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Original Article

Biochemistry Section

# Circulating Protein Carbonyls, Antioxidant Enzymes and Related Trace Minerals among Preterms with Respiratory Distress Syndrome

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## ABSTRACT

**Introduction:** Information about oxidative stress in preterms with Respiratory Distress Syndrome (RDS) is defective, so various researches in this area are required, which may open new roads in understanding the pathogenesis of the disease, hence provide additional helpful therapeutic approaches.

**Aim:** To assess and compare the plasma level of protein carbonyls as a marker for oxidant status and the antioxidant enzymes; Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPx) and the related trace minerals in the form of Copper (Cu), Zinc (Zn) and Selenium (Se) as markers for antioxidant status, in preterms with and without RDS.

**Materials and Methods:** A hospital-based case-control study was conducted on fifty-seven preterm neonates (37 preterms with RDS and 20 preterms without RDS) admitted to neonatal intensive care unit of Qena University Hospitals after approval of the University Hospital Ethical Committee. Plasma protein carbonyls assay was done using commercially available ELISA assay kit. Plasma Cu, Zn, Se, erythrocyte SOD and GPx activities assays were done using commercially available colorimetric assay kits.

**Results:** Significant higher plasma levels of protein carbonyls and oxidant/antioxidants ratio (protein carbonyls/{SOD+GPx}) with significant lower plasma levels of Zn, Cu, Se, erythrocyte SOD and GPx activities were found in the preterms with RDS when compared with the preterms without RDS ($p<0.001$ for all measured markers for both groups). In terms of birth weights and gestational ages, they were negatively correlated with both plasma protein carbonyls and oxidant/antioxidants ratio and positively correlated with plasma copper, zinc, selenium, erythrocyte SOD and GPx activities in a statistically significant manner. Non-significant correlations were found between the measured oxidative stress markers and the severity of RDS.

**Conclusion:** Oxidative stress may have a contributory role in the development of RDS among preterms. Lower birth weight and prematurity may increase the susceptibity to oxidative stress among such patients.


**Keywords**: Birth weight, Glutathione peroxidase, Prematurity, Superoxide dismutase

## INTRODUCTION

RDS that occurs among the neonates (neonatal RDS) still has therapeutic challenges in the preterm babies. It is associated with inflammatory changes with production of free radicals and occurrence of oxidative stress [1]. Data about oxidative stress in preterm babies having RDS is defective [2]. The lungs of the preterm newborn is specially liable to oxidative stress due to relatively higher Reactive Oxygen Species (ROS) production with lower antioxidant defenses [3]. Oxidative stress can cause damage of cellular proteins, lipids and nucleic acids that may cause cell death and contributes in many disease pathogenesis [4].

Protein carbonyls are the products of the damaging effect of oxidative stress on cellular proteins that stimulates the formation of carbonyls (C=O "ketone" or –COH- "aldehyde" groups) in amino acids of proteins. Protein carbonyls can be used as a perfect oxidative stress marker because they are irreversible, stable in physical condition and unrepairable [5].

Neonatal RDS which occurs due to the deficiency in the development of lung alveoli and lung surfactant may complicate by lack of the antioxidant stores [1]. Antioxidant enzymes such as SOD and GPx increase gradually with the progress of pregnancy; in parallel with the surfactant system maturation [6]. SOD catalyzes the reduction of intracellular ROS through dismutation of the superoxide anion ($O_2^-$) to $H_2O_2$ which is dissociated into $H_2O$ and $O_2$ by catalase. GPx reduces lipid hydroperoxides to their corresponding alcohols and free hydrogen peroxide to $H_2O$ by using the reduced form of glutathione (GSH) as a hydrogen donor [7]. Selenium (Se) is one of the antioxidant trace minerals as it is a component of the GPx [8]. Copper (Cu) also is a component of the enzyme Cu-Zn SOD [9], whereas, Zinc (Zn) is a component of Cu-Zn SOD and also stimulate the formation of metallothionein which is a protein that removes the hydroxide radicals [10]. This study was undertaken to investigate the possible contributory role of oxidative stress in the pathogenesis of RDS among preterm neonates and correlate the measured oxidative stress markers with the severity of RDS, birth weight and gestational age.

