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Oxidative Stress and Anti-Oxidant Markers in Premature Infants with Respiratory Distress Syndrome

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Abstract

BACKGROUND: Neonatal respiratory distress syndrome (RDS) caused by decreased surfactant and structural lung immaturity. The imbalance between oxidative status and antioxidant defence system was suggested to be an important trigger for lung affection with RDS.

AIM: The goal of the current research was to elucidate the significance of the oxidant/ antioxidant status in the pathogenesis of RDS in preterm infants.

PATIENTS AND METHODS: This controlled study included 31 preterm neonates with RDS and 36 healthy preterm neonates. Quantification level of oxidative stress biomarkers; malondialdehyde (MDA) & hydrogen peroxide (H_2O_2) along with antioxidant enzymes activity; catalase (CAT) & superoxide dismutase (SOD) in plasma of healthy premature neonates compared with those with RDS.

RESULTS: status of oxidative stress markers (MDA & H_2O_2) showed a significant increase with decreased levels of antioxidant enzymes activity (CAT & SOD) in neonates with RDS when compared to healthy prematures.

CONCLUSION: The results obtained in this study indicate that the increased oxidative stress accompanied by reduced antioxidant defences may play a significant role in the pathogenesis of respiratory distress in preterm newborns.

Introduction

Oxidative stress is recognised by the imbalance between the augmented reactive oxygen/nitrogen species and the defect in the protective ability of the antioxidants. Free radicals with consequent cellular oxidative damage produced by oxidative stress seem to be key players in the pathogenesis of several new-born diseases, like respiratory distress syndrome, bronchopulmonary dysplasia, patent ductus arteriosus, necrotising enterocolitis, and retinopathy of prematurity, periventricular leukomalacia [1].

Negi et al., [2] reasoned the onset of neonatal respiratory distress syndrome (RDS), previously called hyaline membrane disease (HMD) to the decreased surfactant and structural lungs immaturity. Lung tissue damage which occurs during respiratory distress syndrome is not clearly explained yet, but the implication of oxidative damage due to reactive oxygen and hydrogen species is highly appreciated in the etiopathogenesis of this disorder. Preterm neonates are more prone to oxidative deterioration because of the intracellular antioxidant defence system including the anti-oxidant enzymes up-regulates dramatically during the last trimester of pregnancy, and they have low levels of radical scavengers and metal-binding proteins such as transferrin and ceruloplasmin [3]. They also have reduced antioxidant enzymes activity like catalase and glutathione peroxidase. The imbalance between oxidants and antioxidants results in oxidative damage and this problem occurs due to incomplete or abnormal intrauterine development [4].

Immediately after birth, the sudden increase in oxygen supply leads to overproduction of reactive oxygen species (ROS) and down-regulation of antioxidants. This condition induces the enhancement of cytokines and inflammatory mediators (interleukin 6- interleukin 8- Tumor Necrosis factor -α) expression [5].

Preterm infants, in particular, are exposed to many events leading to increased generation of reactive oxygen species (ROS) such as hyperoxia, mechanical ventilation, inflammation and infection [6]. Surfactant is the primary treatment of RDS in neonates as it reduces hyperoxia-induced lung damage. Surfactant replacement has been found to cause antioxidant and anti-inflammatory responses [7].

Superoxide dismutase (SOD) is the most powerful antioxidant and detoxification enzyme. It catalyses the disputation of two molecules of superoxide anion radical ($O_2^{\cdot-}$) to hydrogen peroxide (H_2O_2) and oxygen molecule (O_2) [8]. Several investigators stated that SOD is the crucial enzyme for a proper respiratory function in animal and cellular models and the deficiency of SOD activity almost induces severe hyperoxic lung injury [9]. Endotracheal administration of surfactant increases the activity of SOD in type II alveolar cells, demonstrating enzyme uptake by liposome during the surfactant recycle process [10].

