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ORIGINAL ARTICLE

Status Of Lipid Peroxidation, Glutathione, Ascorbic Acid, Vitamin E And Antioxidant Enzymes In Neonatal Jaundice Patients

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ABSTRACT

The exact pro-oxidant and antioxidant status in neonatal jaundice is still not clear. To add a new insight to the question, changes in the erythrocyte lipid peroxidation products (MDA), levels of glutathione (GSH), ascorbic acid and plasma vitamin E (non enzymatic antioxidant parameters) and activities of antioxidant enzymes super oxide dismutase (SOD), glutathione peroxidase (GP_X), catalase in erythrocytes were studied in forty-eight neonatal jaundice patients and forty-eight healthy subjects. It was observed that there was a significant increase in erythrocyte MDA levels, activities of SOD, GP_X and a significant decrease in erythrocyte GSH, ascorbic acid, plasma vitamin E levels and catalase activity in patients with neonatal jaundice when compared to controls. The results of our study have shown higher oxygen free radical production, evidenced by increased levels of MDA and decreased levels of GSH, ascorbic acid, vitamin E and catalase activity, supports the oxidative stress in neonatal jaundice patients. The increased activities of antioxidant enzymes may be a compensatory regulation in response to increased oxidative stress. The decreased concentration of the glutathione and antioxidant vitamin status supports the hypothesis that neonatal jaundice is an important causative factor in the pathogenesis of lipid peroxidation. These data reveal that antioxidant defense mechanisms might be impaired in neonatal jaundice patients. These findings also provide a theoretical basis for the development of novel therapeutic strategies, such as antioxidant supplementation.

Key Words:
Malondialdehyde (MDA), glutathione (GSH), ascorbic acid, vitamin E, super oxide dismutase (SOD), catalase, glutathione peroxidase (GP_X), neonatal jaundice.

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Introduction

A homeostasis between rate of formation of free radicals and the rate of their

neutralization of free radicals if not maintained, oxidative damage accumulates and is known as oxidative stress[1]. Neonatal Jaundice is a normal physiological event that is being treated on a belief of pathology. Commonly neonatal jaundice occurs for two reasons. A) Infants have too many red blood cells. It is a natural process for the baby's body to break down these excess red blood cells, forming a large amount of bilirubin. It is this bilirubin causes the skin to take an yellowish colour. B) A newborn's liver is immature and can not process bilirubin as quickly as the baby will be able to when he/she gets older. This slow processing of bilirubin has nothing to do with liver disease. It merely means that the baby's liver is not as fully developed as it will be, and thus, there is some delay in eliminating the bilirubin[2]. Neonatal jaundice affects 60% of full term infants & 80% of preterm infants in the first 3 days after birth [3]. Although transient, the condition accounts for upto 75% of hospital re-admissions in the first week after birth [4]. 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 [5],[6]. 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 [7]. The erythrocytes are particularly prone to the 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 to lipids, proteins or nucleic acids. Alteration in the oxidant – antioxidant profile is known to occur in Neonatal jaundice [8],[9]. 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. Antioxidants are compounds that dispose, scavenge, and suppress the formation of free radicals, or oppose their actions[1] and two main categories of antioxidants are those whose role is to prevent the generation of free radicals and those that intercept any free radicals that are generated [10]. They exist in both the aqueous and membrane compartment of cells and can be enzymes or non enzymes. The human body has a complex antioxidant defense system that includes the antioxidant enzymes super oxide dismutase (SOD), glutathione peroxidase (GP_X) and catalase (CAT). These block the initiation of free radical chain reactions [11]. The non enzymatic antioxidant components consists of molecules such as glutathione (GSH), vitamin E, ascorbic acid and beta-carotene that react with activated oxygen species and thereby prevent the propagation of free radical chain reactions.

In the present study, the following parameters were assessed in the erythrocytes and plasma to elucidate the oxidant-antioxidant status in patients with neonatal jaundice. Erythrocyte malondialdehyde (MDA) levels were measured as thio barbituric acid reacting substances (TBARS) which serves as an index of extent of lipid peroxidation. Erythrocyte glutathione (GSH), ascorbic acid and plasma vitamin E serves as non enzymatic antioxidant parameters. The antioxidant enzymes super oxide dismutase (SOD), catalase, glutathione peroxidase (GP_X) in erythrocytes were estimated. 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.