## MATERIALS AND METHODS

### Study Population

A hospital-based case-control study was conducted on fifty-seven preterm neonates (<37weeks) admitted to neonatal intensive care unit of Qena University Hospitals-South Valley University-Egypt, upon approval of the University Hospital Ethical Committee. The study was carried out from October 2015 to October 2016. Preterm neonates were classified according to the presence or absence of neonatal RDS into two groups: a patient group consisting of 37 preterms with RDS (based on clinical and radiological diagnosis of RDS in the form of presence of signs of respiratory distress according to Downes' score combined with radiological findings on chest X-Ray which include lung collapse and diffuse fine granular densities (grade I),

air bronchograms caused by the atelectatic air sacs (grade II), ground-glass appearance (grade III) or white lungs caused by diffuse bilateral atelectasis (grade IV)) [11,12]; a control group consisting of 20 preterms without RDS, gestational age and sex matched. Any preterm neonates suffering from respiratory distress syndrome associated with any co-morbidity (e.g., sepsis, diseases of the respiratory, cardiovascular, or central nervous system, or those with maternal history of receiving multivitamins or antioxidant drugs), were excluded from the study to decrease any possible confounding factors.

### Ethical Considerations

This study was conducted according to the guidelines laid down in the declaration of Helsinki [13] and all procedures involving human patients were approved by the Ethical Committee of the Faculty of Medicine- South Valley University, Qena, Egypt. Parents of the included neonates were informed about the nature of the study and written consent was taken from each.

### Data Collections

All included preterm neonates of the study were subjected to: thorough history taking including prenatal, natal and postnatal history from the neonates' mothers, general and systemic examination. Gestational age assessment was performed by the new Ballard score using neuromuscular maturity and physical maturity [14]. Chest examination was done to diagnose RDS. Imaging tools in the form of Chest X-ray for all included preterms searching for the radiological findings that diagnose the respiratory distress syndrome were used as mentioned before.

### Laboratory Workup

A 5 cc of venous blood was drawn from the included neonates, within the first few hours after birth and prior to any parenteral or oral nutrition or therapies, on EDTA tubes and were centrifuged at 3500 rpm for 15 minutes. The separated plasma from each tube was transferred into 1 ml cryotubes and stored at -80°C until the time of biochemical assays for the oxidative stress markers, while the white buffy layer was removed and discarded, the erythrocytes were washed by four times of its volume with 0.9% NaCl solution and frozen at -80°C (freezing causes lysis of the erythrocytes) till the time of biochemical analysis of SOD and GPx erythrocyte enzyme activity after centrifugation at 3500 rpm for 15 minutes at 4°C and the supernatant (erythrocyte lysate) was used for the assay.

Plasma protein carbonyls assay was done, according to the manufacture protocol, using commercially available ELISA assay kit supplied by Glory Science Co., Ltd, USA, Catalog number 90485 {using ELISA multiskan EX microplate-photomter, thermo scientific, STAT FAX-2100, USA}.

Plasma copper assay was done using commercially available colorimetric assay kit supplied by Spectrum, Egyptian company of biotechnology (SAE), Catalog No. 232002) [15]; plasma zinc assay was done using commercially available colorimetric assay kit supplied by Spectrum, Egyptian company of biotechnology (SAE), Catalog No. 330002) [16]; plasma selenium assay was done using commercially available colorimetric assay kit, ABC Diagnostic Egypt) [17]; erythrocyte GPx activity assay was done using commercially available colorimetric assay kit supplied by Biodiagnostic, Giza, Egypt (BD), Catalog No. GP 2524 [18]; erythrocyte SOD activity assay was done using commercially available colorimetric assay kit supplied by Biodiagnostic, Giza, Egypt (BD), Catalog No: SD 25 20 [19]. All the previously mentioned assays were done using a T60 UV visible spectrophotometer (PG Instruments Limited, alma park wibtoft, Leicester shreshire, England. LE17SBE. Serial No. 20-1650-01-0010).

Oxidant/antioxidants ratio was calculated by dividing the plasma levels of protein carbonyls (oxidant) to the summation of SOD and GPx levels (antioxidants) for every included preterm.

## STATISTICAL ANALYSIS

Date entry and data analysis were done using SPSS version 19.0 (Statistical Package for Social Science). Data were presented as number, percentage, mean±standard deviation. Chi-square test was used to compare between qualitative variables. Mann-Whitney test was used to compare quantitative variables between two groups and Kruskal Wallis test for more than two groups in case of non-parametric data. Spearman correlation was done to measure correlation between quantitative variables. A p-value was considered statistically significant when p<0.05.

