

**Measurement Of Carnitine In Preterm Neonates
Suffering Respiratory Distress Syndrome And The Effect Of Its Supplement**

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Abstract

Background: Respiratory distress syndrome (RDS) is among the most common diseases of preterm infants. RDS is caused by a decreased production or secretion of pulmonary surfactant. Numerous causes of RDS have been identified, and the factors suspected to be involved in the pathogenesis of RDS are numerous, Carnitine is essential for the fetus and is provided via placental transport. As the gestational age increases, fetal tissues store increasing amounts of carnitine, therefore, preterm infants require exogenous carnitine supplementation for carnitine homeostasis. Treatment with carnitine has shown benefit in the respiratory status of ventilator- dependent adults, as well as stabilization of respiratory parameters and increased physical performance in adult patients with chronic respiratory insufficiency.

Objective: The present study was designed to measure the level of free carnitine in preterm neonates with RDS and to evaluate the efficacy of L-carnitine therapy on those neonates.

Patients and Methods: Forty preterm infants, including 14 females and 26 males. Study group were divided in to 2 groups, group A: received L-carnitin in a dose of 30 mg/ kg/ day for 7 days and group B: did not receive supplementation.

Results: Our results show non statistically significant difference between group A (with Carnitine supplementation) and group B (no supplementation) at day 1. There was statistically significant higher serum carnitine level in group A compared to group B at day 7 (after supplementation). Seven neonates (35%) in group A, and 13 (65%) in group B, needed surfactant administration and MV after 24 hours from admission and this difference was statistically significant. Dose of surfactant was statistically significant lower in group A compared to group B (P= 0.001) and duration of mechanical ventilation was statistically significant lower in group A compared to group B (p= 0.03).

Conclusion: L- carnitine is more deficient in preterm with RDS than preterm without RDS and its supplementation can reduce the need and duration of MV and/ or need and dose of surfactant.

Key words: RDS, Carnitine supplementation, Surfactant, MV

قياس نسبة الكارنيتين في الاطفال المبتسرين المرضى بمتلازمة ضيق التنفس وتأثير اعطائه عليهم

الخلفية: يعتبر مرض متلازمة ضيق التنفس واحد من اخطر وأكثر الحالات شيوعا التي تواجه الاطفال حديثي الولادة خاصة المبتسرين منهم. يتم تصنيع مادة الكارنيتين في الجسم وتلعب دورا مهما في تطور الرئة في الطفل المبتسر عن طريق المشاركة في تكوين السرفاكتانت.

الهدف: قياس مستوى الكارنيتين في الاطفال المبتسرين المرضى بمتلازمة ضيق التنفس وتقييم تأثير اعطائه لذوى المستوى المنخفض منه على مدى تطور المرض او الاستجابة السريعة للعلاج.

طريقة البحث: التحق بالدراسة أربعين من الخدج، بينهم ١٤ من الإناث و ٢٦ من الذكور. وكان عمر الحمل متوسط ٣٣,١ ± ٢,٨ أسابيع. وكان متوسط الوزن عند الولادة ١٠٢٤ ± ٥٠٦,٤ غرام. تم تقسيم مجموعة الدراسة في ل ٢ مجموعات، المجموعة (أ) تلقى الكارنيتين في جرعة ٣٠ ملغ/كغ/ يوم لمدة ٧ أيام والمجموعة (ب) لم تتلق مكملات.

النتائج: كان هناك دلالة إحصائية انخفاض الوزن عند الولادة في مجموعة الدراسة مقارنة مع مجموعة السيطرة. وكانت الولادة القيصرية ذات دلالة إحصائية أعلى في مجموعة الدراسة (٧٥%) مقارنة مع مجموعة التحكم (٤٠%)، ووجد ان نسبة الكارنيتين في المصل أقل دلالة إحصائية في مجموعة دراسة مقارنة بالمجموعة الضابطة. نتائجنا تظهر الفرق غير ذات دلالة إحصائية بين المجموعة (أ) (مع كارنيتين مكملات) والمجموعة الثانية (أى مكملات) في اليوم الاول. هناك ذات دلالة إحصائية أعلى مستوى الكارنيتين المصل في المجموعة (أ) مقارنة مع المجموعة (ب) في يوم السابع (بعد مكملات).

