Lipid peroxidation and hyperbilirubinemia in infants

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This study was performed on blood specimens on 50 infants with hyperbilirubinemia and 16 healthy babies cord blood. Red blood cell-malondialdehyde (RBC-MDA) and plasma vitamin E levels were measured in both groups. Plasma vitamin E levels and vitamin E/total lipid ratio of the two groups were not significantly different (p>0.05) but RBC-MDA levels were increased in hyperbilirubinemia group (p<0.001) (2.17±0.62 nmol MDA/g Hb in patients. 1.02±0.18 nmol MDA/g Hb in controls). The result suggest that high bilirubin levels may activate lipid peroxidation. [Turk J Med Res1993;11(5):231-233]

Key Words: Lipid peroxidation, Hyperbilirubinemia

There a re several pathways in aerobic cells that can lead to the production of oxygen free radicals. The current major candidates for a source of oxygen free radials generation include intracellular production from the mitochondria, autooxidation of catecholamines, the conversion of xanthine dehydrogenase to xanthine oxidase, activated neutrophils, and the arachidonic acid cascade (1).

It has been simply demonstrated that a particularly susceptible target for their action is the cell membrane and that damage to this structure is initiated by the oxidation of polyunsaturated fatty acids (2). Vitamin E is a potent antiperoxidant at the cellular level. The lack of this vitamin results in an accelerated of red cell membrane lipid peroxidation and a shortening of red cell life span (3). Low micromolar amounts of bilirubin-ditaurine or biliverdin inhibits the oxidation of phosphatidyl choline (PC) significantly in a concentration dependent way (4) and one of the antioxidants in human plasma is bilirubin (5,6).

In this study, we examined the erythrocyte lipid peroxidation and plasma vitamin E levels in hyperbilirubinemic infants.

Received: Jan.. 16,1993 Accepted: Aug. 26,1993

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MATERIALS AND METHODS

Malondialdehyde (MDA) were measured in erythrocytes of 50 infants blood (gestational aged 35-40 weeks, weighing 2450-4100 g) with non-hemolytic hyperbilirubinemia (serum total bilirubin levels, % mg 23±2.4) and in erythrocytes from 16 healthy infants (gestational aged 35-40 weeks, weighing 2500-4000 g)cord blood (serum total bilirubin levels % mg 2.5+0.3). The hyperbilirubinemic infants have had no phototherapy or drugs treatment. Vitamin E levels were measured in plasma of the same groups. Blood samples were anticoagulated with heparin. After centrifugation the plasma and buffy layer were removed. We examined vitamin E in plasma using the method of Rindi (7). Total lipid was using the phosphoric acidvanilin reaction (8). Vitamin E/total lipid ratio was calculated as mg/g.

The erythrocytes were washed three times with cold 154 mM NaCI. The Thiobarbituric acid reaction was used to determine lipid peroxidation. Malondialdehyde, an end product of fatty acid peroxidation, can react with thiobarbituric acid to form a colored complex that has maximum absorbance at 532 nm. Thiobarbituric acid reactivity of erythrocytes were determined by the method of Jain (9).

Hemoglobin levels of packed cells were measure dusing Cyanmethemoglobin method (10) and RBC-MDA levels were calculated as nmol MDA/g Hb.

We measured plasma bilirubin levels with caffein-benzoat assay by Encore (Baker) autoanalyser.

Statistical significance of results was determined using the Student's t test and regression analysis.

RESULTS

RBC-MDA, Vitamin E values of hyperbilirubinemic and control groups are listed in Table 1. RBC-MDA levels of patient group were significantly increased (P<0.001) and plasma Vitamin E status and vitamin E/total lipids ratio did not differ among the groups (P>0.05).

Plasma Vitamin E and RBC-MDA levels of samples of hyperbilirubinemic and control groups were not significantly correlated (r—0.01326, P>0.55 in hyperbilirubinemic group; r=-0.058, P>0.05 in control group).

