"A PROSPECTIVE STUDY ON THE CORRELATION BETWEEN BILIRUBIN LEVELS AND RETINOPATHY OF PREMATURITY"

By

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ABSTRACT

There is contrasting data regarding whether bilirubin is protective or toxic during free radical related illness among neonates.160 infants with a gestational age <34 weeks and completed physical examination were enrolled in this study. The infants were divided into two groups based on the whether they had a serum bilirubin level high or low as per the NICE guidelines, a significantly lower TSB was found in neonates with ROP. Lower gestational age, very low birth weight, and prolonged duration of oxygen therapy were associated with a higher occurrence of ROP with the p value being statistically significant. It is concluded that bilirubin may play an antioxidant role *in vivo* as *in vitro*; and protect preterm infant against these free radical related illness like ROP

ABBREVATIONS

ROP	Retinopathy of Prematurity
WHO	World Health Organization
KIDROP	Karnataka Internet Assisted Diagnosis of Retinopathy of Prematurity
RLF	Retrolental Fibroplasia
Sa02	Arterial Saturation of Oxygen
VEGF	Vascular Growth Endothelial Factor
PIGF1	Placental Growth Factor 1
IGF	Insulin Growth factor
GH	Growth hormone
TGF	Transforming Growth Factor
PMA	Post Mensetrual Age
VEGFR	Vascular Endothelial Growth Factor Receptor
CO	Carbon Monoxide
GSH	Glutathione
BVR	Biliverdin
NICU	Neonatal Intensive Care Unit
NICE	National Institute of Clinical Excellence
LMP	Last Menstrual Period
TSB	Total Serum Bilirubin

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INTRODUCTION

Retinopathy of Prematurity (ROP), originally called retrolental fibroplasia, is the leading cause of blindness in children from last fifty years. It was first described in the medical literature in 1942 by Terry. In 1952, Campbell theorized that the condition was caused by the use of oxygen therapy to treat the immature lungs in premature infants.

Retinopathy of prematurity (ROP) is a leading cause of potentially avoidable childhood blindness worldwide. In 2010, an estimated 184,700 preterm babies developed ROP, 20,000 of whom became blind or severely visually impaired from ROP, and a further 12,300 developed mild/moderate visual impairment. More than 65 percent of those visually impaired belonged to moderate income countries.[1]

ROP being a slow spreading global pandemic, reduction in proportion of blindness due to ROP is one of the target of vision 2020.[2] In light of the goals set by the World Health Organization (WHO), Narayana Nethralaya launched the KIDROP program in Karnataka with an aim to tackle infantile blindness due to retinopathy of prematurity. Since 2009, the project has partnered with the National Rural Health Mission, Min. of Health and Family Welfare. By the end of the third quarter of 2012, 18 districts in Karnataka have been included and currently over 81 hospitals are being screened by 3 teams in different geographical zones of the state. The model has also been replicated in parts of Maharashtra and Gujarat with the assistance of the KIDROP team.[3]

Retinopathy of prematurity (ROP) is a vaso-proliferative disease of the premature retina In its more severe forms, it results in severe visual impairment or blindness, both of which carry a high financial cost for the community but also a high individual cost by affecting the normal motor, language, conceptual, and social development of the child[4-5]

Preterm delivery is associated with hyaline membrane disease requiring oxygen. The need for oxygen predisposing them to hyperoxia.[6-7]Studies have shown have shown that premature neonates are unable to augment their antioxidant enzyme activities when exposed to hyperoxia. .[8] Increased predisposal to hyperoxia and the inability to augment their antioxidant activities makes premature neonates highly susceptible to oxygen free radical injuries like retinopathy of prematurity, intraventricular hemorrhage, Broncho pulmonary dysplasia, necrotizing enterocolitis.

As early as 1959, it was suggested that bilirubin might be an antioxidant. Bilirubin can suppress oxidation of lysosomes at oxygen concentrations that are physiologically relevant. Bilirubin can act as an important cyto protector of tissues that are poorly equipped with antioxidant defense systems. [9-10]

Although elevated bilirubin can be harmful, the possible antioxidant role of bilirubin was firstly shown by some *in vitro* studies more than two decades ago. However, human studies have resulted in controversial findings. Despite some investigations showed a direct protective relationship between serum level of bilirubin and the potential antioxidant capacity in preterm neonates ,[11-17] some other

studies have introduced elevated bilirubin as a considerable risk factor for free radical related illnesses in preterm neonates specially ROP .[18-21]

A recent trend in neonatology advocates initiating prophylactic phototherapy to premature infants[22], starting prophylactic phototherapy leads to fall in levels of unconjugated bilirubin causing a fall in antioxidant levels and may predispose them to free oxygen radical mediated injuries like ROP. Evaluation of the antioxidant role of bilirubin in preventing oxygen radical mediated injuries is important in order to achieve less neonatal mortality and morbidity.

OBJECTIVES OF THE STUDY

1. To correlate occurrence of retinopathy with serum bilirubin levels in premature neonates requiring, or, not requiring phototherapy.

2. To study the burden of Retinopathy of prematurity in neonates admitted to RLJH.

REVIEW OF LITERATURE

Retrolental Fibroplasia (RLF, later named retinopathy of prematurely - ROP) represents a watershed in modern neonatology. Unfortunately, in spite of the fact that oxygen was discovered as a risk factor of ROP five decades ago we still are not able to control this disease. However, we now have satisfactory knowledge of this disease which may contribute to development of the therapy to prevent ROP.

Role of oxygen

In 1954 Ashton and Cook were the first to establish that oxygen is important in halting retinal blood vessel development.[23] Several investigations have recently shown a relationship between a high oxygen saturation and ROP.[24-28] At SaO2 > 93% the risk for severe ROP increases. Some of these studies demonstrated, increase in lung problems like chronic lung disease among infants nursed in a high oxygen saturation. [29-30]

Some studies have shown that, fluctuation in SaO2 may contribute to ROP. Exposure to alternating hypoxia and hyperoxia causes severe proliferative retinopathy in the newborn rat, and especially when fluctuations occur at a relatively high level.[31-32]

ROP and Angiogenic Factors

During 14-15 weeks of gestation, Retinal vessel growth begins from the optic nerve and progresses peripherally and anteriorly. This progressing vasculature is accompanied by astrocytes which senses the oxygen level and secrete Vascular Endothelial Growth Factor (VEGF) as a response to hypoxia.[32, 34]

Today it is know that hypoxia induces VEGF production which leads to neovascularization of the border between vascularized and non-vascular retina. Hyperoxia suppresses VEGF, which can be prevented by Placental Growth Factor1 (PIGF-1), a ligand specific for VEGF-receptor1.[33, 35-37]

Stages of ROP development

ROP Phase 1

With premature birth, normal vascular development that would occur in-utero stops, and some developing vessels are lost. At front of these vessels VEGF is however, dependent on insulin like growth factor-1 (IGF-1), which is transported across the placenta. IGF-1 is thus a no-hypoxic regulating factor critical to the development of ROP. [38-39] In ROP Phase-1 triggered during premature birth, IGF-1 is not maintained at in utero levels, and drops dramatically.[38] IGF-1 is usually low after preterm birth. Preterm neonates who later develop ROP have low IGF-1 levels from birth.[38-39] Oxygen therapy after birth causes hyperoxia of the immature retina and suppresses VEGF predisposing ROP

ROP Phase 2

With maturation, the non-vascularized retina shows increased Metabolic Activity and therefore becomes hypoxic. Hypoxia leads to high VEGF inducing neo-vascularization. This is similar to other Proliferative Retinopathies and leads to ROP phase 2, occurring around 32-34 weeks post Conception.[39] However, as the infant matures IGF-1 rises slowly. IGF-1 level reaches this threshold at around 34 weeks post conception and if VEGF levels are high, neovascularization continues.

Growth hormone (GH) is known to be a factor non-related to oxygen that plays a role in regulation of neo-vascularization. GH suppressed neo-vascularization is mediated through inhibition of IGF-1 .[37-38] Transforming growth factor β (TGF-β) inhibits hyperoxia induced VEGFR reduction. TGF-β1 protects retinal capillaries from hyperoxia-induced loss. TGF-β1 and the VEGFR ligand PIGF-1 further increases protection from hyperoxia induced degeneration .[40-41]

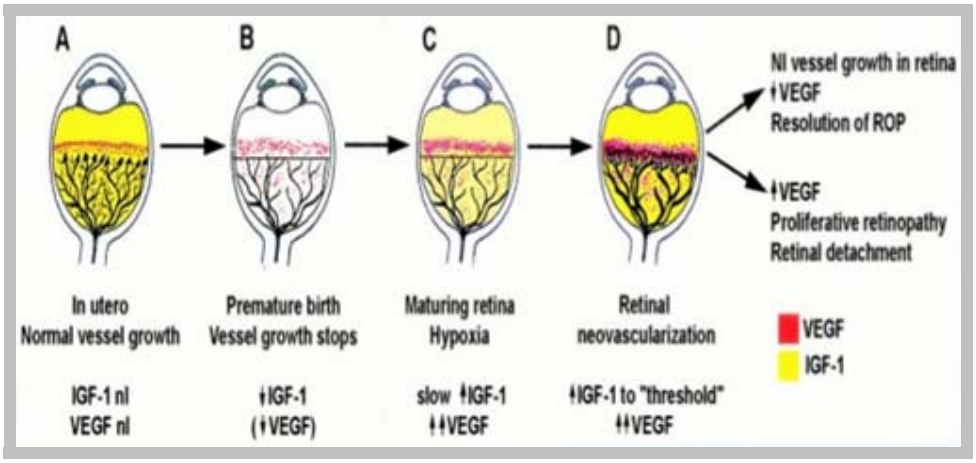


Fig: 1 Diagram Representing Pathogenesis for development of ROP

Factors contributing to development of ROP

Apart from oxygen being the main causative factor, other factors that obstruct the normal development of retinal vascularization in premature newborns, complement each other.[42-43.] These factors are:

- Parameters of immaturity which are given by birth weight and gestational weeks,

- Parameters of general health and many diseases such as distress syndrome, lung atelectasis, pneumonia, intracranial hemorrhage, sepsis, enterocolitis, anemia, transfusions and other disorders of general condition.

The severity of general condition of premature infants is an indication for oxygen therapy. Low weight and low gestational age imply the structural and enzymatic immaturity, which predisposes ROP.[44]

Screening for ROP[45,46]

Development and progression of ROP relates to the baby's postmenstrual age (PMA), that is number of weeks since conception.[46] The timing of screening and window of opportunity for treatment depends on this rather than the baby's post-birth age, especially in the extremely preterm infants.

Guidelines:

- Screen all infants born at <32 weeks of gestational age or weighing <1501 g.
- Babies born before 27 weeks are screened at 30-31 weeks of PMA.
- Babies born between 27-32 weeks OR weighing <1501 g are screened at 28-35 days of postnatal age.
- Screening is weekly or fortnightly according to clinical findings and is carried out by ophthalmologists with a specialist interest in these problems.

Duration of screening

- In babies without ROP, eye examinations may be stopped when vascularization has extended into zone III, usually after 36 weeks of PMA.
- In babies with ROP that does not require treatment, screening can be stopped when the ROP is clearly seen to be regressing on two successive examinations

Disease classification[47]

Classification of ROP was agreed in 1984.[48] It was revised in 2005. There are a number of descriptors used to characterize the amount of ROP. Management and prognosis depend on the location, ~~the~~ extent, ~~the~~ staging and additional factors.

1) **Location** - The retina is divided into concentric zones centered around the optic disc. There are three of these, zone 1 being the innermost and zone 3 the outermost.
2) **Extent**: Amount of disease - the retina is divided into clock hours and involvement is expressed in number of clock hours affected.
3) **Staging**: There are several progressive stages, each describing increasing severity of the disease. These are:
    a) Stage 0 - No clear demarcation line between the developing but as yet non-vascularized area and the vascularized area.
    b) Stage1 - A demarcation line appears between non-vascularized and vascularized areas.
    c) Stage 2 - The demarcation line becomes raised into a ridge.
    d) Stage 3 - Abnormal neovascularization now occurs.
    e) Stage 4 - Partial retinal detachment.
    f) Stage 5 - Total retinal detachment.

Plus and pre-plus disease

- **'Plus disease'** describes tortuosity and venular dilatation. It is the main factor determining the need for treatment at stage 3:
- Plus disease is defined as increased venous dilatation and arteriolar tortuosity of the posterior retinal vessels in at least two quadrants of the eye.
- It may progress to include iris vascular engorgement, poor pupillary dilation (rigid pupil) and vitreous haze.
- **Pre-plus disease** describes vascular abnormalities of the posterior pole that are insufficient for the diagnosis of plus disease, but that cannot be considered normal.

Aggressive Posterior ROP

It is an uncommon, rapidly progressing, severe form of ROP, usually in zone 1, with plus disease. Historically it was known as 'rush disease'. Its features are:

- Posterior location with prominent plus disease.
- Can progress rapidly without going through the classical stages 1-3.
- The retinal changes are less obvious and more easily missed than in other forms of ROP.
- Without treatment, it can rapidly progress to stage 5.

Complications of ROP[48]

There is an increased risk of less serious ophthalmic problems associated with prematurity - e.g., strabismus and myopia. Patients with regressed ROP have a long-term risk of vitreoretinal diseases such as vitreous haemorrhage.[49] Severe or complete visual impairment may result from ROP, and are linked to ROP severity.

ROP can lead to critical complications:[50]

- Myopia.
- Very poor visual acuity.
- Vitreo retinal fibrosis and abnormal retinal traction.
- Peripheral retinal fibrosis.
- Retinal detachment.
- Secondary angle-closure glaucoma.
- Early cataracts.
- Band keratopathy and corneal opacity.

Treatment of ROP

Present therapy for severe ROP is mainly based on laser retinal ablation of the avascular retina. Such therapy reduces incidence of blindness 25 %. However, treatment does not improve the chance of good visual acuity (>20/40). Such therapy therefore still is inefficient.

A number of antioxidants and nutrients have been tested out. Vitamin A and C supplementation do not reduce the rate of severe ROP. [51, 52] In fact, a high ascorbic acid level at the end of 1st week of life indicates worse outcome. A meta-analyses

including limited studies found a significant reduction in stage 3+ (5.3% to 2.4%) with vitamin E (15-100 mg/kg/d). [53]D-penicillamine is a powerful antioxidant and vasomodulator.[54] Some promising data strongly indicate that this drug may reduce severe ROP.[55] In the future a control of vasoactive substances may be of interest.

- In ROP Phase1- the hyperoxic phase, it may be important to elevate VEGF and IGF-1, and
- In ROP Phase2-the hypoxic phase, VEGF should be lowered.

The target in ROP Phase 1 could be to enhance VEGF receptor-1 (VEGFR 1) by PIGF-1 or TGF-β1. Growth hormone (GH) inhibits IGF-1 and consequently VEGF.

In Phase2 Growth Hormone could reduce IGF-1, direct blockers of VEGFR 1 could also be of interest in this phase. However, in order to be successful with such an approach it is utmost important to know exactly in which ROP phase baby is.

Hence, the most important tool at hand is to control oxygen saturation and maintain a careful balance of bilirubin (a powerful antioxidant) thus preventing free oxygen radical mediated injuries

Bilirubin metabolism and Role as an Antioxidant

Bilirubin is widely known as an end product of heme metabolism. Very high levels of serum bilirubin lead to it accumulation in the brain, causing Kernicterus[56-57] Bilirubin is a secondary degradation product of '*heme*'.

Heme is best known as a constituent of hemoglobin, which is released in association with the breakdown of aging red blood cells. Heme is also contained in a wide range of enzymes and their turnover leads to free heme release.

Free heme can be toxic, so nature evolved a family of heme oxygenase enzymes to degrade heme,[58-59]

- Heme Oxygenase blockade leads to an increased excretion of un metabolized heme in the bile.[60]
- These enzymes cleave the heme ring to form biliverdin.
- Iron, and a 1-carbon fragment as carbon monoxide (CO) which is increasingly appreciated as a neurotransmitter,[61-62] and
- Iron, itself toxic, is excreted from cells by a recently characterized pump.[63-67]

As early as the 1950s, bilirubin was reported to protect against the oxidation of lipids such as linoleic acid and vitamin A.[68-69] In the late 1980s, Ames and colleagues demonstrated that the antioxidant effect of bilirubin exceeds that of vitamin E toward lipid peroxidation.[12-13]

Serum concentrations of bilirubin are high enough to account for a substantial portion of the total antioxidant capacity of serum.[70] Thus; bilirubin might alleviate oxidant stress in the blood.

The intracellular environment is exposed to high concentrations of reactive oxygen species. It is now known that the principal cellular antioxidants such as the peptide glutathione (GSH) protects against most instances of oxidative stress.