## RESULTS

Regarding the demographic data of the included neonates, mean±SD values of gestational ages were (31.41±2.3weeks and 31.85±2.39 weeks) for patient and control groups, respectively, with non-significant differences between patients and controls regarding to gestational age, age on admission, sex and weight [Table/Fig-1].

Regarding the degree of respiratory distress in the patients group, there were 16 (43.3%) preterms had mild degree, 11 (29.7%) preterms had moderate degree and 10 babies were in severe degree (27.0%). Regarding the grade of RDS according to radiological findings, there were 16 (43.3%) preterms had grade I, 11 (29.7%) preterms had grade II, 8 (21.6%) preterms had grade III and 2 (5.4%) preterms had grade IV [Table/Fig-2].

The mean values of oxidant (protein carbonyls) were significantly higher in patients (0.97±0.36 nmol/l) (p-value<0.001) when compared to controls (0.18±0.09 nmol/l) (p-value<0.001). The mean values of antioxidants in patients were significantly lower when compared to controls [Table/Fig-3].

| Variables | Patients (n= 37) | Controls (n= 20) | p-value |
|---|---|---|---|
| **Gestational age: (weeks)** | | | |
| Mean ± SD | 31.41 ± 2.31 | 31.85 ± 2.39 | 0.497 |
| Range | 28.0 - 36.0 | 28.0 - 35.0 | |
| **Age on admission: (hours)** | | | |
| Mean ± SD | 3.05 ± 2.09 | 2.75 ± 1.89 | 0.591 |
| Range | 1.0 - 9.0 | 1.0 - 7.0 | |
| **Sex: No. (%)** | | | |
| Male | 20 (54.1%) | 12 (60.0%) | 0.666 |
| Female | 17 (45.9%) | 8 (40.0%) | |
| **Weight: (kg)** | | | |
| Mean ± SD | 1.48 ± 0.52 | 1.70 ± 0.50 | 0.118 |
| Range | 0.8 - 2.5 | 0.8 - 2.8 | |
| **Down score** | | | |
| Mean ± SD | 5.41 ± 1.09 | 1.95 ± 0.83 | <0.001* |
| Range | 3.0 - 7.0 | 1.0 - 3.0 | |

**[Table/Fig-1]:** Demographic and clinical data of the studied groups.
*p-value <0.05 is considered statistically significant.

| Variables | No. (n= 37) | % |
|---|---|---|
| **Degree of RDS:** | | |
| Mild | 16 | 43.3 |
| Moderate | 11 | 29.7 |
| Severe | 10 | 27.0 |
| **Radiological finding:** | | |
| Grade I | 16 | 43.3 |
| Grade II | 11 | 29.7 |
| Grade III | 8 | 21.6 |
| Grade IV | 2 | 5.4 |

**[Table/Fig-2]:** Degree of respiratory distress syndrome (RDS) and radiological findings among the patients groups.

| Variables | Patients (n= 37) | Controls (n= 20) | p-value |
|---|---|---|---|
| **Protein carbonyls: (nmol/l)** | | | <0.001* |
| Mean ± SD | 0.97 ± 0.36 | 0.18 ± 0.09 | |
| Range | 0.3 - 1.8 | 0.1 - 0.4 | |
| **SOD Activity: (U/gm Hb)** | | | <0.001* |
| Mean ± SD | 12.91 ± 2.81 | 65.32 ± 8.00 | |
| Range | 7.5 - 16.5 | 57.0 - 78.0 | |
| **GPx Activity: (mU/mL)** | | | <0.001* |
| Mean ± SD | 74.93 ± 19.97 | 311.75 ± 56.52 | |
| Range | 41.3 - 109.0 | 194.5 - 370.0 | |
| **Copper: (µmol/l)** | | | <0.001* |
| Mean ± SD | 4.08 ± 1.68 | 9.58 ± 1.09 | |
| Range | 1.4 - 6.4 | 8.0 - 11.0 | |
| **Zinc: (µmol/l)** | | | <0.001* |
| Mean ± SD | 3.37 ± 1.09 | 12.06 ± 2.73 | |
| Range | 0.9 - 5.0 | 6.9 - 15.8 | |
| **Selenium: (ng/ml)** | | | <0.001* |
| Mean ± SD | 47.65 ± 5.78 | 79.75 ± 6.55 | |
| Range | 39.0 - 60.0 | 71.0 - 90.0 | |
| **Oxidant/ anti-oxidant ratio:** | | | <0.001* |
| Mean ± SD | 0.0122 ± 0.0065 | 0.0005 ± 0.0002 | |
| Range | 0.0040 - 0.0309 | 0.0002 - 0.0010 | |

