INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

BIOCHEMICAL CORRELATION BETWEEN NEONATAL HYPERBILIRUBINEMIA AND OXIDATIVE STRESS

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ABSTRACT

Neonatal hyperbilirubinemia is known as the visible clinical manifestation of yellowish discoloration of skin and sclera, during the neonatal period, resulting from deposition of bilirubin in the neonatal bodies. Jaundice is observed during the 1st week in approximately 60% of term neonate and 80% of preterm neonate. In present study, we try to investigate and to determine the possible correlation between serum bilirubin levels with oxidative stress and antioxidant status in the Neonatal Hyperbilirubinemia patient with age and sex matched controls. Measured Thiobarbituric acid reacting substance (TBARS) in the serum of neonate (with hyperbilirubinemia) & normal controls as parameters of oxidative stress. To measure reduced glutathione, superoxide dismutase of the neonatal Hyperbilirubinemia patient and normal controls as parameters of antioxidant status. SGPT, ALP also measured between case , control, Serum Thiobarbituric acid reacting substances (TBARS) react with thiobarbituric acid in an acidic medium to form a pink colour compound, this product is extracted in organic solvents such as butanol and its absorbance are read at 532nm.Serum Bilirubin is measured by Diazo Method. Alkaline Phosphatase is measured By CAMP dependent Kinetic Technique. Serum SGPT is measured by Kinetic Method. Reduced Glutathione is measured by Semiautomated DTNB Technique. Serum Superoxide Dismutase (SOD) by modified Kakkar Methods. SGPT, ALP Marker of hepatic injury between case, control. This is Hospital based case control study. Neonatal hyperbilirubinemia is most common in neonate.These correlation studies show increased level of bilirubin increases serum TBARS level (significant at the 0.01 level), increased SOD (Correlation is significant at the 0.05 level, decreased reduced glutathione (Correlation is significant at the 0.05 level.) Hyperbilirubinemia of neonate increases the parameters of oxidative stress, We suggest regular monitoring of these patients for these parameters to avoid impeding harmful effects of oxidative stress.

Keywords: Hyperbilirubinemia, reduced glutathione and Superoxide dismutase.

INTRODUCTION

Neonatal Jaundice or Neonatal Hyperbilirubinemia is a yellowing of the skin and other tissues of a newborn baby. A Bilirubin level more than 5mg/dl manifest Clinical Jaundice in Neonate and it affects 60 % of full-term Neonate and 80 % of preterm neonate in the first 3 days after birth[1]. This condition accounts for up to 75 % of hospital readmission in the first week after birth. Commonly Neonatal Jaundice occurs due to increased Bilirubin on liver cells, increased erythrocyte volume, increased enterohepatic circulation of bilirubin, increased erythrocyte survival, defective hepatic uptake of bilirubin from plasma, decreased ligandin, decreased binding of y-proteins by other anions, defective bilirubin conjugation, decreased UDPG activity, defective bilirubin excretion.

A neonate liver is immature and cannot process bilirubin as quickly as the baby will be able to when he or she gets older[2]. This slow processing

of bilirubin has nothing to do with liver disease. It merely means that neonate's liver is not fully developed as it will be and thus there is some delay in eliminating the bilirubin[3]. Red blood cells are extremely susceptible to lipid peroxidation since they are rich in unsaturated membrane lipids, have rich supply of oxygen and transitional metal catalyst. Neonatal erythrocyte membrane is more susceptible to oxidative damage due to its predominant pro oxidant potential[4].

The erythrocytes are particularly prone to free radical damage, since the membrane lipids are very rich in polyunsaturated fatty acids which play an essential role in generating free radicals.

Free radicals, primarily the reactive oxygen species, superoxide and hydroxyl radicals which are highly reactive having an unpaired electron in an atomic or molecular orbit are generated under physiological conditions during aerobic metabolism. As free radicals are potentially toxic, they are usually inactivated or scavenged by antioxidants before they can inflict damage of lipids, proteins or nuclei. Alteration in the oxidant- antioxidant profile is known to occur in neonatal jaundice.