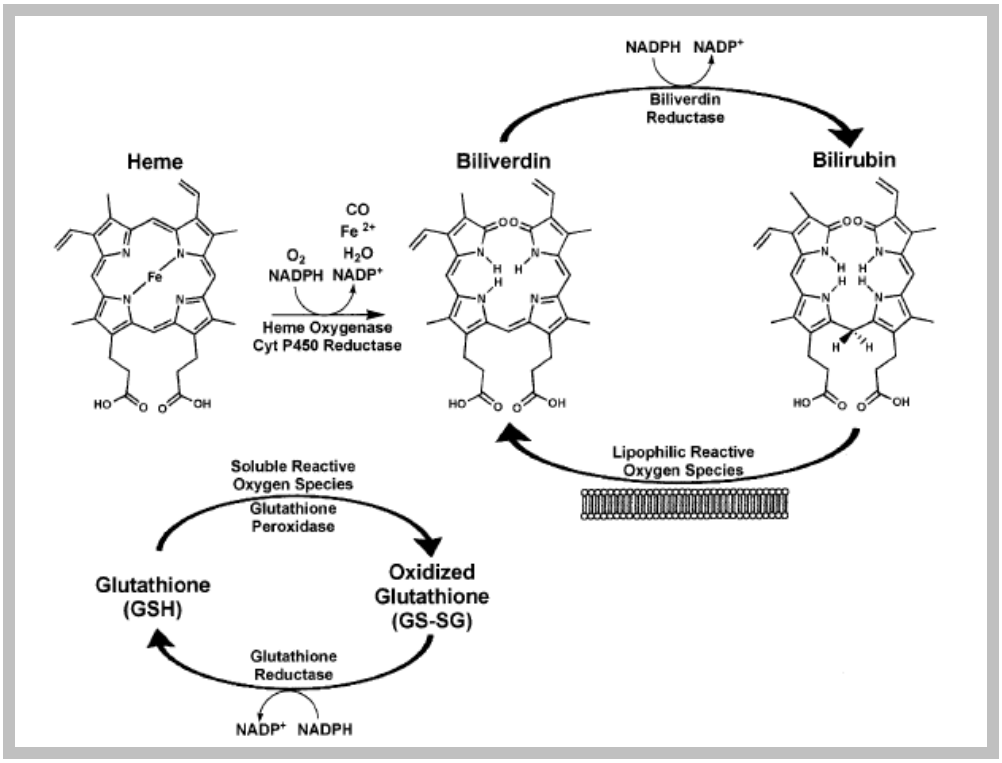


Figure 2: Diagram showing the Mechanism of antioxidant property of Bilirubin

Oxidation-reduction cycles for bilirubin and GSH

Lipophilic reactive oxygen species act directly on bilirubin, leading to its oxidation to biliverdin. BVR catalyzes the reconversion of biliverdin to bilirubin, permitting bilirubin to detoxify a 10 000-fold excess of oxidants. Soluble oxidants are detoxified by GSH, a cycle that requires 2 enzymes, GSH peroxidase and GSH reductase

Clinical significance of antioxidant property of bilirubin

There has been a lot of debate about the clinical implications on the antioxidant role of bilirubin. Some studies have shown a positive effect of elevated serum bilirubin in preventing against oxygen free radical mediated injuries while some studies have shown no effect on the role of bilirubin.

In a retrospective study conducted by Dan D Gaton and coauthors between 1984-1988 analyzed the medical records of 154 preterm neonates less than 34 weeks and less than 1500 grams. They calculated the mean bilirubin during the first 14 days of life and concluded that there was no apparent protective effort of bilirubin in development of ROP. They postulated that as the main pathological effect of ROP is ischemia of peripheral retinal areas due to impaired blood supply, it is reasonable to assume that bilirubin levels in the affected regions are low. It is therefore possible that these low bilirubin levels may be insufficient for the ant oxidative potential effect of bilirubin.[71]

In a prospective study conducted by Hegyi T et al in 1994 where 27 preterm neonates of gestational age 27/+-3 weeks was compared to 57 controls of identical birth weight and gestational age it was concluded that preterm neonates with a higher serum bilirubin levels had lesser incidence of oxygen radical mediated diseases like intraventricular hemorrhage, retinopathy, bronchopulmonary dysplasia, and necrotizing enterocolitis.[72]

In a retrospective study conducted by Jean Claude frauchere in 1994 where 12 preterm neonates less than 32 weeks with advanced ROP was matched for gestational age with 12 preterm neonates with no ROP,serum bilirubin levels were analysed during day 1 to day 8 and it was concluded that bilirubin had no beneficial effect in preventing ROP.[73]

In prospective study conducted in Italy by Romeo MG and other coauthors during 1991-1992 where 219 preterm neonates were analyzed. Serum bilirubin levels were calculated from day 2 to day 7 of life. Results showed that bilirubin levels are higher in neonates which would develop ROP at any stage than the control group. The results therefore conclude that bilirubin doesn't have any role in preventing ROP.[74]

In another prospective study conducted in Michigan, USA by Mitchel H Dejonge and other co authors in 1998 where 157 preterm neonates were analyzed between gestational ages 23-26 weeks found no definitive relation between bilirubin levels and ROP.[75]

In a prospective study conducted in Hong Kong by Lam BCC in 1998 where all preterm neonates less than 32 weeks admitted to neonatal intensive care unit were analyzed it was found that the incidence of ROP was 28% which was comparatively less than the incidence of ROP as per studies conducted by Palmar in 1991 (Multicenter trial of cryotherapy for ROP) where 4540 neonates were analyzed and the incidence of ROP was found to be 65.8%. Similar high incidence for ROP was demonstrated in studies conducted by JE Garoll in 1994 in Sweden where rate of incidence of ROP was 47.4%. It was proposed that hyperbilirubinemia is a common problem among Chinese neonates[76-77] but whether the lower incidence of ROP was attributable to bilirubin remained unclear.[78]

In a study conducted by Alfreid Kohlschutter and other co-workers in 2001 at France measured the susceptibility of newborn plasma to in vitro oxidation in micro samples from 57 neonates and 18 adults, the levels of bilirubin- a powerful antioxidant were higher and levels of poly unsaturated fatty acids was lower in the in the neonatal sample. Plasma oxidizability correlated positively for polyunsaturated fatty acids and negatively for bilirubin. The study hence conclude that plasma is better protected in neonates against oxidation injury than adults owing to higher concentration of bilirubin and lower content of oxidizable lipids.[79]

In a study conducted by Shigeharu Hosono and other coworkers at Japan in 2001 where 76 preterm neonates at 24-25 weeks of gestation were analyzed. Daily bilirubin levels were measured between day 1 to day 14 of life and patients were grouped according to severity of ROP and the results demonstrated there was no protective effect of bilirubin in development of ROP.[80]

In an article published in Pediatrics by Thomas W Sedalk and others showed that bilirubin has got antioxidant properties and undergoes cellular protection by a biliverdin reductase anti oxidative cycle.[81]

In an Indian study conducted by Shakeeb Sahab and other coworkers in PGIMER Chandigarh in 2008 analyzing the oxidant and antioxidant status in term neonates blood samples were grouped into 4 groups based on the serum bilirubin concentration. The concentration of Super oxide dismutase and total antioxidant capacity of plasma was investigated. It was found that the total antioxidant capacity of plasma was higher with a higher concentration of bilirubin. This clearly showed that at higher concentration bilirubin induces the total antioxidant capacity of plasma.[82]

In a prospective, analytic, cross-sectional, case-control study conducted in Iran by Seyedeh Fatemeh Khatam in 2001 where 60 preterm newborns with birth weights less than 2000 g**,** and gestational age less than 34 weeks admitted to the NICU were included in the study There were no significant differences between sex, serum bilirubin level, sepsis, episodes of apnea, blood exchange transfusion, blood transfusion, and the duration of NPO, duration of hospitalization and use of mechanical ventilation. There were significant differences between gestational age, birth weight, episodes of hyperoxia, acidosis and ROP (P<0.001). There was also a significant difference between the mean episodes of hypocarbia and hypercarbia,

duration of oxygen therapy and duration of exposure to phototherapy light in two groups.[83]

In another prospective observational study conducted at Iran where 173 preterm neonates less than 37 weeks were analyzed. Potential risk factors, like gestational age , birth weight, supplemental oxygen therapy, acute respiratory distress syndrome, sepsis, blood transfusion, and phototherapy were assessed. The incidence of ROP was found to be 19.1% and it was concluded that higher levels of bilirubin were found to be associated with a lower incidence of ROP.[84]

In another analytical retrospective longitudinal study conducted by Seyed Mohammad Fereshtehnejad and other coworkers in 2011 at Iran evaluating the possible anti oxidative role of bilirubin protecting from free radical related illnesses in neonates. Seventy one infants with gestational age (GA) of <32 weeks and/or birth weight (BW) of <1500 *g*, who survived beyond 4 weeks and completed physical examinations were enrolled in this study. The infants were divided into two groups based on the presence or absence of advanced retinopathy of prematurity (ROP). The study supported the beneficial role of bilirubin against ROP.[85]

In a retrospective study conducted by Joanna S Kao and other coworkers in 2011 evaluating the possible effects of bilirubin and human milk in protection from retinopathy of prematurity was undertaken at Loa university USA ,739 neonates .were studied in the group from 2000-2009. The study showed that bilirubin may have a protective role in development of ROP where as human milk showed no effect on the occurrence of ROP.[86]

In a cross sectional study conducted by Majid Abrishami and other coworkers in Iran where 960 preterm neonates less than 32 weeks were studied. Oxygen free radicals play an important role in ROP development. Serum bilirubin within the first days of life has antioxidant effects. By reducing the levels of this antioxidant, phototherapy is likely to intensify ROP. The study further states that the indications for phototherapy use (and especially prophylactic phototherapy) in preterm infants should be revised and carefully followed.[87]

MATERIALS AND METHODS

Study hospital

R L Jalappa hospital Kolar is a tertiary level hospital catering to the local needs of the people of Kolar district. The neonatal unit of the hospital has two fully functional level III Neonatal intensive care units. Being the only level 3 neonatal unit in an area of 70 sq. km the unit admits approximately 2000 sick neonates annually.

Preterm deliveries contribute to approximately 10 % of the total NICU admissions annually.

Study population

Preterm neonates admitted to the neonatal unit with gestational age less than 34 weeks as determined by the last menstrual period of the mother, Ballard's score or 1st trimester scan from December 2013-february 2014 were registered for the study.

Criteria for inclusion of the preterm neonates into the study:

Neonates with gestational age less than 34 weeks admitted to the neonatal unit over a period of one year from December 2013 to February 2015 were included in the study.

Criteria for exclusion of the preterm neonates to be considered under the sample

Preterm neonates having conjugated hyperbilirubinemia defined as bilirubin levels more than 2 mg/dl or pathological jaundice requiring exchange transfusion were excluded for the sample size.

Study design

Evaluation of the role of bilirubin in preventing retinopathy of prematurity is a prospective cohort study design approach

Study tools

I) Performa (Annexure)

A structured Performa was prepared to collect information on variables to assess the risk factors of development of retinopathy of prematurity. The studied preterm neonates were divided into 2 categories group one having high bilirubin levels requiring phototherapy as plotted by the NICE guidelines and group two having serum bilirubin levels within physiological limits not requiring phototherapy. Other factors thought to be important in development of ROP like duration of oxygen therapy, gestational age, and sepsis, mechanical ventilation were documented.

The information required to complete the Performa was obtained by taking due consent from the parents and reviewing the neonatal case sheets on a daily basis.

Using the Performa the following assessment was carried out

A) Assessment of the gestational age

The gestational age was documented as per the last menstrual period of the mother in absence of LMP, Ballard's scoring system or 1st trimester scan was used to document the gestational age of the baby.

B) Assessment of risk factor for development of ROP

Duration of oxygen therapy, use of mechanical ventilation, sepsis and role of antenatal steroids thought to be important in the development of ROP was collected.

2) Ophthalmic examination

The preterm neonates in both the groups were subjected to an ophthalmic examination by a pediatric ophthalmologist .The retina examination revealed what stage of ROP the babies were. The ophthalmologist was unaware as to which group the baby belonged-either high bilirubin or normal bilirubin in order to remove bias.

Operational definitions

1. **Preterm baby**- defined as a gestational age less than 34 weeks because the risk of development of ROP was higher in this group of babies.
2. **High bilirubin**-defined as elevated levels of bilirubin higher than phototherapy zone as determined by the NICE guidelines (**ANNEXURE).**
3. **Low bilirubin-**defined as bilirubin levels below the phototherapy zone as determined by NICE guidelines **(ANNEXURE).**
4. **Very high bilirubin-**defined as bilirubin levels in exchange zone.
5. **ROP**- ROP is described by its location in the eye (the zone), by the severity of the disease (the stage) and by the appearance of the retinal vessels (plus disease). The first stage of ROP is a demarcation line that separates normal from premature retina. Stage 2 is a ridge which had height and width. Stage 3 is growth of fragile new abnormal blood vessels. As ROP progresses the blood vessels may engorge and become tortuous (plus disease)[1]. Any baby with ROP stage 1 or above or any plus disease was thus taken to suffer from ROP.

6. **Threshold ROP**- any baby with stage 3 zone 1 or any stage with plus disease was taken as threshold ROP.
7. **Pre threshold ROP**- any baby having stage 1 or stage 2 with zone 1 without plus disease was taken as pre threshold ROP
8. **Prolonged oxygen requirement**-defined as the duration of oxygen supplementation in order to reach a $SpO_{2<}$91% at room air
9. **Sepsis**-defined as either culture positive sepsis or a positive sepsis screen

STUDY METHODOLOGY

After obtaining permission from the institutional review board and written informed consent from the parents of the patient all the detailed information was entered in the Performa. Consent was taken from the parents of all the 160 preterm neonates enrolled in the study to detect the serum bilirubin levels at 48hours of life along with other investigations like renal function test and serum electrolytes which are a routine in neonatal care. Due to ethical considerations the serum bilirubin could not be done on a daily basis. the decision to reassess the serum bilirubin levels was made on the clinical signs of neonatal jaundice as supported by the NICE guidelines **(ANNEXURE)** in case serum bilirubin were detected multiple times the mean bilirubin value was taken and entered in the master chart. During the first 7 days of life, the babies were observed for development of neonatal jaundice. On day 21 of life the neonates were subjected to an ophthalmic examination and were followed up routinely for a maximum period of 6 months. The levels of retinopathy among preterm neonates were compared between the groups which had high bilirubin levels warranting the need for phototherapy to those preterm neonates who had bilirubin levels below phototherapy range.

Figure 3 :IMAGE SHOWING PHOTOTHERAPY BEING ADMINISTERED TO A PRETERM BABY.

Figure 4 : IMAGE SHOWING THE ROP EXAMINATION BEING CONDUCTED AT OUR NICU.

Statistical Analysis

The relevant statistical tests were applied for measuring the significance and association parameters. The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1 ,Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables.

RESULTS AND ANALYSIS

Study design: **A prospective cohort study**

Table 1: Distribution of the studied new born based on sex

Gender	No. of patients	%
Male	**82**	**51.0**
Female	**78**	**49.0**
Total	**160**	**100.0**

Out of studied preterm neonates males were 51% (n=82) and 49 % were females (n=78)

Graph 1: Distribution of the studied new born based on sex

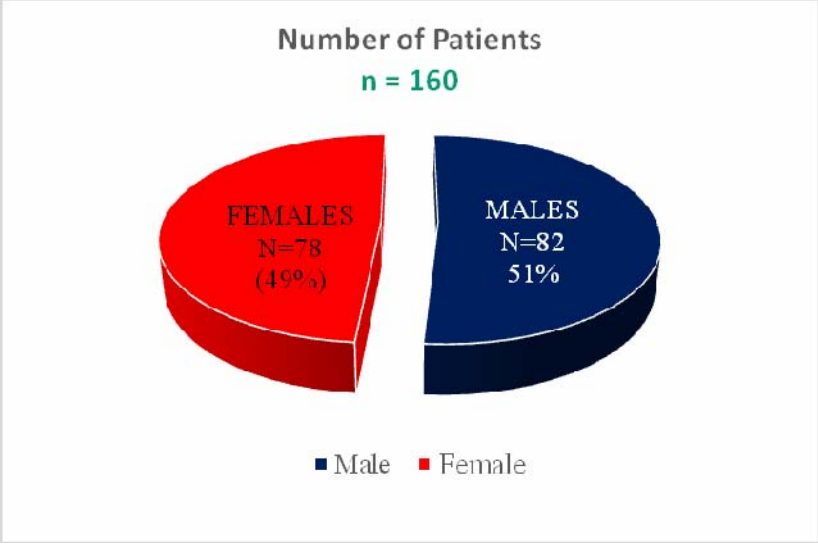


Table 2: Distribution of the studied new born as per birth (weight in grams)

Birth Weight (grams)	No. of patients (n=160)	Mean and standard deviation
<1000	3 (1.8%)	**1578±280 grams**
1001-1500	48(30%)	
1501-2000	106(66.2%)	
2001≤2500	3(1.8%)	
Total	**160**	

The table clearly shows the distribution of the studied newborn based on the birth weight.The mean birth weight of the studied population was 1578±280 grams.

Graph 2: Distribution of the studied new born as per birth weight in grams

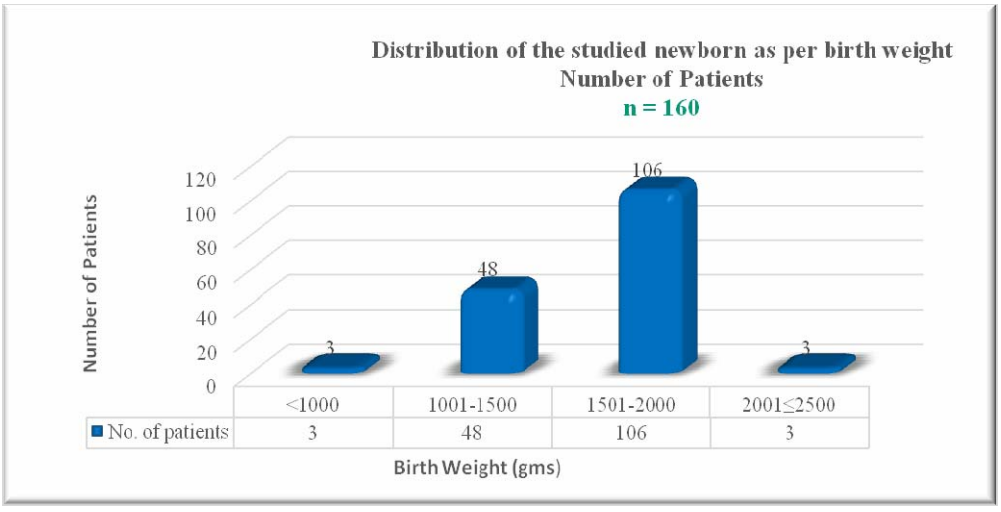


Table 3: Distribution of the studied new born as per period of gestation

Period of Gestation (in weeks)	No. of patients (N=160)	Mean gestational age and standard deviation
28-30	14(8.7%)	**32.26±1.4 weeks**
31-32	66(41.3%)	
33 ≤ 34	80(50%)	
Total	**160**	

Out of the entire studied population majority of preterm neonates 91% were between 31-34 weeks of gestation. The mean gestational age of the studied population was 32.26±1.4 weeks.

