

The background of the cover features a close-up, slightly blurred photograph of a kitchen scale and a burrito. The scale is white with a purple dial and a red needle. The burrito is wrapped in a white tortilla and filled with green lettuce, brown meat, and a red sauce. The text is overlaid on a semi-transparent purple rectangular area.

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Full Length Research Paper

## Serum lipid peroxide and antioxidant vitamins (E, C) in neonates with respiratory distress syndrome

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Respiratory distress syndrome occurs mostly in premature infants with high risk of oxidative stress, free radicals and other reactive species which are constantly generated *in vivo* and cause oxidative damage to DNA and lipid. Antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property, some of it including vitamins C and E. The study was done on sixty preterm neonates with respiratory distress syndrome (RDS), in addition to twenty apparently healthy full term neonates as normal controls to determine the serum levels of the oxidant lipid peroxide and the antioxidant vitamins E and C. The mean levels of serum lipid peroxide which is the end product of lipid peroxidation were found to be higher with variable degrees of significant differences. The raised serum levels of lipid peroxide in neonates with RDS may be due to activated macrophages releasing highly reactive radicals that may cause local disruption of essential structures, and we found that the mean  $\pm$  standard deviation (SD) levels of serum vitamin C and E were lower with variable degrees of significant differences.

**Key words:** Respiratory distress syndrome, vitamin C and E, lipid peroxide free radicals, macrophages.

### INTRODUCTION

Respiratory distress syndrome, also known as hyaline membrane disease occurs mostly in premature infants. The incidence and severity of respiratory distress syndrome are related inversely to the gestational age of the newborn infant (Dizdar et al., 2012). Newborns and particularly preterm infants are at high risk of oxidative stress and they are very susceptible to free radical oxidative damage (Davis et al., 2010). Oxidative stress refers to a state in which an imbalance between pro-oxidants

and antioxidants results in oxidative damage, which has been proposed to play an important role in the pathogenesis of many diseases including respiratory distress syndrome (Da Costa et al., 2012). Oxidative stress presents numerous opportunities for tissue injury through formation of reactive oxygen/nitrogen species, free radicals and other reactive species are constantly generated *in vivo* and cause oxidative damage to DNA and lipid. Evidence for oxidative injury comes from measurements

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**Table 1.** Demographic Data of different neonatal groups GI, GII and GIII.

Demographic data	G I	G II	G III	Significance (P value)		
				I vs II	I vs III	II vs III
Gestational age (wks)	38.3±0.98	34.9±1.2	31.7±1.4	NS	NS	< 0.05*
Sex (M/F)	9/11	14/16	17/13	NS	NS	NS
Birth wt (kg)	3.22±0.45	2.39±0.70	1.72±0.56	< 0.05*	<0.01	<0.05
Length (cm)	49.20±10.8	47.4±4.84	43.6±2.17	NS	< 0.05*	NS
HC (cm)	34.4±0.66	33.4±1.51	31.2±1.84	NS	NS	NS
<b>APGAR score</b>						
1 Min	7.1±0.31	4.6±0.50	2.90±0.55	<0.01**	<0.001***	<0.05*
5 Min	9.16±0.68	7.8±1.00	6.10±1.05	NS	<0.001***	<0.05*

of biochemical markers of lipid per oxidation and protein oxidation. Among important oxidants are lipid peroxide and nitric oxide (Reena et al., 2011). Antioxidants are chemical compounds that can slow or prevent the oxidation of other compounds by elimination of reactive free radical intermediates (Josph et al., 2010). These antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property. These low-molecular-weight antioxidants can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Some of such antioxidants include glutathione, vitamins A and E, superoxide dismutase, catalase and uric acid (Lobo et al., 2010).

## MATERIALS AND METHODS

The study was done on sixty preterm neonates with respiratory distress syndrome (RDS), in addition to twenty apparently healthy full term neonates as normal controls, from the in-patients of neonatal intensive care units of Alazhar University Hospital (Assiut) and Assiut University Hospital, during the period from August, 2013 to February, 2014. Neonates suffering from respiratory distress due to causes other than respiratory distress syndrome as sepsis, diseases of the respiratory, cardiovascular, or central nervous systems were excluded from the study. Parents of the neonates were informed about the nature of the study, and written consents were taken from each. Neonates of our study were classified as Group 1 neonates including 20 apparently healthy full term neonates (G1) as the control group, Group 2 neonates including 30 preterm neonates with RDS and their gestational age from 34 to 36 weeks (G2), and Group 3 neonates including 30 preterm neonates with RDS and their gestational age from 30 to 33 weeks (G3).