Catalase (CAT) is the common antioxidant enzyme that utilises oxygen. It catalyses the degradation of hydrogen peroxide (H_2O_2). Both superoxide dismutase (SOD) and catalase (CAT) are on the top of the first line of the defence system. This line is essential in the entire antioxidant defence mechanism [8]. Superoxide dismutase (SOD) and catalase (CAT) activities have been demonstrated in several natural surfactants. They also have scavenger activity against hydrogen peroxide (H_2O_2) [11]. Malondialdehyde (MDA) is the major reactive aldehyde that results from peroxidation of lipids in the biological membranes. It can be used as an indicator of tissue damage caused by reactive oxygen species (ROS). MDA reacts with DNA and modifies RNA, proteins and other biological molecules, and this leads to tissue destruction [12].

The goal of the current research was to elucidate the significance of the oxidant / antioxidant status in the pathogenesis of RDS in preterm infants. This could be achieved through estimation of the plasma levels of oxidative stress indicators (MDA, H_2O_2) and detection of plasma activity of the antioxidant indices (SOD, CAT).

Patients and Methods

A case-control study was conducted on a total of 67 neonates recruited from Neonatology Department of El-Galaa Teaching Hospital for Obstetrics and Gynecology, Cairo, Egypt. (Between April 2018 and October 2018). This study was approved by the Medical Ethical Committee of the National Research Centre, Egypt. Written informed consent for all participants was collected from their parents. The case (RDS) group consisted of 31 preterm babies ≤ 37 weeks with a diagnosis of RDS. The control group included 36 healthy preterm newborns. Exclusion criteria of the study were an infection, intracranial haemorrhage, surgical problems and hemolytic diseases.

Respiratory distress syndrome (RDS) was diagnosed with the presence of typical clinical and radiological signs of the disease in preterm infants. Clinically, if they have tachypnea, grunting and cyanosis with several hours of birth required mechanical ventilation and typical radiographic findings on the chest X-ray. The characteristics of the newborns including gestational age, weight, Apgar score, surfactant replacement, were obtained from Hospital sheets.

Two mL of heparinised venous blood samples were withdrawn from the neonates during the first 72 hours after birth. Blood specimens were processed to separate plasma samples which were stored at -20°C.

Determination of plasma levels of oxidative stress markers

I Malondialdehyde (MDA):

Principle: Thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) in the acidic medium at a temperature of 95°C for 30 min to form the thiobarbituric acid reactive product. The absorbance of the resultant pink product can be measured at 534 nm Total thiobarbituric acid reactive materials are expressed as MDA [13].

II Hydrogen Peroxide (H_2O_2):

Principle: In the presence of peroxidase (HRP), H_2O_2 reacts with 3, 5-dichloro -2-hydroxybenzensulfonic (DHBS) acid and 4-aminophenazon (AAP) to form a chromophore, which is measured at 510 nm [14].

$$2H_2O_2 + DHBS + AAP \xrightarrow{HRP} \text{Quinoneimine Dye} + 4H2O$$

Determination of plasma antioxidant enzyme activity

I Catalase (CAT):

Principle: Catalase reacts with a known quantity of H_2O_2. The reaction is stopped after exactly one minute with catalase inhibitor.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

In the presence of peroxidase (HRP), remaining H_2O_2 reacts with 3, 5-Dichloro -2- hydroxybenzene sulfonic acid (DHBS) and 4-aminophenazone (AAP) to form a chromophore with a colour intensity measured at 510 nm inversely proportional to the activity of catalase in the original samples.

$$2H_2O_2 + DHBS + AAP \xrightarrow{HRP} Quinoneimine\ Dye + 4H_2O$$

II Superoxide Dismutase (SOD)

Principle: This assay relies on the ability of the enzyme to inhibit the phenazine methosulfate – mediated reduction of nitroblue tetrazolium dye. The resultant colour was measured at 560 nm.

Statistical analysis

Data were collected, verified, coded and analysed using the Statistical Package for Social Science (SPSS) version 23 (SSPS Inc., Pennsylvania, and the USA). Descriptive analysis was performed for demographic and clinical characteristics of the cases. MDA, H_2O_2 levels, and CAT, SOD activities were expressed as mean ± SD. The comparison between the cases and control groups was made using student's t-test. A chi-square test was used for comparison of non-parametric data.