Graph 3: Distribution of the studied new-born as per period of gestation

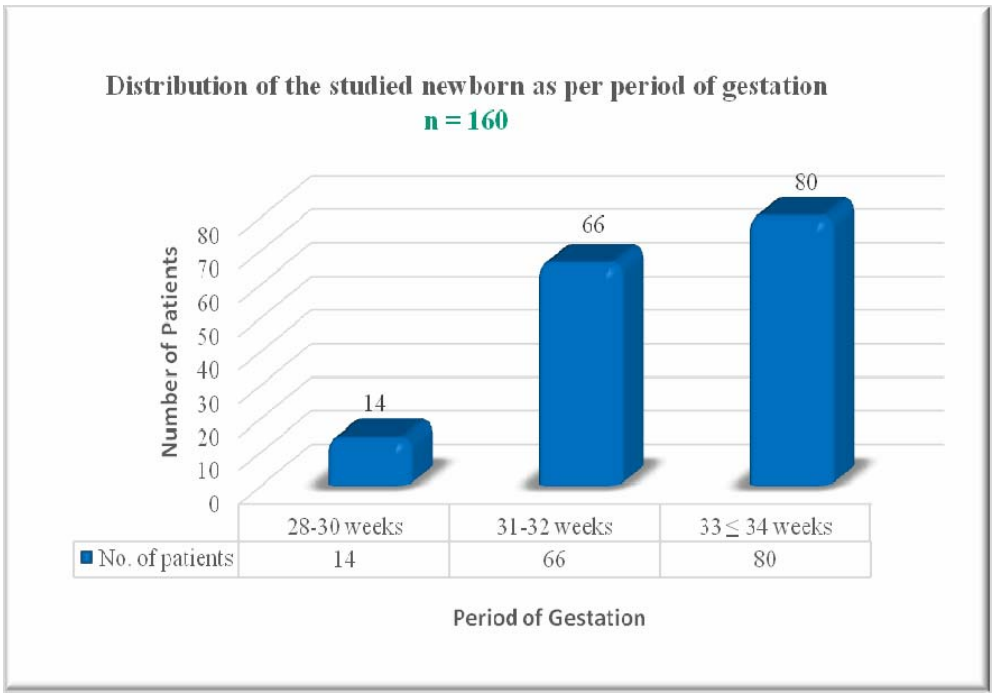


Table 4: Distribution of the studied new born with bilirubin levels and period of gestation

Levels of Bilirubin	Gestational age in weeks							Total (N=160)
	28 weeks (N=3)	29 weeks (N=9)	30 weeks (N=3)	31 weeks (N=22)	32 weeks (N=43)	33 weeks (N=51)	34 weeks (N=29)	
Low	0(0%)	5(55.6%)	2(66.7%)	9(40.9%)	24(55.8%)	22(43.1%)	13(44.8%)	**75(46.9%)**
High	3(100%)	4(44.4%)	1(33.3%)	13(59.1%)	19(44.2%)	29(56.9%)	16(55.2%)	**85(53.1%)**
Total	**3(100%)**	**9(100%)**	**3(100%)**	**22(100%)**	**43(100%)**	**51(100%)**	**29(100%)**	**160(100%)**

P=0.491, Not significant, Fisher Exact test

The table shows the distribution of studied population with bilirubin levels and period of gestation. The table concludes that there is no risk of hyperbilirubinemia in relation to gestational age.

Graph 4: Distribution of the studied new born with bilirubin levels and period of gestation

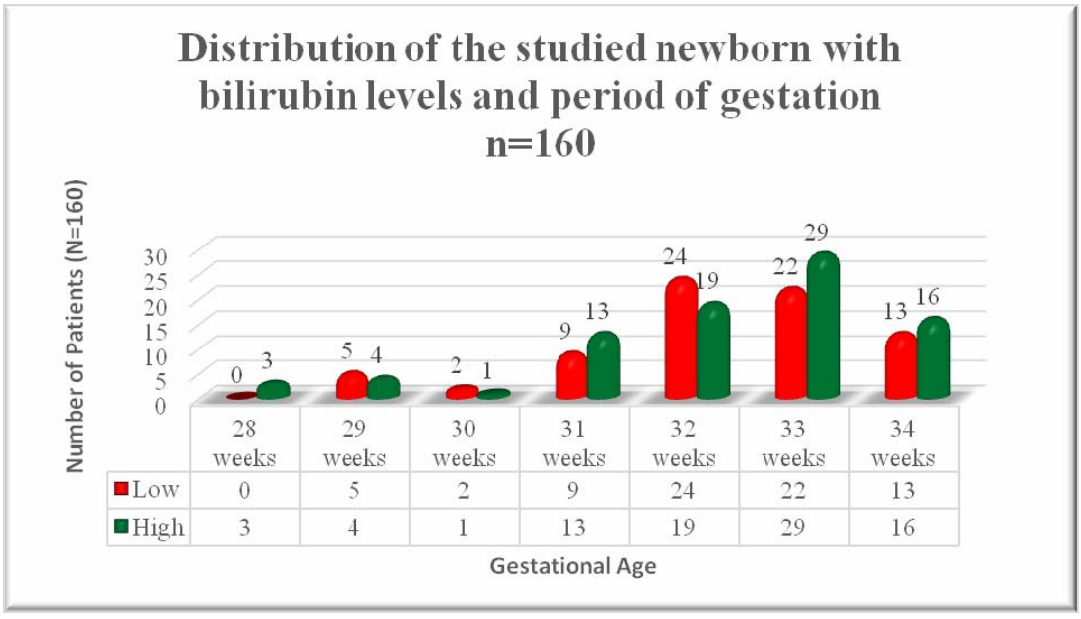


Table 5: Distribution of studied new born with gestational age and duration of oxygen requirement.

Oxygen Requirement	Gestational age in weeks							Total (n=160)
	28 weeks (N=3)	29 weeks (N=9)	30 weeks (N=3)	31 weeks (N=22)	32 weeks (N=43)	33 weeks (N=51)	34 weeks (N=29)	
Less than Equal to 24 hours	0(0%)	1(11.1%)	1(33.3%)	13(59.1%)	34(79.1%)	36(70.6%)	26(89.7%)	**111(69.4%)**
More than 24 hours	3(100%)	8(88.9%)	2(66.7%)	9(40.9%)	9(20.9%)	15(29.4%)	3(10.3%)	**49(30.6%)**
Total	**3(100%)**	**9(100%)**	**3(100%)**	**22(100%)**	**43(100%)**	**51(100%)**	**29(100%)**	**160(100%)**

Table shows the distribution of the studied new born in relation to duration of oxygen requirement and gestational age. The table concludes that preterm neonates with a lower gestational age have a higher duration of oxygen requirement and this difference in duration of oxygen requirement was statistically significant (P<0.001**, significant, Fisher Exact test)

Graph 5: Distribution of studied new born with gestational age and duration of duration of oxygen requirement

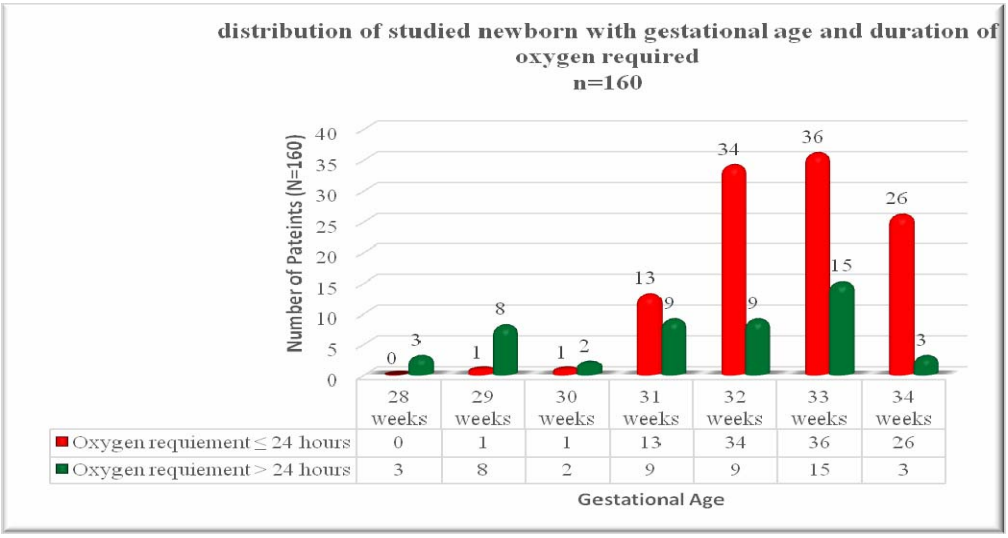


Table 6: Distribution of studied new born with gestational age and occurrence of sepsis

Sepsis	Gestational age in weeks							Total (n=160)
	28 weeks	29 weeks	30 weeks	31 weeks	32 weeks	33 weeks	34 weeks	
Negative N=82	0(0%)	4(44.4%)	2(66.7%)	12(54.5%)	22(51.2%)	22(43.1%)	20(69%)	**82(51.3%)**
Positive N=78	3(100%)	5(55.6%)	1(33.3%)	10(45.5%)	21(48.8%)	29(56.9%)	9(31%)	**78(48.8%)**
Total	**3(100%)**	**9(100%)**	**3(100%)**	**22(100%)**	**43(100%)**	**51(100%)**	**29(100%)**	**160(100%)**

The table shows the distribution of the study population to the occurrence of sepsis. The table shows that the occurrence of sepsis isn't dependent on the gestational age of the baby (P=0.192, Not significant, Fisher Exact test)

Graph 6: Distribution of studied new born with gestational age and occurrence of sepsis

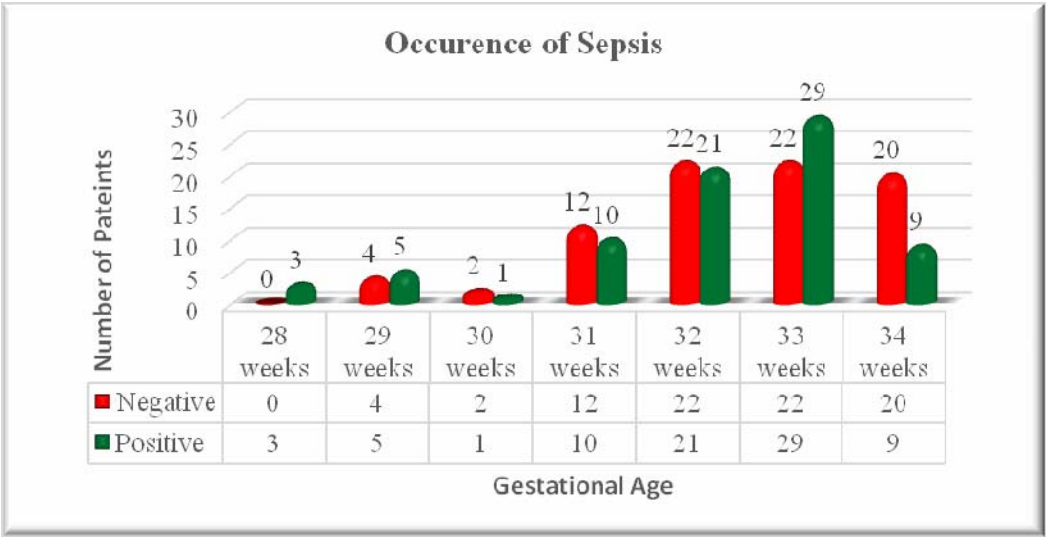


Table 7: Correlation between ROP status and sex of the studied new-born

ROP status	Gender		Total (n=160)
	Female (n=78)	Male (n=82)	
NO ROP	71(91%)	73(89%)	**144(90%)**
ROP	7(9%)	9(11%)	**16(10%)**
Total	**78(100%)**	**82(100%)**	**160(100%)**

The table shows the correlation between ROP status and sex of the studied population. From the table it is seen that there was no correlation between sex and ROP status of the studied population (P=0.673, Chi-Square test)

Graph 7: Correlation between ROP status and sex of the studied new born

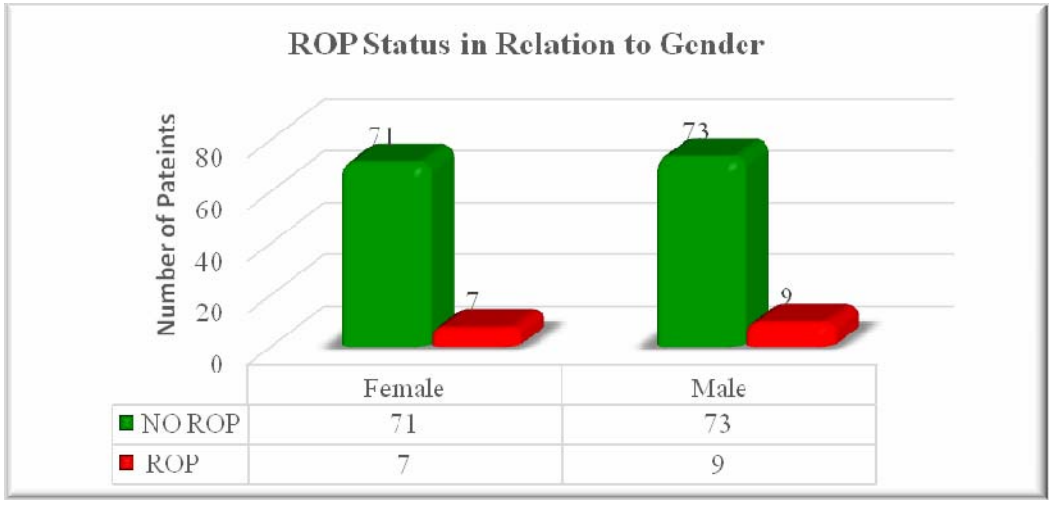


Table 8: Correlation between ROP status and birth weight (in grams) of the studied new born.

Birth weight in grams	No ROP (n=144)	ROP (n=16)
Less than 1500 grams	42	10
1500 grams to 2500 grams N=108	102	6
Total (n=160)	**144**	**16**

The table shows the correlation between ROP status and birth weight of the studied population. The table shows that a higher occurrence of ROP in VLBW babies as compared to low birth weight babies and this difference between birth weights of the studied new born was statistically significant (p value 0.006 chi square).

Graph 8: Correlation between ROP status and birth weight (in grams) of the studied new born.

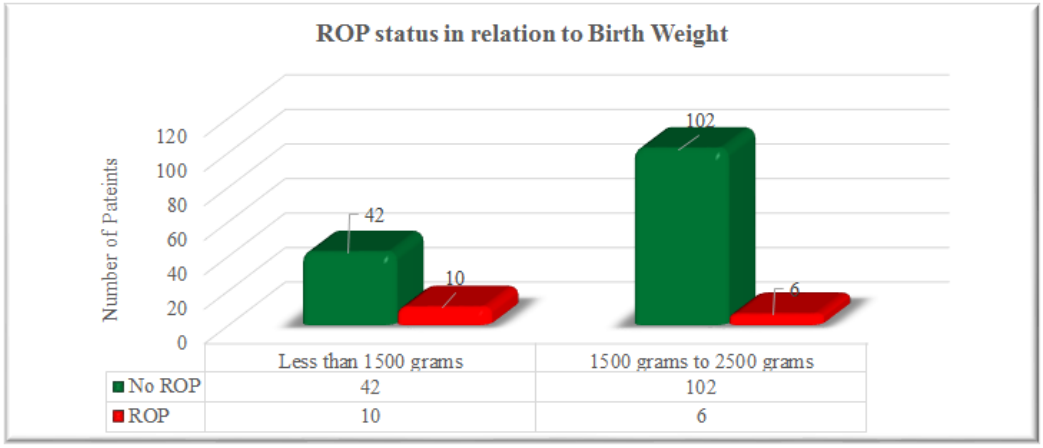


Table 9. Correlation between ROP status and gestational age in weeks of the studied new-born.

ROP Status	Gestational age in weeks			Total n=160
	28-30 weeks (n=15)	31-32 weeks (n=65)	33≤ 34 weeks (n=80)	
NO ROP	7(46.7%)	61(93.8%)	75(93.8%)	**144(90%)**
ROP	8(53.3%)	4(6.2%)	5(6.3%)	**16(10%)**
Total	**15(100%)**	**65(100%)**	**80(100%)**	**160(100%)**

The table shows the correlation between gestational age and ROP status. The table concludes that babies with a lower gestational age had a higher occurrence of retinopathy of prematurity and this difference in gestational age of the studied new-born is statistically significant (P<0.001**, significant, Chi-Square test)

Graph 9: Correlation between ROP status and gestational age in weeks of the studied new-born.

