAL COMPLICATIONS AMONG CASES AND CONTROLS

The following are the various neonatal complications which were significant on univariate analysis of cases and controls:

1. Respiratory distress syndrome: 58.8% of the cases had respiratory distress syndrome compared to 23.8% in the controls which had a high significance ( $p < 0.001$ ).
2. Clinical sepsis: Clinical sepsis was present in 70.5% of the cases where as it was present only in 49.2% of the controls ( $p = 0.171$ ).
3. Hypoxic ischemic encephalopathy was present in 100.0% of cases and 90.4% of controls ( $p = 0.417$ ).
4. Acute kidney injury: Acute kidney injury was considered in the study group if oliguria (urine output  $< 1 \text{ ml/kg}$ ) is present and/or if serum creatinine was elevated 2 standard deviation above the mean value for gestational age or rise in value was  $\geq 0.3 \text{ mg/dl/day}$ . 5.9% of the cases had acute kidney injury where as 6.3% of the controls had it, which was not significant ( $p = 0.999$ ).
5. Convulsions: Presence of convulsion during the period of admission was a very significant factor among cases (5.9%) when compared to the controls (6.3%) ( $p = 0.517$ ).
6. Hypotension: 17.6% of the cases had hypotension requiring inotropic support of atleast one drug. Among controls there were only 12.7% of the babies who required the same. Presence of hypotension was not significant risk factor among the cases ( $p = 0.693$ ).
7. Transfusion of blood and blood products: Transfusion of whole blood or any blood products like packed red blood cells, platelet concentrate and fresh frozen plasma transfusion was considered here. 17.6% of case required any one of the above were as only 14.3% of the controls required it. ( $p = 0.711$ )

**Table 18: Various neonatal complications which were significant on univariate analysis of cases and controls**

Parameter	Cases	Controls	P value	Interpretation
<b>Respiratory distress syndrome</b>				
Yes	10	15	P-value = 0.001	Significant
No	0	48		
<b>Clinical</b>				
Yes	12	31	P-value = 0.171	Non-Significant
No	5	32		
<b>Hypoxic Ischaemic encephalopathy</b>				
Stage 0	17	57	P-value = 0.417	Non-Significant
Stage 1	0	4		
Stage 2	0	2		
<b>Acute Kidney</b>				
Yes	1	4	P-value = 0.999	Non-Significant
No	16	59		
<b>Convulsion</b>				
Yes	1	2	P-value	Non-Significant
No	16	61		
<b>Administration of Blood products</b>				
Yes	3	8	P-value = 0.693	Non-Significant
No	14	55		
<b>Hypotensio</b>				
Yes	3	9	P-value = 0.711	Non-Significant
No	14	54		

#### **VARIOUS NEONATAL COMPLICATIONS WHICH WERE NOT SIGNIFICANT ON UNIVARIATE ANALYSIS OF CASES AND CONTROLS**

Neonatal hyperbilirubinemia, Surfactant administration, patent ductus arteriosus, intraventricular hemorrhage, necrotising enterocolitis, hemorrhagic disease of newborn, anemia, thrombocytopenia, requirement of exchange transfusion, bacterial and fungal sepsis and hypothermia were not significant on univariate analysis.

**Table 18: Various neonatal complications which were not significant on univariate analysis of cases and controls.**

Parameter	Cases	Controls	P value	Interpretation
<b>Neonatal hyperbilirubinemia</b>				
Yes	10	31	0.588	Non-Significant
No	7	32		
<b>Surfactant</b>				
Yes	2	8	0.999	Non-Significant
No	15	55		
<b>Patent ductus</b>				
Yes	1	3	0.999	Non-Significant
No	16	60		
<b>Intra Ventricular Hemorrhage</b>				
Yes	1	5	0.999	Non-Significant
No	16	58		
<b>Necrotising</b>				
Yes	0	2	0.999	Non-Significant
No	17	61		
<b>Hemorrhagic Disease of Newborn</b>				
Yes	1	5	0.999	Non-Significant
No	16	58		
<b>Anaemia</b>				
Yes	2	10	0.999	Non-Significant
No	15	53		
<b>Hypothermi</b>				
Yes	0	3	0.999	Non-Significant
No	17	60		
<b>Exchange</b>				
Yes	2	3	0.286	Non-Significant