الكلمات الدالة: متلازمة ضيق التنفس، الخدج، الكارنيتين.

Introduction:

Respiratory distress syndrome (RDS) is among the most common diseases of preterm infants. RDS is caused by a decreased production or secretion of pulmonary surfactant.⁽¹⁾ Numerous causes of RDS have been identified, and the factors suspected to be involved in the pathogenesis of RDS are numerous.⁽²⁾ In recent years, studies have identified the presence of low serum carnitine levels in preterm newborns with RDS.⁽³⁾

Carnitine is a hydrophilic amino acid derivative synthesized from lysine, which serves a crucial function in β oxidation as it transports long chain fatty acids across the mitochondrial membrane.⁽⁴⁾ Carnitine is essential for the fetus and is provided via placental transport. As the gestational age increases, fetal tissues store increasing amounts of carnitine; however, preterm infants are at an increased risk of carnitine deficiency due to low levels of α -butyrobetaine hydroxylase, a catalyzing enzyme in the final step of the carnitine biosynthetic signaling pathway (Czeszyńska, 1993). Therefore, preterm infants require exogenous carnitine supplementation for carnitine homeostasis.⁽⁵⁾

Pulmonary surfactant production is an important process in fetal lung maturation. Antenatal carnitine administration has been shown to be effective in inducing pulmonary surfactant production and lung maturation in both fetal rats and humans.⁽⁶⁾ As carnitine is an integral component of the membrane phospholipid fatty acid turnover in human cells, it is possible that carnitine causes lung maturation via membrane phospholipid repair activity.⁽⁷⁾

Aim:

To measure the level of free carnitine in preterm neonates with RDS and evaluate the effect of its supplementation to these preterm neonates.

Methodology:

✧ Study Population: This study was conducted as cross sectional, interventional study, including 40 preterm infants (26 males and 14 females) who were admitted to Maternity hospital, Ain Shams University and El Zyton hospital with Respiratory distress syndrome (RDS) over a period of 24 months between January 2011 to December 2012. Ten preterm infants (7 males and 3 females) without RDS, were enrolled as control group. Patients were divided into 2 groups: Group A: included 20 preterm infant (28- 36 wks) who developed RDS and received L- carnitine supplementation. Group B: included 20 preterm infants (28- 36 wks) who developed RDS and did not receive L- carnitine supplementation.

✧ Inclusion Criteria:

1. Preterm infants with gestational age between 28 and 36 wks with clinical and radiological criteria of RDS.
2. Both genders: male and female.
3. During the 1st 6 hours after delivery.
4. Both mode of deliveries (cesarean section or vaginal delivery).

✧ Exclusion Criteria:

1. < 28 weeks or >36 weeks.
2. Received asphyxia surfactant in early post delivery.

3. Neonates with apparent congenital anomalies.
4. Neonates with clinical and/or laboratory picture of sepsis.
5. Neonates with birth asphyxia or congenital pneumonia.
6. Death Before One Week.
7. Neonates with maternal medical conditions:
8. With Evidence Chorioamnionitis.
9. Perinatal Infection.
10. Any endocrinal diseases such as DM, Thyroid or Adrenal dysfunction.
11. Received any hormonal therapy or drugs as dexamethasone during pregnancy.