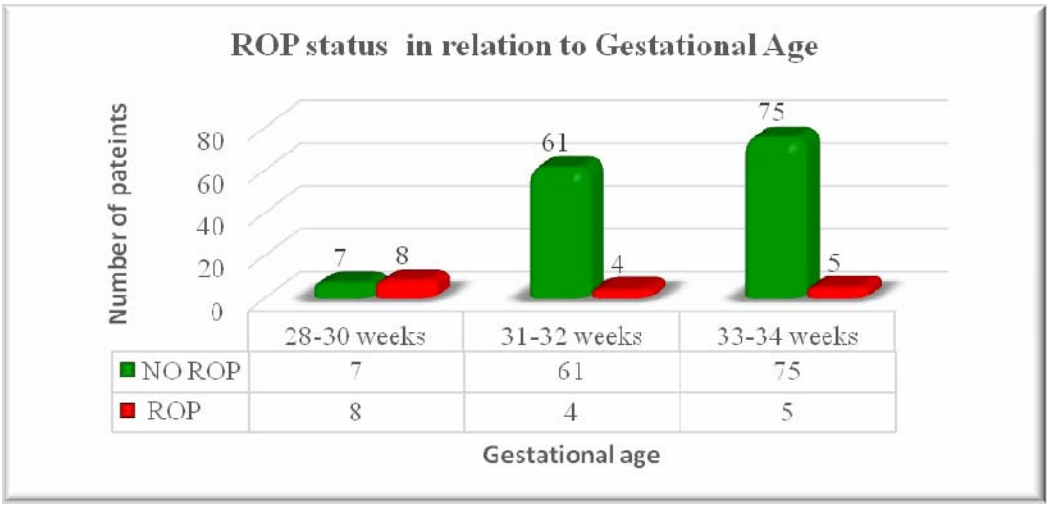


Table 10: Correlation between ROP status and sepsis in the studied new born

ROP Status	SEPSIS		Total (n=160)
	Positive(n=82)	Negative(n=78)	
NO ROP	73(89%)	71(91%)	**144(90%)**
ROP	9(11%)	7(9%)	**16(10%)**
Total	**82(100%)**	**78(100%)**	**160(100%)**

The table shows the co relation between sepsis and ROP status of the studied neonates. From the table it is seen that there is no correlation between sepsis and ROP status in the studied new-born (p value0.673, Not significant, Chi-Square test)

Graph 10: correlation between ROP status and sepsis in the studied new born

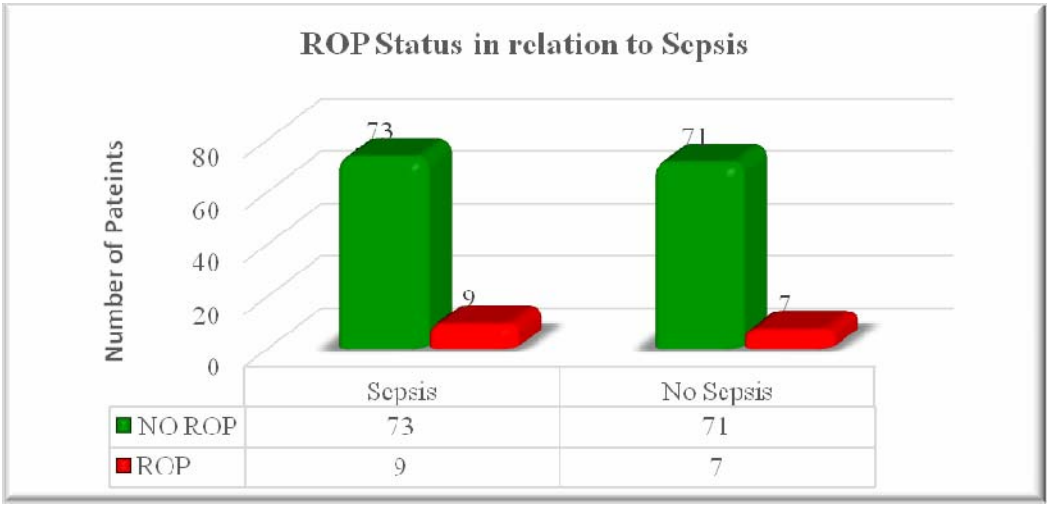


Table 11: Burden of ROP in patients studied

Burden of ROP	No. of patients n=160	%
No ROP	144	**90.0**
ROP	16	**10.0**
Total	**160**	**100.0**

Table shows the burden of ROP in the studied population. The table shows the burden of ROP in neonates less than 34 weeks of gestation as 10%

Graph 11: burden of ROP in patients studied

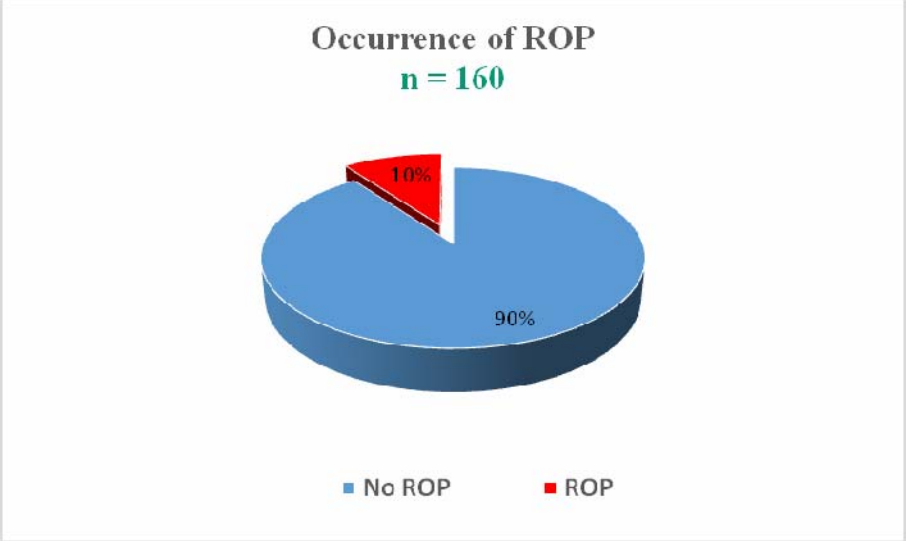


Table 12: table showing the severity of ROP in the study population

SEVERITY OF ROP	NUMBER OF PATIENT (N=16)	PERCENTAGE
Pre-threshold ROP	14	87%
Threshold ROP	2	13%
Total	16	100%

Graph 12: Graph showing the severity of ROP in the study population

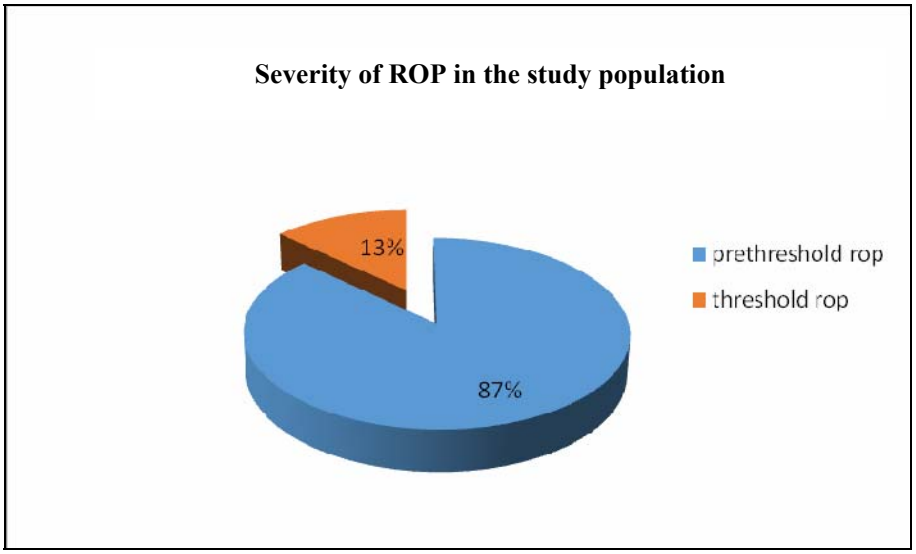


Table 13: Correlation between ROP status and duration of oxygen requirement in the studied new-born

Oxygen of Requirement	Burden of ROP		Total N=160
	No ROP N=144	ROP N=16	
Less than Equal to 24 hours (N=111)	107(74.3%)	4(25%)	**111(69.4%)**
More than 24 hours (N=49)	37(25.7%)	12(75%)	**49(30.6%)**
Total	**144(100%)**	**16(100%)**	**160(100%)**

The table shows the correlation between duration of oxygen requirement and ROP status of the eye. From the table it is shown that there was a higher occurrence of ROP in babies who required oxygen for a longer duration and this difference between the duration of oxygen requirement is statistically significant (P<0.001**, significant, Chi-Square test)

Graph 13: Correlation between ROP status and duration of oxygen requirement in the studied new-born

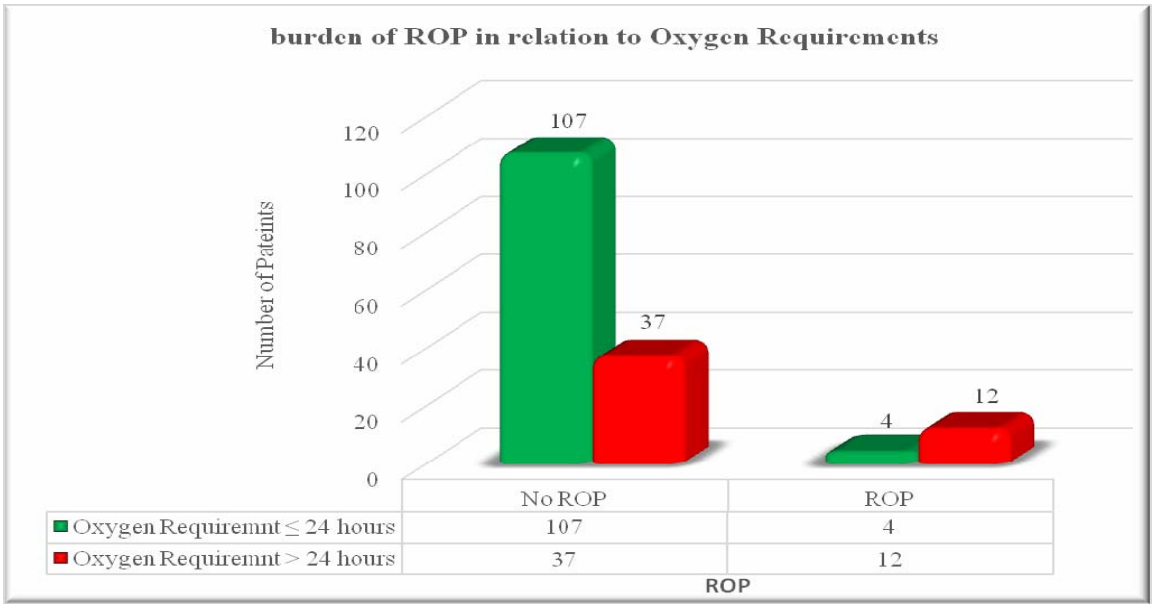
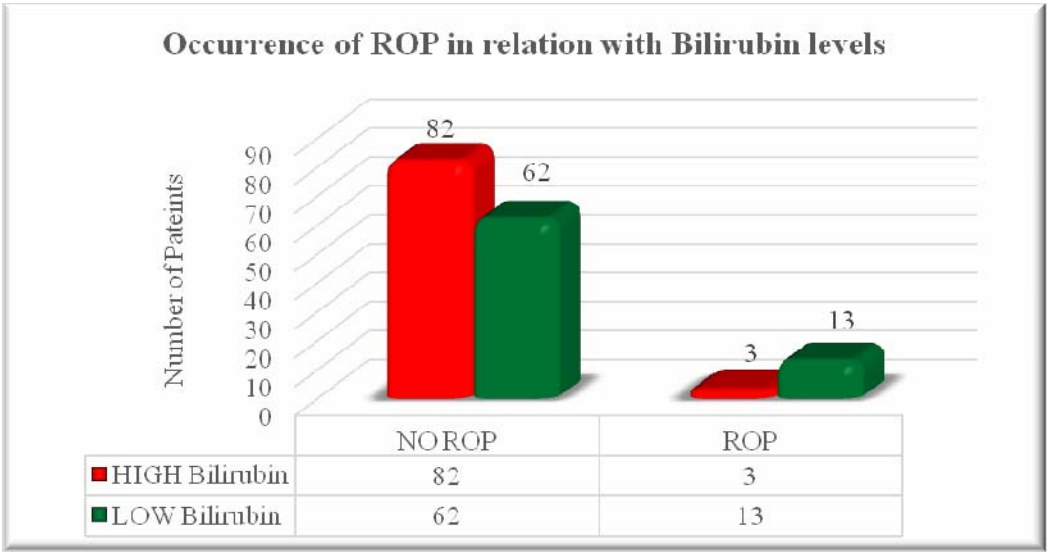


Table 14: Correlation between bilirubin levels and the risk of developing ROP

BILIRUBIN LEVELS	Occurrence of ROP		Total (n=160)
	NO ROP (N=144)	ROP (N=16)	
HIGH (N=85)	82(56.9%)	3(18.8%)	**85(53.1%)**
LOW (N=75)	62(43.1%)	13(81.3%)	**75(46.9%)**
Total	**144(100%)**	**16(100%)**	**160(100%)**

The table shows the correlation between bilirubin levels and ROP. From the table it is seen there was a less occurrence of ROP among neonates having a high bilirubin levels (P=0.005**, significant, Chi-Square test)

Graph 14: correlation between bilirubin levels and the occurrence of ROP



Statistical Methods: Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. The following assumptions on data is made, Assumptions: 1.Dependent variables should be normally distributed, 2.Samples drawn from the population should be random, Cases of the samples should be independent

Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups.

Significant figures

+ Suggestive significance (P value: $0.05<P<0.10$)

* Moderately significant (P value: $0.01<P \leq 0.05$)

** Strongly significant (P value: $P \leq 0.01$)

Statistical software: The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1 ,Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc**.**

DISCUSSION

Study of the correlation between bilirubin levels and retinopathy of prematurity was a prospective cohort study carried out in a tertiary care hospital during period of Jan 2014 to march 2015. In this study n=160 preterm neonates less than 34 weeks were included.

Baseline characteristics

Out of 160 studied cases 51 % (n=82) were males and 49 %(n=78) were females **(table 1)** which is consistent with study carried by Majid Abrishami et al. (2013) which had 56% males and 44 % females in their study group.[87]

Out of 160 preterm neonates the mean birth weight of the studied new born was 1560 grams (**table 2**) which is higher than the mean birth weight as compared to study conducted by Joanna S. Kao et al.[86] The difference in the mean birth weight between this study and others mainly attributable to the higher number of babies between 33-34 weeks of gestational age.

Out of 160 preterm neonates the mean gestational age of the studied preterm was 32.3 weeks(**table 3)** which is consistent with study carried by Seyed Mohammad Fereshtehnejad et al.[85] and by Majid Abrishami et al. (2013)[87] where mean gestational age was 30.54 and 30.17 weeks respectively

Table 15: Showing the baseline characters in this study in comparison with other studies

	Present Study	Joanna S. Kao et al (2011) (n=132)	Majid abrishami et al. (2013) (N=122)	Seyed Mohammad Fereshtehnejad et al (2011) (N=71)
Male	**51%**	55%	44%	56%
Female	**49%**	45%	56%	44%
Birth weight	**1560 grams**	962 grams	1250 grams	1234 grams
Gestational age	**32.3 weeks**	27 weeks	30.54 weeks	30.17 weeks

The difference in period of gestation and birth weight in our study is mainly due to the higher number of babies in gestational age between 32-34 weeks in our study.

Risk factors for development of ROP

The study found a higher occurrence of ROP among preterm neonates less than 31 weeks of gestation and it was concluded that babies with a lesser gestational age have a higher occurrence of ROP as compared to late preterm infants and this difference in occurrence of ROP among neonates was statistically significant(p value<0.001). This data is consistent with data published by Neeraj Gupta et al 2010.[88] which also found a higher incidence of ROP till 32 weeks of gestation

The study also demonstrated a higher occurrence of ROP in preterm neonates less than 1500 grams and it was concluded that babies with a very low birth weight have a higher occurrence of ROP as compared to low birth weight babies. This difference in occurrence of ROP among neonates was statistically significant (p value=0.006) this data is consistent with study conducted by Majid Abrishami et al[87] A higher duration of oxygen therapy was required in extremely premature neonates <30 weeks of gestation. The increased duration of oxygen therapy is mainly attributable to the higher incidence of hyaline membrane disease in these neonates.

Oxygen was the main causative factor for development for ROP. Neonates who were exposed to prolonged duration of oxygen therapy had a higher occurrence of ROP. This difference in occurrence of ROP among neonates was statistically significant (p value <0.001 which is strongly significant). Prolonged duration of oxygen supply is known to be the most risk factor for development of ROP. The same results were published in study conducted by Joanna S. Kao et al[86], Majid Abrishami et al[87], Seyed Mohammad Fereshtehnejad et al.[85]

Sepsis had no role for development of ROP (p value =0.673) which is not significant) This data is consistent with a study by Seyed Mohammad Fereshtehnejad et al who also proved that sepsis was not contributory factors for development of ROP.[85]

The preterm neonates were exposed to develop exaggerated physiological jaundice requiring phototherapy. The risk of developing exaggerated neonatal jaundice was highest at 28 weeks of gestation where 100 % babies developed jaundice and the risk decreased with advancing gestational age. This result is consistent with data published by Maisels MJ et al [88] and Koerner F et al. [89]

The burden of ROP in preterm neonates less than 34 weeks admitted to our hospital was 10 % (table number 11). The occurrence of threshold ROP was 12.5% whereas the occurrence of pre threshold ROP was 87.5 % (table 12). The incidence of ROP varied with gestational age of the baby, for babies less than 31 weeks the burden of ROP was 53.4 %. This data is consistent is consistent to data published by Neeraj Gupta et al 2010.[88]

Role of bilirubin in preventing ROP

Both the groups were compared with relation to oxygen requirement sepsis birth weight and period of gestation which are also considered as important risk factors in development of ROP.[9]

Table 16: Showing comparison of risk factors between the two groups for development of ROP

Risk Factors	**Group 1 (n=85) High Bilirubin**	**Group 2 (n=75) Low Bilirubin**	**P Value**
Oxygen Requirements			
• **> 24 hrs.**	20(25.3%)	23(30.7%)	**0.459**
• **< 24 hrs.**	59(74.7%)	52(69.3%)	
Sepsis			
• **Positive**	44(51.8%)	37(49.3%)	**0.759**
• **Negative**	41(48.2%)	38(50.7%)	
Gestational Age in weeks Mean	33±1.34 weeks	34±1.42 weeks	**similar**
Birth weight in grams mean	1607±180 grams	1562±160 grams	**similar**

It is clearly evident from this table that the two groups were identical in all aspects like oxygen requirement, sepsis, gestational age, and birth weight they were only different with relation to levels of bilirubin.

There was a lesser risk of development of ROP in babies having a higher bilirubin level as compared to babies with low bilirubin levels (p value=0.005 which is strongly significant)

3 babies developed ROP in a group of 85 neonates with a higher bilirubin value. None of these 3 babies had threshold ROP which required treatment. There was a higher occurrence of ROP in the group with a lower bilirubin value. In the group with low bilirubin 13 babies developed ROP out of 75 babies in which 2 babies had threshold ROP that required treatment.

Table 17: showing the bilirubin value of babies who developed ROP in the group with low bilirubin in comparison with cut off limits as per NICE guidelines.

Gestational Age in weeks	Number of babies having ROP N=13	Bilirubin level (mean) in mg/dl	NICE guidelines cut off limit to start treatment in mg/dl
29	5	7.44±0.4	11.1
30	1	8.4	11.6
31	1	9.6	12.28
32	2	7.4±0.2	12.86
33	4	7.87±0.3	13.45

The above table shows level of serum bilirubin in babies in the group with low bilirubin that developed ROP. This table determines the cut off limit below which there is a higher occurrence of ROP.

Table 18: Showing the Bilirubin levels in babies who developed ROP in higher Bilirubin group in comparison to the cut off limits by NICE guidelines.

