EVALUATION OF PLASMA D-LACTATE LEVEL IN PRETERM INFANTS WITH NECROTIZING ENTEROCOLITIS

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ABSTRACT:

Background: *Necrotizing enterocolitis (NEC) is a life - threatening condition of the intestines that manifests solely in premature newborns. Early identification of developing NEC is still challenging, as the current radiology and laboratory tests lack sufficient accuracy.*

Aim of the work: *The current study aimed to assess the role of plasma D-lactate as a diagnostic and prognostic biomarker for NEC.*

Patients and Methods: *The study encompasses 30 preterm neonates diagnosed according to clinical and radiographic criteria as NEC, in addition to 30 preterm growing neonates as matched controls. An assay of plasma D- lactate levels was done by Enzyme-linked immunosorbent assay (ELISA).*

Results: *The plasma D-lactate levels of NEC patients were significantly higher than those of the controls ($p < 0.01$). The levels of plasma D lactate also showed a statistically significant increase with worse prognosis of Bell's stage of the patients ($p < 0.01$). The best cut-off point of D-lactate to identify cases with NEC was > 5.4 µmol/ml with diagnostic sensitivity and specificity of 100%.*

Conclusion: *Measuring plasma D-lactate levels could be useful as a diagnostic biomarker in the early stage of NEC. Moreover, D-lactate levels can be correlated with the severity of the disease.*

Keywords: *NEC, Preterm, D-lactate, Biomarker.*

INTRODUCTION:

Prematurity is a significant health problem. Complications arising from premature birth are the primary contributor to mortality among children below the age of five [(1)]. Necrotizing enterocolitis (NEC) is a serious gastrointestinal problem predominantly impacting infants born prematurely[(2)]. The pathophysiology of the disease probably occurs as a result of innate immune response against the intestinal microbiota from the premature infant's intestinal tract, resulting in inflammation and injury of the intestinal tract[(3)]. Currently, the identification-ion of NEC depends on clinical and radiographic observations. Once babies are diagnosed with definitive NEC (Bell's stage 2), substantial intestinal damage is prone to develop. So, earlier diagnosis and appropriate treatment of the condition could potentially hinder the progression of the disease[(4&5)]. Achieving an accurate early diagnosis is hampered by the absence of sensitive diagnostic noninvasive tests[(6)].

Plasma lactate level serves as a marker of intestinal flora normal metabolism. Progressive ischemic injury to the mesentery with mucosal damage and the disruption in the homeostasis of the intestinal flora lead to bacterial translocation and may cause an increase in serum lactate levels[(7)].

AIM OF THE WORK:

Our study aimed to study the levels of plasma D-lactate in preterm infants diagnosed with NEC in comparison to age matched controls, and also to correlate D-lactate levels with severity of the intestinal disease.

PATIENTS AND METHODS:

This is a comparative cross-sectional case-control study that was carried out at the Neonatal Intensive Care Unit (NICU), at Ain Shams University Hospitals. The study was conducted for 6 months duration. The study included 60 preterm neonates who were categorized into 2 groups (group A and group B). Group A (patients' group) included 30 preterm neonates with proven NEC; diagnosed with radiological and /or clinical criteria. Group B (Controls' group) included 30 preterm infants admitted to NICU as grower babies after exclusion of NEC, sepsis, systemic inflammatory response syndrome, or an inborn error of metabolism. The control subjects were selected to be postnatal age and sex-matched with the patients. Infants with major congenital anomalies and those who needed recent surgery were excluded.

All participants were subjected to history taking (including antenatal, perinatal, and postnatal period complications, indications for admission, and symptoms of the diseases) and clinical examination including(vital signs, general and abdominal examinations; emphasizing on the presence of feeding intolerance, measurement of waist circumference, intestinal sounds, passage of stool, presence of gastric residual, abdominal tenderness, and rigidity). NEC disease stage was determined according to Bell's staging criteria into neonates with an early diagnosis of suspected NEC (stage I) or definite NEC (stages II or III)[8].

Laboratory investigations were done including plasma D-lactate level assessment. Blood was sampled by venipuncture and centrifuged to prepare plasma that was stored at -20°C. Plasma D-lactate concentration was measured using a commercially available double-antibody sandwich (non-competitive) enzyme-linked immunosorbent assay (the test kit was produced in the Shanghai Sunred Biological Technology Co., Ltd., Shanghai, China). After adding 40 uL of each sample to the test wells, we added both 10 uL of D-lactate antibody and 50 uL of Streptavidin HRP. Then the plate was sealed with an adhesive strip and incubated at 37°C for 60 minutes. Following three wash cycles, any residual wash solution was eliminated by either aspirating or decanting. Each well then received 50 uL of chromogen solution A and 50 uL of chromogen solution B followed by a ten- minute incubation. Finally, 50 uL of stop solution was introduced into each well. Optical density was read at 450 nm utilizing a microtiter plate reader within a 15- minute timeframe. Plasma D-lactate levels were quantified by comparison with a predefined set of standards. Plasma D-lactate levels were measured twice for patients; first at enrollment in the study (once diagnosed with NEC) and a second sample after one week. Plasma D-lactate is measured once for controls at enrollment in the study.

