BLOOD L-ARGININE LEVEL IN EARLY RESPIRATORY DISTRESS SYNDROME

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Abstract:
Objective: To study the blood L-arginine level in cases of premature neonates suffering from Respiratory Distress Syndrome (RDS) and correlate it with the severity and the prognosis of RDS.

This study included 30 premature neonates with respiratory distress syndrome and 10 healthy premature neonates as control group from neonatal intensive care unit (NICU), Pediatrics department, Benha University Hospitals during the period from February to December 2011. All the studied neonates were subjected to: complete clinical evaluation, plain X-ray chest examination with determination of the grade of RDS according to Downes scoring system. Laboratory work-up included serum L-arginine assay and arterial blood gases (ABG). Follow up for 1 month to all cases were done to detect their outcome. Results: In our study the mean blood L-arginine level in preterm with RDS was significantly lower than that of normal preterm (30.1±6.1 versus 81.8±5.6, p< 0.001). L-arginine level significantly increased on the third day of life in the cases, while there is no significant difference between first and third day regarding control group. Our result demonstrated significantly reduced blood L-arginine levels in severe cases of RDS (Downes score > 7) compared to other cases, with highly significant negative correlation between L-arginine level and the severity of RDS.

Conclusion: lower level of L-arginine was associated with the severe form of neonatal respiratory distress syndrome, while there was no relation to the prognosis of RDS in premature infants.
Introduction

Neonatal respiratory distress syndrome (RDS) refers to respiratory compromise presenting at or shortly after birth specifically as a result of a deficiency of pulmonary surfactant, an endogenous detergent that serves to decrease the surface tension within alveoli, thereby preventing alveolar collapse. Neonatal RDS affects approximately 1\% of all live births; however, not all infants are at equal risk (1).

Low plasma L-arginine concentrations have been described in children with various pathological conditions such as in premature infants with necrotizing enterocolitis (NEC) (2), children with asthma (3), and in sickle cell crisis (4). Newborns with persistent pulmonary hypertension have decreased plasma arginine concentration (3). The L-arginine-NO signaling pathway has emerged as one of the key second messenger systems involved in the regulation of normal blood pressure, vascular resistance, preservation of endothelial function and protection against ischemic-reperfusion damage (5).

Aim of the work

Is to estimate the blood L-arginine level in cases of premature neonates suffering from Respiratory Distress Syndrome (RDS) and correlate it with the severity and the prognosis of RDS.

Subjects and Methods

This study was implemented during the period from February to December 2011 in neonatal intensive care unit (NICU), Pediatrics department, Benha University Hospitals on 30 premature neonates with respiratory distress syndrome (group I); they included 15 males and 15 females. Their mean gestational age was 32 ± 2 weeks and mean
weight 1.9 ± 0.2 kg. In addition to 10 normal preterm infants served as control (group II) {Preterm infants who developed RDS in the first 6 hours of life were enrolled in the study group (group I) while preterm that did not develop RDS served as the control group (group II)}. All cases were followed up for 1 month and case which not improved and depended on oxygen therapy was considered as bronchopulmonary dysplasia (BPD). An informed consent was taken from parents of the cases before the study.

All the studied cases were subjected to:

1- Complete clinical evaluation. 2- Plain chest X-ray examination (postero-anterior view) with determination of the grade of RDS according to Downes scoring system.

3- Laboratory work-up included arterial blood gases (ABG) and serum L-arginine assay. Three ml peripheral blood samples were drawn under sterile aseptic techniques in the first 2 hours of life in test tube contained EDTA and 2nd sample after 72 hours.

All blood samples were centrifuged for 15 minutes at 1000xg at 2-8°C within 30 minutes of collection and plasma samples were stored at -20°C until analysis. This assay employs the competitive inhibition enzyme immunoassay technique using ELISA kits of human arginine(ARG) Uscescn Life Science Inc. Wuhan. A polyclonal antibody specific for human arginine has been pre-coated onto a microplate. A competitive inhibition reaction is launched between HRP labeled human arginine and unlabeled human arginine (Standards or samples) with the pre-coated antibody specific for human arginine. The more the amount of human arginine in samples, the less the HRP labeled human arginine bound by pre-coated antibody. The substrate solution is added to the wells,
respectively, and the color develops in opposite to the amount of human arginine bound in the initial step. The color development is stopped and the intensity of the color is measured.

**Statistical analysis:**

The collected data were statistically analyzed using SPSS version 12. For quantitative data, the range, mean and standard deviation were calculated. The difference between two means was analyzed using the students (t) test. For comparison between more than two means, the F value of analysis of variance (ANOVA) was calculated. Pearson's correlation coefficient (r) was calculated to test the association between two variables. For qualitative data, the number and percent distribution were calculated. Z-value was used as a test of significance.

