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ORIGINAL ARTICLE

Metabolomics in Context of Alpha-Ketoglutarate and Lactate in Neonates with Birth Asphyxia

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SUMMARY

Background: **Neonatal birth/perinatal asphyxia is a critical condition that can adversely affect many different bodily tissues, particularly the brain; depending on duration and severity of asphyxia, leading to difficulties and lifelong disabilities. These can be avoided by early detection of the biochemical derangements and prompt intervention. Serum alpha-ketoglutarate (α-KG) and cord blood lactate have been found to be associated with birth asphyxia and may have potential to act as biomarkers for birth asphyxia.**
Methods: **Serum levels of α-KG and cord blood lactate were estimated in 34 birth asphyxiated neonates with clinical evidence of asphyxia. The levels were also analyzed in 46 apparently healthy controls, and data was compared among different groups by using appropriate statistical analysis. Serum α-KG was estimated by enzyme-linked immunosorbent assay (ELISA) and cord blood lactate by blood gas autoanalyzer (BGA) in the serum samples.**
Results: **Serum α-KG levels were found to be increased in birth asphyxiated neonates as compared to healthy controls (p-value = 0.06). Correlation of serum α-ketoglutarate (ng/mL) levels with outcome (discharged/expired) in birth asphyxiated neonates was not found to be statistically significant (r value = 0.156, p-value = 0.384). A statistically significant correlation was not found between severity of birth asphyxia and levels of serum α-ketoglutarate (ng/mL) (r value = 0.029, p-value = 0.86). Also, correlation of cord blood lactate levels (mmol/L) with severity in birth asphyxiated neonates was not found to be statistically significant (r value = 0.326, p-value = 0.10). Correlation between cord blood lactate levels (mmol/L) and outcome in birth asphyxiated neonates (discharged/ expired) was not found to be statistically significant (r value = 0.03, p-value = 0.87), while correlation of cord pH levels and severity of birth asphyxia in cases was found to be highly statistically significant (r value = -0.60, p-value < 0.01)**
Conclusions: **Serum α-KG and cord blood lactate bear the potential to act as biomarkers in neonates with birth asphyxia.**
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KEYWORDS

birth asphyxia, alpha-ketoglutarate (α-KG), cord blood lactate

LIST OF ABBREVIATIONS

ABG - arterial blood gas
ADHD - attention deficit hyperactivity disorder
α-KG - alpha-ketoglutarate
ANOVA - analysis of variance test

ATP - adenosine triphosphate
BE - base excess
BGA - blood gas autoanalyzer
ELISA - enzyme-linked immunosorbent assay
HIE - hypoxic ischemic encephalopathy
IVH - intraventricular hemorrhage
LAMA - left against medical advice
LSCS - lower segment caesarian section
PVL - periventricular leukomalacia
TCA cycle - tricarboxylic acid cycle
WHO - World Health Organization

INTRODUCTION

Birth asphyxia, in general, is one of the leading causes of neonatal brain injury, infant morbidity, and mortality globally in the face of improved antenatal and peripartum care. It affects around four million neonates worldwide per year, causing one million deaths [1]. The pathophysiology of asphyxia typically results in fetal hypoxia, hypercarbia, and acidosis as a result of the disruption of placental blood flow. A recent meta-analysis has shown good association of cord arterial blood gas (ABG) abnormalities (pH < 7.0 and base deficit ≥ 16 mmol/L) with short-term outcomes consisting of mortality, stage of hypoxic ischemic encephalopathy (HIE), intraventricular hemorrhage (IVH), or periventricular leukomalacia (PVL) and long-term adverse outcomes (cerebral palsy) [2].

As per a study on metabolomics, estimation of low molecular weight metabolites present in the body seem to be a promising novel approach in the search of new biomarkers. A targeted approach consisting of investigation of pre-known and expected metabolites were identified to understand the biological pathways involved in HIE for characterizing the levels of severity in HIE cases [3].