All neonates of the study were subjected to thorough history taking including prenatal, natal and postnatal history. General and neurological examinations were done. Gestational age assessment was performed by the new Ballard score using neuromuscular maturity and physical maturity (Ballard et al., 1991). Chest examination was done to diagnose RDS, patients are considered to have RDS if they have signs of respiratory distress and decreased air entry on auscultation of the chest combined with radiological findings on chest X-ray (Evrin et al., 2011). Radiographic findings

in chest X-ray include fine reticular shadowing throughout both lungs with accentuation of the air bronchogram or white lung appearance in severe cases (Falah and Raed, 2008). The following investigations were done to all patients and controls including:

1. Routine investigations including complete blood count (CBC), erythrocyte sedimentation rate (ESR), blood glucose level and serum electrolytes Na, K, Ca.
2. Specific investigations including assessment of the serum levels of the oxidant lipid peroxide and the antioxidant vitamins E and C.

Serum lipid peroxide is determined by the reaction of thiobarbituric acid with lipid peroxide in acidic medium at temperature of 95°C for 30 min to form thiobarbituric acid reactive product. The absorbance of the resultant pink product can be measured at 534 nm (Ohkawa et al., 1979) and (Meihuipan et al., 2004). Serum vitamin C level is determined by use of the reagent 2,6-dichlorophenol-indophenol. The absorbance of the sample can be measured at 520 nm (Valko et al., 2007). Serum vitamin E level (alpha tocopherol) was measured by use of the reagents absolute ethanol-aldehyde, xylene, ferric chloride solution and standard solution of D-L-alpha tocopherol, the result of the test can be measured at 460 and 520 nm (Valko et al., 2007). Data were expressed as mean ± SD. SSPS curve was used for analysis of data.

## RESULTS

Table 1 showed a statistically very highly significant differences regarding apgar score at 1 and 5 min in between G1/GII, G1/GIII and GII/GIII. Also it showed variable degree of significant differences regarding to: Gestational age/weeks, weight/kg, length/cm and head circumference/cm in-between GI and RDS neonatal groups (GII and GIII) but insignificant difference in-between GII and GIII. Table 2 showed highly significant difference in blood glucose level with variable degree of significance in blood parameters (ESR, mean corpuscular volume (MCV), platelets) and electrolytes (Ca only) in between GIN to GIIN and GIN to GIIIN but insignificant difference in other remaining elements in between different subjected neonatal groups. Table 3 shows that

**Table 2.** Mean  $\pm$  SD of routine investigations of different studied groups GI, GII and GIII.

Variable	G I	G II	G III	Significance (P value)		
				I vs. II	I vs. III	II vs. III
Blood glucose level(mg/dl)	79.20 $\pm$ 17.38	65.22 $\pm$ 13.14	51.90 $\pm$ 26.01	<0.01**	<0.001***	<0.01**
ESR (mm/h)	8.80 $\pm$ 2.68	19.06 $\pm$ 3.57	18.07 $\pm$ 3.66	<0.01**	<0.01**	NS
<b>Complete blood count (CBC)</b>						
Hb (g/dl)	16.50 $\pm$ 1.72	15.17 $\pm$ 3.52	14.65 $\pm$ 2.79	NS	NS	NS
RBCS (mm <sup>-3</sup> )	4.95 $\pm$ 0.32	4.63 $\pm$ 0.85	4.91 $\pm$ 0.95	NS	NS	NS
HCT%	45.12 $\pm$ 9.80	43.66 $\pm$ 10.87	43.29 $\pm$ 10.89	NS	NS	NS
MCV (fL)	99.94 $\pm$ 1.42	107.6 $\pm$ 6.73	105.5 $\pm$ 6.31	<0.05*	<0.05*	NS
MCH (g/dl)	34.68 $\pm$ 1.26	34.99 $\pm$ 3.56	32.37 $\pm$ 3.79	NS	NS	NS
MCHC(g/dl)	34.75 $\pm$ 1.16	32.41 $\pm$ 2.10	32.76 $\pm$ 2.26	NS	NS	NS
platelets ( $\times$ 10 <sup>9</sup> /L)	285.50 $\pm$ 2.41	203.6 $\pm$ 42.84	201.9 $\pm$ 54.74	<0.01**	<0.01**	NS
WBCs(mm <sup>-3</sup> )	10.42 $\pm$ 3.73	9.93 $\pm$ 4.03	9.64 $\pm$ 3.27	NS	NS	NS
<b>Serum electrolyte</b>						
Ca (mg/dl)	9.72 $\pm$ 0.89	8.86 $\pm$ 1.04	7.93 $\pm$ 0.95	NS	<0.05*	NS
Na (mmol/L)	141.24 $\pm$ 1.09	140.62 $\pm$ 9.40	142.41 $\pm$ 3.34	NS	NS	NS
K (mmol/L)	4.34 $\pm$ 1.03	5.20 $\pm$ 0.79	5.30 $\pm$ 0.87	NS	NS	NS