More ever the body's defense mechanisms would play an important role in the formation of antioxidants and try to minimize the damage, adapting itself to the above stressful situation. Antioxidants are compounds that dispose, scavenge & suppress the formation of free radicals or oppose their actions & two main categories of antioxidants are those whose role is to prevent the generation of free radicals that are generated[5].

They exist in both the aqueous & membrane compartment of cells & can be enzymes or non-enzymes. The human body has a complex antioxidant defense system that includes the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase, catalase (CAT). These block the initiation of free radical chain reaction[6].

In the present study, the following parameters were assessed in the erythrocytes and serum to elucidate the oxidant – antioxidant states in patient with neonatal jaundice. Erythrocyte malondialdehyde (MDA) levels were measured as Thiobarbituric acid reacting substance (TBARS) which serves as an index of extent of lipid peroxidation. The antioxidant enzymes superoxide dismutase (SOD), reduced glutathione in erythrocytes was estimated & serum bilirubin, Alkaline phosphatase, SGPT, were measured. The present study is an attempt to examine oxidative stress and the status of the protective antioxidants under condition of stress due to the neonatal jaundice. The scientific literature linking neonatal hyperbilirubinemia to oxidative stress and antioxidant level has rapidly expanded but there is paucity of data establishing the correlation between oxidative stress and neonatal hyperbilirubinemia. Some literature shows hyperbilirubinemia increased oxidative stress. Lack of detailed study on this association from our country has promoted us to undertake the present study. So we hypothesize that changes in serum bilirubin level may influence the antioxidant system and oxidative stress in newborn patient. Therefore, we shall make efforts to investigate the possible correlation between serum bilirubin level and serum oxidative stress levels of newborn and controls.

MATERIAL AND METHODS

This is case control study. Study area was department of Paedriatic Medicine, Burdwan Medical College & Hospital , indoor newborn with hyperbilirubinemia patients. Sample size is 100. 50 patient selected as Case with less than 28 days age, Bilirubin more than >5 mg/dl, No congenital anomaly& physically stable. Duration of study was 1 year. 50pt selected as Control. After explaining the study, a written informed consent was obtained from every case control subject's parent.

Parameters Studied

Thiobarbichuric Acid Reactants (TBARS)

TBARS react with Thiobarbituric acid in an acidic medium to form a pink colour compound. This product is extracted in organic solvents such as butanol and its absorbance is read at 532nm. **Alkaline Phosphatase** by CAMP dependent Kinetic Technique, **Serum Bilirubin** by Diazo method. **Serum SGPT** by Kinetic Method. **Reduced Glutathione** by semiautomated DTMB Technique. **Plasma Superoxide Dismutase (SOD)** by modified Kakkar Method

Study Tools

Autoanalyser (by XL 600) Spectrophotometer (double beam) SPECTRONIC 21. Boiling water bath. Routine laboratory glass wares ,PVC wires and Micropipettes.

Study Technique

1) Serum TBARS, Plasma SOD, Plasma REDUCED GLUTATHIONE by Absorption spectrophotometry
2) Serum Bilirubin, Alkaline phosphatase, SGPT by Autoanalyser (by XL 600)
3) Records will be maintained about clinical diagnosis.
4) Informed consent form will be completed and duly signed by patients.

5) Comprehensive history will be taken from both case and control groups.

Statistical Analysis

Comparison of variables between case and control was done by mean and standerd deviation, independent t- test, correlation studies between different parameters of both groups

The statistical analyses were performed using Statistic version 6 and SPSS version 17.

Ethical permission

The study protocol strictly adhered to the revised Helsinki declaration for human studies and institutional ethical clearance was obtained before the start of the study.

