Can biochemical markers predict the severity of hypoxic-ischemic encephalopathy?

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Hypoxic-ischemic encephalopathy (HIE) due to perinatal asphyxia remains an important cause of neonatal morbidity and mortality. The aim of this study was to investigate the predictive values of biochemical parameters, including serum creatine kinase (CK), lactate dehydrogenase (LDH), uric acid (UA), and lactate, in newborns with HIE. A total of 94 patients who were diagnosed with HIE were prospectively enrolled into the study. According to the Sarnat and Sarnat classification, 29 (30.9%) patients had Stage I, 36 (38.3%) Stage II, and 29 (30.9%) Stage III HIE. When CK, LDH, UA, and lactate were used together in order to determine the stage of HIE, specificity and sensitivity were calculated to be 87% and 94%, respectively. Measurement of serum CK, LDH, lactate, and UA levels together is a promising method in determining the stage of hypoxia in the laboratory before clinical manifestations occur so that hypothermia treatment can be initiated earlier.

Key words: hypoxic-ischemic encephalopathy, creatine kinase, lactate dehydrogenase, uric acid, lactate.

Hypoxic-ischemic encephalopathy (HIE), the most severe outcome of perinatal asphyxia, is a syndrome characterized mainly by abnormal muscle tone and reflexes, an altered level of consciousness, and commonly by convulsions[1]. These changes occur due to cerebral hypoxia, which a newborn may develop for a variety of reasons. HIE remains a common cause of death in newborns, and newborns who survive are prone to developing serious neurological disorders such as cerebral palsy[2].

The gold standard in the diagnosis of asphyxia is the clinical assessment of the neurological state of newborns in the early postnatal period. The widely used staging system, known as the Sarnat and Sarnat scoring system following a revision, is considered to be an important method in the initial assessment of newborns with asphyxia and in the prediction of the prognosis[3]. According to this staging system, HIE is divided into three stages based on the level of consciousness, muscle tone, posture, deep tendon reflexes, presence of myoclonus, appearance of the pupils, presence of seizure, duration of symptoms, and results of conventional EEG[4]. Nowadays, researchers work on numerous biochemical markers so as to use them in the diagnosis of perinatal asphyxia and HIE in order to initiate hypothermia treatment as soon as possible. Therapeutic hypothermia is beneficial to term newborns with Stages II and III HIE, and cooling reduces mortality and major disability in survivors. However, to the best of our knowledge, only a few among these markers are specific to the stages of HIE, and could thus possibly be used in staging the disease.

The objective of this study was to investigate the predictive values of biochemical parameters, including serum creatine kinase (CK), lactate dehydrogenase (LDH), uric acid (UA), and lactate, in newborns with HIE.

Material and Methods

This is a prospective case series study focusing on a total of 94 patients who were diagnosed with HIE and monitored in the Neonatal Intensive Care Unit (NICU) of Dr. Sami Ulus Obstetrics and Gynecology, Children's Health and Disease Training and Research

Hospital between January 2012 and May 2013. This study was performed with permission from the local Ethics Committee of Keçiören Teaching and Research Hospital, Ankara, Turkey (Approval number: 327).

A medical condition that met one or more of the criteria set by the American College of Obstetrics and Gynecology and the Society of Obstetricians and Gynecologists of Canada was accepted as perinatal asphyxia[5,6]. Patients with a gestational age of <36 weeks and those with major congenital malformation, chromosomal abnormalities, metabolic disorders, congenital viral infection, birth trauma, and septic shock were not included in our study. Gestational age, birth weight, and risk factors for HIE (absence of antenatal care, intrauterine growth restriction, diabetic mother, fetal distress, prolonged rupture of membranes, oligohydramnios and preeclampsia, vacuum-assisted vaginal delivery, cesarean section, abnormal position, umbilical cord entanglement, ablatio placenta, placenta previa, meconium at birth, APGAR score, the need for resuscitation) were also recorded. The patients with HIE were divided into three groups (Stage I: mild, Stage II: moderate, and Stage III: severe) according to the Sarnat and Sarnat staging system within 48-72 hours following their admission to the NICU[3]. Blood samples were taken from all the patients in these three groups within 15 minutes following their admission to the NICU, and these samples were analyzed for blood gas and whole blood count parameters, blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), CK, LDH, lactate, and UA levels. The predictive values of these biochemical markers in determining the stage of HIE were assessed.