Period of gestation	Bilirubin value observed	NICE cut off limits	Treatment received
29 weeks	12.1 mg/dl	11.1 mg/dl	Phototherapy
31 weeks	13 mg/dl	12.28 mg/dl	Phototherapy
33 weeks	14.5 mg/dl	13.45 mg/dl	Phototherapy

None of the babies had Bilirubin levels that required exchange transfusion in the study group. (Very High Bilirubin)

All the 3 babies who developed ROP in the group with high bilirubin had peak serum bilirubin higher than the cut off limits as per NICE guidelines. Hence it is recommended that more judicious approach to be followed towards lowering bilirubin in preterm and infants to protect them from free radical based disorders.

A few in vivo investigations have been previously valuated the association between serum bilirubin and free radical based illnesses.

We displayed a significant lower TSB observed in the infants suffering from advanced ROP. This data is consistent with reports published by Joanna S. Kao et al[86], Majid Abrishami et al[87], Seyed Mohammad Fereshtehnejad et al.[85]

In a study by Milner et al. with a similar study population in the year 2003, it was indicated that elevated peak bilirubin levels were not protective against severe ROP.[19] Even it was shown peak bilirubin was indeed a risk factor for severe ROP. However, Milner *et al.* have further declared that peak bilirubin levels were not associated with increased risk for ROP in the subgroup of infants with prolonged oxygen requirement. As an explanation, they added that sicker infants might be less capable of mounting an effective response to free radical insult by up regulating bilirubin production. They finally concluded the relation between oxygen requirement and ROP may prevent variations in bilirubin levels.

Our study supported the beneficial role of bilirubin against ROP which is in agreement with earlier researches.[21,91] while opposed by other reports.[71,73,80] Similarly, in another study by Belanger *et al.* the protective antioxidant role of bilirubin was demonstrated in term infants.[69] Experimental investigations in neonatal Gunn rats exposed to hyperoxia demonstrated that serum bilirubin protects against serum oxidative damage in the first days of life .[14] Despite the controversial findings of different studies in this subject, it is now accepted that bilirubin is one of the body's natural antioxidants, contributing up to 10% to 30% of the total antioxidant capacity of premature infants.[92,93]

On one hand, bilirubin is toxic to neurons at high concentrations; on the other hand, it has proven to be neuro protective against oxidative injury at Nano molar concentrations .[94] In the other words, although very high levels of serum bilirubin are known to be toxic, there is uncertainty about the risks and benefits of moderate serum

bilirubin values such as physiological hyperbilirubinemia and of the use of phototherapy to reduce the bilirubin values especially in preterm infants.

We support that parallel to physiological bilirubin elevation during early neonatal period probably to compensate for impaired immunity especially among premature infants, free radical related illness may further attenuate antioxidant system by utilizing bilirubin. Our findings adds support to the concept that bilirubin may possess some beneficial as well as toxic properties. According to our study, it is recommended that more judicious lowering of bilirubin in preterm and very low birth weight infants may protect them from free radical based disorders.

CONCLUSION

- The burden of ROP in neonates less than 34 weeks was 10 %, of which 60% were in gestational age less than 31 weeks.
- Lower gestational age (<31 weeks), very low birth weight infants (<1500 grams) and prolonged duration of oxygen therapy (>24 hours) were associated with a higher occurrence of ROP.
- There was a lower occurrence of retinopathy of prematurity in neonates with a higher bilirubin level.
- There was no correlation between sepsis and the burden of ROP.

SUMMARY

This was a prospective cohort study designed to correlate occurrence of retinopathy with serum bilirubin levels in premature neonates requiring and not requiring phototherapy. NICE guidelines which define the cut off limits for initiation of phototherapy were taken as reference for the study. Those neonates with bilirubin level above phototherapy zone were classified as high bilirubin and others were taken as below the phototherapy zone.

160 Preterm neonates less than 34 weeks of gestation were recruited for the study after they satisfied inclusion criteria.

In the study 51 % of neonates were males while females accounted for 49 %.The mean gestational age of the studied newborn was 32.26±1.4 weeks. The mean birth weight of the studied newborn was 1578±280 grams. Preterm neonates are extensively prone to prolonged oxygen therapy which is the main contributory factor to development of ROP.

The entire study population was grouped into two groups.

- Preterm neonates in group 1 were having high bilirubin values above the cut off limit as determined by the NICE guidelines during first 7 days of their NICU admission.
- Preterm neonates in group 2 were having bilirubin values less than the cut off limits as determined by the NICE guidelines.

The two groups were identical with regard to gestation age, birth weight, duration of oxygen requirement (p value=0.459) and sepsis (p value 0.634). These factors have shown to significant risk factors in developing ROP in previous studies. Statistical analysis like chi square test and fisher extract test were done to calculate the correlation of bilirubin levels to retinopathy of prematurity. Analysis of the data revealed that there was a lower risk of developing ROP in preterm babies having a higher bilirubin value (p value=0.004).

The burden of retinopathy of prematurity in the study population was 10 % and this burden varied with the period of gestation. There was a higher occurrence of ROP at gestational age less than 31 weeks (p value<0.001), very low birth weight of less than 1500 grams. (p value=0.006), prolonged duration of oxygen therapy defined as more than 24 hour (p value<0.001).

Sepsis had no correlation with the burden of retinopathy of prematurity (p value =0.673)

Our findings add support to the concept that bilirubin may possess some beneficial as well as toxic properties. According to our study, it is recommended that judicious lowering of bilirubin in preterm and very low birth weight infants may protect them from free radical based disorders.

LIMITATION OF THE STUDY

Due to ethical issues we could not measure serum bilirubin levels for all babies on a daily basis. After estimating serum bilirubin value at 48 hours of life we relied on clinical evidence for re assessing the bilirubin value based on the NICE guidelines. . A mean serum bilirubin level over a period of 7 days would have been a better indicator as compared to peak bilirubin levels.

We relied on pulse ox meter reading for deciding on the duration of oxygen supplementation required. In an ideal scenario an intra-arterial blood gas analysis would have had a better predictive value.

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WHAT IS RETINOPATHY OF PREMATURITY? The retina is the inner lining of the eye that receives light and turns it into messages that are sent to the brain. If one thinks of the eye as being like a camera, the retina functions as the film. Blood vessels that supply the retina are one of the last structures of the eye to mature; they have barely completed growing when a full-term baby is born. This means that a premature infant's retina is not yet completely developed. For reasons not yet fully understood, the blood vessels in the immature part of the retina may develop abnormally in some premature infants. This is called retinopathy of prematurity (abbreviated ROP).

When ROP develops, one of three different things can happen:

1. In most babies who develop ROP, the abnormal blood vessels will heal themselves completely, usually during the first year of life.

2. In some babies the abnormal blood vessels heal only partially. In these infants, nearsightedness, lazy eye or a wandering eye commonly develops. Glasses may be required early in life. In some cases a scar may be left in the retina, resulting in vision problems that are not entirely correctable with glasses.

3. In the most severe cases, the abnormal blood vessels form scar tissues which pull the retina out of its normal position in the back of the eye. This problem results in a severe loss of vision. Fortunately, there is treatment that may minimize severe vision loss. Occasionally, despite all treatment, this condition can lead to blindness.

WHAT ABOUT YOUR BABY'S EYES?

The Neonatologist taking care of your infant can give you more information and will arrange a meeting with the Ophthalmologist for

additional details if you wish.

Based on the eye exam performed on your infant, (only the checked information applies to your infant):

A._____ Your infant's eyes have mature blood vessels and have no risk for developing ROP. He/she should have another eye exam by an Ophthalmologist in six months. Other eye diseases, such as crossed eyes, lazy eye and extreme nearsightedness, occur more frequently in premature infants and may not become apparent until the infant is older. It is your responsibility to arrange this follow-up exam for your baby. An appointment has been made for _____(DATE)_____.

B._____ Your baby does not have ROP but could develop problems later because the retinal blood vessels are still not fully mature. Your baby has been scheduled for an ROP exam again on___(DATE)_____.

C_____ Your baby has early ROP. The ROP is not severe and does not require treatment at this time. To watch for possible serious developments, your baby has been scheduled for an ROP exam again on ___(DATE)_____.

Hospital number

Name of the pt

ANNEXURES

A PROSPECTIVE STUDY ON THE CORRELATION BETWEEN BILIRUBIN LEVELS AND RETINOPATHY OF PREMATURITY

INFORMED WRITTEN CONSENT.

I /we the patients attenders have been explained about condition of the baby.

I/we have been explained by the treating doctor about the details of the study being performed on my baby

I/we have been explained about details of ROP (retinopathy of prematurity)

I/we hereby willingly give consent for participating in the study.i hereby give consent to detect bilirubin level on day 3 of my baby as a screening for neonatala jaundice.

I/we have clearly been explained by the treating doctor that I/we can withdraw for the study at any time I want

I/we have clearly been explained that at any time my decision of withdrawing from the study or not giving consent for the study shall not influence the care for my baby

I/we are giving consent willingly and not under any compulsion.

Guardian signature **Doctors Signature**

PERFORMA FOR THE BABY

1)NAME-

2)GESTATIONAL

AGE

3)SEX

4)HOSPITAL

NUMBER

POG	DAY 1	DAY2	DAY3	DAY4	DAY5	DAY6	DAY 7
OXYGEN REQUIRED							
SERUM BILIRUBIN LEVEL							
PHOTO THERAPY REQUIRED							

OPTHALMIC EXAMINATION

CONATCT NUMBER

DATE OF REGISTERING-

DATE OF 1ST ROP EXAMINATION

RISK

DATE OF REGISTRATION	1ST VISIT	2ND VISIT	3RD VISIT	4TH VISIT	5TH VISIT	6TH VISIT
Contact number						
Name						
STAGE OF ROP						

DISCHARGE INSTRUCTIONS: ABOUT YOUR PREMATURE BABY'S EYES

This information explains the need for follow up care.

WHAT IS RETINOPATHY OF PREMATURITY? The retina is the inner lining of the eye that receives light and turns it into messages that are sent to the brain. If one thinks of the eye as being like a camera, the retina functions as the film. Blood vessels that supply the retina are one of the Iast structures of the eye to mature; they have barely completed growing when a full-term baby is born. This means that a premature infant's retina is not yet completely developed. For reasons not yet fully understood, the blood vessels in the immature part of the retina may develop abnormally in some premature infants. This is called retinopathy of prematurity (abbreviated ROP).

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1. In most babies who develop ROP, the abnormal blood vessels will heal themselves completely, usually during the first year of life.
2. In some babies the abnormal blood vessels heal only partially. In these infants, nearsightedness, lazy eye or a wandering eye commonly develops. Glasses may be required early in life. In some cases a scar may be left in the retina, resulting in vision problems that are not entirely correctable with glasses.
3. In the most severe cases, the abnormal blood vessels form scar tissues which pull the retina out of its normal position in the back of the eye. This problem results in a severe loss of vision. Fortunately, there is treatment that may minimize severe vision loss. Occasionally, despite all treatment, this condition can lead to blindness.

WHAT ABOUT YOUR BABY'S EYES?

The Neonatologist taking care of your infant can give you more information and will arrange a meeting with the Ophthalmologist for

additional details if you wish.

Based on the eye exam performed on your infant, (only the checked information applies to your infant):

A._____ Your infant's eyes have mature blood vessels and have no risk for developing ROP. He/she should have

another eye exam by an Ophthalmologist in six months. Other eye diseases, such as crossed eyes,

lazy eye and extreme nearsightedness, occur more frequently in premature infants and may not

become apparent until the infant is older. It is your responsibility to arrange this follow-up exam for your

baby. An appointment has been made for _____(DATE)_____.

B._____ Your baby does not have ROP but could develop problems later because the retinal blood vessels

are still not fully mature. Your baby has been scheduled for an ROP exam again

on___(DATE)_____.

C_____ Your baby has early ROP. The ROP is not severe and does not require treatment at this time. To

watch for possible serious developments, your baby has been scheduled for an ROP exam again on ___(DATE)_____.

Hospital number

Name of the pt

NHS
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Neonatal jaundice

Treatment threshold graphs

Graphs for assessing whether to treat neonatal jaundice by phototherapy or exchange transfusion

Clinical Guideline Implementation Tools

2010

NICE clinical guideline 98

NHS
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The NCC-WCH and the Guideline Development Group (GDG)would like to thank Dr Giles Kendall MBBS, BSc(hons), MRCPCH PhD Academic Clinical Lecturer Neonatal Medicine University College London / University College London Hospital NHS Foundation Trust, T J Cole, Professor of Medical Statistics, MRC Centre of Epidemiology for Child Health, UCL Institute of Child Health and Janet Rennie, Consultant and Senior Lecturer in Neonatal Medicine, Elizabeth Garrett Anderson Institute for Women's Health, University College London NHS Foundation Trust London for allowing the GDG to adapt their excel spreadsheet in developing the treatment threshold graphs included in this guideline.

Treatment threshold graphs for neonatal jaundice - Instructions

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These treatment threshold graphs will help healthcare professionals assess whether babies with jaundice should be given phototherapy or exchange transfusion. Please access the graphs directly from the NICE website to ensure that you are using the correct version of them.

Click on the 'Treatment threshold graphs' tab to access the graphs. The sheet contains a treatment graph for each gestational age. Before printing, use the drop-down menu that is marked in red to choose the graph for the correct gestational age for each baby with jaundice.

Print off the graph and keep it with the baby's notes. Plot the baby's bilirubin level on the graph each time it is measured, against the baby's age. Each line on the horizontal (x) axis is equal to 6 hours and each line on the vertical (y) axis is equal to 10 micromol/ litre. Assess whether the threshold for either phototherapy or exchange transfusion has been reached. Refer to the NICE neonatal jaundice guideline for detailed recommendations about the treatment of neonatal jaundice www.nice.org.uk/guidance/CG98/QuickRefGuide. Shade the 'single' or 'multiple' cells to show the type of phototherapy that the baby is receiving on each day.

Following a query to NICE about how the treatment threshold graphs for babies with jaundice should be used, please note: The graph that reflects the baby's actual gestational age should continue to be used until the baby is 14 days old. The baby's 'corrected' gestational age should not be taken into consideration, and you should not move up to the next graph when the baby is 7 days old. For example, for a baby of 35 weeks' gestation, the 35-week gestation graph should be used until the baby is aged 14 days. Please note that the NICE guideline does not cover treatment with phototherapy and exchange transfusion for babies older than 14 days. Trusts should therefore agree their own policy about when to treat babies over 14 days with phototherapy and exchange transfusion.

The NICE neonatal jaundice guideline and all implementation tools can be found at www.nice.org.uk/guidance/CG98

Treatment threshold graph for babies with neonatal jaundice

NHS
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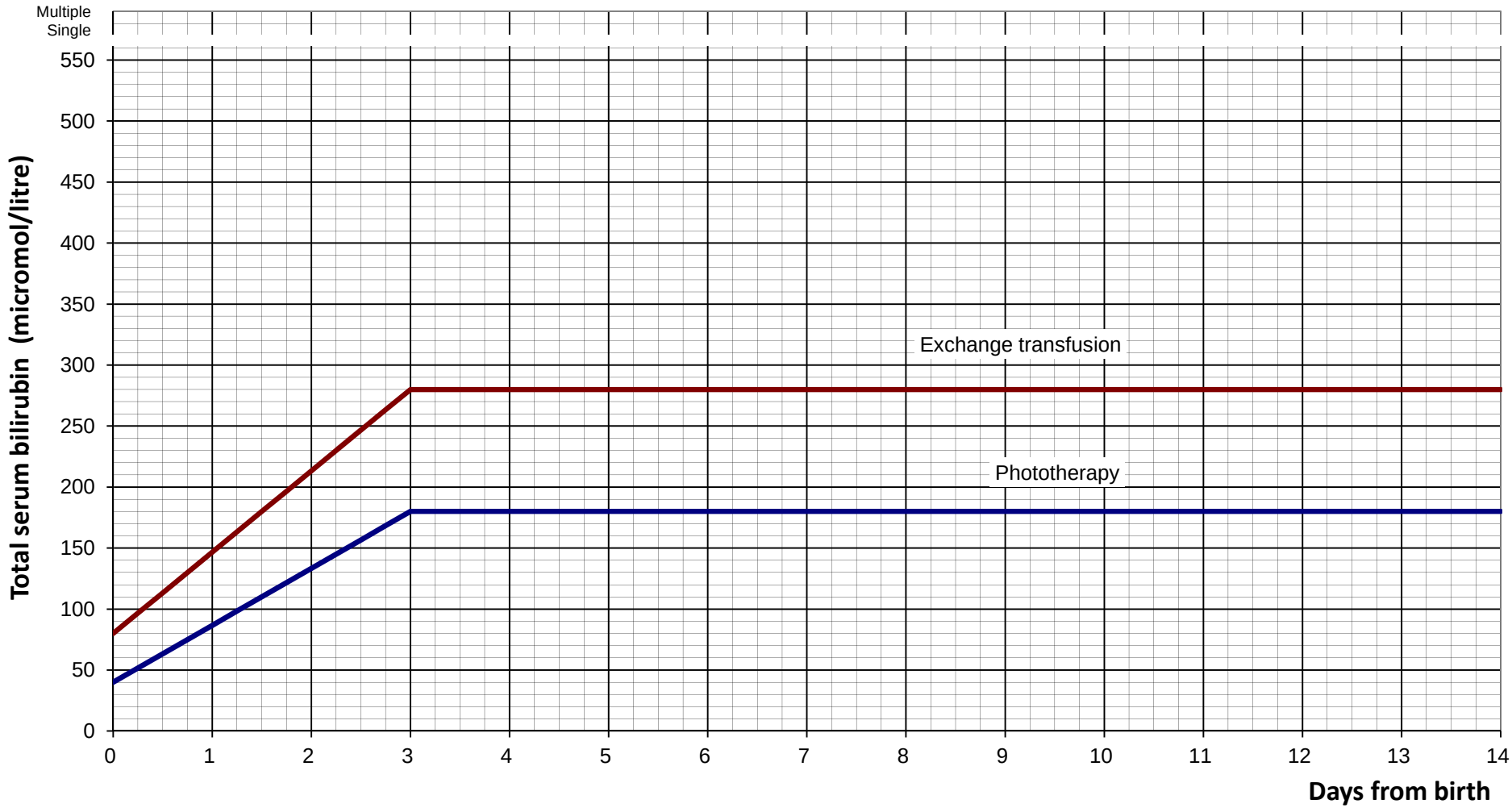
Baby's name _____ Date of birth _____