**[Table/Fig-3]:** Comparison between mean plasma levels of oxidant and antioxidants among the studied groups.
*p-value <0.05 is considered statistically significant

Among the included patients, there were significant negative correlations between protein carbonyls with SOD activity (r =-0.422, p-value <0.01); GPx activity (r =-0.426, p-value <0.01); plasma copper (r =-0.413, p-value <0.05); plasma zinc (r =-0.548, p-value <0.001) and plasma selenium (r = -0.422, p-value <0.01). Significant positive correlations were observed for SOD with GPx activities (r =0.935, p-value <0.001); plasma copper (r =0.953, p-value <0.001); plasma zinc (r =0.786, p-value <0.001) and plasma selenium (r = 0.849, p-value <0.001). Significant positive correlations between GPx activity with plasma copper (r =0.903, p-value <0.001); plasma zinc (r =0.717, p-value <0.001); and plasma selenium (r =0.847, p-value <0.001) [Table/Fig-4] were also observed.

The Down score had non-significant correlations with each of protein carbonyls, SOD, GPx, oxidant/anti-oxidant ratio, copper, zinc and selenium [Table/Fig-5]. Also, there were non-significant differences in the mean plasma levels of the measured oxidative stress markers and the degree of RDS severity according to the radiological findings [Table/Fig-6].

Regarding the correlations between birth weight and the measured oxidative stress makers, among the included patients, there were significant negative correlations between birth weight with plasma protein carbonyls (r=-0.614, p-value<0.001) and oxidant/ antioxidant ratio (r=-0.790, p-value<0.001). There were significant positive correlations between birth weight and SOD activity (r =0.803, p-value<0.001); GPx activity (r =0.755, p-value<0.001); plasma copper (r =0.817, p-value<0.001); plasma zinc (r=0.898, p-value<0.001) and plasma selenium (r=0.803, p-value<0.001) [Table/Fig-5].

Regarding the correlations between gestational age and the measured oxidative stress markers, among the included patients, there were significant negative correlations between gestational age with plasma protein carbonyls (r=-0.421, p-value <0.01) and oxidant/ antioxidant ratio (r=-0.703, p-value<0.001) respectively. While, there were significant positive correlations between gestational age with SOD activity (r=0.925, p-value <0.001); GPx activity (r=0.961, p-value<0.001); plasma copper (r =0.886, p-value<0.001); plasma zinc (r=0.668, p-value <0.001) and plasma selenium (r=0.806, p-value <0.001) [Table/Fig-7].

## DISCUSSION

The protein layer of the lung is the target for ROS and protein oxidation reactions [1,20]. The fluid of the associated pulmonary oedema in RDS is rich in proteins which are the target for ROS [21]. Also, ROS interacts with lung surfactant causing decrease in the normal lung function [22].

Regarding the measured oxidative stress biomarkers in the present study, the mean values of oxidant (plasma protein carbonyls) were significantly higher, while, the mean values of antioxidant enzymes (SOD and GPx activities) and plasma levels of related trace elements (copper, zinc and selenium) were significantly lower in preterms with RDS versus preterms without RDS. In agreement with these findings, a study done by Negi R et al. and another study done by with Buonocore G et al., reported that significantly higher concentration of plasma protein carbonyls were observed among preterm babies with RDS [1,20]. Also, a study done by El-Masry HM et al., reported a significant decrease in the erythrocytic activities of SOD and GPx in RDS preterms compared with the controls [2]. As the antioxidant enzyme system is gradually developed during the last trimester of pregnancy, the preterm babies are more susceptible to the occurrence of oxidative stress [23]. A study done by Falciglia HS et al., reported significant association of increased

