Applied Medical Informatics
Original Research Vol. 25, No. 3-5/2009, pp: 47-54

The Behaviour of Protein Carbonyls in Newborns with Birth Respiratory Distress and Asphyxia

Melinda MATYAS[1], Gabriela ZAHARIE[1], Antonia POPESCU[1], Ligia BLAGA[2]

[1] Department of Neonatology, 1st Clinic of Obstetrics and Gynaecology, "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca.
[2] Department of Neonatology, 2nd Clinic of Obstetrics and Gynaecology, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca.
E-mail(s): melimatyas@yahoo.com

* Author to whom correspondence should be addressed; Tel.: +4-0264-592771.

Abstract: *Objective*: A prospective study was carried out in premature newborns with respiratory distress syndrome (RDS) and asphyxia at birth in order to identify and analyze the effects of RDS on proteins. *Material and Methods*: Protein peroxidation was studied using the Reznick spectrophotometric method. The study group included 14 premature newborns with respiratory distress and asphyxia at birth. The control group included 13 newborns that were born on term, eutrophic and healthy. The determinations were carried out using venous blood. Statistical data analyses were performed using Statistica software. The comparisons between groups (study and controls) were performed by applying parametric and non-parametric tests according with the type of distribution. *Results*: Statistically significant correlations were found between the value of protein carbonyls (PC) and the weight of premature newborns in the case group ($p < 0.05$), as well as between the PC value and the presence of respiratory distress due to surfactant deficiency in the study group. The average PC value in the study group was higher in the third day as compared with the first day. The PC value was significantly higher in the control group as compared with study group. *Conclusion*: The results of our study revealed that the respiratory distress in the premature newborn and oxygen therapy stimulate the peroxidation of proteins.

Keywords: Premature newborn; Respiratory distress; Protein carbonyls.

Introduction

Protein carbonyls represent early protein oxidation markers [1,2]. The oxidation of amino acids from the protein structure occurs under the action of oxygen reactive species (ORS) and lipid peroxides [2,3]. Thus, proteins are degraded and their functioning is altered.

In the neonatal period, oxidative stress is favoured by several circumstances such as oxygen therapy, which is largely used in intensive care units to treat respiratory distress due to surfactant deficiency (RDS) in premature newborns. Besides its beneficial effects, oxygen may have harmful effects when it is used excessively [1,4,6]. The oxygen contributes to the formation of ORS, which influence the morphologically and functionally immature structures of the premature newborn and contribute to the pathogenesis of so-called "free radical diseases" : retinopathy of the premature, intraventricular and periventricular haemorrhage, ulcerative necrotic enterocolitis and bronchopulmonary dysplasia (BPD) [1]. Other disorders are RDS and asphyxia.

The pathogenesis of these disorders is based on the rapid formation of the ORS, which surpasses the detoxification capacity of anti-oxidative defence systems.

|We conducted a prospective study in premature newborns with RDS and asphyxia at birth in order to identify and analyze the effects of ORS on proteins.

Material and Method

Cohort

14 premature newborns admitted in our clinic between Nov 2003-Dec 2006 were included in our study.

The inclusion criteria were:

1. Gestational age between 28 and 35 weeks
2. Associated RDS due to surfactant deficiency and/or
3. Asphyxia at birth associated or not with anoxic ischemia (encephalopathy)

Exclusion criteria were defined as:

1. Premature newborns without RDS or asphyxia
2. Term newborns, gestational age over 38 weeks
3. Post-mature newborns, gestational age over 42 weeks

The control group consisted of 13 term newborns, eutrophic and healthy. All post-mature newborns or term newborn with associated pathologies were excluded.

Methods

Protein carbonyls were determined using the Reznick spectrophotometric method with dinitrophenylhydrazine. Two venous blood samples were collected from each newborn in the study group: one in the first and the other in the third day of life. One venous blood sample was collected from controls in their first day of life. Astrup parameters were also measured in the studied group.

Asphyxia at birth was measured based on the Apgar score at 5 minutes. Three levels of asphyxia were established: severe asphyxia (Apgar score at 5 minutes = 1, 2 or 3), moderate asphyxia (Apgar score at 5 minutes = 4, 5 or 6), and mild asphyxia (Apgar score at 5 minutes = 7).

