ASSOCIATION OF PLASMA LEVEL OF THIOBARBITURIC ACID REACTIVE SUBSTANCES WITH EXTENT OF HEPATOCELLULAR INJURY IN PRETERM INFANTS WITH CHOLESTATIC JAUNDICE

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ABSTRACT

The present study was designed to determine the association between extent of hepatocellular injury and plasma level of thiobarbituric acid reactive substances (TBARS) in pre term infants with cholestasis. Preterm infants (<35 weeks gestation) admitted to the neonatal intensive care unit were enrolled (with their parents informed consent) in either the 'cholestasis' group (if their direct bilirubin was >2 mg/dl) (n = 25) or in the control group (n = 16). Blood samples for measurement of TBARS, direct bilirubin and transaminases were obtained within 24 hours of enrollment. The cholestasis and control groups were comparable with respect to gestational age, birth weight and Apgar score. Serum direct bilirubin, SGOT (EC 2.6.1.1) and SGPT (EC 2.6.1.2) levels were significantly high in the cholestasis group. Plasma levels of TBARS in cholestasis group were correlated with SGOT ($F=276.92$; $P<0.0001$) and SGPT ($F=355.17$; $P<0.0001$) and differed significantly between cholestatic and control infants. Our findings suggest that oxidative stress in preterm infants with cholestasis is associated with hepatocellular injury.

KEY WORDS

TBARS, Bilirubin, Transaminase, Cholestasis, Infants

INTRODUCTION

Reactive oxygen species (ROS) are highly reactive oxidizing agents belonging to the group of free radicals. They are compounds with one, or more unpaired electrons. ROS are incriminated in the causation of several neonatal diseases (1). Oxidative stress is the result of imbalance between the formation of ROS and inactivation of these species by antioxidant defense system (2). It causes hepatic tissue injury. Lipid peroxidation products are increased in the cholestatic liver (3).

The purpose of the study was to determine whether hepatocellular injury in preterm newborn infants with cholestasis is associated with systemic oxidative stress. The extent of liver injury was measured by estimating serum

glutamate oxaloacetate transaminase (SGOT, EC 2.6.1.1) and serum glutamate pyruvate transaminase (SGPT, EC 2.6.1.2). In order to quantify extent of lipid peroxidation, we measured thiobarbituric-acid-reacting substances (TBARS). The most abundant of these substances is MDA, an aldehydic lipid peroxidation product formed by the action of ROS on lipid membranes. The identification of ROS as potential markers of liver injury in cholestatic preterm infants may aid in the diagnosis and management of those at highest risk for ongoing liver impairment.

MATERIALS AND METHODS

Patients: Preterm infants (<35 weeks' gestation) born at Central Referral Hospital (Gangtok, INDIA) and admitted to the hospital's neonatal intensive care unit were serially screened during the course of hospitalization for entry into the 'cholestasis' study group. Infants with major congenital anomalies (including all gastrointestinal and liver anomalies) or with congenital or acquired infection were excluded. The criteria for entry into the cholestasis group were direct bilirubin >2 mg/dl. If the infant met these criteria, the parents' informed

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consent for entry into the study was requested. During study period, 30 infants qualified for the cholestasis group, and informed consent was obtained from the parents of 25. When each eligible infant with cholestasis was enrolled, a preterm infant without cholestasis was matched for gestational age, birth weight, and severity of respiratory illness, and the parents' informed consent was requested to enroll the infant as a control subject. For the 25 infants enrolled in the cholestasis group, matched controls were identified for 24. Parental consent could not be obtained for 8, so 16 infants constituted the control group. Study personnel obtained demographic and medical information from infant's medical records.

Analytical methods: Blood samples were often obtained from the umbilical vein catheter to reduce the number of painful interventions. Infusion contamination was prevented by aspirating the infused solution and at least 0.5 ml blood from the catheter. Biochemical assays were carried out with whole blood serum. Heparinised (200 units) blood sample was used for the hemolysate preparation. All analyses were carried out within six hours of blood sampling to avoid the effects of storage

Preparation of hemolysate: Heparinised whole blood was centrifuged at 1000g for 10 minutes at 2° C. Plasma was separated. Packed erythrocyte was washed with cold normal saline thrice and in each case centrifuged at 15000g for 15 minutes at 2°C. Washed, packed erythrocyte was hemolysed by the addition of chilled double distilled water. The hemolysate was centrifuged at 13000g for 1 hour at 2°C.