✧ Diagnosis of RDS:

1. Clinical: onset of tachypnea (respiratory rate exceeding 60 breaths/min) within 6 hours after birth, persistence of tachypnea for at least 12 hours.⁽⁸⁾
2. Radiological: radiological signs of at least one of the following in the chest x- ray:
 - a. Prominent central vascular markings.
 - b. Widened interlobar fissures of pleural fluid.
 - c. Symmetrical perihilar congestion.
 - d. Hyperaeration as evidenced by flattening and depression of the diaphragmatic domes or increased anteroposterior diameter or both.⁽⁸⁾

Written-informed consent was obtained from the parents of the infants. The protocol of the present study was approved by the ethical committee of the IPGS, Ain Shams University.

Methods:

1. All the patients were directed to Complete History taking; Thorough clinical examination and Assessment of gestational age using the criteria of the new Ballard score.⁽⁹⁾
2. Assessment Of Ventilator Requirement.
3. Vital data including: temperature, heart rate, respiratory rate and blood pressure
4. Complete examination including: Chest examination, Cardiac examination, Abdominal examination, Neurological examination and O₂ saturation by pulse oximetry.

Estimation Of Carnitin Level:

Carnitine levels were measured in the plasma samples collected from all patients during the first 6 h following birth and on day 7 following birth. Standard carnitine kits (Z13010; Eureka Lab Division S.r.l., Chiaravalle, Italy) were used for the analysis of carnitine levels. The present method facilitated the analysis of the plasma levels of L-carnitine base (free) via a specific derivatization solution, separation by high pressure liquid chromatography (Agilent 1100; Agilent Technologies GmbH, Waldbronn, Germany) and subsequent quantification using fluorimetry.

In group A (interventional group), all patients (RDS and non-RDS) received daily carnitine treatment (30 mg/ kg/ day, 3 times/ day)

beginning from 6 h to 7 days. Carnitine was administered intravenously during parenteral feeding, and then via the enteral route when the patient began enteral feeding.

Statistical Analysis:

Data were collected, revised, verified then edited on P.C, All the statistical analyses were performed Statistical Package version-20 for the Social Sciences (SPSS). The results of quantitative data are expressed as the mean and standard deviation (mean± SD). The results of qualitative data are expressed as number and percentage. Unpaired t- test was used to compare a quantitative variable between two independent groups in parametric data. Chi square test was used to compare between two independent qualitative variables. Levels of statistical significance were set as: P> 0.05: considered as non significant. P< 0.05: considered as significant. P< 0.01: considered as highly significant.

Ethical Aspects:

Approval by the Ethics Committees of the Institute of Postgraduate Childhood Studies And the Egyptian Ministry of education, in addition to parental informed Consent were obtained.

Results:

There was non- significant difference between both study groups regarding GA, birth weight, gender and APGAR score at baseline Table (1).

Table (1): Comparison between studied groups regarding neonatal descriptive data

		Group A N= 20	Group B N= 20	T	Sig.
GA (Wk)	Mean±SD	32.9±1.9	33.4±2.5	- 0.712	0.240 (NS)
	Min- Max	29- 36	27- 35		
Birth Weight (Gm)	Mean±SD	1550±516.4	1510±559.2	0.235	0.592 (NS)
	Min- Max	(2155- 1050)	(2050- 950)		
		N (%)	N (%)	X	Sig.
Sex	Male	13 (65.0)	13 (65.0)	0.000	1.000 (NS)
	Female	7 (35.0)	7 (35.0)		
APGAR	1 min (median IQ)	7 (5- 8)	7 (5- 9)	0.000	1.00 (NS)
APGAR	5 min (median IQ)	9 (6- 9)	9 (6- 9)	0.000	1.00 (NS)

Our results show non statistically significant difference between group A (with Carnitine supplementation) and group B (no supplementation) at day 1. There was statistically significant higher serum carnitine level in group A compared to group B at day 7 table (2).