Respiratory distress due to surfactant deficiency was another pathology investigated in the studied group. It had three degrees of severity: mild, moderate and severe based on Silverman score

Statistical analysis

Statistical data analyses were carried out using Statistica. The data which proved to be normally distributed were compared using parametric tests; the quantitative variables on which the hypothesis of normality was rejected and the qualitative variables were subject of comparison using non-parametric tests (Wilcoxon for paired samples; Mann-Withney for independent samples) [13]

Results

The average weight of patients included in study group was of 2015.71 g.

Severe asphyxia occurred in four (28.57%) cases, moderate asphyxia in one case (7.14%) and mild asphyxia in 4 (28.57%) cases. Five newborns from study group did not present asphyxia at birth.

In the study group, AIE occurred in two out of the four newborns with severe asphyxia.

Mild RDS (respiratory distress) prevailed (five cases, 35.71%), followed by the moderate RDS (thre cases, 21.43%) in the study group (Table 1).

Mechanical ventilation was used to treat moderate and severe forms of RDS in two patients (14%).

In the study group, five patients (36%) presented intra-ventricular hemorrhage. Twelve patients were diagnosed with hyperbilirubinaemia (86%), and 10 of them required phototherapy for its treatment

Table 1. Incidence of RDS due to surfactant deficiency in the studied group

RDS	Absolute frequency	Relative frequency	CI 95%
Severe	2	14.29	[0.51 – 42.35]
Moderate	3	21.43	[7.65 – 49.49]
Mild	5	35.71	[14.80 – 63.78]
Absent	4	28.57	[7.65 – 56.63]
Total	14	100	

CI 95% = 95% confidence interval associated with relative confidence

The Pearson correlation coefficient was used to quantify the relationship between quantitative variables when the data proved to be normal distributed; the Spearman correlation was employed for quantitative non-normal distributed and qualitative variables. The results are illustrated in Tables 2 and 3.

Table 2. Pearson correlation for the quantitative variables

			Day 1						Day 3					
		W	FiO_2	pH	pCO_2	pO_2	SaO_2	PC	FiO_2	pH	pCO_2	pO_2	SaO_2	PC
Day 1	W	1												
	FiO_2	-0.39	1											
	pH	0.12	-0.39	1										
	pCO_2	-0.64*	0.21	-0.28	1									
	pO_2	0.64*	-0.16	-0.07	-0.45	1								
	Sa_2	0.89*	-0.29	-0.03	-0.62*	0.78*	1							
	PC	0.60*	-0.02	-0.15	-0.46	0.44	0.68*	1						
Day 3	FiO_2	-0.51	0.46	-0.66*	0.65*	-0.15	-0.47	-0.22	1					
	pH	-0.04	-0.31	0.03	0.15	-0.08	-0.12	-0.28	-0.15	1				
	pCO_2	0.03	0.24	-0.72*	0.46	0.27	0.13	0.09	0.55*	0.10	1			
	pO_2	-0.59*	0.31	-0.18	0.12	-0.34	-0.39	-0.27	0.30	-0.12	0.00	1		
	SaO_2	-0.23	0.06	-0.20	-0.04	-0.36	-0.13	-0.08	0.22	-0.39	-0.02	0.54*	1	
	PC	0.78*	0.02	-0.07	-0.51	0.49	0.74*	0.86*	-0.37	-0.20	0.12	-0.42	-0.33	1

* $p < 0,05$; $n_{valid} = 14$; W=weight, FiO_2 =concentration of oxygen to which the newborns were exposed to; pH= Astrup value; pCO_2 = partial pressure of carbon dioxide; pO_2 =partial pressure of oxygen; SaO_2 =oxygen saturation; PC=protein carbonyls