Determination of plasma thiobarbituric-acid-reactive substances (TBARS): The extent of lipid peroxidation was determined by assaying malondialdehyde (MDA) formation according to the method of Sinnhuber *et al.* (4). To 0.5 ml of the serum, 0.5 ml of 30% trichloro acetic acid (TCA) and 100 µl of 1% TBA reagent were added. The tubes were covered with aluminum foil and kept in an ice-cold water bath for 10 min followed by centrifugation at 3000 rpm for 15 min. The absorbance of the supernatant was read at 540 nm at room temperature against blank. The concentration of MDA was read from standard calibration curve plotted using 1, 1, 3, 3' tetra-ethoxy propane. The extent of lipid peroxidation was expressed as nmol MDA formed / ml using a molar extinction coefficient for MDA of $1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$.

Determination of serum bilirubin, SGOT and SGPT: Quantitative determinations of serum bilirubin, SGOT and SGPT levels were performed by spectrophotometry using reagent kit (Randox Laboratories Ltd, UK) at the clinical laboratory, Central Referral Hospital, Gangtok.

Statistical analysis: The data were expressed as mean $\pm$ SD and analyzed for significant differences by one-way analysis of variance. Simple regression analysis was used to assess the correlation between parameters. Value of $p < 0.05$ was considered statistically significant.

RESULTS

Twenty-five infants were enrolled in the cholestasis group and 16 infants served as controls. The cholestasis and control groups were comparable with respect to gestational age and birth weights as well as Apgar scores.

Serum bilirubin, SGOT and SGPT levels were significantly high in the cholestasis group than in the control group (Table 1). Plasma TBARS levels were also elevated significantly in the cholestasis group (Table 1) and it significantly correlated with serum SGOT ($F=276.92$; $P < 0.0001$) and SGPT ($F=355.17$; $P < 0.0001$). However it didn't correlate with serum bilirubin ($F=0.7293$; $P < 0.4019$).

Table 1
Blood levels of transaminases, bilirubin and TBARS in preterm infants studied.

	Control (n=16)	Cholestasis group (n=25)
SGOT (IU/L)	8.75 $\pm$ 1.61	32.6 $\pm$ 4.66*
SGPT (IU/L)	20.25 $\pm$ 2.46	70 $\pm$ 3.04*
Direct bilirubin (mg/dL)	1.22 $\pm$ 0.10	3.06 $\pm$ 0.15*
TBARS (nmol/mL)	2.48 $\pm$ 0.10	5.46 $\pm$ 0.50*

Values are mean $\pm$ SD

Statistical comparison was done between control and cholestasis group;

* $P < 0.05$

DISCUSSION

We found that serum transaminases, bilirubin and TBARS are elevated in preterm infants (<35 weeks' gestational age) with cholestasis. In addition, increase in plasma TBARS level, which are proportional to lipid peroxidation and oxidant stress, is positively correlated with serum transaminase levels suggesting the association of extent of lipid peroxidation (as indicated by elevated TBARS) with hepatocellular injury in preterm infants.

Although there is ample evidence that oxidant stress follows cholestasis, oxidative injury in the liver may be induced by mechanisms that are independent of cholestasis (5) like inflammation. Activated Kupffer cells and neutrophils release

ROS in response to inflammatory cytokines in the liver (6) which in turn inactivate proteins, disrupt DNA, and oxidize lipids (7). As preterm newborn infants exhibit an imbalance between ROS generating and scavenging (antioxidant) systems they may be more susceptible to oxidative injury (8).

Along with elevated serum transaminases, infants with cholestasis displayed elevated plasma TBARS. This suggests that cholestasis and hepatic impairment in preterm infants are induced by mechanisms that are dependent on oxidant-mediated hepatocellular injury (9). TBARS measurements provide a measure of extent of lipid peroxidation and, as such, may provide a direct assessment of the progression of liver injury at the cellular level. Furthermore, the association between TBARS levels and liver injury does not necessarily indicate causality. Despite physiologic evidence that ROS play a central role in tissue injury during inflammation, it is possible that elevated TBARS occurs secondary to other mechanisms of hepatocellular injury (10). Larger longitudinal and/or interventional (e.g. antioxidant administration) studies will be necessary to determine whether there is a causal relationship between lipid peroxidation and hepatocellular injury in preterm infants.

Present study suggests that oxidant stress is associated with hepatocellular injury in preterm infants. Serum level of TBARS was significantly high in infants with cholestasis than control infants suggesting that cholestasis in preterm infants is induced, in large part, by mechanisms that are dependent on oxidant-mediated hepatocellular injury.

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