**Table 3.** Mean  $\pm$  SD of oxidants and antioxidants of different studied groups GI, GII and GIII.

Variable	G I	G II	G III	Significance (P value)		
				I vs. II	I vs. III	II vs. III
<b>Oxidants</b>						
Serum lipid peroxide (nmol/ml)	9.92 $\pm$ 3.51	15.17 $\pm$ 9.93	19.82 $\pm$ 3.65	<0.01**	<0.001***	<0.05*
<b>Antioxidant vitamins</b>						
Serum ascorbic acid (vit. C) (mg/L)	34.90 $\pm$ 16.82	22.05 $\pm$ 6.50	17.30 $\pm$ 5.25	<0.001***	<0.001***	<0.01**
Serum vit. E (mg/L)	4.81 $\pm$ 0.34	2.56 $\pm$ 0.72	1.98 $\pm$ 0.62	<0.01**	<0.001***	<0.05*

very highly significant difference ( $P < 0.001$ ) for serum oxidants and vitamin C while highly significant difference ( $P < 0.01$ ) for serum vitamin E.

## DISCUSSION

Balanced status between the serum levels of oxidants and antioxidants represent the main target of human body metabolism for normal healthy cellular growth and development. Newborns, especially preterm or low birth weight or even full term healthy births are associated with an increased risks from oxidants over burden, which places these infants at higher risk of cellular injury but the protective compensatory mechanisms of different human body cells through several antioxidants either individually

or cumulatively, keep the normal healthy cellular growth and developmental pattern in different human body organs and tissues after birth (Davis and Auten, 2010).

The aim of this study was to evaluate the serum levels of lipid peroxide as an oxidant agent in addition to serum levels of vitamin C and E as antioxidants in subjected different neonates with respiratory distress syndrome (RDS), compared to full term healthy neonates as a control group. As regard the demographic data of growth parameters of subjected neonates in our study, it showed lower mean  $\pm$  SD levels of birth weight with variable degrees of significant differences in comparing GIII to GII and in comparing both groups to control group and also lower mean  $\pm$  SD of length in group  $\Pi$ 1 compared to control group. These results were in agreement with Geoffrey et al. (2005), Saker et al. (2008), Bental et al.

2011 and Jeng et al. (2012).

Regarding Apgar score at one/five minutes in different subjected neonatal groups, in the present study, it showed very highly lower significant differences when comparing GII and GIII to control group, and to each other. Our results were in concordance with previous researches by Kumar et al. (2012), Dizdar et al. (2012) and Davis and Auten (2010). These results revealed the impact and effectiveness of balanced status regarding oxidants/antioxidants in different studied neonates and maturity state.

Blood glucose levels were significantly lower in GIII compared to GII and also when comparing both groups to control group. It was stated by Kayıran and Gürakan (2010) that the pre-term babies had a lower mean blood glucose concentration in the first few postnatal hours compared with term babies. As regard complete blood picture, we found significant lower counts of blood platelets in RDS subjected neonates (GII and GIII) compared to control group. This finding is confirmed by several studies reported a much higher incidence of thrombocytopenia among sick neonates admitted to the neonatal intensive-care unit (NICU) than in the general neonatal population (18 to 35% vs < 1%, respectively) (Castle et al., 1986; Mehta et al., 1980). The highest incidence was found among the smallest and most premature infants, with approximately 70% of neonates born at a weight < 1000 g developing thrombocytopenia at some point during their hospital stay (Christensen et al., 2006). The MCV of red blood cells showed higher volume of RBCs in hypoxic RDS neonates but within normal neonatal limited volumes in the present study including GII and GIII compared to control group. Calcium level is significantly lower in G111 compared to control group. Other blood investigated parameters showed variable degrees of insignificant differences including hemoglobin Hg, RBCs count, WBCs count, hematocrite value MCH, MCHC, sodium and potassium between all neonatal groups of our study.

In the present study, the mean levels of serum lipid peroxide which is the end product of lipid peroxidation were found to be higher with variable degrees of significant differences when comparing G111 and G11 to control group and also in G111 compared to G11. The raised serum levels of lipid peroxide in neonates with RDS ( G11 and G111) may be due to activated macrophages releasing highly reactive radicals that may cause local disruption of essential structures (membrane lipids, deoxyriboneucleic acid and proteins) and hence tissue destruction (Nair et al., 2008). Our results were in concordance with (Kumar et al., 2008; Evrim et al., 2011; Da Costa et al., 2012).

In our study, we found that the mean  $\pm$  SD levels of serum vitamin C and E were lower with variable degrees of significant differences when comparing GIII and GI

with GI and with each other. The role of anti-oxidant vitamins (C and E) in the maintaining of physiological process of fetal growth during pregnancy was reported by Wang et al. (2009). Brion et al. (2003) confirmed that preterm infants have lower antioxidant vitamins in their serum compared with term controls. Vitamin E is decreased as a result of vitamin C deficiency. This is explained by Ashok et al. (2008) and Traber (2006) who reported that Ascorbate could regenerate tocopherol from the tocophoroxy radical.


### Conflict of interest

Authors declare that there are no conflicts of interest.

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