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Research article

ESTIMATION OF MALONDIALDEHYDE AND VITAMIN-E LEVELS IN NEONATAL HYPERBILIRUBINEMIA BEFORE AND AFTER PHOTOTHERAPY

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ABSTRACT

Background: Hyperbilirubinemia is a common and benign problem in neonates worldwide. It is observed during the 1st week of life in approximately 60% of term neonates and 80% of preterm neonates. Phototherapy is most widely used as therapy for unconjugated hyperbilirubinemia. Phototherapy is related to oxidative stress and lipid peroxidation. The present study is undertaken to establish the relation between anti-oxidant status and the marker of lipid peroxidation in neonatal hyperbilirubinemia before and after phototherapy. **Objectives:** To estimate the levels of MDA (malondialdehyde) and vit-E levels in neonatal hyperbilirubinemia before and after phototherapy. **Material and Methods:** A total of 30 patients were eligible for the study who met the inclusion and exclusion criteria. Blood sample was collected from neonates of preterm/full term age 1 to 10 days with hyperbilirubinemia undergoing phototherapy. Total Bilirubin, Direct Bilirubin, MDA and vit-E levels were estimated in serum by thiobarbituric acid (TBA) method and Backer and Frank's Method respectively. **Results:** The present study showed increase in Total bilirubin, Direct bilirubin, MDA and decrease in vit-E levels before phototherapy when compared to control group and same subjects after phototherapy showed decrease in Total bilirubin and Direct bilirubin, MDA and a further decrease in vit-E levels. **Conclusion:** From these results it is concluded though phototherapy had a beneficial effect in treatment of neonatal hyperbilirubinemia, supplementation of vit-E is necessary in addition to phototherapy.

Keywords: Malondialdehyde, Vitamin-E, Neonatal hyperbilirubinemia, Phototherapy.

INTRODUCTION

Hyperbilirubinemia is a common and benign problem in neonates worldwide. Neonatal hyperbilirubinemia is observed during 1st week of life in approximately 60% of term infants and 80% of preterm infants. The incidence of jaundice is higher in breast-fed babies than in formula-fed ones [1].

Newborns appear jaundiced when the serum bilirubin level is >7 mg/dL [2]. Although transient, the condition accounts for up to 75 % of hospital re-admissions in 1stweek after birth [3]. However hyperbilirubinemia in the newborn period can be associated with severe illness such as hemolytic disease, metabolic and endocrine disorders, anatomic abnormalities of the liver and infections [4].

Phototherapy is most widely used as therapy for unconjugated hyperbilirubinemia [5,6]. It has recently been reported that phototherapy cause oxidative stress and lipid peroxidation [7].

The generation of free radicals is a normal physiological process but increased production of free radicals acts on lipid to cause lipid peroxidation. The cells have evolved a number of counter acting antioxidant defenses [8].

Uncontrolled lipid peroxidation is known to play an important role in pathogenesis of many neonatal complications. Excessive generation of free radicals, depressed antioxidant status or imbalance in peroxidation and free radical scavenging system might play an important role in neonatal hyperbilirubinemia. Antioxidants are compounds that dispose, scavenge and suppress the formation of free radicals or oppose their action [9].

The present study is undertaken to establish the relation between antioxidant status and the marker of lipid peroxidation in neonatal hyperbilirubinemia before and after phototherapy.

Malondialdehyde (MDA) is a metabolic product of polyunsaturated fatty acids widely used as an indicator of peroxidation [10, 11].

Vitamin-E is most important chain breaking antioxidant and protects membrane lipids from free radical damage. The primary function of vitamin- E as an antioxidant is prevention of non-enzymatic oxidation of cell components, for example poly-unsaturated fatty acids, by molecular oxygen and free radicals.

Antioxidant activity in the serum of term neonates is lower than that of adults and is still lower in preterm and low birth weight babies as compared to term babies [12]. Supplementation of natural antioxidants like alpha-tocopherol, ascorbic-acid may be beneficial in preventing neonatal complications.

MATERIALS AND METHODS

Study design & Place: The present case-control study was carried out in SVS Medical College of Mahabubnagar district during March 2009 to March 2011.