Results

The characteristics of all studied neonates and their mothers are illustrated in Table (1). The case (RDS) group composed of 31 preterm neonates with a mean gestational age 31.2 ± 3.2 weeks having a mean birth weight 1740.3 ± 720.0 gm. The control group composed of 36 preterm neonates with mean gestational age 34.3 ± 1.1 weeks–and a mean birth weight 2248.6 ± 147.1 gm. New-borns in RDS group are more premature and have lower birth weight in comparison to the control group (P < 0.005). There is no significant difference in the maternal age between neonates with respiratory distress syndrome and controls (P > 0.05). The mean birth weight and Apgar scores at 1 and 5 minutes and the gestational age are highly significantly lower in cases (RDS) than controls (p < 0.005).

Most preterm neonates with RDS delivered by C.S (80.6%), have no history of premature rupture of membranes (71.0%) and 58.1% of them have a history of multiple pregnancies.

Twenty preterms with RDS (64.5%) received surfactant therapy. Twenty preterms with RDS (64.5%) were on mechanical ventilation.

Table 1: Characteristics of the studied neonates and their mothers

	Case (RDS) group (n = 31)	Control group (n = 36)	t-Test	P
	Mean ± SD	Mean ± SD		
Maternal age (years)	28.97± 7.9	29.4 ± 3.4	*t* = 0.329	0.743
Gestational Age (weeks)	31.2 ± 3.2	34.3 ± 1.1	*t* = 5.348	0.000
Birth weight (gm)	1740.3 ± 720.0	2248.6 ± 147.1	*t* = 4.141	0.000
Apgar score 1st min	3.9 ± 2.05	7.72 ± 0.97	*t* = 10.059	0.000
Apgar score 5 min	6.5 ± 1.9	8.7 ± 097	*t* = 6.248	0.000
	No (%)	No (%)		
Gender:				
Male	24 (77.4%)	14 (38.9%)	χ^2 = 10.073	0.002
Female	7 (22.6%)	22 (61.1%)		
Delivery mode:				
Vaginal	6 (19.4%)	17 (47.2%)	χ^2 = 5.738	0.017
CS	25 (80.6%)	19 (52.8%)		
Multiple pregnancies:				
Yes	13 (41.9%)	6 (16.7%)	χ^2 = 5.235	0.022
No	18 (58.1%)	30 (83.3%)		
Premature rupture of membranes:	9 (29.0%)	2 (5.6%)	χ^2 = 6.690	0.010
Yes	22 (71.0%)	34 (94.4%)		
No				
Surfactant:				
Yes	20 (64.5%)	-	-	-
No	11 (35.5%)			

χ^2 = chi-square; t = t-test.

The data in Table 2 represented the plasma levels of oxidative stress markers and the activity of plasma antioxidant enzymes in neonates with RDS and control counterparts. There is a highly significant elevation in the levels of the oxidative stress markers (MDA, H_2O_2) in the RDS group versus the control one (p < 0.005). The highly significant drop in the activity of the antioxidant enzymes (CAT, SOD) is noted in the RDS group relative to the control group (p < 0.005).

Table 2: The oxidant and antioxidants markers in prematures with RDS and control group

	Case (RDS) group Mean ± SD (n = 31)	Control group Mean ± SD (n = 36)	t	p
Oxidative stress markers:				
MDA mmol/L	7.074 ± 1.88722	2.367± 1.3459	11.753	0.000
H_2O_2 mμ/L	0.78100 ± 0.2498	0.25900 ± 0.109	8.274	0.000
Antioxidants markers:				
SOD U/ml	186.596 ± 47.936	267.244 ± 33.476	-6.218	0.000
Catalase U/L	352.939 ± 68.421	571.217± 117.812	-7.962	0.000

The correlation between clinical parameters of RDS cases is depicted in Table 3. The gestational age shows a highly significant positive correlation with birth weight, maternal age, Apgar score at 1 and 5 minutes (P < 0.005). On the contrary, it shows a highly significant negative correlation with surfactant therapy (P < 0.005). Surfactant therapy shows a highly significant positive correlation with gestational age and birth weight (P < 0.005).