DISCUSSION

The reduction of oxygen to water via H_2O_2 can lead to formation oxygen free radical which are toxic to living organisms (2). The antioxidants in aqueous phase of human plasma include ceruloplasmin, albumin (the protein itself and possibly also albumin bound bilirubin), ascorbic acid, transferrin, haptoglobin and hemopexin (5). It is clear that a very small quantity of oxygen free radicals (superoxid anion, singlet oxygen etc.) can cause the peroxidation of large quantities of fatty acids (11,12). Vitamin E is the most effective in early stages of membrane damage (2). Stocker et al. (4), demonstrated that bilirubin and biliverdin can act synergistically with membrane-bound vitamin E to prevent lipid peroxidation initiated in the lipid phase. Therefore bile pigments are powerfull peroxyl radical scavengers.

Hyperbilirubinemia remains as one of the most pathological condition in the newborn. The possibility of low grade brain damage due to bilirubin toxicity continue to be of great interest to the clinician. Bilirubin toxicity leads to inhibition of phosphorylation, partial inhibition of respiration, bilirubin encephalopathy, electron-microscopic pathologic alterations in the brain (13), impaired neurodevelopmental outcome (14), shortening of red cell life span (15) etc. Vitamin E deficiency in ifants, especially the prematures leads to increased red blood cell susceptibility to hemolysis* (16). The RBC-MDA assay reflects adequacy the overlapping antioxidant systems of RBC, including glutathione, glutathione peroxidase, glutathione reductase, catalase, selenium, vitamin E and methemoglobin reductase (17). In our study the levels of RBC-MDA of hyperbilirubinemic infants were higher than those of the controls and this findings could not be attributed to vitamin E deficency, since the levels of vitamin E showed no significant difference between the two groups.

Table 1. RBC-MDA and plasma vitamin E levels of hyperbilirubinemic and control infants (X±SD)

	Hyperbilirubinemic group	Control group	P
n	50	16	
Male/Female	28/22	10/6	
Vitamin E (mg/dL)	0.68±0.07	0.70±0.08	>0.05
Vit E/total lipid (mg/g)	1.30+0.23	1.25±0.24	>0.05
RBC-MDA (nmol MDA/g Hb)	2.17±0.62	1.02±0.18	<0.001

Aykac et al. (18) showed that NADPH-induced microsomal lipid peroxidation was significantly inhibited in the livers of cholestatic rats. Values of the study group are as follows: total bile acids (umol/L) 111.4±20.7 and total bilirubin (mg/dL) 5.10±3.40.

Petukhov et al. (19) demonstrated that RBC-lipid peroxidation processes were markedly activated in 32 patients with obstructive jaundice which led to changes in the red cell membrane properties reduction of their electric charge, and in the metabolic shifts promoting cell destruction. Babin et al. (20) reported that the mean lipid peroxide values was nearly 4 times higher than the mean control value in cholestatic children with syndromatic paucity of interlobular bile ducts and the plasma lipid peroxide levels were inversly correlated with the vitamin E status (vit.E/total lipid). Most of the total and free acid variations observed were the greatest in patients with severe jaundice. They concluded that the dietary fat malabsorption and the increased lipid peroxidation could partly explain their results. In our patients there was no problem of fat malabsorption and no difference between the two groups regarding plasma vitamin E status (vit. E/total lipid). The increasing effect to bilirubin on lipid peroxidation may be due to its high concentrations. Future research must aim to clarify the basic mechanisms for bilirubin toxicity in the brain and bilirubin effect on membrane integrity need to be studied in more detail.

Yeni doğanlarda lipid peroksidasyonu ve hiperbilirubinemi

Yeni doğan 50 hiperbilirubinemik ve 16 sağlıklı doğmuş bebeğin kord kanı bu çalışmada kullanıldı. Her iki grupta da eritrosit-içi malondialdehit (RBC-MDA) ve plazma vitamin E seviyesi ölçüldü. Plazma vitamin E seviyesi ve vitamin E/total lipid oranı her iki grupta farklı bulunamadı (P>0.05), fakat RBC-MDA seviyesi hiperbilirubinemik grupta artmış olarak bulundu (2.17±0.62 nmol MDA/g Hb hasta grubunda, 1.02±0.16 nmol MDA/g Hb kontrol grubunda; P<0.001).

Bu sonuçlar, yüksek bilirubin seviyesinin lipid peroksidasyonunu aktive edebileceğini göstermektedir. [Turk J Med Res 1993, 11(5):231-233]

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