Methods

The study was conducted in department of biochemistry, Dr. Pinnamaneni Siddhartha Institute of Medical Sciences & Research Foundation, Chinoutpally, Gannavaram (Mandal), A.P, INDIA. The study was carried out on forty eight full term jaundiced neonates with appropriate weight, delivered normally in the labour room of Dr.PSIMS &RF general hospital, Chinoutpally. It was ensured that the jaundice was of non-hemolytic with the help of relevant investigations. Bilirubin levels were monitored for all babies at 24 hour interval. All the parameters were estimated in these babies. The results were compared with those of forty eight neonates that did not develop jaundice in the neonatal period who comprised the control group. Informed consent was taken form parents before drawing blood. Due permission was obtained from the ethical committee of the Dr.PSIMS&RF General Hospital, Chinoutpally before the start of the work.
The controls and patients were divided into two groups.

Group 1: Forty-eight healthy age & sex matched babies as Controls.
Group 2: Forty-eight Neonatal jaundice patients.

The heparinised venous blood samples obtained from these subjects were used for the analysis. Plasma was separated by centrifugation at 1,000 g for 15 minutes. Separated plasma was used for the estimation of vitamin E. The buffy coat was removed and the packed cells were washed three times with physiological saline. The erythrocyte suspension was prepared by the method of Dodge et al.,[12] modified by Quist (13). The packed cells were used for the analysis of GSH, ascorbic acid, MDA, SOD, catalse, GP_X. Serum bilirubin was estimated by bilirubinometer. Erythrocyte GSH was estimated by the method of Beutler et al [14] using di thio bis nitro benzoic acid (DTNB). Ascorbic acid levels were estimated in plasma by the method of Tietz [15]. Plasma vitamin E levels were estimated by the method of Baker H et al [16]. Erythrocyte MDA was determined as the measure of thio barbituric acid reactive substances (TBARS) [17]. SOD (EC 1.15.1.1) activity was determined in the hemolysate according to the method described by Murklund and Murklund (18) with some modifications as described by Nandi and Chatterjea [19]. Catalase (EC 1.11.1.6) activity was measured in the hemolysate by the method of Sinha [20] and the activity of glutathione peroxidase (GPX, EC 1.11.1.9) was measured as described by Paglia and Valentine [21] in erythrocytes. All reagents used were of analytical reagent grade. DTNB and thio barbituric acid were obtained from sigma chemicals, St.Louis, MO. Statistical analysis between group 1 (controls) and group 2 (patients) was performed by the student t – test using the stat -view package. The data were expressed as mean $\pm$ SD. $P < 0.05$ was considered as significant.

[Table/Fig 1]: The mean + SD values of serum bilirubin, malondialdehyde (MDA), glutathione, ascorbic acid, vitamin E, super oxide dismutase (SOD), catalase and glutathione peroxidase (GPX) in controls and neonatal jaundice patients.

Parameters	Group1 (controls) n=48	Group2 (Patients) n=48
Bilirubin (mg/dl)	1.86 ± 0.26	16.86 ± 3.23 **
Glutathione (mg/gm of Hb)	43.66 ± 2.99	28.84 ± 1.86 **
Ascorbic Acid (mg/dl)	1.59 ± 0.33	1.54 ± 0.22 **
Vitamin E(μmoles/L)	12.20 ± 0.32	11.08 ± 0.17 *
MDA(nmoles/gm of Hb)	9.90 ± 0.56	10.52 ± 0.84 **
SOD(U/gm of Hb)	495.88 ± 29.23	523.03 ± 29.33 *
Catalase(U/gm of Hb)	10.57 ± 0.32	9.74 ± 0.16 ***
GP_X (U/gm of Hb)	75.85 ± 1.83	87.48 ± 1.16 **

* P < 0.01 compared to controls ** P < 0.001 compared to controls
*** P < 0.05 compared to controls

Results

The mean $\pm$ SD of serum bilirubin, erythrocyte GSH, ascorbic acid, MDA, SOD, neonatal jaundice patients. Impaired antioxidant defense and increased lipid catalase, GP_x , plasma vitamin E were indicated in the [Table/Fig 1]. There was a statistically significant increase in the erythrocyte MDA levels in neonatal jaundice patients compared to controls. The activities of erythrocyte antioxidant enzymes SOD and GP_X were significantly increased in group2 compared to group1. The levels of erythrocyte GSH, ascorbic acid, plasma

vitamin E and catalase activity were significantly decreased in patients with Neonatal jaundice compared to controls.