## COMPARISON OF VARIOUS LABORATORY PARAMETERS AMONG CASES AND CONTROLS

**Table 19:** Comparison of various laboratory parameters among cases and controls

Parameter	Cases	Controls	P value	Interpretation
<b>C- Reactive</b>				
<6	5	14	0.750	Non-Significant
>6	12	47		
<b>Platelet</b>				
<1.5	10	16	0.041	Significant
>1.5	7	40		
<b>Mean</b>				
Mean	13.41	13.63	0.751	Non-Significant
SD	2.25	2.63		
<b>Fungal</b>				
Yes	2	7	0.999	Non-Significant
No	15	56		
<b>Bacterial</b>				
Yes	4	13	0.749	Non-Significant
No	13	50		

## COMPARISON OF VARIOUS PARAMETERS OF SEPSIS AMONG CASES AND CONTROLS

Distribution of CRP did not differ significantly between cases and controls (P-value =0.750).

Distribution of Plate count differs significantly between cases and controls (P-value =0.041).

Distribution of Mean Hb did not differ significantly between cases and controls (P-value =0.751).

Distribution of Fungal sepsis did not differ significantly between cases and controls (P-value =0.999).

Distribution of bacterial sepsis did not differ significantly between cases and controls (P-value =0.749).



**MULTIPLE LOGISTIC REGRESSION ANALYSIS**

On multivariate analysis, GA, duration of hospital stay, day of establishment of feeds, apnea, need for resuscitation and duration of oxygen administration were found to be independent risk factors.

**Table 20: Independent Predictors of ROP (Multivariate analysis).**

Factors	P-value
Resuscitation	0.034 (Significant)
Apnea	0.001(Significant)
Duration of Stay in hospital	0.001 (Significant)
Lower Gestational Age	0.015 (Significant)
Days of Establishment of OGF	0.001 (Significant)
Duration of Oxygen	0.003 (Significant)

**NEED FOR RESUSCITATION AMONG CASES AND CONTROLS**

47.1% of the cases needed resuscitation compared to 17.5% of the controls. Resuscitation was a significant and independent risk factor of ROP (P-value = 0.021).

**Table 21: Need for resuscitation among cases and controls**

	8 (47.1)	11 (17.5)
NO	9 (52.9)	52 (82.5)
P-value = 0.021 (Significant)		

**DISTRIBUTION OF APNEA AMONG CASES AND CONTROLS**

58.8% of the cases had at least one episode of apnea. There was no episode of apnea among the controls.

Apnea was an independent risk factor for ROP. Table 22 shows the same.

**Table 22: Distribution of Apnea among cases and controls**

RESPONSE	CASES	CONTROLS	P-VALUE	INTERPRETATION
Yes	10	0	0.001	Significant
No	7	63		

**DURATION OF STAY IN THE HOSPITAL AMONG CASES AND CONTROLS**

Majority of the cases (82.4%) had duration of hospital stay more than 13 days but the most of the controls (77.8%) had a period of stay less than 13 days.

**Table 23: Duration of stay in the hospital among cases and controls**

Duration of stay (Days)	Cases	Controls	P-value
<10	1	11	0.001 (Signifiant)
10 – 11	2	26	
12 – 13	0	12	
>13	14	14	
Total	17	63	

**COMPARISON OF MEAN DURATION OF ESTABLISHMENT OF FEEDING AMONG CASES AND CONTROLS**

Any type of enteral feeding of breast milk in the form of nasogastric or orogastric feeding, pallada or direct breastfeeding was established at a significantly earlier period among controls when compared to the cases (P value<0.01).

**Table 2 Comparison of mean duration of establishment of feeding among cases and controls.**

PARAMETER		CASES (n=17)	CONTROL S (n=63)
Days of establishment of feeds (OG)	M	4.47	3.21
	S	2.50	1.31
	P Value <0.01 (Significant)		

**Discussion**

Significance of ROP screening lies in the fact that ROP is the most common cause of childhood blindness which is preventable. The primary prevention of ROP can be done by limiting the exposure to antenatal, natal and postnatal risk factors which are proposed to contribute to the increased incidence as well as severity of ROP. Secondary prevention of ROP is done by timely screening and early treatment to prevent blindness that can occur in severe ROP which were missed at the screening and are not treated. So the secondary prevention of ROP is given utmost importance in the WHO. VISION 2020 programme.<sup>2</sup>