| Variables | | Protein Carbonyls (nmol/l) | Superoxide dismutase Activity (U/gm Hb) | Glutathione peroxidase activity (mU/mL) | Oxidant/ anti-oxidant ratio | Copper (µmol/l) | Zinc (µmol/l) |
|---|---|---|---|---|---|---|---|
| SOD activity (U/gm Hb) | r-value | -0.422 | | | | | |
| | p-value | 0.009* | | | | | |
| GPx activity (mU/mL) | r-value | -0.426 | 0.935 | | | | |
| | p-value | 0.009* | <0.001* | | | | |
| Oxidant/ anti-oxidant ratio | r-value | 0.911 | -0.701 | -0.720 | | | |
| | p-value | <0.001* | <0.001* | <0.001* | | | |
| Copper (µmol/l) | r-value | -0.413 | 0.953 | 0.903 | -0.685 | | |
| | p-value | 0.011* | <0.001* | <0.001* | <0.001* | | |
| Zinc (µmol/l) | r-value | -0.548 | 0.786 | 0.717 | -0.717 | 0.765 | |
| | p-value | <0.001* | <0.001* | <0.001* | <0.001* | <0.001* | |
| Selenium (ng/ml) | r-value | -0.422 | 0.849 | 0.847 | -0.646 | 0.866 | 0.756 |
| | p-value | 0.009* | <0.001* | <0.001* | <0.001* | <0.001* | <0.001* |

**[Table/Fig-4]:** Correlations between the studied oxidative stress markers.
*p-value <0.05 is considered statistically significant

| Variables | Birth weight (kg) | | Down score | |
|---|---|---|---|---|
| | r-value | p-value | r-value | p-value |
| Protein carbonyls (nmol/l) | -0.614 | <0.001* | -0.197 | 0.242 |
| Superoxide dismutase activity (U/gmHb) | 0.803 | <0.001* | -0.280 | 0.094 |
| Glutathione peroxidase activity (mU/mL) | 0.755 | <0.001* | -0.232 | 0.168 |
| Oxidant/ anti-oxidant ratio | -0.790 | <0.001* | -0.022 | 0.895 |
| Copper (µmol/l) | 0.817 | <0.001* | -0.210 | 0.211 |
| Zinc (µmol/l) | 0.898 | <0.001* | -0.185 | 0.274 |
| Selenium (ng/ml) | 0.803 | <0.001* | -0.276 | 0.099 |

**[Table/Fig-5]:** Correlations of oxidative stress markers with birth weight and Down score.
*p-value <0.05 is considered statistically significant.

| Variables | Radiological findings | | | p-value |
|---|---|---|---|---|
| | Grade I (n=16) | Grade II (n=11) | Grade III/ IV (n=10) | |
| | Mean ± SD | Mean ± SD | Mean ± SD | |
| Protein carbonyl (nmol/l) | 1.05 ± 0.37 | 0.98 ± 0.28 | 0.83 ± 0.41 | 0.21 |
| Superoxide dismutase activity (U/gm Hb) | 12.77 ± 3.05 | 13.38 ± 2.69 | 12.64 ± 2.79 | 0.85 |
| Glutathione peroxidase activity (mU/mL) | 72.99 ± 20.04 | 78.98 ± 19.82 | 73.57 ± 21.45 | 0.64 |
| Oxidant/ anti-oxidant ratio | 0.0136 ± 0.0068 | 0.0114 ± 0.0047 | 0.0108 ± 0.0079 | 0.44 |
| Copper (µmol/l) | 4.09 ± 1.68 | 4.21 ± 1.79 | 3.92 ± 1.72 | 0.96 |
| Zinc (µmol/l) | 3.18 ± 1.11 | 3.59 ± 0.67 | 3.43 ± 1.44 | 0.599 |
| Selenium (ng/ml) | 46.88 ± 5.16 | 48.09 ± 5.59 | 48.40 ± 7.24 | 0.83 |

**[Table/Fig-6]:** Comparison between mean plasma levels of oxidant and antioxidants among the patient group according to the radiological grading of RDS.
*p-value <0.05 is considered statistically significant.

| Variables | Gestational age (weeks) | |
|---|---|---|
| | r-value | p-value |
| Protein Carbonyls (nmol/l) | -0.421 | 0.009* |
| Superoxide dismutase activity (U/gmHb) | 0.925 | <0.001* |
| Glutathione peroxidase activity (mU/mL) | 0.961 | <0.001* |
| Oxidant/ anti-oxidant ratio | -0.703 | <0.001* |
| Copper (µmol/l) | 0.886 | <0.001* |
| Zinc (µmol/l) | 0.668 | <0.001* |
| Selenium (ng/ml) | 0.806 | <0.001* |

**[Table/Fig-7]:** Correlations between gestational age and oxidative stress markers.
*p-value <0.05 is considered statistically significant.