Discussion

The results indicate that there is increase in free radical generation and antioxidant defense is impaired in peroxidation have been reported in neonatal jaundice patients [8].

In the present study the lipid peroxidation product i.e. malondialdehyde (MDA) levels have been increased significantly in erythrocytes of the neonatal jaundice patients than that in control group. This may show the presence of increased oxidative stress. Rise in MDA could be due to increased generation of reactive oxygen species (ROS) due to the excessive oxidative damage generated in these patients. These oxygen species in turn can oxidize many other important biomolecules including membrane lipids. The raised MDA level reflects the oxidative injury due to neonatal jaundice, which is attributed to free radical formation that abstracts hydrogen atoms from lipoproteins causing lipid peroxidation, of which MDA is the main product [22],[23]. The membrane phospholipids, specifically polyunsaturated fatty acids are converted to MDA by peroxidation, which can be analysed by reactivity to thiobarbituric acid. Increased levels of thiobarbituric acid reaction products have been found in the erythrocytes of neonatal jaundice patients[8].

We observed a significant decrease in the levels of erythrocyte reduced glutathione (GSH), ascorbic acid and plasma vitamin E (non enzymatic antioxidant defense system) in Neonatal jaundice patients when compared to controls. GSH, vitamin E, and ascorbic acid are important chain breaking antioxidants responsible for scavenging the free radicals and suppression of peroxidation in aqueous and lipid region of the cell [24],[25]. The decrease in the levels of these non enzymatic antioxidant parameters may be due to the increased turnover, for preventing oxidative damage in these patients suggesting an increased defense against oxidant damage in Neonatal jaundice patients. Similar reports of decreased GSH, ascorbic acid and vitamin E levels in neonatal jaundice patients were reported by various studies [9],[26].

In our study the erythrocyte antioxidant enzyme i.e. super oxide dismutase (SOD) & glutathione peroxidase (GP_x) activities have been increased significantly in patients with neonatal jaundice patients compared to controls. The increased activity of SOD may be indicative of increased superoxide generation by whichever mechanism like increased catecholamine metabolism. SOD is the important antioxidant enzyme having an antitoxic effect against super oxide anion. The over expression of SOD might be an adaptive response and it results in increased dismutation of superoxide to hydrogen peroxide. In neonatal jaundice. But on the other hand, low erythrocyte SOD activities have also been reported in various studies [27]. GP_X, an oxidative stress inducible enzyme plays a significant role in the peroxyl scavenging mechanism and in maintaining functional integration of the cell membranes [28]. The rise in the activity of GP_X could be due to its induction to counter the effect of increased oxidative stress. GP_X provides an effective protective mechanism against cytosolic injury because it eliminates H_2O_2 and lipid peroxides by reduction utilizing GSH. Decrease in antioxidant enzyme status was reported in various studies [26],[29].

In the present study, we have observed a significant decrease in the activity of Catalase in patients with Neonatal jaundice compared to controls. Catalase is the enzyme which protects the cells from the accumulation of hydrogen peroxide by dismutating it to form water and oxygen or by using it as an oxidant in which it works as a peroxidase [30]. A decrease in Catalase activity in neonatal jaundice patients as compared with normal healthy subjects was also observed [29],[31].

The results of our present study have shown higher oxygen free radical production & decreased Catalase activity, supports the higher oxidative stress hypothesis in neonatal jaundice patients. The increased activities of antioxidant enzymes may be a compensatory regulation in response to increased oxidative stress. The results demonstrate that jaundice can produce an increased oxidative stress which can be counteracted by increase in antioxidant response as evidenced by increase in antioxidant enzymes. It is evident from the study that increased oxidative stress in neonatal jaundice babies leads to decrease in the levels of antioxidants like GSH, vitamin E and ascorbic acid and disturb their metabolism, that weaken their ability to fight the growing stress. Intense oxidative stress and decreased antioxidants may contribute to neural cell death and alter the erythrocyte membrane structure processing in neonatal jaundice. So, the treatment with antioxidants in the initial stages of the disease may be useful as secondary therapy to prevent the oxidative damage and deterioration of the neural tissues in Neonatal jaundice patients. Further studies are needed to use antioxidants such as vitamin E, ascorbic acid, beta – carotene as secondary therapy in addition to current drug therapy in neonatal jaundice.

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