Alpha-ketoglutaric acid (also known as 2-oxo-glutaric acid or 2-oxopentanedioic acid) is a low molecular mass metabolite found in all living species. Alpha-ketoglutarate (α-KG), an anion of alpha-ketoglutaric acid, is an endogenous crucial metabolite that has antioxidant properties. It is an important component in the Krebs cycle, influencing its entire pace. It is a nitrogen scavenger and substance that promotes protein synthesis while inhibiting protein breakdown in muscles. It is a precursor of glutamine, an essential metabolic fuel for gastrointestinal tract cells. It can be utilized in clinical applications to improve bone tissue formation by decreasing protein catabolism and increasing protein synthesis [4]. Lactate is produced by astrocytes through aerobic glycolysis and is crucial for brain function. Extracellular lactate is transported to neurons, where it serves as an alternate substrate to glucose (the astrocyte-neuron lactate shuttle) [5,6]. Hyperlactatemia is now recognized as a helpful measure for predicting disease severity and death [7,8]. As part of the stress response, cerebral hyper glycolysis stimulates lactate generation and metabolism as a substitute fuel source for glucose; however, when combined with low oxygen tissue tension, raised lactate and lactate:pyruvate ratio suggest genuine metabolic crisis [9]. Lactate is increasingly being evaluated as a possible predictor of the severity of neurologic injury. Elevated cerebral lactate and low glucose levels indicate ischemia in the presence of hypoxia and predict adverse outcomes [10].

α-KG delays age-related disease. It has been found to prolong the lifespan and reduce the morbidity in elderly mice [11]. Neonatal hypoxia and HIE cases were associated with lower α-KG levels in urine. α-KG has been found to function as neuroprotective agent in ischemic pathology [12]. It is commonly known as the immune nutrition factor and is crucial to the overall immune metabolism. It exhibits anti-inflammatory effects by mediating metabolic and epigenetic reprogramming [13]. It can dissolve well in water, does not show toxic properties, and its water solutions have high stability. Although α-KG is an intracellular metabolite, some of its amount is found in blood plasma. In old people, the levels of α-KG in the blood plasma were found to be significantly lower as compared with young people [14]. The literature available on these functions of α-KG is very limited, and not much research has been done on human subjects regarding metabolomics. Some animal models are there, but the common drawback in all animal models is the validity of the obtained metabolomics pattern to humans; therefore, further studies need to be done in this area [15-17]. So, the present study has been planned to measure levels of the metabolite, α-KG, in neonates suffering from birth asphyxia as well as healthy controls and to study their association with mortality or stage of HIE.

MATERIALS AND METHODS

The present study was conducted in the Department of Biochemistry in collaboration with the Department of Neonatology and Department of Microbiology of the institute. The study population included a total of 80 newborns delivered in the labor room, out of which 34 were birth asphyxiated neonates and 46 were uneventfully delivered neonates serving as controls.

A total of 80 samples were taken for this study, after obtaining written informed consent and institutional ethical approval. They were divided into the following groups:

Group I (cases): 34 neonates with gestational age ≥ 30 weeks, having birth asphyxia with Apgar score ≤ 7 at 1 minute, with one or more risk factors for asphyxia and neurological injury including arterial cord pH < 7.4, and needing intubation and cardiopulmonary resuscitation at birth, were included in the study as cases.

They were further divided into two subgroups: Group IA neonates with birth asphyxia progressing to HIE (n = 23) and Group IB neonates with birth asphyxia not progressing to HIE (non-HIE) (n = 11).

Group II (controls): Apparently healthy uneventful delivered neonates (n = 46).

Neonates with gross congenital anomalies were excluded from the study. The outcome criteria consisted of mortality and stage of HIE.

Six milliliters of umbilical cord arterial blood sample from fetal side after cord clamping was collected aseptically from all the subjects soon after the delivery.

For routine biochemical investigations, serum samples were analyzed on the autoanalyzer using standard methods. Cord arterial blood gas (cord ABG) analysis, including lactate, were done on automated blood gas analyzer (BGA). Serum was separated and α-KG levels were analyzed using sandwich-enzyme linked immunosorbent assay (ELISA) method [18].

Statistical analysis

The data was compiled and analyzed using suitable statistical methods. All categorical variables were expressed in terms of percentages and proportions. The quantitative variables, such as age, were described in terms of mean ± standard deviation, as appropriate. The chi-squared test was used to analyze the association between sociodemographic and laboratory variables with clinical features in neonates with birth asphyxia. The parametric Student's *t*-test or analysis of variance (ANOVA) were used for quantitative comparisons between different groups, respectively. Pearson's test was used for correlational analysis between quantitative variables. A p-value less than 0.05 was considered statistically significant for statistical analysis purposes.