Ethical approval: After obtaining approval from institutional ethical committee and informed consent from parents were taken.

Sample size: 30 in each group.

Inclusion Criteria: Cases: 30Preterm / full-term Neonates of age 1 to 10 days with hyper bilirubinemia (>11 mg/dl) undergoing phototherapy treatment (before and after phototherapy).

Controls: 30 Preterm / full-term Neonates of age 1 to 10 days with hyperbilirubinemia(<10 mg/dl) and not advised for phototherapy treatment.

Grouping: Control group (n=30), Test group(n=30)

Exclusion Criteria: Neonates of diabetic mother.

Sample Collection: About 4 ml of blood is collected from peripheral vein by vene puncture under aseptic conditions into a sterile tube. The serum was separated by centrifugation at 3000 rpm and stored at 4^{o}c until analysis was carried out.

Methods of estimation: Neonates with bilirubin > 11 mg/dl and those who were recommended for phototherapy treatment samples were collected. The following biochemical parameters were analyzed with their respective methods before and after phototherapy and compared with the control group neonates. Estimation of total bilirubin and direct bilirubin: Bilirubin was estimated by Diazo method of Pearlman and Lee Method, Bilirubin reacts with diazotized sulphanillic acid in acidic medium to form pink coloured azobilirubin with absorbance directly proportional to bilirubin concentration [13].

Estimation of malondialdehyde: MDA is estimated by thiobarbituric acid method (TBA). Autoxidation of unsaturated fatty acids involve the formation of semi stable peroxides which undergo a series of reaction to form short chain aldehydes like malondialdehyde. One molecule of MDA reacts with TBA with the elimination of two molecules of water to yield pink crystalline pigments with absorption maximum at 535 nm[14].

Estimation of Vitamin-E: Vit-E is estimated by Baker and Frank's method. Serum tocopherol can measure by their reduction of ferric to ferrous ions which then form a red colour complex with α, α-dipridyl. Tocopherols and carotenes are first extracted into xylene and the absorbance is read at 460nm to measure the carotenes. A second reading is made after addition of ferric chloride at 520nm for vitamin E [15].

Statistical analysis: Data was presented as mean and standard deviation (mean ± SD). Means and standard deviations are compared between control and case groups by unpaired student 't'test and between two case groups (before phototherapy and after phototherapy) by paired student't' test. A p-value of < 0.05 and $p<0.001$ was considered statistically significant. Descriptive statistical analysis was carried out in the present study by using SPSS, 20version software.

RESULTS

A total of 60 neonates of age 1 to 10 days were included in the study. Out of these 30 neonates with

hyperbilirubinemia but not recommended phototherapy treatment and 30 neonates with hyperbilirubinemia and recommended for phototherapy were included. In Case group blood samples were collected before and after phototherapy and results were analyzed. The mean and standard deviation values of all data and demographic changes are tabulated. Table-1 the biochemical profile in cases (before phototherapy and after phototherapy) and controls.

Total bilirubin levels and Direct bilirubin were compared between the controls and cases (before and after phototherapy) showed statistically significant and the Total bilirubin values and direct bilirubin values of case group were decreased after phototherapy.

The mean and standard deviation values of MDA are shown in table-1 It shows that the MDA levels are raised in hyperbilirubinemia neonates before phototherapy when compared with control group neonates and decreased after phototherapy.

The mean and standard deviation values of Vit-E are shown in table no-1, it shows that the Vit-E levels are less in hyperbilirubinemia neonates before phototherapy when compared with control group neonates and decreased after phototherapy.