Statistical analysis:

The data collected were coded, processed, and analyzed with Statistical Package for Social Sciences (SPSS) version 26 for Windows® (IBM, SPSS Inc, Chicago, IL, USA). Categorical data were presented as number and percentage and analyzed by the Chi-square test. Values were expressed as mean and standard deviation in case of parametric data, and as median and interquartile range in case of skewed data. The comparison between two groups regarding quantitative parameters was done by using independent t-test for data with normal distribution, and Mann-Whitney test for data

with non-parametric distribution. Comparison between more than two groups regarding quantitative parameters was done by using Kruskal-Walli's test. Wilcoxon Ranks signed test was used for the comparison between two paired groups regarding quantitative parameters with non- parametric distribution. P value > 0.05 was considered non-significant. P value < 0.05 was considered significant. P value < 0.01 was considered highly significant. Receiver operating characteristic curve (ROC) was used to detect the best cut off point, sensitivity, and specificity.

Ethical consideration:

The study is conducted following Helsinki Standards as revised in 2013[16]. The study was conducted after obtaining approval from the local ethics committee, Faculty of Medicine, Ain-shams University, and obtaining written/oral informed consent from the legal guardians of included cases and controls (FMASU MS 151/2022)

RESULTS:

60 subjects were included in our study. They were divided into two groups: patients' group (n = 30), and controls' group (n = 30). The demographic data and characteristics of all studied subjects are included in Table 1. It shows that there was no statistically significant difference between the controls and patients regarding demographic data and characteristics.

Table 1: Comparison between controls and patients regarding demographic data and characteristics

		Controls (No. = 30)	Patients (No. = 30)	Test Value	P-value
Sex	Male	18 (60.0%)	13 (43.3%)	1.669*	0.196
	Female	12 (40.0%)	17 (56.7%)		
Age at 1st sample (days)	Mean ± SD	18 ± 3	17 ± 3	1.084#	0.283
	Range	12 – 24	11 – 23		
Birth weight (kg)	Mean ± SD	2.02 ± 0.22	1.96 ± 0.24	1.049#	0.299
	Range	1.58 – 2.46	1.48 – 2.44		
Maternal Risk Factor	No	23(76.7%)	19(63.3%)	1.270*	0.260
	Yes	7(23.3%)	11(36.7%)		
Consanguinity	No	17(56.7%)	19(63.3%)	0.278*	0.598
	Yes	13(43.3%)	11(36.7%)		

* Chi-square test, # Independent t-test

Table (2) shows the modified Bells' staging of the studied patients. The table also shows that from the thirty studied patients; four patients (13.3%) were died.

		Patients No. = 30
Modified Bell's stage	Ia	6 (20.0%)
	IIa	7 (23.3%)
	IIIa	3 (10.0%)
	Ib	5 (16.7%)
	IIb	8 (26.7%)
	IIIb	1 (3.3%)
Disease Outcome	Discharged	26 (86.7%)
	Died	4 (13.3%)

Table 2: Bell's staging and Disease outcome among the studied patients

Table (3) shows comparison between the levels of plasma D-lactate of the first samples of both patients and controls. It revealed that D-lactate levels were significantly higher in cases than controls.

Table 3: D-lactate levels in patients versus controls.

D-Lactate 1st sample (µmol/ml)	Controls No. = 30	Patients No. = 30	Test Value	P-value
Median (IQR)	4.11 (1.99 – 4.96)	7.11 (6.79 – 11.82)	6.654**	<0.01

** Mann-Whitney test

Table (4) shows that there was statistically significant increase in the level of plasma D-lactate of the 1st and 2nd samples of patients' group with the increase in modified Bells' grading.