Data were recorded using the Statistical Package for the Social Sciences (SPSS) 15.0 (Chicago, IL, USA). Descriptive analyses were performed. Mean (standard deviation [SD]) or median (interquartile range [IQR]) values were given according to distribution of the data. Correlations between the biochemical markers were tested using Pearson or Spearman test, as appropriate. In the intergroup comparison of the three groups, one-way ANOVA test was used for normally distributed variables; in the intragroup comparison of each of the three groups, the Scheffé test was used whenever the variances were equal ($p<0.05$) according to Levene's test, and Tamhane's T2 test was used whenever the variances were not equal ($p>0.05$). For variables that did not follow a normal distribution between the three groups, Kruskal-Wallis test was used. In the intergroup comparison of qualitative data, chi-square test was used. A receiver operating characteristic (ROC) curve analysis, wherein the "state variable" was set to HIE Stage ≥II, meaning advanced HIE stage, was performed to determine the cutoff values of CK, LDH, UA, and lactate levels. Accordingly, specificity, sensitivity, positive predictive value (PPV), negative predictive value (NPV), and accuracy rate were calculated. $p<0.05$ was considered statistically significant.

Results

A total of 94 patients, with a male rate of 57.4 (n=54), were included in the study. The mean gestational age and birth weight were $39^{1/7}\pm2.0$ weeks and 3307 ± 540 g, respectively. The cesarean rate was 53.2% (n=50). APGAR scores at 1 and 5 minutes were 4 (0-9) and 7 (2-10), respectively. According to the classification of Sarnat and Sarnat, 29 (30.9%) patients had Stage I, 36 (38.3%) Stage II, and 29 (30.9%) Stage III HIE.

There was no statistically significant difference among the three groups with regard to sex, gestational age, birth weight, type of delivery, age at NICU admission, and antenatal risk factors ($p>0.05$). Patients with Stage III HIE had lower 5-minute APGAR scores than those with Stage I and Stage II HIE ($p=0.001$); there were no difference between Stage I and Stage II HIE (Table I).

The intergroup comparison of blood parameters showed that among the blood gas parameters, pH, HCO_3 and base excess (BE) levels were the lowest in patients with Stage III HIE ($F=9.345$, $p=0.0001$; $F=5.345$, $p=0.006$; and $F=14.647$, $p=0.0001$, respectively). No statistically significant difference was detected among the three groups with regard to hemoglobin (Hb) and leukocyte levels. However, platelet count was the lowest in the group of patients with Stage III HIE ($F=0.648$, $p=0.525$; $F=2.687$, $p=0.074$; and $F=6.630$, $p=0.002$, respectively).

Among the biochemical parameters, the levels of BUN and creatinine were the highest in

Table I. Demographic Data of the Patients

	Stage I (n=29)	Stage II (n=36)	Stage III (n=29)	p
Gender (female/male)	13/16	13/23	14/15	0.588
Gestational age (weeks)	38.9 (36.0-41.0)	38.7 (36.0-41.0)	39.5 (36.0-41.0)	0.159
Birth weight (grams)	3288±540	3409±599	3198±451	0.291
Mode of birth (V/CS)	11/18	21/15	12/17	0.204
Maternal risk factors				
GDM	1 (3.4%)	1 (3.4%)	1 (3.4%)	0.984
Preeclampsia	3 (10.3%)	0	1 (3.4%)	0.117
PROM	1 (3.4%)	1 (3.4%)	2 (6.9%)	0.692
APGAR score (5 minutes)	7 (5-10)	6 (2-10)	5 (2-10)	0.001
Admission age in NICU (hours)	3 (2-6)	2 (2-5)	2 (1-5)	0.234

C/S: Cesarean section. GDM: Gestational diabetes mellitus. NICU: Neonatal intensive care unit. PROM: Premature rupture of membranes. V: Vaginal.