**Results:**

Table (1): *Demographic characters of the studied groups.*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study groups</th>
<th>Group I (n = 30)</th>
<th>Group II (n = 10)</th>
<th>*Test of significance</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age</td>
<td>Group I</td>
<td>32 ± 2</td>
<td>33 ± 2</td>
<td>1.364</td>
<td>0.178</td>
</tr>
<tr>
<td>(weeks)</td>
<td>Group II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>Group I</td>
<td>1.9 ± 0.2</td>
<td>2 ± 0.3</td>
<td>1.202</td>
<td>0.236</td>
</tr>
<tr>
<td></td>
<td>Group II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male/ Female</td>
<td>Group I</td>
<td>15/15</td>
<td>6/4</td>
<td>0.176</td>
<td>0.567</td>
</tr>
<tr>
<td>Mode of delivery</td>
<td>Group II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(VD/CS)</td>
<td>Group I</td>
<td>9/21</td>
<td>3/7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Group II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
*Unpaired t-test for gestational age and weight. * Chi-square test for neonatal sex and mode of delivery.

VD: vaginal delivery. CS: cesarean section

No statistically significant difference regarding the demographic criteria of both groups.
Table (2): *Comparison between both groups regarding blood L-arginine levels in the 1st day and 3rd day.*

<table>
<thead>
<tr>
<th>Group</th>
<th>1st day level X ± S.D</th>
<th>3rd day level X ± S.D</th>
<th>Test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases (30)</td>
<td>30.1 ± 6.1</td>
<td>60.2 ± 1.1</td>
<td>26.59</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Control (10)</td>
<td>81.8 ± 5.6</td>
<td>80.1 ± 5.2</td>
<td>0.703</td>
<td>0.490</td>
</tr>
<tr>
<td>P value</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (2) shows that the mean blood L-arginine level in preterm babies with RDS was significantly lower than that of normal preterm (30.1±6.1 versus 81.8±5.6), and demonstrated significantly increase blood L-arginine level on the third day of life in cases, while there is no significant difference between first and third day in control group.

Our measures demonstrated significantly reduced blood L-arginine levels in severe cases of RDS (Downes score > 8) compared to less severe cases (fig.1) and there was statistically highly significant negative correlation between blood L-arginine levels and the severity of RDS {Pearson correlation co-efficient (r) -0.629} (fig. 2).
Our results pointed out that there was no statistically significant difference between L-arginine levels either on first or third days measures regarding the prognosis of our cases [24 case (80%) improved, development of bronchopulmonary dysplasia (BPD) in 3 cases (10%) and 3 cases (10%) died]

There were positive significant correlations between L-arginine levels and pH, and PO2, of premature infants, whiles the correlation with HCO3 not significant. Meanwhile, there was negative significant correlation between L-arginine and PCO2.

Also, we found positive correlation between L-arginine levels and gestational age and weight of premature infants, but without statistical significant difference.
Discussion

The demographic data of our patients showed equal frequency of males and females. This disagree with Ingemarrson (2003) (6) who reported higher RDS in males and also that deaths occurring secondary to respiratory distress syndrome are greater for males. The results of Ingemarrson (2003) (6) regarding death of RDS neonates were agreed with our results as the death rate was higher in male preterm (2/3). The majority of our patients were delivered by caesarean section (70 %), this is in agreement with the study of Borkowski and Mielniczuk (2007) (7) who found that odds for RDS are increased by cesarean section; they added that cesarean section before labor versus cesarean after onset of labor increased additionally odds for RDS.
In our study, the mean blood L-arginine level in preterm babies with RDS was significantly lower than that of normal preterm infants (30.1±6.1 versus 81.8±5.6). This finding could be due to various possible mechanisms; one of them is based on a "consumption" theory, as Canpolat et al., 2005 (8) said that infants with RDS might use more L-arginine for producing more nitric oxide (NO) to reduce pulmonary vascular tone via nitric oxide synthetase than would patients without RDS. Another possible mechanism involves patients' genetic variability and polymorphisms, related to enzymes with a role in L-arginine or NO production. This second theory was used in genetic polymorphism research where neonatal pulmonary hypertension patients were included, and nitric oxide synthetase gene was investigated. However, there was no relationship between polymorphism of the nitric oxide synthetase gene and neonatal pulmonary hypertension (9). It was speculated that in stressed environment (i.e., after hypoxia) , prolonged inhibition of nitric oxide synthetase activity produces activation of leukocytes, capillary leak, the release of secondary mediators, and subsequent regulation of vascular tone (10).

Our results agree with the results of El-Sayed et al., (2011) (11), who pointed out decrease serum L-arginine in preterm infants with RDS. Also Canpolat et al., 2005 (8)
demonstrated reduced blood L-arginine concentrations in preterm infants with RDS compared to control group.

In our study there was significantly increase blood L-arginine level on the third day of life in the cases, while no significant difference between first and third day in control group. These results in concordance with Zamora et al (1998) (12), who measured L-arginine in preterm infants with RDS in first, third, 7th and 14th day of life and pointed out increased L-arginine with advanced neonatal period. This may be due to mechanical ventilation or improvement in severity of RDS.