In this era of improving standards of neonatal care, ROP is becoming a significant problem in developing countries like India. Though there are data from the different urban and rural areas of India, reports from large randomised multicentric trials are lacking from our country. So there is a scarcity of data on the epidemiology of ROP from the Indian sub continent.<sup>85</sup>

Studies from developed countries have reported that although the clinical spectrum and incidence of ROP is not similar in all the units, there is an overall decrease in the incidence of the disease wherever there is an ongoing surveillance programme.<sup>86</sup> So timely screening is a very important aspect in management of ROP. Incidence of ROP

The incidence of ROP in the present study is 21.3% Various studies have shown that about 9.4%-25.4% of babies with gestational age 32wk or less develop some degree of ROP.

**Table 32: Comparison of incidence of ROP with other studies**

<b>INDIAN STUDIES</b>	<b>GESTATIONAL AGE (wk)</b>	<b>BIRTH WEIGHT(gm)</b>	<b>INCIDENCE</b>
Maheshwari 1996 <sup>23</sup>	≤35	≤1500	20%
Patil 1997 <sup>24</sup>	≤32	≤1250	17.5%
Dutta 2003 <sup>25</sup>	≤32	≤1750	21%
Gupta 2004 <sup>18</sup>	≤32	≤1250	21.7%
Chaudhari 2009 <sup>26</sup>	≤32	<1500	22.3%
Present Study	<34	<1750	21.3%

<b>INTERNATIONAL STUDIES</b>	<b>GESTATIONAL AGE (wk)</b>	<b>BIRTH WEIGHT(gm)</b>	<b>INCIDENCE</b>
Chye 1999 <sup>27</sup>	≤37	≤1250	15%
Conrath 2001 <sup>29</sup>	≤32	≤1750	9.4%
Nair 2003 <sup>30</sup>	≤32	≤1500	25.4%
Austeng 2009 <sup>20</sup>	<27	-	72.7%

Studies in the literature usually use a cut-off point of a BW of 1,250gm or 1,500gm or 1,750gm, a GA of 28wk or 32 wks, or both. Using a BW of 1750gm or less, a GA of 34 wk or less, or both as criteria for inclusion in this study, explains the similar incidence of ROP when compared to other Indian studies.

The overall incidence of ROP in the present study is 21.3%. Patil<sup>24</sup> et al reported the overall incidence of ROP as 17.5% and there were no cases of severe ROP. They studied 40 babies with <32wk or < 1250gm. Maheshwari<sup>23</sup> et al. in 1996 reported overall incidence as 20% and severe ROP as 7%. They studied 66 babies with <35wk or < 1500gm. Gupta et al in 2003 reported overall incidence as 21.7% and severe ROP as 5%. They studied 60 babies with ≤ 35wk or ≤1500gm. Dutta<sup>25</sup> et al screened 108 babies of ≤32 wk or ≤1700gm and reported overall incidence as 21%. However, in most instances it is not possible to compare studies, as the inclusion criteria are different.

The incidence of ROP in our study would have increased if the screening was done only in babies weighing <1300gm or in babies <32wk of GA at birth. Screening of babies with a GA of <34wk and/or <1750gm BW in this study has made the incidence of ROP comparable to other Indian studied.

Inclusion criteria of ROP Screening if changed to lower limit of GA or BW ( $\leq 30$ wk and  $\leq 1250$ gm) would make screening more cost effective and detect the more severe stages of ROP easily enough to permit treatment, reduce unnecessary examinations and avoid wastage of time and manpower.<sup>87,88</sup> But there are high chances of missing ROP cases which can lead to sequelae which are avoidable with screening and early treatment. In the present study all the babies who were  $\leq 28$ wk of GA developed ROP. All the babies who had a BW  $\leq 1000$ gm developed stage 2 ROP which was the maximum stage of ROP in this study.

### SEVERITY OF ROP

Most of the studies consider stage 3 and above as severe ROP. The percentage of severe ROP among various stages of ROP is depicted in the box below.

**Table 33: Comparison of severity of ROP with other studies**

Study	Maheshwari <sup>2</sup>	Rekha <sup>1</sup>	Patil <sup>24</sup>	Gupta <sup>1</sup>	Austeng <sup>2</sup>	Present
	3			8	0	
Severe ROP(%)	7	8	0	5	34.8	0

In our study there were no stages of ROP above stage 2, which was similar to study conducted by Patil et al. This could be explained by the fact that the screening programme and surveillance for the risk factors was good in our hospital.