Table 3. Spearman correlation for the quantitative and qualitative variables

	Day 1							**Day 3**					
	W	FiO_2	pH	pCO_2	pO_2	SO_2	PC	FiO_2	pH	CO_2	O_2	SO_2	PC
GA	0.86	-0.60	0.13	-0.56	0.40	0.74	0.28	-0.43	0.17	-0.01	-0.41	0.05	0.40
G	0.53	-0.45	0.27	-0.41	0.25	0.12	0.11	-0.13	0.39	-0.11	-0.41	-0.47	0.19
Asphyxia	0.38	-0.06	0.11	-0.41	0.45	0.44	0.49	0.01	-0.17	0.02	-0.19	0.14	0.36
TP	-0.38	0.32	-0.46	0.38	0.17	-0.15	-0.03	0.48	-0.36	0.45	0.39	0.47	-0.31
ImAb	0.00	-0.05	-0.16	0.08	-0.31	0.03	-0.61	-0.16	0.24	0.13	0.08	0.32	-0.35
Dex	-0.07	0.07	0.39	0.03	0.38	-0.15	-0.24	0.33	0.25	-0.07	-0.22	-0.36	-0.24
RDS	0.84	-0.58	0.13	-0.60	0.33	0.85	0.43	-0.66	0.24	0.03	-0.45	0.11	0.56
CH	0.30	-0.45	0.36	-0.39	-0.36	0.25	-0.02	-0.54	0.50	-0.26	0.00	0.43	0.13
Hipotr	-0.07	0.07	0.39	0.03	0.38	-0.15	-0.24	0.33	0.25	-0.07	-0.22	-0.36	-0.24
Hbr	0.38	-0.34	0.26	-0.36	-0.18	0.08	0.33	-0.16	0.29	-0.26	-0.05	0.11	0.41
AIE	0.56	-0.05	0.05	-0.46	0.31	0.56	0.41	-0.43	0.03	0.03	-0.50	-0.32	0.61
AB	0.36	-0.52	-0.04	-0.16	-0.13	0.18	-0.02	-0.25	0.41	0.02	-0.13	0.34	-0.05
UV	0.63	-0.70	0.46	-0.39	0.18	0.50	0.41	-0.25	0.22	-0.22	-0.08	0.17	0.47
VM	-0.61	0.47	-0.13	0.10	-0.15	-0.22	-0.10	0.05	-0.45	0.00	0.63	0.51	-0.25

PC=protein carbonyls; pO_2 = partial pressure of oxygen; SaO_2 = oxygen saturation; pCO_2 = partial pressure of carbon dioxide; pO_2 = partial pressure of oxygen; GA=gestational age; G=gender; TP=toxic pregnancy; ImAb=imminent abortion; Dex=prophylactic administration of dexamethasone, RDS=respiratory distress syndrome caused by surfactant deficiency; CH= cerebral hemorrhage; AIE= anoxic ischemic encephalopathy; MV=mechanical ventilation; AB=antibiotherapy; UV=phototherapy;

Regression equations and their associated parameters were determined in order to test the linear relationship between the PC variable and weight at birth, FiO_2, pH, pCO_2, pO_2, SaO_2 for the samples selected in both the first and third day of life. The results are illustrated in Table 4.

Table 4. ANOVA test results for OC an SaO_2

Model	Sum of squares	Degrees of freedom	Median of squares	F	p
Regression	18.921	1	18.921	10.259	0.008(a)
Residues	22.131	12	1.844		
Total	41.052	13			

Predictors: (Constant), SaO_2 day 1
Dependant variable: PC day 1

The PC value was significantly higher in cases with AIE as compared with those without this pathology (see Figure 1).