Table (2) Serum Carnitine level in studied groups before and after supplementation

		Group A N= 20	Group B N= 20	T	Sig.
		Mean±SD	Mean±SD		
Day 1		21.1±3.2	20.8±4.1	0.258	0.797 (NS)
Day 7		18.9±1.8	12.4±3.6	1.251	<0.0001(HS)

Seven neonates (35%) in group A, and 13 (65%) in group B, needed surfactant administration and MV after 24 hs from admission and this difference was statistically significant. Dose of surfactant was statistically significant lower in group A compared to group B (P= 0.001) and duration of mechanical ventilation was statistically significant lower in group A compared to group B (p= 0.03).

Table (3) Surfactant and MV use in studied groups

	Group A N= 20	Group B N= 20	x	Sig.
	N (%)	N (%)		
Need for surfactant and MV after 24 hs	7 (35)	13 (65)	4.9123	0.02 (S)
	Mean±SD	Mean±SD	t	Sig.
Dose of surfactant (amp)	1.1±0.7	2.0±0.9	3.533	0.001 (HS)
Duration of MV (days)	4.4±3.4	5.9±2.9	2.138	0.03 (S)

There was statistically significant negative correlation between serum carnitine and GA.

There was no statistically significant correlation between serum carnitine and birth weight, APGAR score, maternal age and parity.

Table (4) Correlation between serum carnitine and neonatal and maternal data

		GA	Birth Weight	APGAR 1min	ABGAR 5 Min	Maternal Age	Parity
Serum Carnitine	r	- 3.061	- 0.069	0.189	0.779	- 0.161	- 0.193-
	Sig	0.04*	0.671	0.242	0.122	0.454	0.232

Discussion:

Carnitine is stored in fetal tissues in increasing amounts over the latter part of gestation. Premature infants are at increased risk of tissue carnitine deficiency because of the immaturity of the carnitine biosynthetic pathways. In addition, reduced placental transfer and reduced intakes from breast milk or conventional parenteral nutrition solutions also contribute to carnitine deficiency in this population.⁽¹⁰⁾ Treatment with carnitine has shown benefit in the respiratory status of ventilator-dependent adults, as well as stabilization of respiratory parameters and increased physical performance in adult patients with chronic respiratory insufficiency.⁽¹¹⁾

The present study was designed to measure the level of free carnitine in preterm neonates with RDS and to evaluate the efficacy of L-carnitine therapy on those neonates. Forty preterm infants, including 14 females and 26 males, were enrolled in the present study. The mean gestational age was 33.1± 2.8 weeks (range, 28- 36 weeks). The average birth weight of the infants was 1524± 506.4 g; the mean birth weight for the control group infants was 1790± 549.2 g.

In the current study, study group were divided in to 2 groups, group A: received L- carnitin in a dose of 30 mg/ kg/ day for 7 days and group B: did not receive supplementation.

Similar to our results, in Ozturk et.al.,⁽³⁾ study, a dose of 30 mg/ kg/ day was used. On the other hand Crill et.al.⁽¹²⁾ used L-carnitine at a dosage of 20 mg/kg/day for all infants.

Using higher doses compared to ours, Iafolla et.al.,⁽¹³⁾ in a case series, showed a dramatic decrease in episodes of apnea and periodic breathing in infants with carnitine deficiency following 48 hours of treatment with 100 mg/ kg/ day of oral carnitine. There have been no serious side effects reported with carnitine supplementation in preterm infants.

Among preterm infants, Bonner et.al.,⁽¹⁴⁾ found that short- term low L- carnitine supplementation doses (50 to 70 mmol/ kg/ day) have been shown to improve lipid tolerance, ketogenesis, and short- term weight gain.

Our results show non statistically significant difference between group

A (with Carnitine supplementation) and group B (no supplementation) at day 1. There was statistically significant higher serum carnitine level in group A compared to group B at day 7 (after supplementation).

Similar to our results, Ozturk et.al.,⁽³⁾ found that following comparison of serum carnitine levels, no significant difference was observed on day 1 of measurements; however, on day 7 the carnitine levels were significantly higher in the experimental groups, as compared with the control groups (P= 0.02).