RESULTS

The mean age of mothers of enrolled neonates in the control group was 24.13 ± 3.96 years (18 - 32 years) and in the case group, it was 24.65 ± 4.25 years (18 - 35 years). The parity of mothers of neonates was comparable in both groups, as 41.18% (n = 14) of cases had multigravida mothers and 58.82% (n = 20) of cases had primigravida mothers; 43.48% (n = 20) of controls had multigravida mothers and 56.52% (n = 26) had primigravida mothers. The distribution of neonates (cases) was comparable in both lower segment caesarean section (LSCS) and vaginal mode of delivery; 50% (n = 17) of mothers of cases delivered by LSCS and 50% (n = 17) delivered by vaginal route. For mothers of controls, 2.17% (n = 1) delivered by LSCS and 97.83% (n = 45) delivered by vaginal route. Based on the chi-squared test, the difference in the mode of delivery in both groups was found to be highly statistically significant ($\chi^2 = 17.534$, p-value < 0.05).

The mean Apgar scores in cases were 2.94 ± 1.45 (0 - 7) in the first minute and 5.70 ± 1.38 (3 - 9) in the first five minutes. However, in controls it was 7 (0 - 7) in the first minute and 9 (7 - 10) in the first five minutes. Out of 34 birth asphyxiated neonates, 29.41% (n = 10) were diagnosed with moderate birth asphyxia and 70.59% (n = 24) were diagnosed with severe birth asphyxia. Out of total 34 birth asphyxiated neonates, 79.41% (n = 27) were discharged, 17.65% (n = 6) expired, and 2.94% (n = 1) left against medical advice (LAMA). It was also found that 67.65% (n = 23) of birth asphyxiated neonates had HIE and the remaining 32.35% (n = 11) were non-HIE. The mean gestational maturity was 37.26 ± 2.94 weeks (30 - 42.7 weeks) in cases and 38.56 ± 1.70 weeks (34 - 41 weeks) in controls. The mean birth weights were 2.27 ± 0.63 kg (1.32 - 3.5 kg) in cases and 2.68 ± 0.44 kg (1.6 - 3.66 kg) in controls. Based on the *t*-test, the difference between both the groups for both the variables was found to be statistically highly significant (p-value < 0.01). Distribution of mean serum α-ketoglutarate (ng/mL) levels in controls and according to outcome (discharged/expired) in birth asphyxiated neonates (cases), is shown in Table 1 and distribution of cord blood lactate (mmol/L) levels in controls and cases is shown in Table 2. Correlation of serum α-ketoglutarate (ng/mL) levels with outcome (discharged/expired) in birth asphyxiated neonates was not found to be statistically significant (r value = 0.156, p-value = 0.384). A statistically significant correlation was not found between severity of birth asphyxia and levels of serum α-ketoglutarate (ng/mL) (r value = 0.029, p-value = 0.86). Also, correlation of cord blood lactate levels (mmol/L) with severity in birth asphyxiated neonates was not found to be statistically significant (r value = 0.326, p-value = 0.10). Correlation between cord blood lactate levels (mmol/L) and outcome in birth asphyxiated neonates (discharged/expired) was not found to be statistically significant (r value = 0.03, p-value = 0.87), while the correlation of cord pH levels and severity of birth asphyxia in cases was found to be highly statistically significant (r value = -0.60, p-value < 0.01) (Graph 1). Correlation between serum α-ketoglutarate levels and plasma lactate levels was not found to be statistically significant (r value = -0.021, p-value = 0.916) in cases, as shown in Table 3.

DISCUSSION

The neonatal period accounts for 45% of all deaths in children under the age of five worldwide. Perinatal asphyxia is responsible for almost one-quarter of all neonatal deaths worldwide [1]. The World Health Organization (WHO) defines birth asphyxia as the inability to begin and sustain breathing or spontaneous respiration at birth. It deprives the newborn of oxygen, causing physical harm to vital organs, most notably the brain. The newborn brain is highly sensitive to damage. In most cases, birth asphyxia is incurable, and those who survive usually suffer from irreversible neurodevelopmental sequelae such as cognitive and motor deficits in the short and long term. Around 25% of survivors of asphyxia suffer hypoxic-ischemic encephalopathy (HIE)

Table 1. Distribution of mean serum α-ketoglutarate (ng/mL) levels in controls and according to outcome in birth asphyxiated neonates (cases).