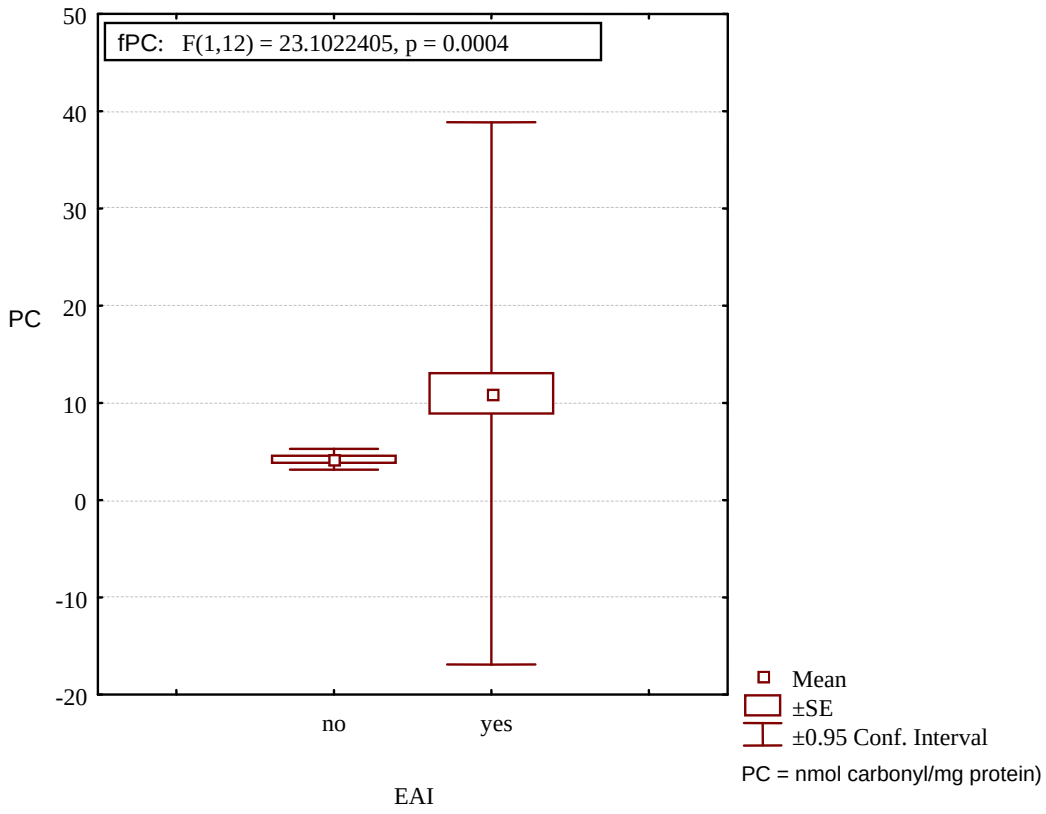

Figure 1. PC value in cases with and without AIE

The statistical analysis revealed a significant differences between the PC in the study group compared to the control group, both for the first and the third day values (p=0.00005 and 0=0.00653 respectively) These results are illustrated in Figure 2.

The comparisons with the normal values in adults were included in Table 5.

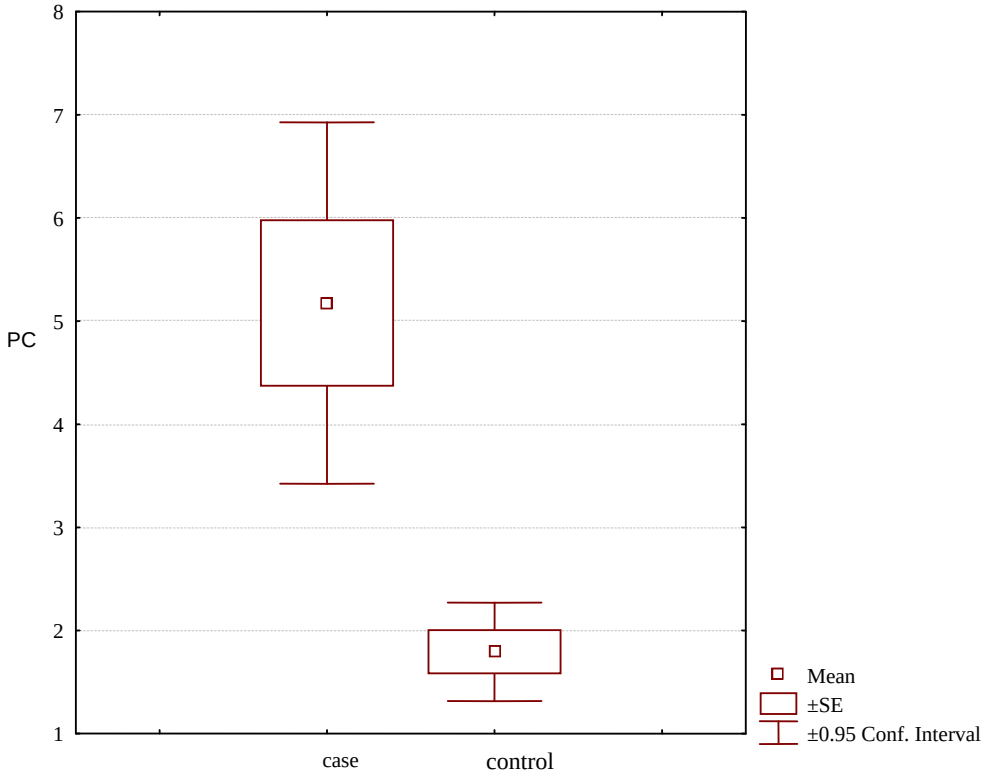

Figure 2. Comparison of PC in the studied and control group ($p < 0.001$) *Legend:* s-study group; c-control group