Table II. Laboratory Data (Blood Gas, Complete Blood Count, Liver and Kidney Function Tests) of the Patients According to the HIE Stages

	Stage I (n=29)	Stage II (n=36)	Stage III (n=29)	F	X^2	p (within groups)	p (between groups)
pH**	7.31±0.09	7.20±0.19	7.10±0.20	9.345		0.0001	p_{1-2}:0.060 p_{2-3}:0.107 p_{1-3}:0.001
BE**	-8.3±5.1	-10.9±5.9	-16.7±6.9	14.647		0.0001	p_{1-2}:0.155 p_{2-3}:0.003 p_{1-3}:0.001
Bicarbonate**	16.2±5.2	14.6±5.9	11.6±5.1	5.345		0.006	p_{1-2}:0.584 p_{2-3}:0.089 p_{1-3}:0.004
Hemoglobin (g/dl)**	16.3±2.4	16.4± 2.3	15.7±3.1	0.648		0.525	
Leukocyte (/mm^3)**	23727.6± 8341.5	20452.8± 8334.9	25575.9± 10507.6	2.687		0.074	
Thrombocyte (/mm^3)**	234068.9± 76801.2	241888.9± 85940.1	177000.0± 60331.8	6.630		0.002	p_{1-2}:0.973 p_{2-3}:0.002 p_{1-3}:0.008
BUN (mg/dl)*	7 (1-18)	11 (4-57)	11 (7-74)		10.616	0.005	p_{1-2}:0.038 p_{2-3}:0.323 p_{1-3}:0.001
Creatinine (mg/dl)**	0.8±0.2	0.9±0.3	1.5±1.2	6.878		0.002	p_{1-2}:0.901 p_{2-3}:0.011 p_{1-3}:0.005
AST (IU/L)*	64 (30-214)	102 (23-1031)	200 (38-4004)		20.778	0.0001	p_{1-2}:0.030 p_{2-3}:0.005 p_{1-3}:0.001
ALT (IU/L)*	16 (5-306)	25 (10-525)	89 (11-916)		17.667	0.000	p_{1-2}:0.003 p_{2-3}:0.041 p_{1-3}:0.001
Albumin (g/dl)**	2.8±0.3	2.9± 0.3	2.7±0.5	3.049		0.052	
Fibrinogen (mg/dl)*	259 (171-452)	155 (58-399)	127 (50-426)		6.901	0.032	p_{1-2}:0.015 p_{2-3}:0.448 p_{1-3}:0.016

*: Kruskal-Wallis test, **: One-way ANOVA test.

ALT: Alanine aminotransferase. AST: Aspartate aminotransferase. BE: Base excess. BUN: Blood urea nitrogen. CRP: C-reactive protein.

Table III. Laboratory Data of the Patients According to the HIE Stages

	Stage I (n=29)	Stage II (n=36)	Stage III (n=29)	F	X^2	p (within groups)	p (between groups)
CK (mg/dl)*	617 (160-3480)	2159 (47-13092)	2664 (238-39252)		13.323	0.001	p_{1-2}:0.002 p_{2-3}:0.694 p_{1-3}:0.001
LDH (IU/L)*	649 (348-1096)	1015 (355-5252)	1729 (580-7865)		19.021	0.001	p_{1-2}:0.009 p_{2-3}:0.028 p_{1-3}:0.001
UA (mg/dl)**	6.3±1.7	6.9±2.4	9.8±3.2	16.917		0.001	p_{1-2}:0.691 p_{2-3}:0.001 p_{1-3}:0.001
Lactate (mmol/L)*	4.4 (1.5-15.6)	4.5 (0.5-25.4)	8.3 (2.6-102.0)		15.753	0.001	p_{1-2}:0.954 p_{2-3}:0.001 p_{1-3}:0.001

*: Kruskal-Wallis test, **: One-way ANOVA test.
CK: Creatinine kinase. LDH: Lactate dehydrogenase. UA: Uric acid.

patients with Stage III HIE (χ^2 =10.616, p=0.005; F=6.878, p=0.002; respectively). Although the levels of ALT and AST were the highest in patients with Stage III HIE, the levels of albumin did not differ in the three groups (χ^2 =17.667, p=0.0001; χ^2 =20.778, p=0.0001; F=3.049, p=0.052, respectively). Fibrinogen levels were the lowest in patients with Stage III HIE when compared with Stage I and Stage II HIE (χ^2=6.901, p=0.032) (Table II).