		Controls	Cases		p-value
Serum α-ketoglutarate (ng/mL) levels	mean ± SD	0.0336 ± 0.0152	0.05521 ± 0.0749		0.06
	range	0.0157 - 0.100	0.021 - 0.036		
			cases: discharged	cases: expired	p-value
			0.06 ± 0.083	0.03 ± 0.003	0.38
Correlation between serum α-ketoglutarate (ng/mL) levels and severity in birth asphyxiated neonates			r value		p-value
			0.029		0.86
Correlation between serum α-ketoglutarate (ng/mL) levels and outcome in birth asphyxiated neonates (discharged/expired)			0.156		0.384

Table 2. Distribution of cord blood lactate (mmol/L) levels in controls and cases.

		Controls	Cases		p-value
Cord blood lactate (mmol/L) levels	mean ± SD	4.19 ± 2.36	10.51 ± 3.93		0.00005
	range	1.8 - 11.5	3.7 - 18.4		
			cases: discharged	cases: expired	p-value
			10.21 ± 3.47	10.52 ± 5.44	0.87
Correlation between cord blood lactate levels (mmol/L) and severity in birth asphyxiated neonates			r value		p-value
			0.326		0.10
Correlation between cord blood lactate levels (mmol/L) and outcome in birth asphyxiated neonates (discharged/expired)			0.03		0.87

Table 3. Correlation of cord blood lactate (mmol/L) and serum α-ketoglutarate (ng/mL) levels in cases.

	Cases	
Correlation between cord blood lactate (mmol/L) and serum α-ketoglutarate (ng/mL)	r value	p-value
	-0.021	0.916

and neurological problems [1]. Perinatal asphyxia has been linked to newborn morbidity and mortality all over the world. Therefore, there is a need to identify novel factors involved in development and progression of birth asphyxia and HIE. As the role of α-KG has been recognized in the pathogenesis of birth asphyxia, this prospective study was conducted to evaluate the role of α-KG in neonates with birth asphyxia.

In the present study, mean cord pH levels were 7.06 ± 0.15 (6.835 - 7.366) in cases and 7.34 ± 0.09 (7.1 - 7.512) in controls, and the difference between the groups was found to be statistically highly significant (p-value < 0.01). A study found the mean cord blood pH to be 7.18 ± 0.14 in cases and 7.27 ± 0.13 in controls, with p-value = 0.001 [19]. In another study, a statistically significant association was found between umbilical artery cord blood pH value < 7.10 and attention deficit hyperactivity disorder (ADHD) when compared with umbilical artery cord blood pH values of 7.20 or higher. Furthermore, when umbilical artery cord blood pH values were examined as a continuous variable, a tendency of an increasing risk of ADHD with decreasing umbilical artery cord blood pH values was observed [20].

A study conducted in three Romanian hospitals found that out of 21,224 newborns, 124 instances satisfied the criteria for IA (hypoxia and fetal acidosis) between 2010 and 2012. The umbilical cord pH was consider-