Table 4: Plasma D-lactate in different modified Bells' grades

Modified Bells' stage	Lactate 1 (µmol/ml) Median (IQR)	Test Value	P-value	Lactate 2 (µmol/ml) Median (IQR)	Test Value	P-value
Ia	6 (5.48 – 6.79)	23.196 ¤	<0.01	4.54 (3.8 – 5.8)	19.792¤	<0.01
Ib	6.71 (6.7 – 6.8)			5.6 (5.51 – 5.8)		
IIa	7.1 (6.8 – 7.87)			6.26 (6.1 – 6.56)		
IIb	12.16 (7.75 – 13.22)			6.28 (5.82 – 7.03)		
IIIa	15.17 (7.49 – 15.76)			13.29 (8.33 – 19.3)		
IIIb	28.26 (28.26 – 28.26)			23.27 (23.27 – 23.27)		

¤ Kruskal-Walli's test

By ROC curve analysis, it was shown that plasma D-lactate can detect patients with NEC at the cut off >5.4 µmol/ml with sensitivity of 100.0%, specificity of 100.0% and area under curve (AUC) of 100% Diagram (1).

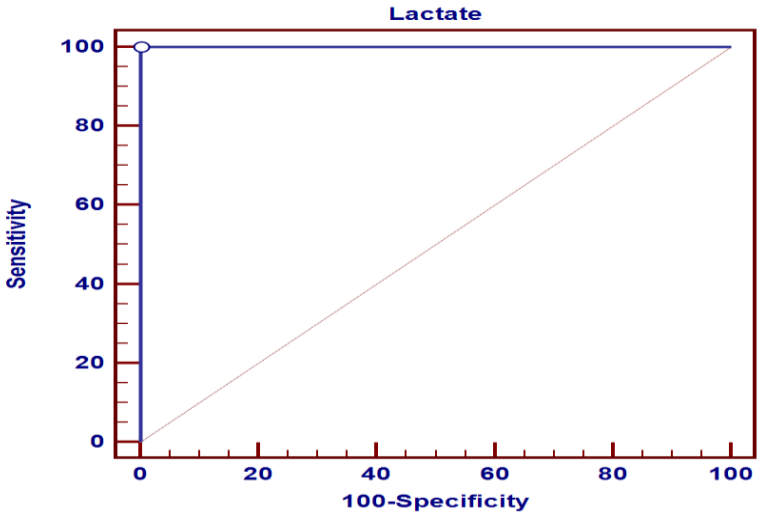

Diagram 1: Receiver operating curve (ROC) for Plasma D-Lactate Level in µmol/ml to detect cases with NEC.

Table (5) shows that levels of plasma D-lactate of both first and second samples were significantly higher in died than discharged patients with P-value <0.05 and <0.01 respectively. As regard the discharged patients; The table also shows that the levels of D-lactate of the second samples were significantly lower than those of the first

samples with p-value <0.01, while no statistically significant difference was found between d-lactate levels of the 1st and 2nd samples in died patients with p-value >0.05.

Table 5: Correlation between patients' outcome and Plasma D-Lactate levels.

		Discharged patients. No. = 26	Died patients. No. = 4	Test value	P-value
Lactate (1st sample) (µmol/ml)	Median (IQR)	7.04 (6.71 – 7.87)	15.46 (11.33 – 22.01)	-2.502**	<0.05
Lactate (2nd sample) (µmol/ml)	Median (IQR)	5.93 (5.51 – 6.31)	16.29 (10.81 – 21.28)	-3.174**	<0.01
Willcoxon Ranks signed test	Z	-4.457	-0.365		
	P-value	<0.001	0.715		

** Mann-Whitney test

DISCUSSION:

Necrotizing enterocolitis (NEC) stands as a primary contributor to morbidity and mortality in premature infants[9]. Several risk factors have been identified, encompassing small for gestational age, premature rupture of membranes, assisted ventilation, sepsis, and hypotension[10]. Additional risk factors encompass formula feeding, exposure to acid suppression medication, and the use of antibiotics[11]. Early accurate detection of NEC represents a major objective of clinical practice, but this is hindered by the absence of a sensitive test to early diagnose this condition[12]. Lactate plasma level serve as an indicator of the normal metabolic processes of the intestinal flora. The pathogenesis of NEC is characterized by progressive development of ischemic damage to the mesentery and alterations in the equilibrium of the intestinal flora leading to bacterial translocation. This in turn may result in elevated plasma lactate levels[13].

Herein this study we investigated plasma D lactate levels in NEC patients and matched controls. The study included 30 preterm neonates diagnosed with NEC and 30 preterm matched neonates who were admitted into NICU as grower babies.