Hospital number _____ Time of birth _____ Direct Antiglobulin Test _____

Click below and choose gestation

28 **weeks gestation**

Shade for phototherapy Baby's blood group _____ Mother's blood group _____



Treatment threshold graph for babies with neonatal jaundice

NHS
National Institute for
Health and ClinicalExcellence

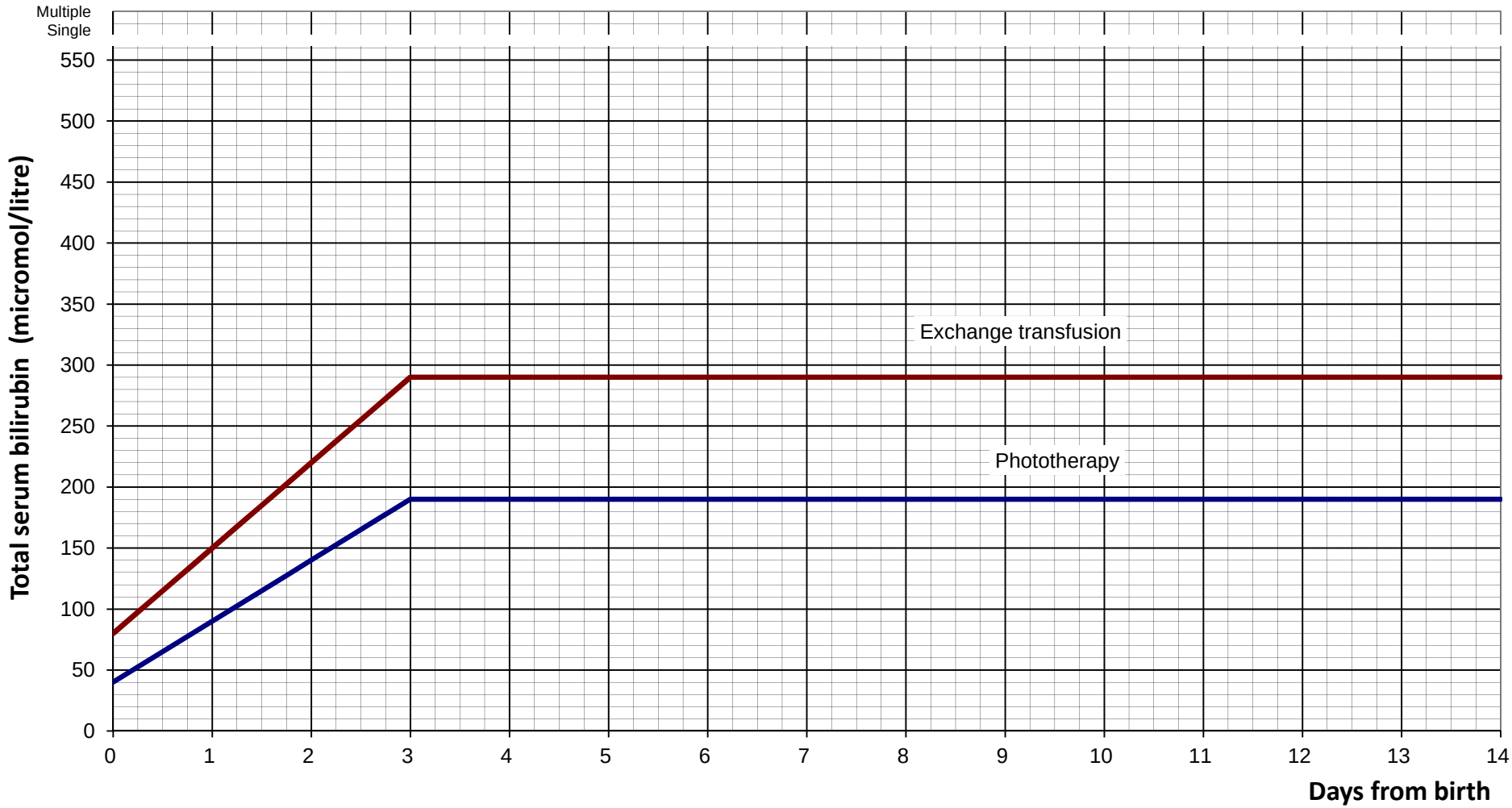
Baby's name _____ Date of birth _____

Hospital number _____ Time of birth _____ Direct Antiglobulin Test _____

Click below and choose gestation

29 **weeks gestation**

Shade for phototherapy Baby's blood group _____ Mother's blood group _____



Treatment threshold graph for babies with neonatal jaundice

NHS
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Health and ClinicalExcellence

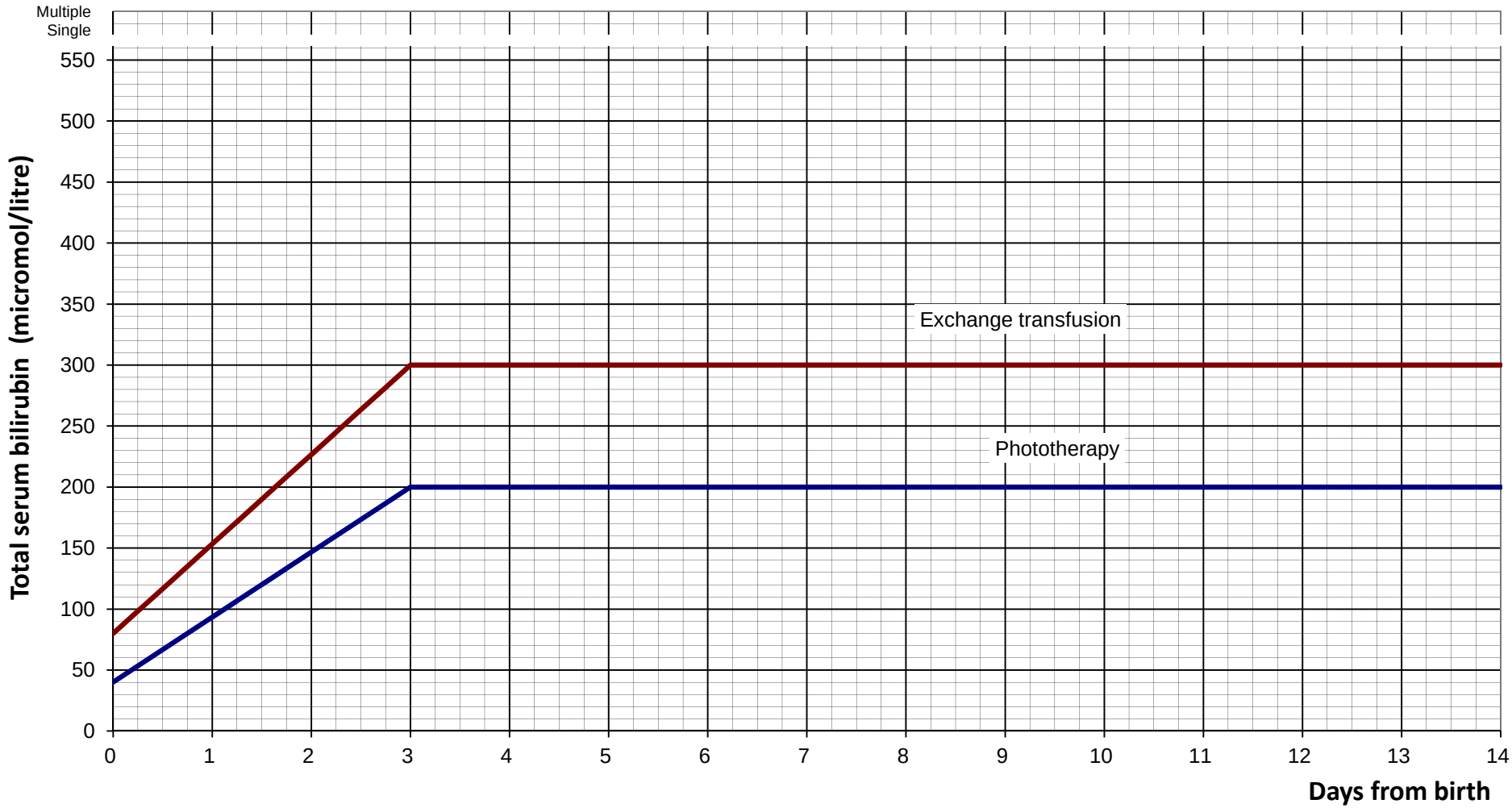
Baby's name _____ Date of birth _____

Hospital number _____ Time of birth _____ Direct Antiglobulin Test _____

Click below and choose gestation

30 **weeks gestation**

Shade for phototherapy Baby's blood group _____ Mother's blood group _____



Treatment threshold graph for babies with neonatal jaundice

NHS
National Institute for
Health and ClinicalExcellence

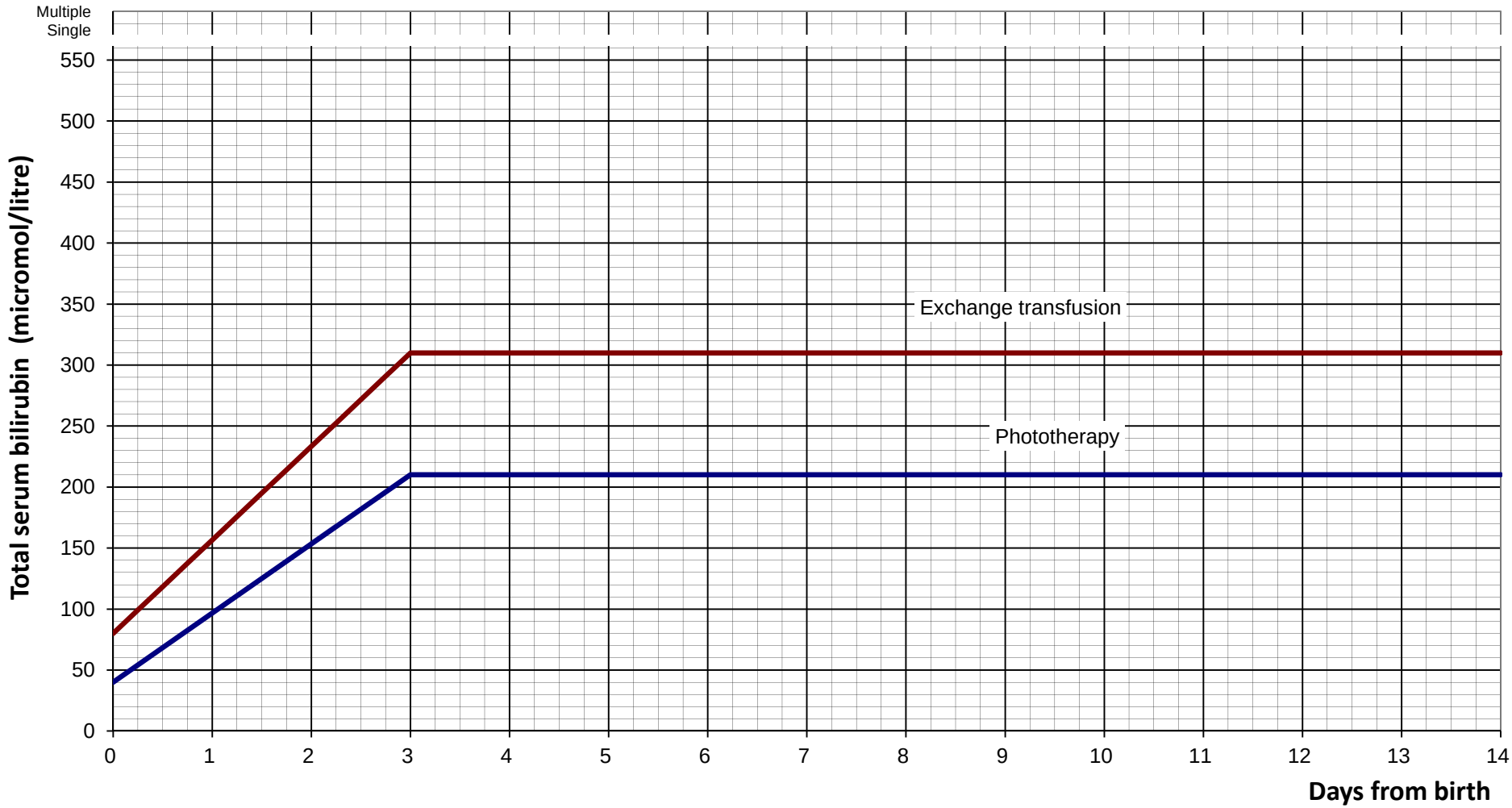
Baby's name _____ Date of birth _____

Hospital number _____ Time of birth _____ Direct Antiglobulin Test _____

Click below and choose gestation

31 **weeks gestation**

Shade for phototherapy Baby's blood group _____ Mother's blood group _____



Treatment threshold graph for babies with neonatal jaundice

NHS
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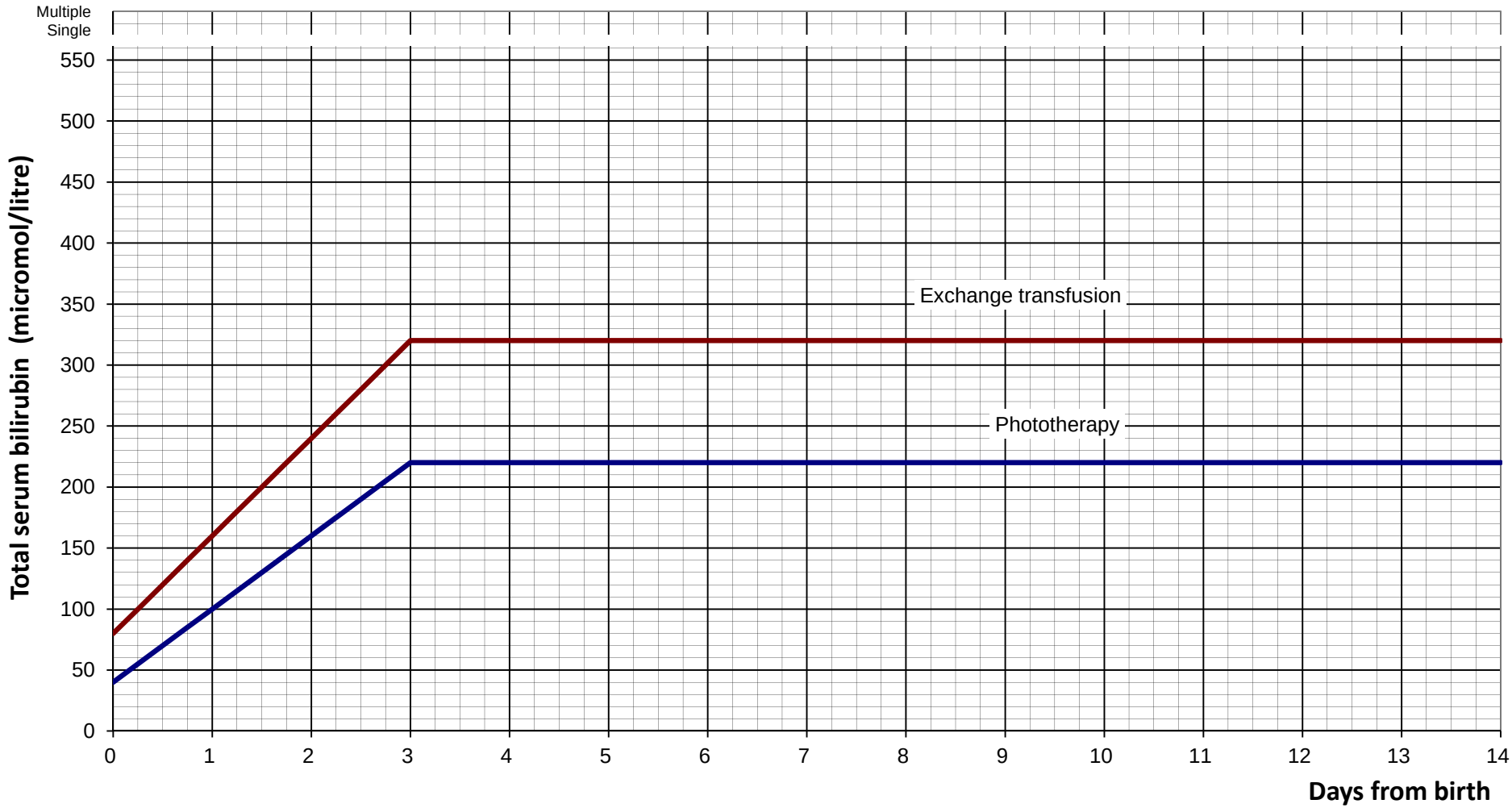
Baby's name _____ Date of birth _____