### SIGNIFICANT RISK FACTORS IN VARIOUS STUDIES

Though accumulating evidence indicates that ROP is a multifactorial disease, immaturity of retina and a period of hyperoxia are the main contributing etiological factors in the pathophysiology of ROP.<sup>25</sup> In our study, the incidence of ROP was significantly inversely proportional to both birth weight( $p=0.001$ ) and gestational age ( $p<0.001$ ).

On univariate analysis, the duration of oxygen administration, mean of maximum and minimum SpO<sub>2</sub>, need for oxygen supplementation, clinical sepsis, apnea, RDS, HIE, mean APGAR at first and fifth minute of life, acute kidney injury, convulsions, positive CRP, administration of blood and its products and hypotension are significantly associated with development of ROP.

### **LOW BIRTH WEIGHT AND PREMATURITY**

The prevalence of ROP was more among VLBW neonates and the risk is inversely proportional to BW and GA in studies conducted by Maheshwari et al.<sup>23</sup> The mean gestational age of the cases was 29.93wk  $\pm$ 2.18wk and the controls were 32.42wk  $\pm$  0.89wk. The range of gestational age was 27 wks – 34wks among cases and 29wk-34wk among controls. Mean birth weight of the ROP cases were 1340gms and non ROP babies was 1480gms. Incidence and severity of ROP increased as the birth weight decreased.

### **OXYGEN ADMINISTRATION**

The duration of oxygen administered was an independent risk factor for development of ROP (p=0.001). 23.8% of babies who received oxygen therapy developed ROP in the present study and nearly 50 %of the babies on oxygen therapy developed the disease in other studies.<sup>18,85</sup> Though cases were exposed to hyperoxia and hypoxia more than the controls, it was not found to be a significant factor in causing ROP. This can be explained due to the close monitoring of babies on oxygen therapy by pulse oximetry and arterial blood gas analysis in our unit. The causal link between ROP and supplemental oxygen has been confirmed by controlled trials and clinical studies.<sup>14,85</sup> However, a safe level of oxygen usage has not been defined. Preliminary work has suggested that continuous oxygen monitoring may reduce the incidence of ROP. In present study oxygen administration is a significant risk factor for development of ROP but not an independent risk factor on multivariate analysis.

### **ANTENATAL MATERNAL STEROID INTAKE**

A study conducted by Rosemary et al showed that antenatal steroid administration by the mother had a protective effect against ROP in the neonates.<sup>16</sup>But in our study it was not a significant risk factor.

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**LOW APGAR SCORE AND HYPOXIC ISCHAEMIC ENCEPHALOPATHY (HIE)**

Preterm babies who had a lower APGAR at 1 minute had a higher risk of having ROP in the study conducted by Shah et al.<sup>48</sup> In our study Mean APGAR scores at 1 minute and 5 minutes were significantly lower among case when compared to the controls, signifying that neonates with perinatal asphyxia are at risk of ROP. Distribution of various stages of HIE was significant among the cases but was not an independent risk factor for ROP.

**RDS**

RDS is significant risk factor in the present study but not an independent risk factor on multivariate analysis. Gupta et al.<sup>18</sup> and associates reported ROP in 33.3% of babies with RDS. In our study, 40% of babies among cases had RDS, which is almost comparable to the other studies mentioned.

**SURFACTANT ADMINISTRATION**

Surfactant used to treat hyaline membrane disease has been shown to reduce the risk of ROP but it did not significantly reduced the incidence of ROP in the present study ( $p=0.12$ ). It may be due to the fact that very few cases among babies having RDS had surfactant therapy.

**MULTIVARIATE ANALYSIS OF THE RISK FACTORS**

In study conducted by Chaudhari<sup>26</sup> et al septicemia ( $P<0.001$ ), apnea ( $P=0.0001$ ) and oxygen therapy ( $P=0.031$ ) were independent risk factors In our study on multivariate analysis, GA, duration of hospital stay, day of establishment of feeds, apnea, need for resuscitation and duration of oxygen administration were found to be independently significant risk factors.