RESULTS

Table 1: Independent sample t test for different Parameters in case and control groups

Parameters	Case n-50 mean (SD)	Control n-50 Mean (SD)	T	P- value
Age (Days)	7.58	6.32	2.070	0.041
Serum bilirubin (mg/dl)	16.32 (6.07)	2.29 (0.75)	16.200	<0.001
Serum TBARS (nmol/ml)	12.79 (3.99)	8.60 (1.37)	6.940	<0.001
Plasma SOD (unit/ml)	27.90 (3.05)	10.07 (3.33)	15.725	<0.001
Reduced glutathione (µg/ml)	3.10 (1.21)	8.58 (0.99)	-24.34	<0.001
Serum SGPT (U/L)	47.52 (17.17)	46.22 (6.44)	-0.557	0.57
Serum ALP (U/L)	128.30 (37.31)	126.78 (31.28)	-0.533	0.06

The demographic profiles of all the subjects understudy were analysed. Both controls and cases were in the similar age group. When the age groups were analyzed according to days significant difference was observed between the Case and Control.

The results of the present study indicates as shown in Table 1

a. Serum bilirubin level in Cases are significantly higher than the Controls p-value < 0.001 by independent t -test.
b. TBARS levels in cases are found significantly higher in case than the controls (p-value <0.001) by independent t test.
c. Plasma SOD level in cases are found significantly higher than the control (p-value<0.001) by independent t test.
d. Plasma reduced glutathione in cases are found significantly lower than the control (p value <0.001).
e. Serum SGPT, ALP levels are not significantly raised between case and control.

Table 2: Correlation of Serum Bilirubin with other parameters

Parameters	Pearsons correlation (R-value)	Significance
Serum TBARS	0.951	< 0.010
Plasma SOD	0.322	0.029
Reduced glutathione	- 0.290	0.040

Correlation is significant at the 0.01 level (2 tailed) and Correlation is significant at the 0.05 level (2 tailed)

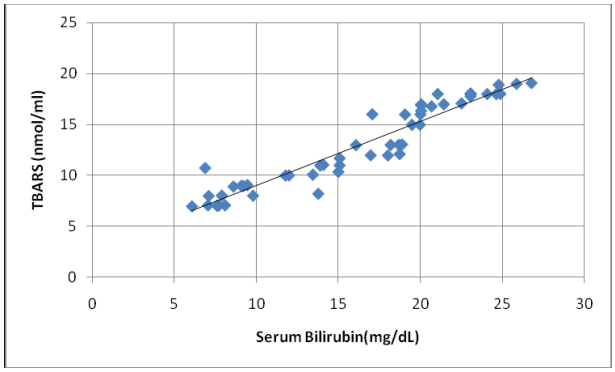

Fig. 1: Scatter diagram showing distribution of serum bilirubin and TBARS level in cases

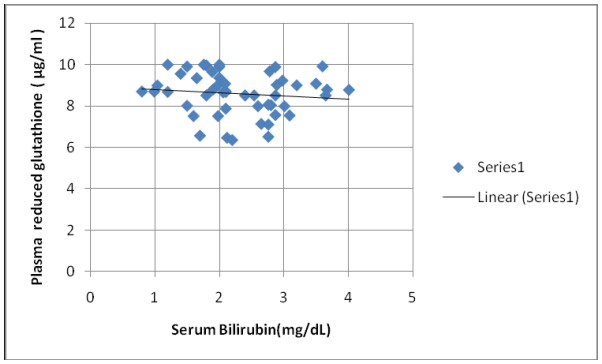

Fig. 2: Scatter diagram showing distribution of Serum Bilirubin and reduced Glutathione in cases