Hospital number _____ Time of birth _____ Direct Antiglobulin Test _____

Click below and choose gestation

32 **weeks gestation**

Shade for phototherapy Baby's blood group _____ Mother's blood group _____



Treatment threshold graph for babies with neonatal jaundice

NHS
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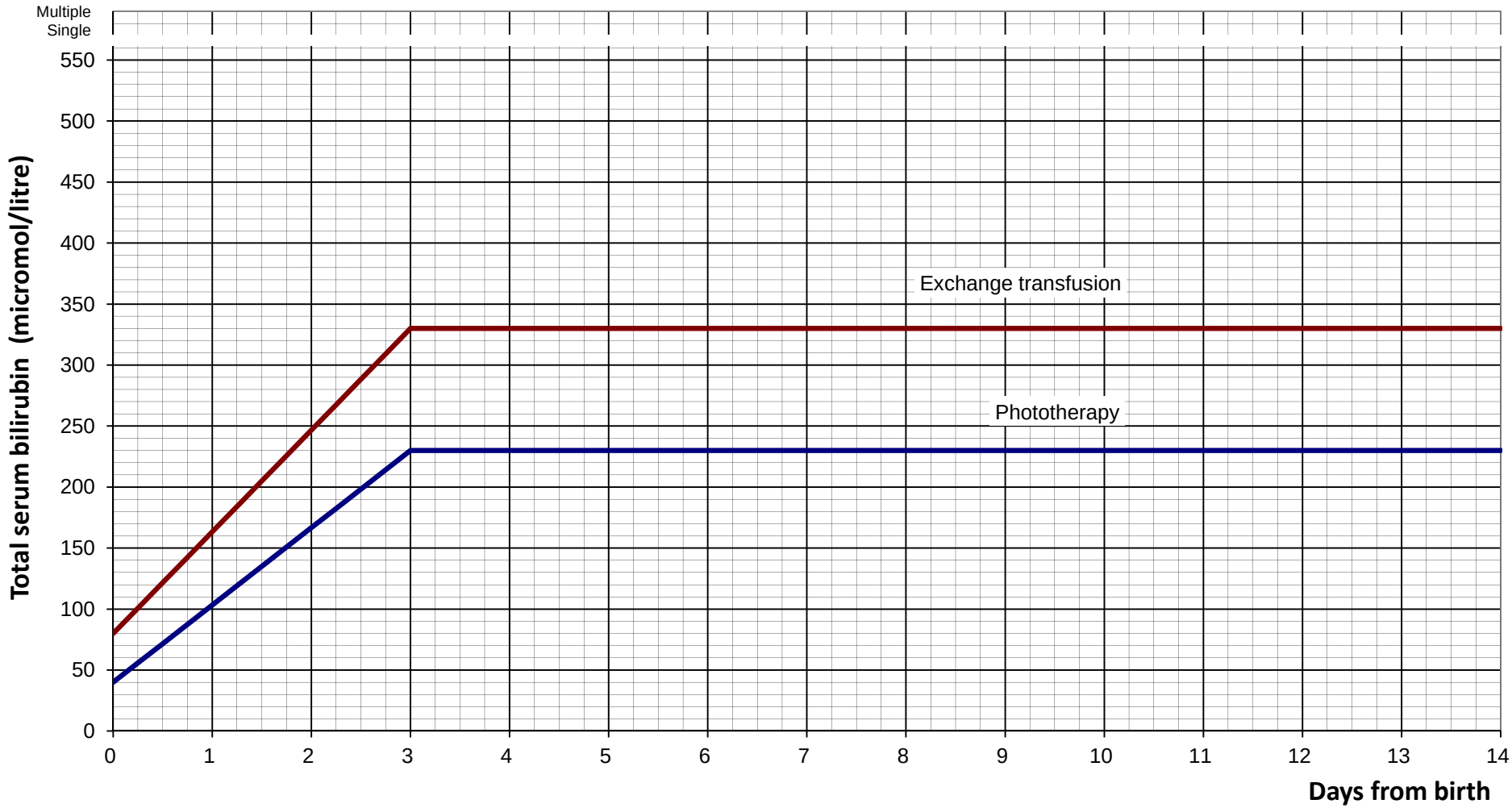
Baby's name _____ Date of birth _____

Hospital number _____ Time of birth _____ Direct Antiglobulin Test _____

Click below and choose gestation

33 **weeks gestation**

Shade for phototherapy Baby's blood group _____ Mother's blood group _____



Treatment threshold graph for babies with neonatal jaundice

NHS
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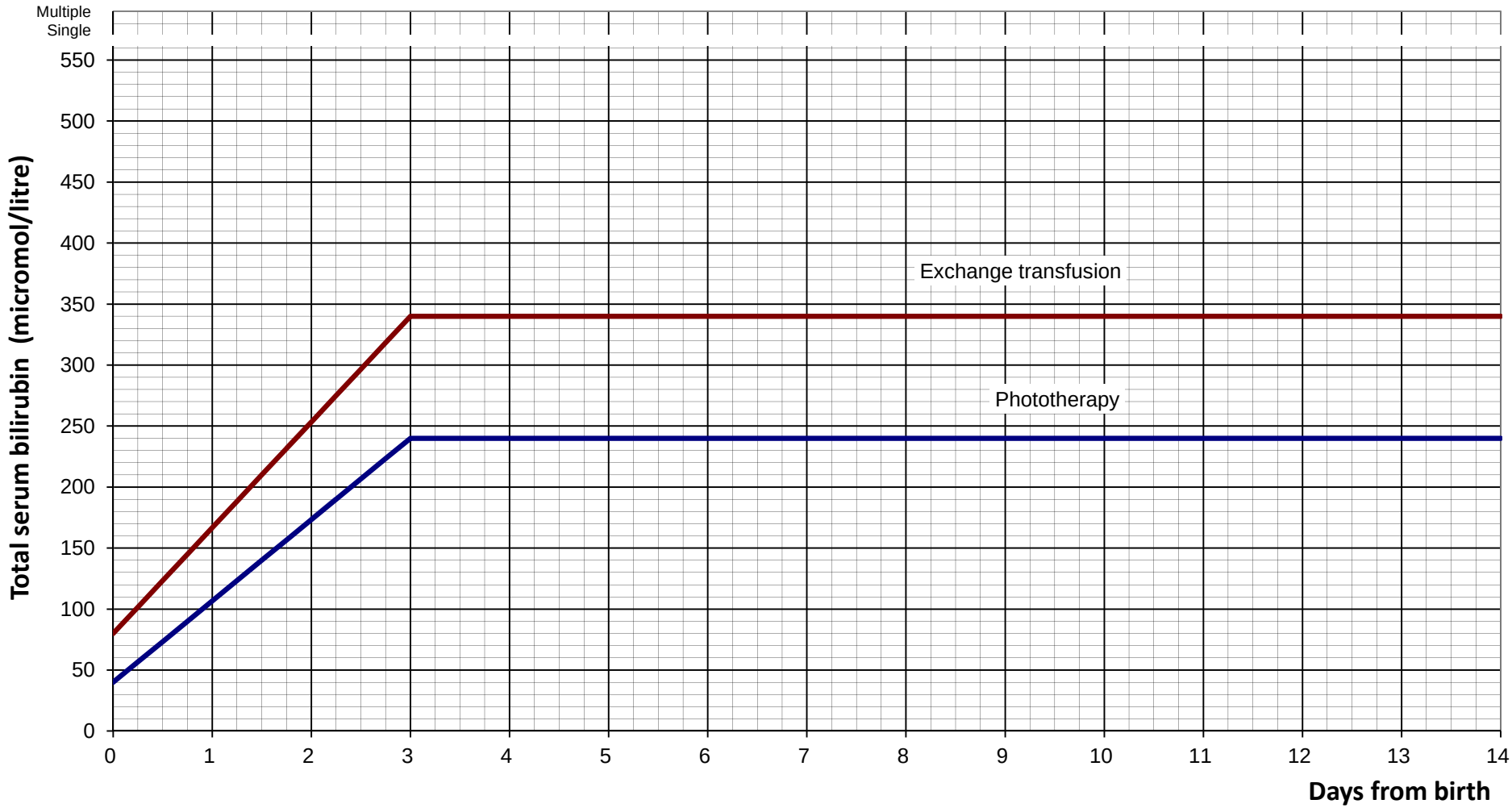
Baby's name _____ Date of birth _____

Hospital number _____ Time of birth _____ Direct Antiglobulin Test _____

Click below and choose gestation

34 **weeks gestation**

Shade for phototherapy Baby's blood group _____ Mother's blood group _____



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Where to find the guidance

The NICE neonatal jaundice guideline contains recommendations about the recognition, assessment and treatment of neonatal jaundice.

You can download the following documents from www.nice.org.uk/ guidance/CG98.

- The NICE guideline – all the recommendations
- The full guideline – all the recommendations, details of how they were developed and summaries of the evidence they were based on
- The quick reference guide – a summary of the recommendations for healthcare professionals (www.nice.org.uk/guidance/CG98/QuickRefGuide)
- 'Understanding NICE guidance' – a version of the guideline for parents and carers

For printed copies of the quick reference guide or 'Understanding NICE guidance' phone NICE publications on 0845 003 7783 or email publications@nice.org.uk and quote:

- N2143 – (quick reference guide)
- N2144 – ('Understanding NICE guidance')

Other implementation tools are available from the NICE website:

- slide set
- parent information factsheet
- audit tools
- costing tools

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www.nice.org.uk

KEY TO MASTER CHART

BW **Birth Weight**

POG **Period of Gestation**

Requiring Phototherapy

1 Yes

2 No

Right Eye Status

1 Tar

2 Almost Vascular

RZ **Right Zone**

1. Zone 1
2. Zone 2A
3. Zone P
4. Zone 3

RP **Right Eye Plus**

1 No Plus

2 Plus

LS **Left Eye Status**

1 Tar

2 Almost Vascular

LZ - Left Eye Zone

1 Zone 1

2 Zone 2A

3 Zone P

4 Zone 3

LP Left Eye Plus

1 No Plus

2 Plus

Final Zone of ROP

1 Zone 1

2 Zone 2

3 Zone 3

MASTER CHART

Sl No	NICU	ID	DOB	Name	Date	Sex	BW	POG	levels of bilirubin	oxygen requirement	Sepsis	requiring phototheraphy	RS	RZ	RP	LS	LZ	LP	final diagnosis	final zone of rop	eye plus
1	RLJ		6/3/2014	Ishrath Begum	13/3/2014	male	1750	33	14.3	20	positive	1	1	2	1	1	2	1	fully vascularised	3	1
2	RLJ	988969	9/2/2014	Ibrath Sulthana	13/2/2014	female	1680	33.3	14.5	10	positive	1	1	2	1	1	2	1	almost vascular	3	1
3	RLJ	403923	11/6/2014	Nagamani	26/6/2014	female	1450	33	16.3	16	negative	1	1	2	1	1	2	1	fully vascularised	3	1
4	RLJ	28141	11/7/2014	Lavanya M	17/7/2014	female	1890	34	15	2	positive	1	1	2	1	1	2	1	fully vascularised	3	1
5	RLJ	990483	13/2/2014	Padma	20/2/2014	male	2050	34	15.8	12	negative	1	1	2	1	1	2	1	fully vascularised	3	1
6	RLJ	1018672	15/7/2014	Supriya Anand	24/7/2014	female	1250	31	13.5	36	negative	1	1	A	1	1	2	1	fully vascularised	3	1
7	RLJ	1018643	15/7/2014	Mallika N	24/7/2014	female	1800	34	11	5	positive	2	1	2	1	1	2	1	fully vascularised	3	1
8	RLJ	991966	18/2/2014	Savitha A	6/3/2014	male	1240	32	15	48	positive	1	1	2	1	1	2	1	almost vascular	1	1
9	RLJ	396240	21/2/2014	Uma Rani	13/3/2014	female	1920	33	8.2	36	negative	2	4	1	1	4	1	1	stage 2	1	1
10	RLJ	22724	27/6/2014	Shalini	3/7/2014	male	1420	33	9.2	48	positive	2	1	2	1	1	2	1	almost vascular	3	1
11	RLJ	995850	28/2/2014	Girija	6/3/2014	male	2000	34	17	20	negative	1	1	2	1	1	2	1	fully vascularised	3	1
12	RLJ	23256	29/6/2014	Lahari	10/7/2014	male	1240	32	16	36	negative	1	1	2	1	1	2	1	fully vascularised	3	1
13	RLJ			Roopa R	9/1/2014	female	1780	33	14	12	positive	1	1	2	1	1	2	1	almost vascular	1	1
14	RLJ	988316	7/2/2014	Manjula Suresh	13/2/2014	female	1200	29	7.2	72	negative	2	1	1	1	1	1	1	stage 3	1	1
15	RLJ	1005443	26/3/2014	Rita kumari	3/4/2014	male	1700	33	10	30	negative	2	1	3	1	1	3	1	fully vascularised	3	1
16	RLJ	1007202	1/4/2014	Reshma Afsar	10/4/2014	male	1800	34	18.5	12	negative	1	1	3	1	1	4	1	fully vascularised	3	1
17	RLJ	877467	2/1/2014	Anuradha	9/1/2014	male	1600	33	17	28	positive	1	1	3	1	1	4	1	fully vascularised	3	1
18	RLJ	977408	2/1/2014	Nagalakshmi	9/1/2014	female	1780	34	15	36	positive	1	1	3	1	1	4	1	fully vascularised	2	1
19	RLJ		2/5/2014	Rashmi Harish	8/5/2014	female	2000	33	6.4	22	negative	2	1	3	1	1	4	1	fully vascularised	3	1
20	RLJ	996877	4/3/2014	Sunitha E	13/3/2014	male	1900	31	14	34	negative	1	1	3	1	1	4	1	fully vascularised	3	1
21	RLJ	997284	4/3/2014	Nethravathi A	13/3/2014	male	1900	33	15.6	32	positive	1	1	3	1	1	4	1	almost vascular	3	1
22	RLJ	401033	4/4/2014	Geetha N	29/5/2014	female	1650	32	10.4	10	positive	2	3	3	1	3	4	1	fully vascularised	3	1
23	RLJ	1008524	4/4/2014	Savithri N	29/5/2014	female	1750	33	7.2	28	negative	2	4	1	1	4	1	1	stage 2	1	1
24	RLJ	2302	4/5/2014	Nageena Taj 1	8/5/2014	male	1800	34	17.3	36	negative	1	1	3	1	1	4	1	fully vascularised	3	1
25	RLJ	988301	6/2/2014	Roopa K M	13/2/2014	female	1750	33	15.2	12	positive	1	1	3	1	1	4	1	fully vascularised	3	1
26	RLJ	14548	6/6/2014	Amaravathy	26/6/2014	male	1800	33	16	48	positive	1	1	3	1	1	4	1	fully vascularised	3	1

MASTER CHART

27	RLJ	25976	6/7/2014	Sunanda C	24/7/2014	male	1400	32	14	16	negative	1	2	3	1	2	4	1	fully vascularised	3	1
28	RLJ	988923	8/2/2014	Gayathri M	6/3/2014	male	1100	29	7.6	72	positive	2	4	1	1	4	1	1	stage 2	1	1
29	RLJ	842014	8/4/2014	Dr. Swapna	8/5/2014	male	1900	34	15.2	2	negative	1	2	1	1	2	4	1	fully vascularised	3	1
30	RLJ	14960	8/6/2014	Sushma	3/7/2014	female	1680	33	16	10	negative	1	1	3	1	1	4	1	fully vascularised	2	1
31	RLJ	998983	9/3/2014	Lalitha Suresh	13/3/2014	male	2100	34	18.2	6	negative	1	1	3	1	1	4	1	fully vascularised	3	1
32	RLJ	998967	9/3/2014	Munilakshmi V	13/3/2014	female	1900	33	10.04	24	positive	2	1	3	1	1	4	1	fully vascularised	3	1
33	RLJ	403773	10/5/2014	Tabassum	29/5/2014	female	2000	33	9.2	21	positive	2	1	2	1	1	4	1	almost vascular	3	1
34	RLJ	967708	13/10/2013	Pavithra	16/1/2014	female	1800	31	15.3	32	negative	1	3	3	1	3	4	1	fully vascularised	3	1
35	RLJ	1001165	14/3/2014	Hemavathi N	20/3/2014	female	1200	29	12.1	72	positive	1	1	1	1	1	1	1	stage 2	1	1
36	RLJ	1001164	14/3/2014	Sarala R	3/4/2014	male	1650	31	9.2	8	positive	2	1	3	1	1	4	1	fully vascularised	3	1
37	RLJ	972394	16/12/2013	Kamalamma	2/1/2014	male	1700	31	14	12	positive	1	1	3	1	1	4	1	fully vascularised	3	1
38	RLJ	991175	16/2/2014	Renuka 2	27/2/2014	female	1800	32	15.5	24	positive	1	1	3	1	1	4	1	fully vascularised	2	1
39	RLJ	991174	16/2/2014	Renuka 1	27/2/2014	female	1900	34	10.02	8	negative	2	1	3	1	1	4	1	almost vascular	3	1
40	RLJ	175964	17/10/2013	Sabina Taj	1/5/2014	female	1800	34	9.06	6	negative	2	3	3	1	3	4	1	fully vascularised	3	1
41	RLJ	1002835	19/3/2014	Meera Rao	27/3/2014	male	1200	29	7	72	positive	2	1	1	1	1	1	1	stage2	1	1
42	RLJ	403947	19/6/2014	Umadevi P G 2	3/7/2014	male	1750	33	11	32	positive	2	1	3	1	1	4	1	fully vascularised	3	1
43	RLJ	3303884	20/3/2014	narsima	24/4/2014	male	1600	34	16.2	4	negative	1	4	1	1	1	1	1	almost vascular	3	1
44	RLJ	1003025	20/3/2014	Parvathamma	3/4/2014	male	1650	32	15.2	21	positive	1	1	3	1	1	4	1	fully vascularised	3	1
45	RLJ	31165	20/7/2014	Mamatha N	24/7/2014	female	1800	33	14.8	16	negative	1	1	2	1	1	4	1	almost vascular	3	1
46	RLJ	974282	21/12/2013	Philomina	2/1/2014	female	1300	31	13	22	positive	1	1	3	1	1	4	1	fully vascularised	1	1
47	RLJ	1003795	21/3/2014	Shashikala	27/3/2014	female	1900	34	17	8	negative	1	1	3	1	1	4	1	fully vascularised	3	1
48	RLJ	393118	22/1/2014	Shymala	8/5/2014	male	1800	33	15.8	20	negative	1	3	3	1	3	4	1	fully vascularised	3	1
49	RLJ	1004169	22/3/2014	Arshiya Taj	10/4/2014	female	1750	33	9.9	32	negative	2	1	3	1	1	4	1	fully vascularised	3	1
50	RLJ		23/12/2013	Anitha Devi 1	20/3/2014	female	1600	33	8.4	16	negative	2	3	3	1	3	4	1	fully vascularised	3	1
51	RLJ		23/12/2013	Ruksana Kouser	20/3/2014	male	1300	31	9.6	36	negative	2	1	1	1	1	1	1	stage 2	1	1
52	RLJ	399707	23/3/2014	Savitha Nandish	10/4/2014	male	1280	30	14.5	22	positive	1	1	3	1	1	4	1	fully vascularised	3	1
53	RLJ	1004224	23/3/2014	rukmani	27/3/2014	female	1360	31	16.5	12	positive	1	1	2	1	1	4	1	almost vascular	1	1
54	RLJ	984095	24/1/2014	pooja	30/1/2014	male	920	28	15	72	positive	1	1	3	1	1	4	1	fully vascularised	3	1
55	RLJ	984101	24/1/2014	geetha	30/1/2014	male	1050	29	14.2	36	positive	1	1	3	1	1	4	1	fully vascularised	3	1