Performing comparative analysis of L-arginine levels in respect to demographic parameters, we found reduced L-arginine levels in premature infants less than 34 gestation weeks compared to more than 34 gestation weeks and this difference was statistically significant. As regard clinical parameters, our measures demonstrated significantly reduced blood L-arginine levels in severe cases of RDS (Downe score > 8) compared to less severe cases and there was highly significant negative correlation between blood L-arginine levels and the severity of RDS measured by Downe score. These results agree with the study of Canpolat et al., (2005) (8) who demonstrated that blood L-arginine concentration was inversely related to increasing severity of RDS. This finding most likely reflects the consumption of L-arginine or could be explained by a generalized increase in catabolism in these
infants. On other hand El-Sayed et al., (2011) (11), in their study on 71 premature infants (gestational age 29-35w) with and without RDS demonstrated no relation between blood L-arginine levels and the severity of RDS.

Regarding the prognosis of premature infants with RDS, our results demonstrated improvement in 24 cases (80%), development of bronchopulmonary dysplasia (BPD) in 3 cases (10%) and 3 (10%) cases died. Our results pointed out that there was no statistically significant difference between L-arginine levels either on first or third day regarding the prognosis of our cases. These results were in concordance of the study of El-Sayed et al., (2011) (11) who demonstrated no relation between blood L-arginine levels and the subsequent complications and pointed out that L-arginine was lower in infants with RDS but it did not differ between infants who developed BPD and those who did not. Heckmann et al., (2004) (13) demonstrated although it was shown in premature infants that severe cases of RDS had lower concentrations of blood L-arginine, infants who later developed BPD were reported to have normal blood L-arginine concentrations after birth. These finding can be explained as the pathway that connects hypoxia with pulmonary hypertension does not necessarily depends on L-arginine as hypoxia can directly inhibit the activity of NO synthetase enzyme system and impact the pulmonary vascular pressure (14).
Conclusion: Lower level of L-arginine was associated with the severe form of respiratory distress syndrome in premature neonate, while there was no relation to the prognosis of RDS.

References:


مستوى الأرجينين في متلازمة صعوبة التنفس المبكرة عند الأطفال

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يعتبر الأرجينين مادة هامة في جسم الإنسان سواء في حالة الصحة وحالة المرض، فهو
يُساعد جسم الإنسان على التخلص من المواد السامة، كما يُساعد على رفع مقاومة الجسم وتعمل في
تكوين العديد من الهرمونات المفيدة لجسم الإنسان. ومن الوظائف الأساسية للأرجينين أن يُدخل في
صناعة مادة أكسيد النيتريك الذي يعتبر باسط مهم للأوعية الدموية. كما وجد أن نقص مستوى مادة
الأرجينين في دم الأطفال في حالات مرضية كثيرة مثل التهابات القولون الداخلي والأزمة الصدرية
وارتفاع ضغط الدم الرئوي خاصية في الأطفال حديثي الولادة والمبسرين.

الهدف من الدراسة: مقارنة بين مستوى مادة الأرجينين في دم الأطفال حديثي الولادة الذين يعانون
من متلازمة سوء التنفس ومقارنتهم بالأطفال حديثي الولادة الطبيعيين.

وُضِعت الدراسة في قسم الأطفال وحدة العناية المركزية لحديثي الولادة بمستشفى بنها الجامعي. غُطي
30 حالة من الأطفال المتسرين الذين يعانون من متلازمة صعوبة التنفس ومقارنتهم مع 10 أطفال
اصحاح وتم عمل اثني لكل طفل:

1- تاريخ الحمل والولادة لكل طفل 2- فحص طبي كامل
2- أخذ عينة في خلال أول ساعتين بعد الولادة ثم بعد 72 ساعة لقياس مستوى الأرجينين في
الدم مع قياس غازات الدم.

وقد أظهرت النتائج أن متوسط مستوى الأرجينين في دم الأطفال حديثي الولادة كان
30.1±5.6 في حالات المتلازمة لضيق التنفس و30.1±5.6 في الحالات الطبيعية،
وهذا الاختلاف ذو دلالة إحصائية. كما وجد أن متوسط مستوى الأرجينين في دم الأطفال حديثي
الولادة في حالات المتلازمة لضيق التنفس الشديدة كان أقل من متوسط مستوى الأرجينين في دم
الأطفال حديثي الولادة الآخرين (27.1 في الحالات الشديدة) وهذا الاختلاف ذو دلالة إحصائية.
وقد خلصت الدراسة إلى أن مستوى الأرجينين في الدم يكون أقل في حالات متلازمة ضيق
التنفس عند حديثي الولادة وبالخصوص في الحالات الشديدة، بينما لم يرتبط انخفاض مستوى الأرجينين
بوفيات هذه الحالات.