

Free Radical Status in Retinopathy of Prematurity

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Received: 25 May 2011 / Accepted: 10 November 2011 / Published online: 25 December 2011
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Abstract Retinopathy of prematurity (ROP) is a major cause of blindness in children. Free radicals are implicated in the development of this retinopathy. We studied the role of free radicals in ROP and enrolled 60 preterm neonates at 30–32 weeks age. Thirty neonates predisposed to development of ROP, were placed in study group and 30 normal neonates in control group. Malondialdehyde and antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPX) were measured in blood spectrophotometrically. Both the groups were followed-up to 40–42 weeks age. Serum MDA levels, erythrocyte SOD and plasma GPX were significantly high in study group at 30–32 weeks as compared to control group. At follow up visit significant increase in MDA level and decrease in SOD and GPX level among the study group was seen. This disturbance in equilibrium of oxidant and antioxidant status initiates an inflammatory process in retinal tissue leading to development of ROP.

Keywords Free radicals · Antioxidants · Retinopathy of prematurity

Introduction

Free radicals are highly reactive and short lived species as they contain unpaired electrons in their outer orbital. Free hydroxyl radical, superoxide anion and nitric oxide are important free radicals produced from metabolic reactions, irradiation and leakage from electron transport chain in the body [1]. Free radicals can react with cellular lipids, proteins and nucleic acids, leading to local injury and eventual organ dysfunction. In low to moderate concentration they play an important physiological role in apoptosis, vascular tone, hormone regulation, immunocompetence and adaptive response to enzymes [2]. However, in higher concentration they are harmful. Free radicals have been implicated in the development and progression of several age related and inflammatory diseases [3]. Excessive production of free radicals from lipid peroxidation and deficient antioxidant system has been associated with certain neonatal diseases like bronchopulmonary dysplasia, retinopathy of prematurity (ROP) and intraventricular haemorrhage [4].

Retinopathy of prematurity (ROP) is a major cause of blindness in children. Incidence of ROP is more common in premature infants exposed to high concentration of oxygen as it causes generation of free radicals. This initiates an inflammatory process in retinal tissue leading to ischemia followed by reperfusion injury to the retina [5]. The other contributing factors implicated in the pathogenesis of ROP are hypoxia, apnoea, sepsis, intraventricular haemorrhage, and low birth weight of neonate [6].

Free radical damage in the body is prevented by antioxidant system that includes enzymes like superoxide dismutase, glutathione peroxidase, catalase and non enzymatic defences like vitamin E, A etc. It has been shown that premature infants have poorly developed antioxidant

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systems and therefore may be at higher risk of free radical damage [7].

This study was undertaken to assess the role of free oxygen radicals and antioxidant system involved in development of ROP in premature neonates.

Materials and Methods

A prospective case controlled study was conducted in the Departments of Biochemistry, Ophthalmology and Paediatrics of Lady Hardinge Medical College and associated Smt. Sucheta Kriplani hospital and Kalawati Saran Children hospital, New Delhi with informed written consent and after institutional ethical clearance.

Sixty preterm neonates born between 30 and 32 weeks of gestation, having birth weight $\leq 1,500$ – $2,000$ gm with unstable clinical course were included in the study. Babies with gross congenital abnormalities were excluded from the study. Thirty neonates predisposed to ROP were placed in study group and 30 neonates not predisposed to ROP were placed in control group. All the preterm neonates were managed according to standard neonatal intensive care unit (NICU) protocol. Antenatal, natal and postnatal history were taken including details of nutrition, oxygen supplementation and ventilatory support.

Screening and follow up of babies for retinopathy of prematurity was done by indirect ophthalmoscopy as per Cryotherapy for retinopathy of prematurity (CRYO-ROP) recommendations [8, 9]. Retinal vascular findings were recorded, posterior pole was examined first and then periphery of retina was examined using indentation technique. Indirect ophthalmoscopy and markers of oxidative stress were done at the time of enrolment in the study i.e. 32–34 weeks and followed up at 40–42 weeks.