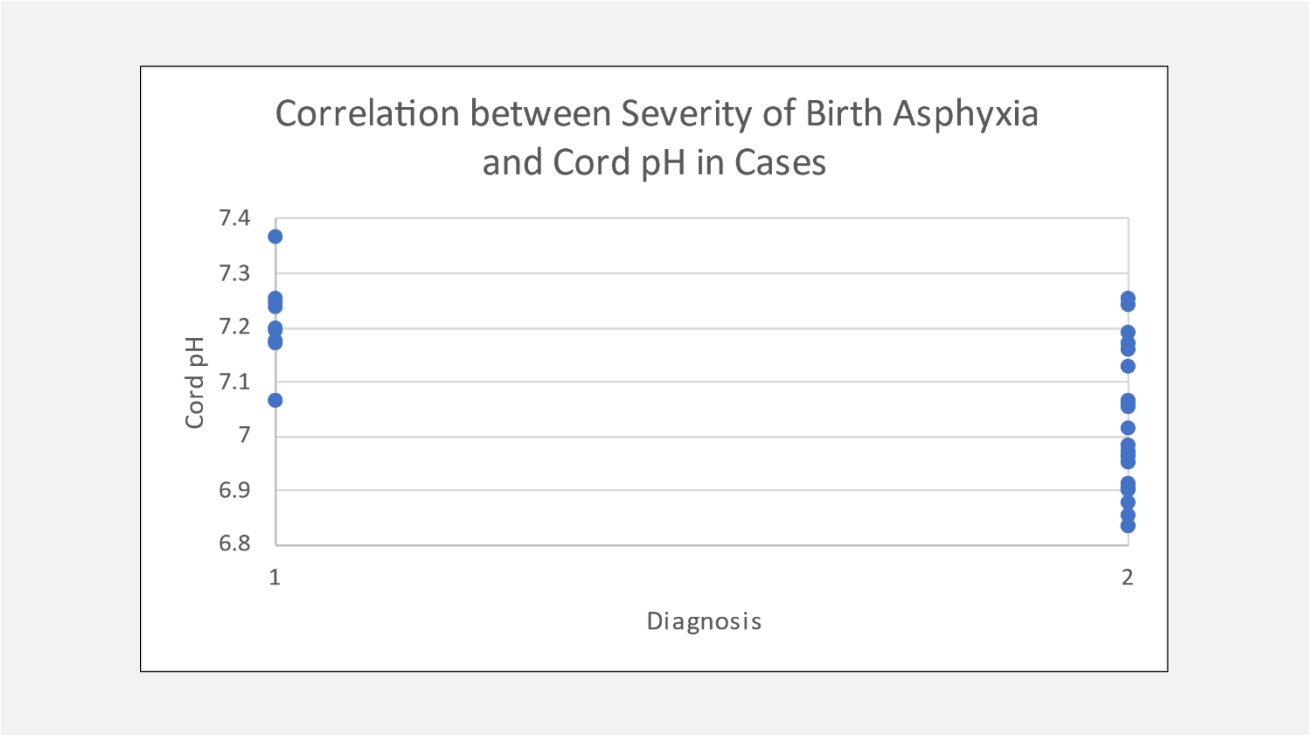

Figure 1. Scattered diagram showing correlation between levels of cord pH and severity of birth asphyxia in cases. * Moderate = 1 and severe = 2 *****

ably lower ($p < 0.001$) in the I A group, with a mean of 7.17 (7.05 - 7.32) versus 7.28 in the control group. Lactate levels were substantially higher ($p < 0.001$) in the I A group, with a mean of 5.34 mmol/L (2.35 - 8.75 mmol/L) compared to the control group's mean of 2.78 mmol/L [21]. Acidosis is characterized by low umbilical cord pH or an elevated umbilical base deficit, measured as a negative base excess (BE) at birth. Hypoxia due to impaired blood supply to the fetus in the early stages leads to respiratory acidosis that is characterized by a decrease in pH but normal BE. Following these initial events, if hypoxia continues, there will be a shift to anaerobic metabolism, resulting in the formation of lactic acid and increase in BE. Lactate builds up under aerobic settings due to β2 stimulation caused by defective mitochondrial processes and sympathetic activation. This can occur after sepsis, excessive use of β2 agonists, or severe trauma [22-25]. Excessive adrenergic stimulation can increase glucose metabolism and release pyruvate and lactate, which can be detected in the plasma. Studies using cerebral microdialysis have shown that lactate concentrations can rise in the brain and that lactate can be used as an alternative fuel source for brain tissue [26]. Thus, high cord blood lactate levels can be correlated with fetal acidosis and asphyxia [21].

Neonatal hypoxia disrupts the tricarboxylic acid (TCA) cycle due to oxygen deprivation, resulting in metabolite buildup, including α-KG and lactate. As the severity of birth asphyxia grows, the level of α-KG also rises. Neonates with HIE had higher levels of α-KG compared to those without the condition. Babies who had been discharged had greater α-KG levels due to reactivation of aerobic pathways after therapy, but those who died did not have this reactivation.

In another study, it was calculated that, high urinary organic acid concentrations were present within the clusters, with either good or poor outcome. It was further revealed that there were only eight organic acids found to be statistically significant between the two groups. Concentrations of ethylmalonate, 3-hydroxy-3-methylglutarate, 2- hydroxy-glutarate, and 2-oxo-glutarate/α-KG (mean ± SD in good outcome group was 1.435 ± 0.757 and in poor outcome group, it was 1.128 ± 0.874, with p-value < 0.05) were significantly higher in urine samples of infants with good outcome (admitted to neonatal intensive care unit) than compared with poor outcome [27].