Table 5. Comparison of PC in the studied group and in adults

Parameter	**day 1**	**day 3**	**control**
Sample median	4.91	5.18	1.79
Population median	1.18	1.18	1.18
Standard deviation in population	0.34	0.34	0.34
Sample size	14	14	13
Standard error of sample median	0.09	0.09	0.09
Parameter of Student's t test	41.09	43.96	6.51
Significance threshold	0.05	0.05	
Degrees of freedom	13	13	12
P (test significance)	**3.78E-15**	**1.58E-15**	**2.90E-05**

Discussion

Lung proteins are attacked by ORS in the first 3-4 days of life. When RDS is present, pulmonary oedema occurs because of increased permeability of cell membranes. The fluid in the oedema is rich in proteins, which represent the ideal target for ORS. In order to initiate the oxidative attack, ORS inactivates alpha-1 protease thus causing an imbalance in the lung protease – antiprotease system [1,5,6].

Reactive oxygen species also interact with pulmonary surfactant as well as with other protein and lipid structures thus delaying the normal functioning of the lung. Therefore surfactant administration before the initiation of mechanical ventilation diminishes the severity of lung lesions by providing consistent ventilation [14].

Other risk factors of oxidative stress include haemolysis after birth, which occurs in almost all premature newborns (jaundice in the premature), and low iron binding antioxidant levels that favour protein destruction [3,4,12].

The analysis of the risk factors of oxidative stress in the premature newborns included in the studied group revealed that the average weight of the female newborns (1520 g) was smaller than the average weight of the male newborns (2511.43 g). The FiO_2 values did not exceed 42% and the Astrup parameters had average values.

The Pearson application was used for quantitative variables. A statistically significant correlation was found between the PC value in the first day and the weight of the newborns in the study group ($r = 0.60$; $p < 0.05$).

This correlation between PC value and weight demonstrates that immaturity plays a key role in the genesis of ORS. Newborns with small weight at birth and small gestational age require neonatal intensive care that may trigger ORS through oxygen therapy, inflammatory response and episodes of ischemia- reperfusion [2,5].

In our study, the Spearman correlation for quantitative and qualitative variables showed a significant correlation between the PC value in the third day and the presence of RDS due to surfactant deficiency ($r=0.56$; $p<0.05$). This confirms that RDS influences protein peroxidation significantly. The protein layer in the lung is the perfect target for ORS and protein oxidation reactions. This protein oxidation process activated by ORS has been proven to contribute to BPD pathogenesis in newborns with RDS [2,7,8,15].

In the multivariate analysis, a statistically significant correlation ($p = 0.008$; $r = 0.679$) was found between the PC value in the first day and the SaO_2 value in the same day. PC was the dependant variable in regression while SaO_2, pCO_2, pH, pO_2, FiO_2 were the predictors (Table 4). Therefore, the oxygenation in the first day of life reflected by SaO_2 influenced the PC value.

According to literature data, the correlation between the value of carbonyl proteins and FiO_2 exist when the FiO_2 is higher than 40% [3,7]. However, such a correlation could not be demonstrated in our study since the average FiO_2 was below 40%.

In cases with AIE, the PC value in the third day was significantly higher ($p=0.0285$) as compared with the newborns without this pathology. Some literature studies described that in the seventh day of life, increased levels of protein oxidation products in plasma occurred in newborns with hypoxia [9,10,16]. Our findings show that oxidative stress in hypoxic newborns is presents in the first three days after birth, and persists up to seventh day of life. ORS release is followed by cytokine production: interleukin -1β and IL-6 without infection. The release of ORS in the CNS in the context of asphyxia seems to be responsible for severe forms of AIE. Hypoxic lesions are increased by the release of non-protein bound iron. When such iron is present, the Fenton reaction generates hydroxyl radicals, which have a powerful toxic effect at cerebral level. The toxicity of ORS in newborns is augmented by the increased production of ORS, the newborn's rapid tissue growth and their poor antioxidative defence. Endothelial lesions, haemostasis anomalies, inflammatory reactions, increase of the anaerobic mechanism occur in the developing brain and are followed by lactic acid accumulation. Oligodendroglioma lesions, astrocytary dysfunctions and synapse anomalies may also occur [9,11,15,16].

In premature newborns the average PC value in the first day was smaller (4.91 nmol/mg) than that registered in the third day (5.175nmol/mg protein). For healthy term newborns, the average PC value was 1.79nmol/mg protein.

In premature newborns, the PC values both in the first ($p=0.000005$) and third ($p=0.00653$) day were significantly higher than the PC value registered in the control group .(above and fig 2) . Therefore, the protein oxidation process was stronger in the study group that included premature newborns with RDS and asphyxia.