RDS occurrence among preterm babies with lower plasma selenium levels [24]. Foetus stores the trace minerals during the late months of pregnancy, so they are at higher risks of trace elements deficiencies which required for normal growth [24,25]. In contrary to our study, Mohammadzadeh A et al., reported non-significant association between umblical cord blood selenium level and occurrence of RDS in preterm neonates [26], which could be explained by the different inclusion criteria and methodology compared with our study.

In the present study, there were negative significant correlations between protein carbonyls with each of the antioxidant enzymes (SOD and GPx) and the related trace elements (copper, zinc, selenium). This was in agreement with Khare M et al., who reported significant negative correlations between protein carbonyls versus Cu and GPx, concluding that the magnitude of the initial oxidative stress was larger than the compensatory capacity of the antioxidants [27].

In the present study, there were positive significant correlations between antioxidant enzymes (SOD and GPx) and the related trace elements (copper, zinc, selenium) with each other's. This was in agreement with Ozturk P et al., who reported significant positive correlations between selenium and GPx [28], which indicates that the oxidative stress was confirmed by the significant decrease in GPx and selenium levels in patients when compared to controls.

Regarding the oxidant/antioxidant ratio in the present study, there were significant higher ratio among patients than controls; this was in agreement with Negi R et al., who observed that increased oxidative stress accompanied by the decrease in the antioxidant defenses and may play a role in the pathogenesis of a number of inflammatory pulmonary diseases including RDS [1]. Also, this was in agreement with other studies, where these studies confirmed that an imbalance between oxidant-antioxidant is associated to the oxidative stress which plays a significant role in RDS [2,20,29,30].

The findings of the present study showed significant negative correlation between gestational age and plasma protein carbonyls. On the other hand, there were significant positive correlations between gestational age with antioxidant enzymes (SOD and GPx) and related trace elements (copper, zinc and selenium). This was in agreement with Pathak R et al., who reported decreased levels of GPx with increased oxidative stress in preterm delivery versus full term delivery [31]. Also, this was in agreement with Piyush G et al., who reported significant lower levels of SOD and GPx with the decrease in the gestational age [32]. Another study done by Javier E et al., reported that the immaturity of the antioxidant defense among newborns especially preterm infants making them more vulnerable to the occurrence of oxidative stress [33].

The finding of the present study showed significant negative correlation between birth weight and plasma protein carbonyls, while there were significantly positive correlations between birth weight with antioxidant enzymes (SOD and GPx) and related trace elements (copper, zinc and selenium). This was in agreement with Buss IH et al., who reported significantly higher protein carbonyls in infants <1500 gm than infants with birth weight >1500 gm [34]. Also, our data are in agreement with Mehrdad M et al., who reported significant positive correlation between birth weight and antioxidants especially SOD [35]. Another study done by Dolapo PO et al., who reported that low birth weight babies might have suffered from more oxidative stress than normal birth weight babies showed that there was negative correlation between birth weight and cord blood oxidative stress [36].

In the present study, the severity of RDS as recorded by Down score was not statistically significant when correlated with mean values of each of protein carbonyls, SOD, GPx, oxidant/anti-oxidant ratio, copper, zinc and selenium, although, a study done by Philip AG et al., reported positive correlation between plasma protein carbonyls and severity score in bronchopulmonary dysplasia, supporting the association between the oxidative stress and the severity of lung disease [37]. The absence of significant correlation between severity of RDS and parameters of oxidative stress in our study may be explained by the prematurity of lung which may be the leading cause of high oxidative stress parameters regardless the severity of disease process. Anatomical and physiological considerations of the human lungs make them vulnerable to oxidative damage, so the research in the field of free radicals and antioxidants will provide insights into the pathogenesis and hence additional therapeutic options to improve the outcome of various lung diseases including the RDS.

## LIMITATION

The relatively limited number of the included neonates which may be attributed to the fact that, the study was conducted in a single center and also difficulty in finding healthy pretems who were without any therapeutic or nutritional intervention for collecting blood samples.

## CONCLUSION

The present study proves that the oxidative stress may have a contributory role in the pathogenesis of RDS among the preterm neonates, which may be attributed to the low birth weight and gestational age, where the stores of antioxidants and trace minerals are lower in such group of patients, as evidenced by our results, so the possible addition of antioxidants as an adjuvant therapy to preterms with RDS has to be investigated as therapeutic trials.

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