The comparison of the levels of CK, LDH, UA, and lactate with regard to the three stages of the disease showed that patients with Stage III HIE had significantly higher levels of CK, LDH, UA, and lactate than the patients with Stage I and Stage II HIE (χ^2 =13.323, p=0.001; χ^2 =19.021, p=0.0001; F=16.917, p=0.0001; and χ^2 =15.753, p=0.0001, respectively) (Table III, Fig. 1).

Table IV shows predictive values for the biochemical parameters, according to cutoff points. When the four parameters were used together in order to determine the stage of HIE, their specificity and sensitivity were calculated to be 87% and 94%, with an accuracy rate of 68%. Cutoff points and area under curve (AUC) values obtained from ROC curve analysis, when Stage ≥II was considered as an advanced stage of HIE, are presented in Table V and Figure 2. It was found that LDH has the highest AUC value.

Discussion

Perinatal asphyxia is characterized by the progressive damage that it causes to all organ systems and particularly to the nervous system. It can take up to 72 hours for neurological manifestations to appear[7]. Therapeutic hypothermia is the only medical treatment with promising long-term neurodevelopmental results, and HIE stage is taken into consideration in decisions about whether to administer this treatment or not[8]. However, because this treatment is effective only when it is administered in the first six postnatal hours, the need for biochemical markers that can be used in determining the HIE stage before clinical manifestations of the disease appear becomes evident.

It is reported in the literature that there is an increase in tubular proteins (notably in beta-2 microglobulins and myoglobulins) along with an increase in BUN and creatinine in the early stages of renal failure secondary to perinatal asphyxia[9,10]. An increase in liver enzymes can also be observed due to the effects of liver damage and hypoxia on other organ systems. This increase becomes more evident as the level of hypoxia increases[11]. In our study, we observed a significant worsening in the results of liver and kidney function tests as the stage of HIE progressed. In their study, Fernandez et al.[12] measured the serum CK-BB (brain isoenzyme) activities of 33 full-term

Table IV. The Specificity, Sensitivity, PPV, NPV, and Accuracy Rate of CK, LDH, UA, and Lactate in Determining the Stage of HIE

	Specificity	Sensitivity	PPV	NPV	Accuracy rate
CK	57%	90%	75%	80%	76%
LDH	56%	79%	76%	62%	71%
UA	48%	78%	73%	55%	68%
Lactate	39%	74%	66%	48%	61%
CK+LDH+UA+Lactate	87%	94%	94%	87%	68%

CK: Creatinine kinase. LDH: Lactate dehydrogenase. NPV: Negative predictive value. PPV: Positive predictive value. UA: Uric acid.

Table V. Cutoff Points and Area Under Curve Values Obtained from ROC Curve Analysis

	Cutoff value	AUC	p	CI 95% Lower-upper limits
CK (IU/L)	1196	0.78	0.001	0.65-0.91
LDH (IU/L)	738	0.81	0.001	0.70-0.92
UA (mg/dL)	6.2	0.75	0.003	0.62-0.88
Lactate (mmol/L)	4.4	0.67	0.046	0.51-0.82

AUC: Area under curve. CI: Confidence interval. CK: Creatinine kinase. LDH: Lactate dehydrogenase. UA: Uric acid.