**Table 20: Independent Predictors of ROP (Multivariate analysis).**

Factors	P-value
Resuscitation	0.034 (Significant)
Apnea	0.001(Significant)
Duration of Stay in hospital	0.001 (Significant)
Lower Gestational Age	0.015 (Significant)
Days of Establishment of OGF	0.001 (Significant)
Duration of Oxygen	0.003 (Significant)

**EVALUATION OF NEED FOR TREATMENT AMONG CASES**

In study by Austeng, 33.3% required treatment. In our study, four babies (23.5%) had stage 2 plus disease and both required laser treatment. This may be due to the fact that our study had wide screening criteria compared to the other study and also there was strict monitoring for the avoidable risk factors and appropriate and timely screening as per the AAP.

**Conclusion**

1. The incidence of ROP in our study is 21.3%(17) babies, 7(41.17%) babies had stage-1 ROP and10 babies (58.8%) had stage-2 ROP.
2. Male to female ratio was 1.29:1.
3. 7 cases had a stage in zone 2 and 10 had a stage in zone 3.
4. The BW of the ROP babies ranged from 620gm-1700 gm while that of non- ROP babies ranged from 1100gm-1750gm. Maximum number of cases had BW ranging from 1000gm-1499gm where as maximum number of controls had BW ranging from 1500gm-1750gm. LBW was significantly associated with increased incidence of ROP (p<0.001).
5. The mean GA of the cases was 30.12wk ± 2.29wk and the controls were 32.43wk ± 0.96wk. Cases had significantly lower mean GA when compared to controls which was statistically significant (P-value<0.001).

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6. Low birth weight and prematurity are important risk factors for ROP.

7. Mean GA at which complete vascularization of retina was evident was  $43.06\text{wk} \pm 2.63\text{wk}$  for cases where as the controls attained complete vascularization at a mean GA of  $40.48\text{wk} \pm 2.50\text{wk}$  which was significantly earlier than the ROP cases ( $P$  value  $< 0.001$ ).

8. On univariate analysis, mean APGAR scores at 1 minute and 5 minutes were significantly lower among case when compared to the controls, signifying that neonates with perinatal asphyxia are at risk of ROP .

9. On multivariate analysis by application of multiple logistic regression models, GA, duration of hospital stay, day of establishment of feeds, apnea, need for resuscitation and duration of oxygen administration were found to be independent risk factor.

10. The following are the various neonatal complications which were significant on univariate analysis of cases and controls:

- Respiratory distress syndrome: 58.8% of the cases had respiratory distress syndrome compared to 23.8% in the controls which had a high significance ( $p < 0.001$ ).
- Clinical sepsis: Clinical sepsis was present in 70.5% of the cases where as it was present only in 49.2% of the controls ( $p = 0.171$ ).
- Acute kidney injury: Acute kidney injury was considered in the study group if oliguria (urine output  $< 1\text{ml/kg}$ ) is present and/or if serum creatinine was elevated 2 standard deviation above the mean value for gestational age or rise in value was  $\geq 0.3\text{mg/dl/day}$ . 5.9% of the cases had acute kidney injury where as 6.3% of the controls had it, which was not significant ( $p = 0.999$ ).
- Convulsions: Presence of convulsion during the period of admission was a very significant factor among cases (5.9%) when compared to the controls (6.3%) ( $p = 0.517$ ).
- Hypotension: 17.6% of the cases had hypotension requiring inotropic support of atleast one drug. Among controls there were only 12.7% of the babies who required the same. Presence of hypotension was not significant risk factor among the cases ( $p = 0.693$ ).
- Transfusion of blood and blood products: Transfusion of whole blood or any blood products like packed red blood cells, platelet concentrate and fresh frozen plasma transfusion was considered here. 17.6% of case required any one of the above were as only 14.3% of the controls required it. ( $p = 0.711$ ).

11. Meticulous fundus examination with indirect ophthalmoscopy should be done in all preterm babies as per the guidelines and screening should be intensified in the presence of factors like apnea, need for resuscitation, oxygen administration, clinical sepsis, RDS, hypoxic ischaemic encephalopathy, low APGAR, acute kidney injury, convulsions, clinical sepsis, positive CRP, administration of blood and its products and hypotension.

### **Study Limitations:**

1. The sample size is small and may not represent all premature babies in the region. Hence, a larger multi-centric study over a longer duration of period is required to establish the true incidence and causal relationship of risk factors associated with ROP in a developing country like India.
2. Lack of long term follow up to assess future ophthalmological sequelae including myopia, cataract, squint and other long term complications associated of ROP.

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