Markers of Oxidative Stress

Four millilitres of heparinized venous blood sample was collected and processed for the isolation of erythrocytes and plasma. The red cells were stored at 4°C and serum samples at -20°C until analysed.

Serum malondialdehyde (MDA) was measured using the colorimetric method described by Placer et al. in 1966 [10], based upon the reaction of thiobarbituric acid (TBA) with MDA, one of the aldehyde products of lipid peroxidation. The absorbance of the MDA-TBA adduct thus produced was measured spectrophotometrically at 532 nm.

The activity of superoxide dismutase (SOD) in erythrocytes was determined by using 2,4 iodophenyl 3,4 nitrophenol 5 phenyltetrazolium chloride, which is based

on the inhibition of INT/formazan dye, brought about by SOD at 505 nm [11].

Glutathione peroxidase (GPX) was measured using Paglia and Valentine method in heparinised blood. GPX catalyses the oxidation of glutathione by cumene hydroperoxide and causes oxidation of NADPH to NADP + and this decrease in absorption was measured spectrophotometrically at 340 nm [12].

Statistical Analysis

The levels of MDA, SOD and GPX were compared between the study and control group by Mann–Whitney *U* test. The observed values for these parameters of oxidative stress were correlated to the maternal anthropometry (weight, height, body mass index), haemoglobin and albumin, and the neonatal anthropometry (weight, length, head circumference) by univariate analysis. Pearson's correlation coefficient $P < 0.05$ was considered significant. The statistical analysis was carried out using SPSS version 17⁺.

Result

We observed that the mean weight gain of pregnant female in study group was 3.35 ± 0.48 kg while in control group it was 4.85 ± 0.83 kg and this difference in two groups was statistically significant ($P < 0.001$). The mean systolic and diastolic blood pressure in study group was 134.93/86.66 mmHg and in control group was 127.07/82.46 mmHg. This difference in BP was statistically significant. Other risk factors like age of mother, haemoglobin level, multiple pregnancy, or mode of delivery, did not have any significant effect on development of ROP.

Mean birth weight in neonates in study group were 1225.67 ± 123.06 gm and in control group were significantly higher i.e. 1323.33 ± 141.26 gm. On further follow up it has been observed that nine out 30 cases in study group were kept on ventilation and 11 neonates develop acidosis while in control group, two out 30 were kept on ventilation and three develop acidosis.

Comparison of free radical levels and antioxidants in both the groups at 30–34 week and in 40–42 week were depicted in Table 1. MDA which is an oxidant is significantly increased in neonates who develop ROP later on and the significant increase was sustained in the follow up visit. The value of SOD and GPX were significantly increased in study group as compared to the control group ($P < 0.001$) at 30–32 week. A comparative serial free radical measurement in study group on follow up at 40–42 week showed that the values of SOD and GPX were significantly decreased.

Table 1 Comparison of blood oxidant and antioxidant levels in study and control groups at 30–34 week and in 40–42 week

Parameter	Time of study (week)	Study group (Mean \pm S.E.)	Control group (Mean \pm S.E.)	<i>P</i> value
MDA (μ M/l)	32–34	10.67 \pm 0.91	8.21 \pm 0.92	<0.001
	40–42	10.87 \pm 0.91	8.92 \pm 0.93	
SOD (U/ml)	32–34	268.93 \pm 36.34	181.67 \pm 30.18	
	40–42	256.80 \pm 39.37	201.33 \pm 30.48	
GPX(U/l)	30–34	7337.7 \pm 953.96	4152.5 \pm 204.46	
	40–42	6989.8 \pm 953.96	4497.5 \pm 196.30	

Discussion

Free radicals have been implicated in the pathogenesis of a wide spectrum of human diseases. Premature infants are probably developmentally unprepared for extrauterine life in an oxygen-rich environment and exhibit a unique sensitivity to oxidant injury. With the advent of therapies designed to combat the injurious effects of free radicals, the role of these highly reactive chemical molecules in the pathogenesis of neonatal diseases needs to be fully determined.