newborns in the 4th and 10th hours of life and discovered that infants who died of severe HIE or developed neurologic sequelae while they were being monitored had significantly higher serum CK-BB activities than infants who did not develop neurological anomalies. Based on this observation, they claimed that a high serum CK-BB activity is a sensitive marker of brain injury. Adhikari et al.[13], on the other hand, showed that 30 newborns with asphyxia (16 severe, 14 moderate) continued to have cerebral edema for more than 48 hours as revealed by cranial ultrasonography, that their serum CK-BB activity was significantly high in the 12th-33rd hours following delivery compared to the control group consisting of healthy newborns, that the sensitivity of cranial ultrasonography in determining the extent of brain damage was 81.2%, that the sensitivity of serum CK-BB activity was 76.4%, and that when used together, these two tests had an accuracy rate of nearly 90% in predicting death and significant neurological damage. In their retrospective study on 97 newborns with asphyxia, Sweet et al.[14] dealt with the predictive value of serum CK-BB levels for conditions like growth retardation, cerebral palsy, vision problems, and deafness or hearing loss caused by perinatal asphyxia in patients with severe HIE. They found that the levels of serum CK-BB increase significantly in patients with severe HIE. However, they calculated the sensitivity of CK-BB for predicting morbidity as 76% and its specificity as 40%. Based on this, they asserted that CK-BB is not a useful marker in the prediction of the neurological results of perinatal asphyxia. In all the above-mentioned studies, the focus was on the levels of CK-BB, which is the brain-specific isoenzyme of CK. Yet, it is a well-known fact that all organ systems, including the cardiovascular and muscular systems, are affected in the hypoxic process. In our study, we preferred to measure total CK levels instead of the levels of the brain-specific isoenzyme of CK because, even though Sarnat and Sarnat staging system is based mainly on the functions of the nervous system, it also involves systems like the cardiovascular and gastrointestinal systems. Relevant studies in the literature seem to focus mainly on the effects of CK-BB levels on neurological morbidity. The results of our study reveal the noteworthy fact that serum total CK levels have a low specificity but high sensitivity in determining the HIE stage. Holzmann et al.[15] showed that fetal scalp blood sampling is an early marker of intrapartum hypoxia, and they claimed that lactate levels may be an earlier marker than pH value when a hypoxic process is present. In a study in which the assessment of a total of 13,735 routine cord acid-base values was analyzed, it was reported that the levels of lactate in the arterial cord blood obtained at birth is as helpful as base