MASTER CHART

56	RLJ	1004278	24/3/2014	ramya	27/3/2014	female	1650	32	16	33	negative	1	1	3	1	1	4	1	fully vascularised	3	1
57	RLJ	24314	24/3/2014	anjanna	10/4/2014	female	1700	33	9.6	24	positive	2	1	3	1	1	4	1	fully vascularised	3	1
58	RLJ	994333	25/2/2014	reshma	13/3/2014	female	1520	32	8.5	32	negative	2	1	3	1	1	4	1	fully vascularised	2	1
59	RLJ	5994	25/6/2014	muskan	3/7/2014	female	1800	33	10.04	36	negative	2	1	3	1	1	4	1	fully vascularised	3	1
60	RLJ	400099	26/3/2014	Hamsa	24/4/2014	female	2100	34	17.2	5	positive	1	2	3	1	2	4	1	fully vascularised	3	1
61	RLJ	1005464	26/3/2014	deepa	1/5/2014	female	1680	32	7.6	12	positive	2	4	1	1	4	1	1	stage 2	1	1
62	RLJ		26/4/2014	jyoti	1/5/2014	male	2000	33	14.5	4	negative	1	1	3	1	1	4	1	fully vascularised	3	1
63	RLJ	2918	26/5/2014	Rekha Manjunath	12/6/2014	female	1200	32	9.2	14	positive	2	1	3	1	1	4	1	fully vascularised	3	1
64	RLJ	22714	26/6/2014	Manjula Suresh	3/7/2014	male	1050	31	9	25	negative	2	1	3	1	1	4	1	fully vascularised	3	1
65	RLJ	10564	27/5/2014	Radha 1	12/6/2014	male	1650	33	8.8	36	negative	2	1	3	1	1	4	1	fully vascularised	3	1
66	RLJ	10565	27/5/2014	abida	12/6/2014	male	1750	32	10	42	positive	2	1	3	1	1	4	1	almost vascular	3	1
67	RLJ	985791	30/1/2014	shreya	3/7/2014	male	1350	33	9.6	38	negative	2	3	3	1	3	4	1	fully vascularised	1	1
68	RLJ	985791	30/1/2014	shweta	1/5/2014	male	1100	30	8.4	48	negative	2	4	1	1	5	1	1	stage 3	1	1
69	RLJ		26/5/2014	Bharathi	2/1/2014	male	1250	29	15.1	72	negative	1	3	3	1	3	4	1	fully vascularised	3	1
70	RLJ		26/6/2014	laksmi	20/3/2014	male	1700	33	14.6	2	positive	1	1	1	1	1	4	1	fully vascularised	3	1
71	RLJ		27/5/2014	manasa	6/3/2014	male	1680	32	14.9	12	positive	1	1	3	1	1	4	1	fully vascularised	3	1
72	RLJ		27/5/2014	Pushpa	20/3/2014	male	1700	34	8	15	negative	2	1	3	1	1	4	1	fully vascularised	1	1
73	RLJ		30/1/2014	Shilpa	20/3/2014	male	1300	32	9.6	18	negative	2	1	2	1	1	4	1	almost vascular	3	1
74	RLJ		30/1/2014	Venkatamma	6/3/2014	female	2100	34	12	6	positive	2	1	3	1	1	4	1	fully vascularised	3	1
75	RLJ		17/9/2014	manjula	17/9/2014	female	1300	31	13.9	26	positive	1	1	2	1	1	2	1	fully vascularised	3	1
76	RLJ	988969	19/9/2014	Sulthana	19/9/2014	male	1800	33	15	8	negative	1	1	2	1	1	2	1	almost vascular	3	1
77	RLJ	403923	19/9/2014	Nagamani	19/9/2014	female	1200	32	14.5	24	negative	1	1	2	1	1	2	1	fully vascularised	2	1
78	RLJ	28141	10/9/2014	Lavanya M	10/9/2014	female	1750	33	16	12	positive	1	1	2	1	1	2	1	almost vascular	3	1
79	RLJ	990483	13/9/2014	Padma	13/9/2014	male	960	28	15.2	72	positive	1	1	2	1	1	2	1	almost vascular	3	1
80	RLJ	1018672	15/9/2014	nagaeni	15/9/2014	female	1100	29	14	24	negative	1	1	2	1	1	2	1	almost vascular	3	1
81	RLJ	1018643	15/9/2014	Mallika N	15/9/2014	male	1680	33	10	8	positive	2	1	2	1	1	2	1	almost vascular	3	1
82	RLJ	991966	18/9/2014	Savitha A	18/9/2014	male	1350	32	16.2	24	negative	1	1	2	1	1	2	1	almost vascular	3	1
83	RLJ	396240	21/9/2014	nagarathna	21/9/2014	female	1700	33	9.6	12	negative	2	4	1	1	4	2	1	stage 2	1	1
84	RLJ	22724	27/9/2014	Shalini	27/9/2014	male	1100	30.5	8.5	26	negative	2	1	2	1	1	2	1	almost vascular	3	1

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85	RLJ	995850	28/9/2014	Girija	28/9/2014	male	1580	33	14.2	24	positive	1	1	2	1	1	2	1	almost vascular	3	1
86	RLJ	23256	29/9/2014	Lahari	29/9/2014	male	1350	32	15	32	positive	1	1	2	1	1	2	1	almost vascular	3	1
87	RLJ			Roopa R		female	1200	31	14.2	12	negative	1	1	2	1	1	2	1	almost vascular	1	1
88	RLJ	988316	7/10/2014	Manjula Suresh	7/10/2014	female	1800	32	10	8	positive	2	1	3	1	1	3	1	fully vascularised	3	1
89	RLJ	1005443	26/10/2014	manjula hm	26/10/2014	male	1200	29	8	72	positive	2	1	1	1	1	1	1	stage 2	1	1
90	RLJ	1007202	1/11/2014	Reshma Afsar	1/11/2014	male	1480	32	9.2	12	positive	2	1	3	1	1	4	1	fully vascularised	3	1
91	RLJ	877467	2/11/2014	Anuradha	2/11/2014	male	1560	33	15.2	8	positive	1	1	3	1	1	4	1	fully vascularised	3	1
92	RLJ	977408	2/11/2014	Nagalakshmi	2/11/2014	female	1580	32	17	6	negative	1	1	3	1	1	4	1	fully vascularised	3	1
93	RLJ		2/11/2014	Rashmi Harish	2/11/2014	female	1550	32	10.04	10	negative	2	1	3	1	1	4	1	fully vascularised	3	1
94	RLJ	996877	4/11/2014	Sunitha E	4/11/2014	male	1600	33	18.4	12	positive	1	1	3	1	1	4	1	fully vascularised	3	1
95	RLJ	997284	4/11/2014	Nethravathi A	4/11/2014	male	1350	31	17	6	positive	1	1	3	1	1	4	1	fully vascularised	3	1
96	RLJ	401033	4/11/2014	Geetha N	4/11/2014	female	1700	34	10	5	negative	2	3	3	1	3	4	1	fully vascularised	3	1
97	RLJ	1008524	4/11/2014	Savithri N	4/11/2014	female	1600	32	7.2	18	positive	2	4	1	1	4	1	1	stage 2	1	1
98	RLJ	2302	4/11/2014	Nageena Taj 1	4/11/2014	female	2000	34	not done	7	negative	1	1	3	1	1	4	1	fully vascularised	3	1
99	RLJ	988301	6/11/2014	Roopa K M	6/11/2014	female	1700	33	15.9	12	positive	1	1	3	1	1	4	1	fully vascularised	3	1
100	RLJ	14548	6/11/2014	Amaravathy	6/11/2014	male	1600	32	17	24	negative	1	1	3	1	1	4	1	fully vascularised	3	1
101	RLJ	25976	6/11/2014	Sunanda C	6/11/2014	male	1550	32	15.2	36	negative	1	2	3	1	2	4	1	fully vascularised	3	1
102	RLJ	988923	8/11/2014	Gayathri M	8/11/2014	male	1100	29	7.4	72	negative	2	4	1	1	4	1	1	stage 2	1	1
103	RLJ	842014	8/11/2014	Dr. Swapna	8/11/2014	male	2000	31	16.2	36	negative	1	2	3	1	2	4	1	fully vascularised	3	1
104	RLJ	14960	8/11/2014	Sushma	8/11/2014	female	1800	32	17	24	negative	1	1	3	1	1	4	1	fully vascularised	3	1
105	RLJ	998983	9/11/2014	Lalitha Suresh	9/11/2014	female	1900	33	14.2	8	positive	1	1	3	1	1	4	1	fully vascularised	1	1
106	RLJ	998967	9/11/2014	Munilakshmi V	9/11/2014	female	1600	32	9.8	12	positive	2	1	3	1	1	4	1	fully vascularised	3	1
107	RLJ	403773	10/11/2014	Tabassum	10/11/2014	female	1750	33	7.8	16	positive	2	1	2	1	1	4	1	almost vascular	3	1
108	RLJ	967708	13/11/2014	Pavithra	13/11/2014	female	1700	33	15	18	positive	1	3	3	1	3	4	1	fully vascularised	3	1
109	RLJ	1001165	14/11/2014	Hemavathi N	14/11/2014	female	1300	31	16.4	16	negative	1	1	3	1	1	4	1	fully vascularised	3	1
110	RLJ	1001164	14/11/2014	Sarala R	14/11/2014	male	2000	34	8.6	12	positive	2	1	3	1	1	4	1	fully vascularised	3	1
111	RLJ	972394	16/11/2014	Kamalamma	16/11/2014	male	1900	32	15	24	negative	1	1	3	1	1	4	1	fully vascularised	3	1
112	RLJ	991175	16/12/2014	Renuka 2	16/12/2014	female	1700	33	15.8	36	negative	1	1	3	1	1	4	1	fully vascularised	1	1
113	RLJ	991174	16/12/2014	Renuka 1	16/12/2014	female	1200	32	8.6	10	positive	2	1	3	1	1	4	1	fully vascularised	3	1

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114	RLJ	175964	17/12/2014	Sabina Taj	17/12/2014	female	1650	33	9.5	18	positive	2	3	3	1	3	4	1	fully vascularised	3	1
115	RLJ	1002835	19/12/2014	Meera Rao	19/12/2014	male	1200	31	8.4	12	negative	2	1	3	1	1	4	1	fully vascularised	3	1
116	RLJ	403947	19/12/2014	Umadevi P G 2	19/12/2014	male	1700	32	9.6	24	positive	2	1	3	1	1	4	1	fully vascularised	3	1
117	RLJ	3303884	20/12/2014	Kantha Priya	20/12/2014	male	1600	31	13	36	negative	1	4	1	1	1	1	1	stage 2	1	1
118	RLJ	1003025	20/12/2014	Parvathamma	20/12/2014	male	1800	32	16.2	36	negative	1	1	3	1	1	4	1	fully vascularised	3	1
119	RLJ	31165	20/12/2014	Mamatha N	20/12/2014	female	1700	33	9.6	12	positive	2	1	3	1	1	4	1	fully vascularised	3	1
120	RLJ	974282	21/12/2013	Philomina	21/12/2013	male	1200	32	10	12	negative	2	1	3	1	1	4	1	fully vascularised	3	1
121	RLJ	1003795	21/12/2014	Shashikala	21/12/2014	female	1660	33	17	18	negative	1	1	3	1	1	4	1	fully vascularised	3	1
122	RLJ	393118	22/12/2014	Shymala	22/12/2014	male	1700	34	16.8	4	negative	1	3	3	1	3	4	1	fully vascularised	3	1
123	RLJ	1004169	22/12/2014	Arshiya Taj	22/12/2014	female	2000	34	9	2	positive	2	1	3	1	1	4	1	fully vascularised	3	1
124	RLJ		23/12/2014	Anitha Devi 1	23/12/2014	female	1600	31	7,4	48	positive	2	3	3	1	3	4	1	fully vascularised	3	1
125	RLJ		23/12/2014	Ruksana Kouser	23/12/2014	male	1750	32	11	24	negative	2	1	3	1	1	4	1	fully vascularised	3	1
126	RLJ	399707	23/12/2014	Savitha Nandish	23/12/2014	male	1800	33	16.5	36	negative	1	1	3	1	1	4	1	fully vascularised	3	1
127	RLJ	1004224	23/12/2014	Kalai selvi	23/12/2014	female	1700	32	15	24	positive	1	1	3	1	1	4	1	fully vascularised	1	1
128	RLJ	984095	24/1/2015	Nimbi 1	24/1/2015	male	920	28	14.2	72	positive	1	1	3	1	1	4	1	fully vascularised	3	1
129	RLJ	984101	24/1/2015	Nimbi 2	24/1/2015	male	1050	33	15.8	24	negative	1	1	3	1	1	4	1	fully vascularised	3	1
130	RLJ	1004278	24/3/2015	Jyothiamma 1	24/3/2015	female	1600	34	17	33	negative	1	1	3	1	1	4	1	fully vascularised	2	1
131	RLJ	24314	24/3/2015	Fazila Begum	24/3/2015	female	1750	33	10.04	24	positive	2	1	3	1	1	4	1	fully vascularised	3	1
132	RLJ	994333	25/2/2015	Amratha	25/2/2015	female	1550	32	9.2	36	positive	2	1	3	1	1	4	1	fully vascularised	3	1
133	RLJ	5994	25/2/2015	Asha Rani	25/2/2015	female	1600	32	8.6	12	negative	2	1	3	1	1	4	1	fully vascularised	3	1
134	RLJ	400099	26/3/2015	Hamsa	26/3/2015	female	1800	33	15.2	8	positive	1	2	3	1	2	4	1	fully vascularised	3	1
135	RLJ	1005464	26/3/2015	Farheen Taj	26/3/2015	female	1700	34	12	4	negative	2	4	1	1	4	1	1	almost vascular	2	1
136	RLJ		26/3/2013	Sheela	26/3/2013	male	1900	34	18	2	negative	1	1	3	1	1	4	1	fully vascularised	3	1
137	RLJ	2918	15/3/2015	Rekha Manjunath	15/3/2015	female	1300	32	9.4	12	negative	2	1	3	1	1	4	1	fully vascularised	3	1
138	RLJ	22714	15/3/2015	Sangeetha	15/3/2015	male	1200	31	9.2	18	positive	2	1	3	1	1	4	1	fully vascularised	3	1
139	RLJ	10564	27/3/2015	Radha 1	27/3/2015	male	1680	33	8.4	12	positive	2	1	3	1	1	4	1	fully vascularised	3	1
140	RLJ	10565	27/3/2015	Radha 2	27/3/2015	male	1580	34	11	1	negative	2	1	3	1	1	4	1	fully vascularised	3	1
141	RLJ	985791	30/1/2014	Chandrakala	30/1/2014	male	1250	32	9.8	24	positive	2	3	3	1	3	4	1	fully vascularised	3	1
142	RLJ	985791	30/1/2014	Chandrakala	30/1/2014	male	1380	33	6.5	36	positive	2	4	1	1	5	1	1	stage 2	1	1

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143	RLJ		24/3/2015	Bharathi	24/3/2015	male	1100	31	15.2	24	positive	1	3	3	1	3	4	1	fully vascularised	3	1
144	RLJ		24/3/2015	Shilpa 2	24/3/2015	male	1780	32	16	12	positive	1	1	3	1	1	4	1	fully vascularised	3	1
145	RLJ		25/2/2015	Lakshmi Prasad	25/2/2015	male	1800	33	17	24	negative	1	1	3	1	1	4	1	fully vascularised	3	1
146	RLJ		25/2/2015	Pushpa V	25/2/2015	male	1580	34	not done	2	negative	2	1	3	1	1	4	1	fully vascularised	3	1
147	RLJ		26/3/2015	Shilpa 1	26/3/2015	male	1260	31	10	24	positive	2	1	3	1	1	4	1	fully vascularised	3	1
148	RLJ		26/3/2015	Venkatrathnamma	26/3/2015	female	1650	32	10	24	positive	2	1	3	1	1	4	1	fully vascularised	3	1
149	RLJ	1001165	14/1/2015	Hemavathi N	14/1/2015	female	1250	32	15.2	12	negative	1	1	3	1	1	4	1	fully vascularised	3	1
150	RLJ	1001164	14/1/2015	Sarala R	14/1/2015	female	1650	33	16	18	positive	1	1	3	1	1	4	1	fully vascularised	3	1
151	RLJ	972394	16/1/2015	Kamalamma	16/1/2015	female	1700	32	not done	12	negative	1	1	3	1	1	4	1	fully vascularised	3	1
152	RLJ	991175	16/1/2015	Renuka 2	16/1/2015	male	1550	34	19	2	positive	1	1	3	1	1	4	1	fully vascularised	3	1
153	RLJ	991174	16/2/2015	Renuka 1	16/2/2015	female	2000	34	not done	12	negative	2	1	3	1	1	4	1	fully vascularised	3	1
154	RLJ	175964	17/2/2015	Sabina Taj	17/2/2015	male	1450	31	7.5	24	negative	2	3	3	1	3	4	1	fully vascularised	3	1
155	RLJ	1002835	19/2/2014	Meera Rao	19/2/2014	female	1300	32	8.6	12	negative	2	1	3	1	1	4	1	fully vascularised	3	1
156	RLJ	403947	19/12/2015	Umadevi P G 2	19/12/2015	female	1400	32	7.2	18	negative	2	1	3	1	1	4	1	fully vascularised	3	1
157	RLJ	3303884	20/12/2014	Kantha Priya	20/12/2014	male	1700	33	14.5	18	negative	1	4	1	1	1	1	1	stage 2	1	1
158	RLJ	1003025	20/2/2015	Parvathamma	20/2/2015	male	1700	34	18	2	positive	1	1	3	1	1	4	1	fully vascularised	3	1
159	RLJ	31165	20/12/2014	Mamatha N	20/12/2014	female	1650	32	10.4	16	positive	2	1	3	1	1	4	1	fully vascularised	3	1
160	RLJ	974282	21/12/2013	Philomina	21/12/2013	female	1200	31	8.2	12	negative	2	1	2	1	1	4	1	almost vascular	3	1