Retinopathy of prematurity (ROP) is a major cause of blindness in children in developed countries. It is a two-phase disease, beginning with delayed retinal vascular growth after premature birth (Phase I). Phase II follows when Phase I-induced hypoxia releases factors to stimulate new blood vessel growth. Both oxygen-regulated and non-oxygen-regulated factors contribute to normal vascular development and retinal neovascularization [13].

We compared demographic features in the study and control group which shows that birth weight and gestational age were inversely proportional to the development and progression of ROP and thus higher in the study group. These findings are in accordance with Patil J et al. which state that immaturity or the lesser weight of neonate is having the greatest association with risk of ROP [14]. Post natal risk factors were compared in study and control group. It was observed that in study group nine neonates required ventilator support compared to two neonates in control group. It has been seen that neonates on ventilation for longer duration have more chances for development of ROP. Presence of acidosis could be associated with higher incidence of ROP.

When maternal factors were compared, a unique finding was seen in the study that mothers who gained lesser weight during pregnancy were significantly more prone to give birth to the infants who developed ROP later on. In

our study we also observed an increase in systolic and diastolic blood pressure during pregnancy was significantly associated with the mothers giving birth to the babies who developed ROP later on.

As shown in Table 1, MDA which is an oxidant and indicator of lipid peroxidation is significantly increased in neonates who develop ROP later on and this increase was sustained in the follow up visit leading to increased oxidative stress and progression of the disease. Two different studies by schlenzig et al. [15] and Inder et al. [16] found MDA excretion in urine and plasma MDA, respectively, to be higher in infants who developed bronchopulmonary disease.

Table 1 depicts that the value of SOD and GPX were significantly increased in study group as compared to the control group ($P < 0.001$). A comparative serial free radical measurement in study group on follow up showed that the values of SOD and GPX were significantly decreased. This clearly shows the disturbed equilibrium between the oxidants and antioxidants, leading to increased oxidative stress in study group. These findings are in accordance with Gupta P et al. [17], who found that oxygen free radical scavenging systems including SOD were lower and MDA a measure of lipid peroxidation was higher in cord blood of small for age neonates.

Premature infants, who have reduced antioxidant defenses, are particularly sensitive to the toxic effects of oxidants that causes tissue injury through the formation of reactive oxygen intermediates and peroxidation of membrane lipids. This in turn induces vasoconstriction in the retina as an early response and leads to vaso-obliteration, neovascularization and retinal traction (retinopathy of prematurity) [18].

Alon et al. [19] showed that increased oxidative stress either due to hyperventilation or hyperoxia causes selective apoptosis of retinal vascular endothelial cells and induces vaso-obliteration. It has been proposed that premature down regulation of vascular endothelial growth factor (VEGF), an endothelial cell-specific mitogen and survival factor, leads to vaso-obliteration of newly formed capillaries [20]. It has also been shown that oxidative stress causes increased expression of pigment epithelium-derived growth factor (PEDF), a potent angiostatic factor, within the retina [21].

The increase in antioxidant levels at 30–32 week in study group as compared to control group is in response to the increased oxidant stress due to ROP which on follow up decreases due to increased consumption of antioxidants to combat the oxidative stress.

In the control group levels of both MDA as well as antioxidant enzymes increased at the time of follow up at 40–42 week as compared to the baseline at 30 week, thus causing the equilibrium of oxidants and antioxidants to be

more in favour of antioxidants and thus protecting against the development of ROP. Hence our study shows that increased oxidative stress in premature infants correlated significantly with the development and progression of ROP.

Hence considering the protective effect of antioxidants, the therapeutic effect of liposomal SOD in an animal model of retinopathy of prematurity was studied by Niesman et al. [22]. It has been seen that on daily supplementation of liposome encapsulated SOD at 6 days of age there is significant increase in retinal SOD activity. There is also reduced oxygen induced vaso-attenuation as evidenced by increased vessel density and decreased vascular area. It has been suggested that supplementation of endogenous antioxidants in oxygen damaged retinal tissue may be a potentially valuable therapeutic strategy.

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