Table1: Comparison of Parameters between control, case before and after phototherapy

Parameter	Control	before phototherapy	After phototherapy	P values$	P values #	Pvalues^
Total bilurubin (mg/dl)	9.6±2.3	14.94±3.2	11.61±2.850	<0.0001	0.004	<0.0001
Direct bilirubin (mg/dl)	0.23±0.06	0.43±0.18	0.200±0.053	<0.0001	0.04	<0.0001
MDA(nmol/ml)	2.443±0.203	3.227±0.227	2.860±0.266	<0.0001	<0.0001	<0.0001
Vit E (mg/dl)	0.293±0.104	0.192±0.091	0.133±0.063	=0.0002	<0.0001	= 0.008

$Comparison between control and before phototherapy, #Comparison between control and after phototherapy, ^ Comparison between before and after therapy * p<0.05 is statistically significant**p<0.001 is highly statistically significant

DISCUSSION

Neonatal hyperbilirubinemia is a common complication in newborn and phototherapy is now the accepted method of treatment which has replaced exchange transfusion in management of neonatal hyperbilirubinemia [16].

Bilirubin is generally regarded as a toxic compound when, in its unconjugated form, it accumulates to abnormally high concentrations in biological tissues and is thus responsible for the development of kernicterus [17,18].

Bilirubin reactions involving free radicals or toxic oxygen reduction products have been well documented: Unconjugated bilirubin scavenges singlet oxygen anions and peroxy radicals and serves as a reducing substrate for peroxidases in presence of hydrogen peroxides or organic hydro peroxides [19,20] .

Although phototherapy has been used on millions of infants worldwide during the past 35 years, in previous studies it has been reported phototherapy as a photodynamic process that can cause peroxidative damage to tissues [21]. It has also been suggested that hydrogen peroxide and free hydroxyl radical (OH·) are responsible for the photoproducts induced chromatid changes [22]. .Furthermore, Ostrea et al reported that the exposure of red cell suspension to phototherapy light in presence of sensitizer (bilirubin) resulted in oxidative injury to the red cell membrane [23]. Recent studies showed that fluorescent light decrease ATPase activity in red blood cell [24].

In a healthy human being, formation and inactivation of reactive oxygen species is balanced at a level at which the compounds can play their physiological role without any toxic side effects. This balance can be unstable in neonatal period following rapid changes in tissue oxygen concentration immature antioxidant mechanism and considerable neonatal developmental changes in antioxidant [25].

As free radicals are potentially toxic, they are usually inactivated or scavenged by antioxidants before they can inflict damage to lipids, proteins or nucleic acids.[26].

In the present study there are significant increased levels of serum total bilirubin and direct bilirubin in case group neonates as compared to control group neonates. Malondialdehyde (MDA) levels have been increased significantly in serum of test group neonates (before phototherapy)than in control group

neonates. Rise in MDA shows the increased generation of reactive oxygen species (ROS) due to excessive oxidative damage generated in neonatal hyperbilirubinemia case. These oxygen species in turn oxidizes many other important biomolecules including membrane lipids. This raised MDA level reflects the oxidative injury in neonatal hyperbilirubinemia, which is attributed to free radical formation that abstracts hydrogen atoms from lipoproteins causing lipid peroxidation of which MDA is the main product.

A significant decrease in the MDA levels was observed in case group (after phototherapy) as compared to same test group neonates (before phototherapy)

In the present study there is a significant decrease in the level of vitamin-E in test group neonates as compared to control group neonates. Vitamin-E is an important chain breaking antioxidant responsible for scavenging the free radicals and suppression of peroxidation products in aqueous and lipid region of the cell [27] .Further significant decrease in vitamin –E was observed in test group neonates after phototherapy as compared to same test group neonates before phototherapy. This decrease in the level of vitamin-E may be to increased turnover, for preventing oxidative damage in these patients [27] .

CONCLUSION

From these results it can be concluded that neonates with hyperbilirubinemia above 7 mg/dl and advised phototherapy showed a significant rise in serum MDA levels and decrease vitamin-E levels. As compared to control group of neonates. The same subjects after phototherapy showed decrease in total bilirubin, indirect bilirubin, MDA levels and a further decrease in vitamin-E levels. From the present study it is concluded though phototherapy had a beneficial effect in the treatment of neonatal unconjugated hyperbilirubinemia but supplementation of vitamin-E and other antioxidants is necessary as secondary therapy in addition to phototherapy to prevent oxidative damage in neonates with hyperbilirubinemia.

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Conflict of Interest: Nil

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