Table 3: Correlation between clinical parameters of RDS cases (31 newborns)

		Gestational Age	surfactant therapy	Birth weight	Maternal age	1st min Apgar score	5min Apgar score
Gestational Age	Pearson Correlation	1	-0.783**	0.910**	0.656**	0.640**	0.638**
	Sig. (2-tailed)		.000	0.000	0.000	0.000	0.000
surfactant therapy	Pearson Correlation	-0.783**	1	-0.638**	-0.367*	-0.450*	-0.390*
	Sig. (2-tailed)	0.000		0.000	0.042	0.011	0.030
1st min Apgar score	Pearson Correlation	0.640**	-0.450*	0.763**	0.685**	1	0.928**
	Sig. (2-tailed)	0.000	0.011	0.000	0.000		0.000
5min Apgar score	Pearson Correlation	0.638**	0.390*	0.711**	0.588**	.928**	1
	Sig. (2-tailed)	0.000	0.030	.000	.001	.000	

**P < 0.005 highly significant; *P < 0.05 significant.

Meanwhile, it shows a significant negative correlation with maternal age and Apgar score at 1 & 5 minutes. The highly significant positive correlation is observed between the Apgar score (at 1 & 5 minutes) and birth weight as well as maternal age ($P < 0.005$).

Discussion

Neonatal respiratory distress syndrome is a major health problem in neonates. It occurs due to immature lungs and requires assisted ventilation with high oxygen concentration. The pathophysiology of RDS is based on the rapid formation of reactive oxygen species (ROS) that inhibit the detoxification capacity of the antioxidative mechanisms [2].

In preterm labour, oxidative stress and other oxidative molecules exceed the antioxidant buffering capacity leading to lung tissue damage [16]. In our study, birth weight and gestational age were significantly lower in RDS cases than controls. The more prematurity, was the less lung surfactant production and more severe RDS. Extremely preterm and very low birth weight infants are considered at particular risk of oxidative stress because of both endogenous and passively acquired exogenous defence systems do not mature enough until late in the third trimester [17].

In the present study, most of the case group (RDS group) were born by caesarean section (CS). The effect of the type of delivery on the oxidative stress experienced by both mother and infant is still not clear. Actually, there is a controversial hypothesis about the mode of delivery and the generation of oxidative stress. Yaacobi et al., [18], stated that there is an increased concentration of malondialdehyde in vaginal delivery and emergency CS after prolonged labour group as compared to elective CS without labour. In contrast, Mutlu et al., [19] demonstrated a conflicting result as their study reported that CS increases total oxidative stress, and oxidative stress indices, and lipid hydroperoxide level.

Gerten et al., [20] concluded that caesarean section is an independent risk factor for RDS development, and the risk is decreased with labour before section. This supports the importance of being certain of neonatal lung maturity before taking the decision of cesarean delivery mainly when done before labor. In our study, as most of the RDS group was born by CS, we were not able to identify whether the increased levels of the oxidative stress were due to the mode of delivery or not. However, Lauire et al., [21] found that distressed neonates born by caesarean section had high MDA concentration, a marker of lipid peroxidation in amniotic fluid and cord blood compared to non-distressed neonates delivered by vaginal route. Nevertheless, we need to investigate the exposure of the mother and neonates to the oxidative stress about the mode of delivery.

In our study, male neonates were more significantly affected by RDS than females. This is in concordance with the study done by Kaltofen et al., [22] who demonstrated that female fetal alveolar cells of the saccular stage of lung development have a higher alveolar Na transport activity compared to age-matched male cells. Besides, male androgens decrease surfactant production and delay lung maturity.

Most neonates in our study had no history of premature rupture of membranes (PROM) and this could be explained by the fact that more than 80% of our neonates were delivered by C.S. Some reactive oxygen species (ROS) during pregnancy may cause direct vasoconstriction or inability to vasodilate placental blood vessels leading to preterm deliveries [23].

Multiple pregnancies in our study were significantly lower than singletons. After considering gestation, twins are not at elevated risk of having RDS except at very early gestation [24].