As expected, the PC values in the study group were also significantly higher than the normal PC value in adults ($p <0.05$) (Table 5).

The findings of this study are according with the literature data.

Due to the relative reduced number of cases included in this study will be necessary to extend the research to larger groups.

Conclusions

The study of ORS effects on proteins confirmed the oxidative stress in newborns with RDS and asphyxia. For these patients, statistically significant correlations were observed between PC value in the third day of life and the occurrence of RDS($r=0.56, p<0.05$), as well as between the PC value in the first day and the SaO_2 value.

The average PC value in the studied group in both the first and the third day were significantly higher than the average PC value in the control group.

Our study suggests that prematurity and asphyxia should be considered risk factors for protein oxidation. Careful monitoring of oxygen exposure in premature newborns with RDS and asphyxia might limit the protein oxidation and improve their outcome.

References

1. Saugstad OD. Update on oxygen radical disease in neonatology. Curr Opin Obstet Gynecol 2001;13:147-53.
2. Levine RL, Williams JA, Stadtman ER, Shacter E. Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol 1994;233:346-57.
3. Vento G, Romagnoli C, Zecca E, Matassa PG, Tortorolo L, De Carolis MP, et al. Increased levels of soluble intercellular adhesion molecule-1, neutrophils and elastase in the lung of preterm infants with bronchopulmonary dysplasia. Prenat Neonat Med 1997;2:348-55.
4. Munkeby BH, Borke WB, Bjornland K, Sikkeland LIB, Borge GIA, Halvorsen B, Saugstad OD. Resuscitation with 100% O2 increases cerebral injury in hypoxemic piglets. Pediatr Res 2004;56(5):783-790.
5. Winterbourn CC, Chan T, Buss IH, Inder TE, Mogridge N, Darlow BA. Protein carbonyls and lipid peroxidation products as oxidation markers in preterm infant plasma: association with chronic lung disease and rethinopaty and effects of selenium supplimentation. Pediatr Res 2000;48:84-90.
6. Buss IH, Darlow BA,Winterbourn CC. Elevated lipid carbonyls and lipid peroxidation products correlating with myeloperoxidase in tracheal aspirates from premature infants.Pediatr Res 2000;47:640-5.
7. Buonocore G, Perrone S, Longini M, Terzuoli L, Bracci R. Total hydroperoxide and advanced oxidation protein products in preterm hypoxic babies. Pediatr Res 2000;47:221-224.
8. Watterberg K, Demers L, Scott S, Murphz S. Chorioamnionitis and early lung inflammation in infants in whom bronchopulmonary dysplasia develops. Pediatrics 1996;97:210-5.
9. Perrone S, Florio P, Longini M, Tanganelli D, Luisi S, Petraglia F. Activin A in newborn with and without hypoxia at birth. Pediatr Res 2002;51:255 A.
10. Perrone S, Bracci R, Buonocore G. New biomarkers of fetal-neonatal hypoxic stress. Acta Paediatr Suppl 2002;438:135-138.
11. Volpe JJ. Neurobiology of periventricular leukomalacia in the premature infant. Pediatr Res 2001;50:553-562.
12. Rogers S, Witz G, Anwar M, Hiatt M, Hegyi T. Antioxidant capacity and oxygen radical diseases in the preterm infant. Arch Pediatr Adolesc Med 2000;154:544-548.
13. Bolboacă SD, Jäntschi L. Assessment of Confidence Intervals used in Medical Studies. AcademicDirect & AcademicPres, Cluj-Napoca, Romania, 2008.
14. Carty J.L, Bevan R, Waller H. The effects of Vitamin C supplementation on protein in healthy volunteers. Biochem. Res. Com. 2000;273:729-735.
15. Winterbourne CC, Buss IH, Chan TP, Planl LD, Clark MA, Widsor JA. Protein carbonyl measurements show evidence of early oxidative stress in critically ill patients. Crit Care Med 2000;28:143-149.
16. Perrone S, De Marco L, Ciccoli L, Tanganelli I, Rossi V, Bracci R et al. Intraerythrocyte non protein bound iron release and diffusion after in vitro hypoxia reoxygenation. Biol Neonate 2001;80:325.

17. Akisu M, Yilmaz D, Tuzun S, and Kultursay N. Antioxidant defense system in newborn undergoing phototherapy. Indian J Pediatr 1999;66:651-655.