We found that plasma lactate levels in NEC patients were statistically significantly higher as compared to those of the controls. These results agreed with Lei et al. and El-Abd Ahmed et al. who found that plasma D-lactate was significantly higher in NEC patients than feeding intolerance or control groups[14,15]. Moreover, Garcia et al. reported an increase in urinary lactate levels in cases of NEC which corresponds to heightened enteric bacterial activity in affected infants[16]. Also this copes with the study done by Herzlich et al. and gave a clue in the differentiation between NEC and spontaneous intestinal perforation (SIP) preterm infants, the study reported that NEC preterm infants showed a combination of increase plasma glucose and lactate levels, while SIP preterm infants showed an increase plasma glucose along with decreased lactate levels[17].

In our study, the best cutoff point of D-lactate to identify cases with NEC was > 5.45 µmol/ml with sensitivity of 100.0%, specificity of 100.0%, suggesting a promising role of this biomarker in the early diagnosis of NEC.

In the current study, there was a statistically significant increase in the lactate levels with the increase in the modified bell's stage of the patients.Within the same line, Lei et al. conducted a study on plasma D-lactate in premature infants suffering from NEC, and they discovered a significant

elevation that correlated directly with the severity of the intestinal disease[14].

We also found that plasma D-lactate levels of both first and second samples were significantly higher in the group of patients who died as compared to those of the discharged patients.This came in the same line with Wang et al. who reported the same finding[18]. In addition, we reported that plasma levels of D-lactate of the second samples of discharged patients decreased significantly as compared to their first samples, while no significant difference was found between the level of D-lactate of first and second samples of the patients who have died. The previous findings highlighted a possible role of plasma D-lactate in predicting the prognosis and outcome of NEC among such patients.

Conclusion:

In premature infants, necrotizing enterocolitis is a common and serious pathology that is associated with a high morbidity and mortality rate. As a marker of intestinal injury, plasma D-lactate levels may aid in the early diagnosis of NEC. In addition, the severity and prognosis of NEC has been shown to have a correlation with D-lactate levels.

Declaration:

Consent for publication:

not applicable.

Availability of data and materials:

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interest:

The authors declare that they have no competing interests.

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Authors' contributions:

Ola G El-Farghali and Mohamed Hussein Metwally analyzed and interpreted the patient data regarding NEC diagnosis and staging. Dina Tharwat Ghanem and Ahmed Hamdy Farid performed the laboratory investigations. Dina Tharwat Ghanem was a major contributor to writing the manuscript. All authors read and approved the final manuscript.

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List of abbreviations

NEC : Necrotizing enterocolitis
NICU : Neonatal intensive care unit
SPSS : Statistical Package for Social Sciences
AUC : Area under curve
ROC : Receiver operating characteristic curve
SIP : Spontaneous intestinal perforation

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تقييم مستوى البلازما D -لاكتات عند الخدج المصابين بالتهاب الأمعاء والقولون الناخر

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الخلفية : التهاب الأمعاء والقولون الناخر هو مرض شديد وربما مميت يحدث حصرا عند الخدج. لا يزال التشخيص المبكر لهذا المرض يمثل تحديًا، حيث تفتقر الأشعة والاختبارات المعملية الحالية إلى الدقة الكافية.

الهدف : تهدف الدراسة الحالية إلى تقييم دور البلازما D-لاكتات كمؤشر حيوي تشخيصي وإنذاري لمرض التهاب الأمعاء والقولون الناخر.

الطرق : شملت الدراسة 30 من الولدان الخدج الذين تم تشخيص إصابتهم بـ التهاب الأمعاء والقولون الناخر بالإضافة إلى 30 من الولدان الخدج الذين ينمون كعناصر تحكم متطابقة. تم إجراء فحص مستويات D- اللاكتات في البلازما باستخدام مقايسة الامتصاص المناعي المرتبط بالإنزيم. وقد تم تحليل البيانات باستخدام الإحصاء الوصفي والاستنتاجي.

النتائج : كانت مستويات D-لاكتات البلازما لدى مرضى التهاب الأمعاء والقولون الناخر أعلى بكثير من تلك الموجودة في المجموعة الضابطة ($p < 0.01$). كما أظهرت مستوياتD - لاكتات في البلازماا زيادة ذات دلالة إحصائية مع التشخيص الأكثر سوءا لمرحلة بيل لدى المرضى ($p < 0.01$). كانت أفضل نقطة قطع لـ D-لاكتات لتحديد الحالات المصابة بـ التهاب الأمعاء والقولون الناخر هي > 5.4 ميكرومول/مل مع حساسية تشخيصية ونوعية 100%.

الاستنتاج : يمكن أن يكون قياس مستويات D-لاكتات في البلازما مفيدًا كمؤشر حيوي تشخيصي في المرحلة المبكرة من التهاب الأمعاء والقولون الناخر. علاوة على ذلك، يمكن أن ترتبط مستويات D-لاكتات بحدة المرض ونتائجه.