The TCA is the central metabolic hub of the cell, being the gateway to the aerobic metabolism of any molecule that can be transformed into an acetyl group or dicarboxylic acid. The increase of these metabolites over the time can be correlated with progressive reactivation of oxygen-dependent adenosine triphosphate (ATP) production pathways after the hypoxic-ischemic event. In the present study, the expired group of neonates had serum α-KG (ng/mL) concentrations between 0.02 - 0.04

ng/mL. The percentages of discharged neonates according to serum α-KG levels for 0.02 - 0.03 ng/mL, 0.03 - 0.04 ng/mL, 0.04 - 0.05 ng/mL, 0.05 - 0.06 ng/mL, and > 0.06 ng/mL were 25.93% (n = 7), 40.74% (n = 11), 11.11% (n = 3), 7.41% (n = 2), and 14.81% (n = 4), respectively, but no statistically significant correlation was found between levels of serum α-KG (ng/mL) and outcome (discharged or expired) of birth asphyxiated neonates (r value = 0.156, p-value = 0.384), which may be due to the small sample size. In a similar study done on a urine sample, high levels of α-KG were reported in the urine of babies who survived both at birth and at 72 hours. It strongly supports the assumption that in these babies the aerobic pathways had been reactivated, while it had not done so in dead babies [28].

Based on the abovementioned observations, it can be concluded that α-ketoglutarate and lactate have the potential to serve as biomarkers for birth asphyxia. They may also help in understanding the biochemical and pathological mechanism of the disease as well as developing newer therapeutic modalities for prevention and treatment. More experimental and clinical studies with larger sample sizes and newborns with known asphyxia onset time are needed to determine the clinical relevance of α-ketoglutarate serum levels in asphyxiated newborns.

Declaration of Interest:

No conflicts of interest exist. All authors have agreed to the submission, and the manuscript hasn't been published in whole or in parts nor has it been submitted anywhere else.

References:

1. Fattuoni C, Palmas F, Noto A, Fanos V, Barberini L. Perinatal asphyxia: a review from a metabolomics perspective. Molecules 2015;20(4):7000-16. (PMID: 25898414)

2. Malin GL, Morris RK, Khan KS. Strength of association between umbilical cord pH and perinatal and long-term outcomes: systematic review and meta-analysis. BMJ 2010;340:1471-84. (PMID: 20466789)

3. Solevåg AL, Zykova SN, Thorsby PM, Schmölzer GM. Metabolomics to Diagnose Oxidative Stress in Perinatal Asphyxia: Towards a Non-Invasive Approach. Antioxidants (Basel) 2021; 10(11):1753. (PMID: 34829624)

4. Bayliak MM, Lushchak VI. Pleiotropic effects of alpha-ketoglutarate as a potential anti-ageing agent. Ageing Res Rev 2021;66: 101237. (PMID: 33340716)

5. Oddo M, Levine JM, Frangos S, et al. Brain lactate metabolism in humans with subarachnoid hemorrhage. Stroke 2012;43(5):1418-21. (PMID: 22343642)

6. Pellerin L, Magistretti PJ. Glutamate uptake into astrocytes stimulates aerobic glycolysis: A mechanism coupling neuronal activity to glucose utilization. Proc Natl Acad Sci U S A 1994;91(22): 10625-9. (PMID: 7938003)

7. Casserly B, Phillips GS, Schorr C, et al. Lactate measurements in sepsis-induced tissue hypoperfusion: results from the surviving sepsis campaign database. Crit Care Med 2015;43(3):567-73. (PMID: 25479113)

8. Singer M, Deutschman CS, Seymour CW, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). JAMA 2016;315:801-10. (PMID: 26903338)

9. Poblete RA, Cen SY, Zheng L, Emanuel BA. Serum Lactic Acid Following Aneurysmal Subarachnoid Hemorrhage Is a marker of disease severity but is not associated with hospital outcomes. Front Neurol 2018;9:593. (PMID: 30083130)

10. Zahra K, Gopal N, Freeman WD, Turnbull MT. Using Cerebral Metabolites to Guide Precision Medicine for Subarachnoid Hemorrhage: Lactate and Pyruvate. Metabolites 2019;9(11):245. (PMID: 31652842)

11. Shahmirzadi AA, Edgar D, Liao C-Y, et al. Alpha-ketoglutarate, an endogenous metabolite, extends lifespan and compresses morbidity in aging mice. Cell Metab 2020;32(3):447-56.e6. (PMID: 32877690)