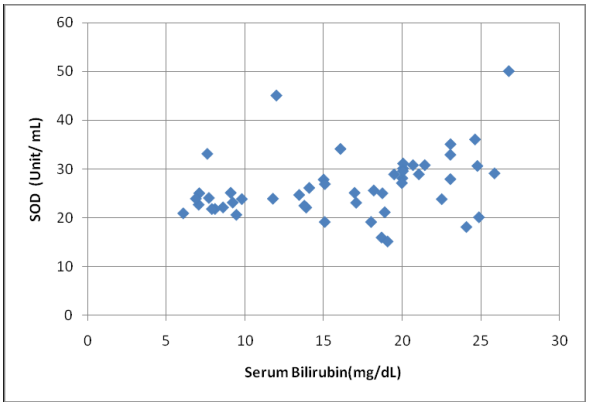

Fig. 3: Scatter diagram showing distribution of serum bilirubin and Plasma SOD in cases

DISCUSSION

Neonatal hyperbilirubinemia affects 60% of full-term infants and 80% of preterm infants in the first 3 days after birth[7]. Although transient, the condition accounts for up to 75% of hospital re-admissions in the first week after birth. Antioxidant activity in the serum of term neonates is lower than that of adults and is re-still lower in preterm and low birth weight babies as compared to term babies. Red blood cells are extremely susceptible to lipid peroxidation since they are rich in unsaturated membrane lipids, have rich supply of oxygen and transitional metal catalysts. Neonatal erythrocyte membrane is more susceptible to oxidative damage due to its predominant pro-oxidant potential The erythrocytes are particularly prone to the free radical damage since and the membrane abstract hydrogen atom from lipoprotein and produces MDA which is main product of lipid perooxidation. The membrane phospholipid contains significantly polyunsaturated fatty acid that converted to MDA by peroxidation which can be analyzed by

reaction of thiobarbituric acid. The results indicates that there is increase in free radical generation and antioxidant defence like as plasma superoxide dismutase, Erythrocytic reduced glutathione impaired in neonatal jaundice patients. In this study, lipid peroxidation product that is (MDA) levels were measured as thiobarbituric acid reacting substance have been significantly increased in case group (Table-1) (P-value < 0.001) than control group. This results supports that neonatal jaundice patients have oxidative stress. (Surapaneni Km et al shows same result, also Davutoglu Met al, SuleYigit et al found that same result, but (Ashok Kumar et al found that negative correlation MDA) Rises of MDA in serum of case group suggest that increase generation of reactive oxygen species who have unpaired electron on their orbit, causes excessive oxidative damage.

These oxygen species in turn can oxidize many other important biomolecules including membrane lipid etc. Raised MDA level reflects the oxidative injury to neonatal jaundice patients. Lipid peroxidation by reactive oxygen species (ROS) or Free radical can lead to formation of cross linked lipid-lipid and lipid-protein adduct and loss of membrane fluidity and integrity[7].

Significant decreased in the level of erythrocytic plasma non enzymatic reduced glutathione in neonatal jaundice patient when compared to control (Table-1) (p- value < 0.001). (Surapaneni Km et al ; shows the same result) Reduced glutathione is important non enzymatic antioxidant that breaks chain and scavenging the free radicals and decreased level of peroxidation in aqueous and lipid region of the cell. The decrease level of this antioxidant parameters may be due to increased production of free radicals, for preventing oxidative damage of these patients suggesting increase defense against oxidant damage in neonatal jaundice patient. Similar reports of decrease reduced glutathione found in many studies[8].