Most preterm with RDS in our study received surfactant therapy. A study was done by Sardesai et al., [25] demonstrated that surfactant therapy decreases air leaks and neonatal mortality significantly. It also contributed to faster weaning from invasive ventilation. Noninvasive surfactant administration techniques (atomization or aerosolisation) may play an important role in the future. Carty et al., [26] mentioned that reactive oxygen species (ROS) might interact with protein and lipid structures of the lung in addition to pulmonary surfactant, leading to the delay of the normal function of the lung. Therefore, surfactant administration is very important before the start of assisted ventilation [27].

Preterm in the present study showed significantly lower Apgar score at 1st and 5 minutes than controls. These results matched with a study done by Negi et al., [2], who stated that a low Apgar score is indicative of perinatal hypoxia.

At the present study, there was a significant positive correlation between Apgar score and both weight and maternal age. A study done by Jerneck and Herbst [28] registered higher Apgar score with birth weight above 5 kg.

MDA is a final product of lipid peroxidation. The present study showed an increased concentration of MDA and H_2O_2 (oxidative stress markers) in neonates with RDS concerning controls. These findings are in concordance with those of Negi et al., [2]. Furthermore, Zahran et al., [29] recorded an increased level of MDA in neonates with RDS, which is consistent with our results. Our results also fit the study of Dizdar et al., [30] which showed an increase in the total oxidant status in preterm infants. These

investigators mentioned that lower total antioxidant status / total oxidant status is associated with increased severity and mortality in these infants.

Nevertheless, in addition to the elevated levels of MDA and H_2O_2, shown in the present study, there was a significant drop in the activity of the antioxidant enzymes (SOD and CAT). This imbalance between elevated oxidative stress factors and the reduced antioxidant enzymes might be a contributing factor to the RDS in new-born. It is worth noting that the total antioxidant status might serve as a prognostic marker in new-borns with RDS and help to distinguish high-risk infants [31], [32]. This hypothesis is greatly supported by Zahran et al., [33] study, which demonstrated that the suppressive activity of SOD, as an antioxidant, may lead to neonatal RDS.

Several studies proved that SOD is a key enzyme for optimum respiration in animals and cellular models. The absence of SOD3 activity increases massive lung tissue damage, while SOD2 overexpression in type II alveolar cells may lead to prolonged survival in a hyperoxic environment [9].

Aerosol delivered SOD improved alveolar development in RDS patients and reduced the occurrence of bronchopulmonary dysplasia caused by prematurity and mechanical ventilation [33]. A previous study of Dani et al., [34] demonstrated that commercial natural lung surfactants contain a significant concentration of SOD and CAT. These surfactants enhance scavenger activity against H_2O_2 and are effective in reducing oxidative lung damage.

It has been shown that while preterm with RDS developing Bronchopulmonary dysplasia (BPD), they typically exhibit increased CAT enzyme activity in the epithelial lung fluid during the first week of life. Those preterms with simple RDS showed decreasing values of oxyradical inflammation markers during the disease course [35].

Antioxidants have a vital role in defence against free radical-induced lung tissue damage in neonates with RDS [2].

Bahbah et al., [36] found that there is no significant reduction in the activity of CAT, but SOD activity is significantly suppressed in the preterm group as compared to controls.

Trindade et al., [37] obtained similar results as ours; they recorded a decrease in the activity of the antioxidant enzymes and an increased in the susceptibility to oxidative stress among premature neonates in comparison to controls immediately after birth. This could be explained by the development of antioxidant enzyme capacity in the third trimester, so the preterm group is at increased risk of oxidative stress [38].

Pure oxygen use during resuscitation should be avoided. Restriction, the usage of mechanical ventilation by early usage of surfactant and nasal continuous positive air pressure, may decrease respiratory tissue damage in RDS patients [39].

In conclusion, the results obtained in this study indicate that the increased oxidative stress accompanied by reduced antioxidant defenses may play a significant role in the pathogenesis of respiratory distress in preterm new-borns.

Further researches are recommended to study the protective role of antioxidant markers against RDS. Antioxidant modalities may be beneficial in the treatment of RDS cases and prevention of bronchopulmonary dysplasia.

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