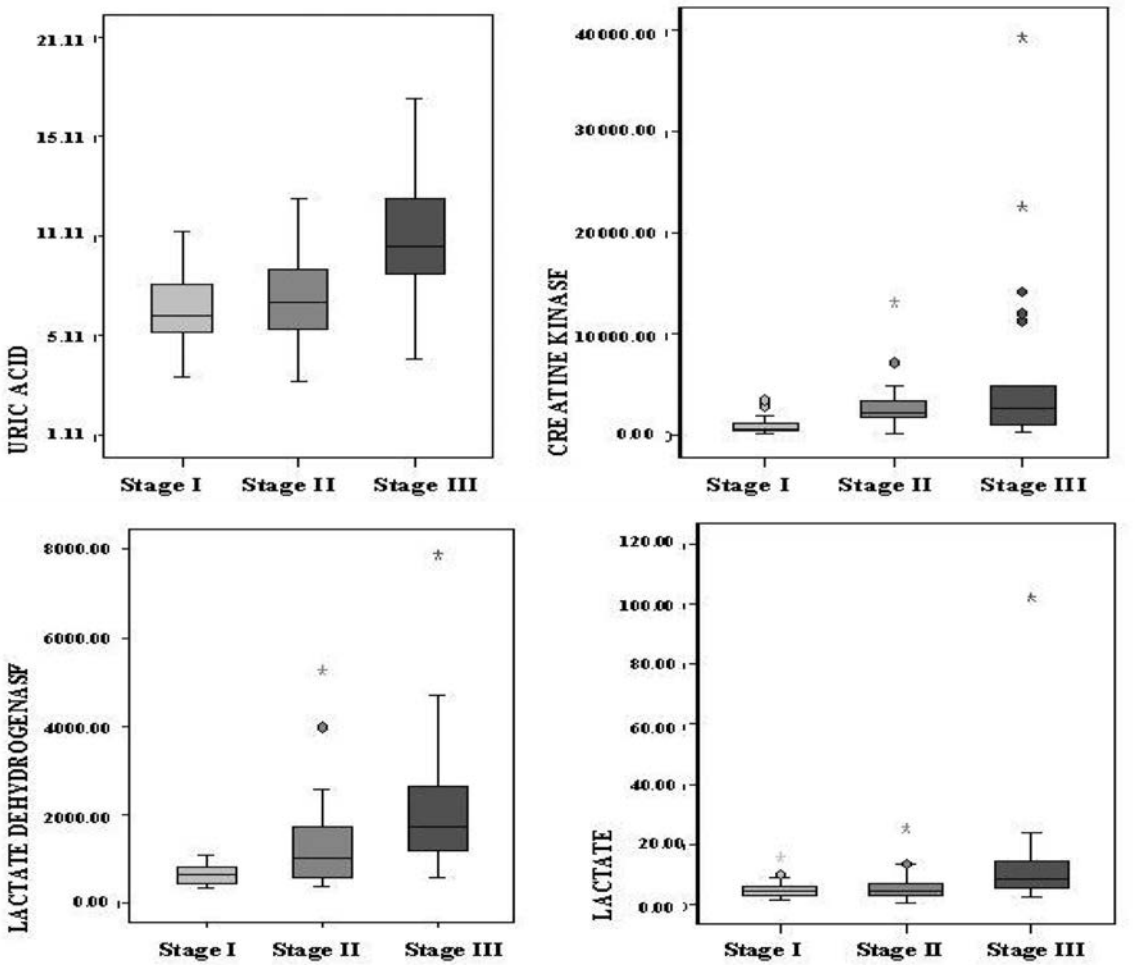

Fig. 1. The comparison of the levels of CK, LDH, UA, and lactate with regard to the three stages of HIE.

deficit in diagnosing a patient with perinatal asphyxia[16]. The above-mentioned studies focus mainly on the diagnosis of HIE. In our study, we assessed serum lactate and LDH levels with regard to the stages of HIE and saw that, as the stage of HIE progressed, the results of the two tests used in measuring serum lactate and LDH levels showed a statistically significant increase. However, it was also observed that when Stage ≥II was accepted as the advanced HIE stage, lactate and LDH levels had low predictive values. To the best of our knowledge, there are only a limited number of studies in the literature that focus on UA levels in perinatal asphyxia. In their studies including 60 newborns with asphyxia and 60 healthy newborns, Basu et al.[17] showed that the newborns with asphyxia had significantly higher plasma UA levels than healthy newborns, and

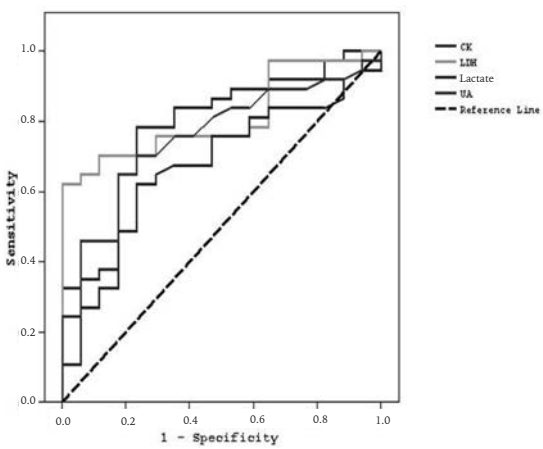

Fig. 2. Sensitivity and specificity of CK, LDH, UA, and lactate in severe HIE.

that there was a positive correlation between UA levels, HIE stage, and APGAR score. Banupriya et al.[18], on the other hand, showed that there was a positive correlation between

urine UA levels, HIE stage, and APGAR score. In our study, we found that serum UA levels increased significantly as the stage of HIE progressed. However, we also found that its specificity and sensitivity in determining the stage of HIE are low.

Creatine kinase (CK), lactate, LDH, and UA are the non-specific markers of hypoxia. In our study, the specificity and sensitivity of these four tests in determining the stage of HIE were calculated to be low when evaluated separately. However, when these tests were used together, their specificity was calculated to be 87%, sensitivity 94%, positive predictive value 94%, and negative predictive value 87%. When Stage ≥II was considered as an advanced stage of HIE, cutoff points were found as follows: CK 1196 IU/L, LDH 738 IU/L, UA 6.2 mg/dl, and lactate 4.4 mmol/L. Based on the results of our study, we can claim that the above-mentioned four tests, which are conducted routinely before the clinical determination of the HIE stage in newborns with perinatal asphyxia according to the Sarnat and Sarnat scoring system and have a high specificity and sensitivity when used simultaneously, can help determine the HIE stage in the laboratory at a much earlier time. Further, determining the cutoff values for these biochemical markers would help clinicians to differentiate Stage I HIE from the advanced stages so that therapeutic hypothermia treatment can be initiated.

In conclusion, HIE is an important cause of mortality and morbidity in newborns. The Sarnat and Sarnat scoring system is a clinical staging system widely used in the initial examination of newborns with asphyxia, in the prediction of prognosis and in determining the correct treatment. Taking into account the fact that even minutes matter when it comes to beginning the hypothermia treatment in a patient with HIE, it appears that the measurement of serum CK, LDH, lactate, and UA levels together is a promising method in determining the stage of hypoxia in the laboratory, before clinical manifestations occur.

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