In my study erythrocyte antioxidant enzyme superoxide dismutase activities have been increased significantly in study group than control group (Table-1) (P-value < 0.001). (Surapaneni et al shows the same result) SOD is important antioxidant enzyme that has antioxidant activities against super oxide anion. Superoxide is produced both accidentally and also as reactive oxygen species required for a number of enzymes catalysed reactions. A family of superoxide dismutase catalyses, increases the reaction between superoxide and water yield oxygen and hydrogen peroxide[9]. This hydrogen peroxide removed by the Catalase and various peroxidases. SOD is increased in case group indicates that overexpression of this enzyme might be an adaptive response and it results in increased dismutation of superoxide to hydrogen peroxide. Low erythrocytic activities are found in many studies. In this study I also examined serum SGPT and ALKALINE PHOSPHATASE between case and control to find any abnormalities exist between them due to hepatic injury. But no abnormalities found between case and control group. SGPT level between case and control group not significant. Table -1 (P-value 0.57). ALP level between case and control is not significant, Table-1 (P-value-0.06). ALP level found in both case & control in normal level. From this results suggests no hepatic injury occurs in neonatal hyperbilirubinemia patients. I have not found any significant studies that supports this result. The scientific literature linking neonatal hyperbilirubinemia to oxidative stress and antioxidant level has rapidly expanded but there is lack of data establishing the correlation between oxidative stress and neonatal hyperbilirubinemia[10].

In my study, I mainly concentrate the correlation studies (between BILIRUBIN level with MDA, SOD, REDUCED GLUTATHIONE) to establish strong relationship between neonatal hyperbilirubinemia with oxidative stress. Correlation studies between bilirubin level and oxidative stress (TBARS) antioxidant status (SOD,REDUCED GLUTATHIONE) of cases found that positive correlation between serum bilirubin and serum TBARS at 0.01 level (Table-2), and positive correlation with SOD, negative correlation with REDUCED GLUTATHIONE (Table-2) at 0.05 level.

Lipids are very rich in polyunsaturated fatty acids which play an essential role in generating free radicals[11].

Free radicals, primarily the reactive oxygen species, superoxide and hydroxyl radicals which are highly reactive having an unpaired electron in an atomic or molecular orbit are generated under physiological conditions during aerobic metabolism[12]. 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. Alteration in the oxidant – antioxidant profile is known to occur in neonatal jaundice[13]. Moreover, the body's defense mechanisms would play an important role in the form of antioxidants and try to minimize the damage, adapting itself to the above stressful situation.

The results indicates that there is a increase in free radical generation and antioxidant defence like as serum superoxide dismutase,

erythrocytic plasma reduced glutathione impaired in neonatal jaundice patients. In this study, lipid peroxidation product that is (MDA) levels were measured as thiobarbituric acid reacting substance, have been significantly increased in case group (Table-1), (P value < 0.001) than control group. This result supports that neonatal jaundice patients have oxidative stress. (Surapaneni Km et al shows same result, also Davutoglu Met al, Sule Yigit et al found that same result, but (Ashok Kumar et al found that negative correlation MDA). Rises of MDA in serum of case group suggest that increase generation of reactive oxygen species who have unpaired electron on their orbit, causes excessive oxidative damage. These oxygen species in turn can oxidize many other important biomolecules including membrane lipid etc. Raised MDA level reflects the oxidative injury to neonatal jaundice. Lipid peroxidation by reactive oxygen species (ROS) or Free radical can lead to formation of cross linked lipid-lipid and lipid-protein adduct and loss of membrane fluidity and integrity[14].

CONCLUSION

Oxidative stress along with altered redox balance is supposed to be an important causative factor for different diseases. Increased lipid peroxidation along with a reduced level of different antioxidant vitamins have been reported in patients with neonatal hyperbilirubinemia. Increase antioxidant enzymes also have been reported[15]. The present study conducted an analysis on lipid peroxidation product, anti-oxidant enzymes and the antioxidant responsible for causing an altered redox status leading to oxidative stress in the neonatal hyperbilirubinemia patients. Serum TBARS was selected as marker of free radical induced lipid peroxidation and, plasma SOD, plasma reduced glutathione were selected as the markers of antioxidant status. The study group included 50 neonatal hyperbilirubinemia patients against properly selected controls. The diagnosis of neonatal hyperbilirubinemia was done by different books. The controls were matched in different aspects with the selected cases. The cases were selected by a simple random method.

Short Communications

Correlation between serum bilirubin levels with oxidative stress and antioxidant status in the Neonatal Hyperbilirubinemia.

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