12. Liu S, He L, Yao K. The Antioxidative Function of Alpha-Ketoglutarate and Its Applications. Biomed Res Int 2018;2018: 3408467. (PMID: 29750149)

13. Liu S, Yang J, Wu Z. The Regulatory Role of *α*-Ketoglutarate Metabolism in Macrophages. Mediators Inflamm 2021;2021: 5577577. (PMID: 33859536)

14. Reinke SN, Walsh BH, Boylan GB, et al. 1H NMR derived metabolomic profile of neonatal asphyxia in umbilical cord serum: implications for hypoxic ischemic encephalopathy. J Proteome Res 2013;12(9):4230-9. (PMID: 23931672)

15. Dabek M, Kruszewska D, Filip R, et al. Alpha-ketoglutarate (AKG) absorption from pig intestine and plasma pharmacokinetics. J Anim Physiol Anim Nutr (Berl) 2005;89(11-12):419-26. (PMID: 16401194)

16. Skappak C, Regush S, Cheung P-Y, Adamko DJ. Identifying hypoxia in a newborn piglet model using urinary NMR metabolomic profiling. PLoS One 2013;8(5):e65035. (PMID: 23741447)

17. Walsh BH, Broadhurst DI, Mandal R, et al. The metabolomic profile of umbilical cord blood in neonatal hypoxic ischaemic encephalopathy. PLoS One 2012;7(12):e50520. (PMID: 23227182)

18. Kaławaj K, Sławińska-Brych A, Mizerska-Kowalska M, et al. Alpha Ketoglutarate exerts in vitro anti-osteosarcoma effects through inhibition of cell proliferation, induction of apoptosis via the JNK and Caspase 9-dependent mechanism, and suppression of TGF-β and VEGF production and metastatic potential of cells. Int J Mol Sci 2020;21(24):9406. (PMID: 33321940)

19. Meena P, Meena M, Gunawat M. Correlation of APGAR score and cord blood pH with severity of birth asphyxia and short-term outcome. Int J Contemp Pediatr 2017;4(4):1325-8. https://www.ijpediatrics.com/index.php/ijcp/article/view/993#:~:text=Correlation%20coefficients%20of%20APGAR%201,asphyxia%20and%20short%2Dterm%20outcome

20. Mikkelsen SH, Olsen J, Bech BH, et al. Birth asphyxia measured by the pH value of the umbilical cord blood may predict an increased risk of attention deficit hyperactivity disorder. Acta Paediatr 2017;106:944-52. (PMID: 28247426)

21. Neacsu A, Herghelegiu CG, Voinea S, et al. Umbilical cord lactate compared with pH as predictors of intrapartum asphyxia. Exp Ther Med 2021;21(1):80. (PMID: 33363591)

22. Adamides AA, Rosenfeldt FL, Winter CD, et al. Brain tissue lactate elevations predict episodes of intracranial hypertension in patients with traumatic brain injury. J Am Coll Surg 2009;209(4): 531-9. (PMID: 19801326)

23. Cureton EL, Kwan RO, Dozier KC, Sabjadi J, Pal JD, Victorino GP. A different view of lactate in trauma patients: protecting the injured brain. J Surg Res 2010;159(1):468-73. (PMID: 19726055)

24. Garcia-Alvarez M, Marik P, Bellomo R. Sepsis-associated hyperlactatemia. Crit Care 2014;18(5):503. (PMID: 25394679)

25. Saadia TA, George M, Lee H. Lactic acidosis and diastolic hypotension after intermittent albuterol nebulization in a pediatric patient. Respir Med Case Rep 2015;16:89-91. (PMID: 26744665)

26. Cesarini KG, Enblad P, Ronne-Engström E, et al. Early cerebral hyperglycolysis after subarachnoid haemorrhage correlates with favourable outcome. Acta Neurochir (Wien) 2002;144(11):1121-31. (PMID: 12434168)

27. Chu CY, Xiao X, Zhou XG, et al. Metabolomic and bioinformatic analyses in asphyxiated neonates. Clin Biochem 2006;39(3):203-9. (PMID: 16460720)

28. Noto A, Pomero G, Mussap M, et al. Urinary gas chromatography mass spectrometry metabolomics in asphyxiated newborns undergoing hypothermia: from the birth to the first month of life. Ann Transl Med 2016;4(21):417. (PMID: 27942508)