It is well-established that carnitine serves a crucial function in cellular metabolic processes, such as aerobic catabolism of glucose and β oxidation of free fatty acids.⁽¹⁵⁾ Due to its role in metabolism, the diaphragmatic muscle requires carnitine for contractility.⁽¹⁶⁾ Premature infants may have lower glycogen levels, and therefore use more carnitine for fatty acid oxidation, leading to a decrease in carnitine levels.

Bonner et.al.,⁽¹⁴⁾ found that supplementation of preterm infants using doses of 50 to 60 mmol/kg/day significantly increases serum carnitine levels to values at least three times those in controls.

Seven neonates (35%) in group A, and 13 (65%) in group B, needed surfactant administration and MV after 24 hs from admission and this difference was statistically significant.

Similar to our results, Ozturk et.al.,⁽³⁾ found that 12 patients (40%) in group I required mechanic ventilation and surfactant replacement therapy, while a significantly greater number of patients (17 patients; 55%) required mechanic ventilation and surfactant therapy in supplementation groups. Similarly Zaharia et.al.⁽¹⁷⁾ reported that 54% of preterm infants >28 weeks of gestational age that received prophylactic NCPAP required mechanical ventilation.

Kurz et.al.,⁽¹⁸⁾ found that maternal carnitine administration induced fetal lung maturity by increasing total phospholipid and dipalmitoylphosphatidylcholine content, phosphatidylcholine species, free carnitine and short chain acylcarnitine levels in fetal rat lungs and Lohninger et.al.,⁽¹⁹⁾ found that antenatal administration of L- carnitine in combination with lower betamethasone doses resulted in a significant decrease in the incidence of RDS in humans.

Dose of surfactant was statistically significant lower in group A compared to group B (P= 0.001) and duration of mechanical ventilation was statistically significant lower in group A compared to group B (p= 0.03).

Similar to our results, Ozturk et.al.,⁽³⁾ found that the duration of mechanical ventilation and the requirement for surfactant therapy was significantly reduced in the groups that received L-carnitine treatment, as compared with the control groups. They concluded that the group treated with L-carnitine required less surfactant, which may decrease the number of complications and provide more cost-effective therapy.

Also similarly Crill et.al.⁽¹²⁾ reported that the requirement for mechanical ventilation and duration of ventilator use were not significantly different between the therapy and placebo groups.

Lohninger et.al.,⁽⁶⁾ found that treatment of pregnant rats with carnitine

resulted in a significant increase in total phospholipids and dipalmitoylphosphatidylcholine levels in fetal rat lungs.

The data demonstrated the efficacy of treatment with L-carnitine and the consequences of carnitine deficiency. The transport of L-carnitine to the lungs of the premature infant likely increases for surfactant synthesis, and this may cause the reduced serum levels of carnitine observed in patients with RDS. Following birth there is a marked increase in energy metabolism. Premature infants with RDS require more energy due to their increased respiratory effort.⁽¹⁵⁾

Our results show statistically significant negative correlation between serum carnitine and GA.

Similar to our results, Meyburg et.al.⁽²⁰⁾ reported a negative correlation between gestational age and serum carnitine concentrations. On the other hand, Ozturk et.al.⁽²⁾ did not establish a correlation between carnitine and gestational age.

Our results show statistically non- significant correlation between serum carnitine and birth weight, APGAR score and parity.

Ozturk et.al.,⁽³⁾ found that serum carnitine levels did show a negative correlation with birth weight and a positive correlation with Apgar scores. This may due to the increased requirement for surfactant synthesis in low birth weight infants as compared with infants with high Apgar scores that exhibit a healthy respiratory pattern. In two previous studies (Ozturk et.al.,⁽²⁾ Korkmaz et.al.⁽⁷⁾) no significant differences were reported between L-carnitine levels and gender, birth weight and Apgar scores.

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