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THE ROLE OF ADENOSINE MONOPHOSPHATE-ACTIVATED PROTEIN KINASE AND HIGH-MOBILITY GROUP BOX 1 PROTEIN IN EARLY DIAGNOSIS OF NEONATAL SEPSIS

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Neonatal sepsis, a significant cause of infant mortality, necessitates early diagnosis for effective intervention. Current diagnostic markers, such as C-reactive protein and procalcitonin, exhibit limited sensitivity and specificity. This study investigates the potential of adenosine monophosphate-activated protein kinase and high-mobility group box1 protein levels as early diagnostic biomarkers for neonatal sepsis. We examined 143 neonates with suspected sepsis, analyzing clinical and laboratory data, including complete blood cell count, C-reactive protein, procalcitonin, fibrinogen, lactate, HCO3, and base excess levels, alongside adenosine monophosphate-activated protein kinase and high-mobility group box1 protein levels using enzyme-linked immunosorbent assay. Our findings indicate elevated adenosine monophosphate-activated protein kinase and high-mobility group box 1 levels in septic neonates compared to non-septic counterparts, with significant correlations between these markers and disease severity. These results suggest that adenosine monophosphate-activated protein kinase and high-mobility group box1 could serve as reliable biomarkers for early sepsis detection, potentially guiding timely and appropriate therapeutic interventions. Further research is recommended to validate these biomarkers' clinical utility in neonatal sepsis diagnosis.

Key words: adenosine monophosphate-activated protein kinase, high-mobility group box1, neonate, sepsis

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РОЛЬ АДЕНОЗИНМОНОФОСФАТ-АКТИВОВАНОЇ ПРОТЕЇНКІНАЗИ ТА ВИСОКОМОБІЛЬНОГО БІЛКА ГРУПОВОГО БЛОКУ 1 У РАННІЙ ДІАГНОСТИЦІ НЕОНАТАЛЬНОГО СЕПСИСУ

Неонатальний сепсис, який є головною причиною смертності новонароджених, вимагає ранньої діагностики для ефективного втручання. Сучасні діагностичні маркери, такі як С-реактивний білок і прокальцитонін, мають обмежену чутливість і специфічність. У цьому дослідженні вивчається рівень аденозинмонофосфат-активованої протеїнкінази та високомобільного білка групового блоку 1 як ранніх діагностичних біомаркерів неонатального сепсису. Ми обстежили 143 новонароджених із підозрою на сепсис, проаналізувавши клінічні та лабораторні дані, включаючи загальний аналіз крові, С-реактивний білок, прокальцитонін, фібриноген, лактат, HCO3 та рівні надлишку основ, а також аденозинмонофосфат-активовану протеїнкіназу за допомогою імуноферментного аналізу. Наші результати вказують на підвищені рівні аденозинмонофосфат-активованої протеїнкінази та високомобільного білка групового блоку 1 у септичних новонароджених порівняно з несептичними новонародженими, зі значною кореляцією між цими маркерами та тяжкістю захворювання. Ці результати дозволяють припустити, що аденозинмонофосфат-активована протеїнкіназа і високомобільний білок групового блоку 1 можуть бути надійними біомаркерами для раннього виявлення сепсису, потенційно визначаючи своєчасні та відповідні терапевтичні втручання. Рекомендується провести подальші дослідження з метою підтвердження клінічної значущості цих біомаркерів при діагностиці неонатального сепсису.

Ключові слова: аденозинмонофосфат-активована протеїнкіназа, високомобільний білок групового блоку 1, неонатальний, сепсис.

According to the World Health Organization's 2017 data, a quarter of the annual 2.7 million neonatal deaths, or 560,000, are attributed to neonatal infections. Of these, 400,000 are due to sepsis and meningitis, while 160,000 are due to pneumonia. Despite advancements in neonatal resuscitation, sepsis remains a leading cause of neonatal mortality worldwide. Neonatal sepsis is reported to occur in every 1000 live births [12]. However, due to the presence of risk factors that can lead to nosocomial sepsis in preterm infants, sepsis is observed in 3 % to 20 % of the neonatal population. Mortality due to neonatal sepsis is significantly dependent on the pathogen and the gestational age of the infants, with mortality rates rising to 20 % in very premature infants [13]. Many of these deaths can be prevented through preventive measures such as early diagnosis, timely examinations, appropriate antibiotic therapy, and continuous monitoring. Early diagnosis is possible through the rapid recognition of clinical signs, symptoms, and syndromes. Although markers such as C-reactive protein (CRP) and Procalcitonin are commonly used in the diagnosis of sepsis, their sensitivity decreases due to various influences. Despite numerous studies on early diagnosis of sepsis, there are still no markers with high sensitivity and specificity for early diagnosis. There is work to improve the diagnosis of early neonatal sepsis in prematurely born children based on determining the diagnostic significance of the serum level trigger receptor (sTREM-1) [1]. Delayed diagnosis of sepsis not only increases morbidity and mortality during

the neonatal period but also negatively impacts the subsequent developmental period of surviving infants, affecting their psychoneurological and physical development, thereby delaying children's social adaptive functions. Therefore, neonatal sepsis is considered not only a problem of neonatology but also one of the current issues in pediatrics and neurology. Early detection of illness and early intervention can help prevent adverse outcomes. Furthermore, finding highly sensitive and reliable markers can reduce unnecessary antibiotic use. Therefore, the search for new, more sensitive and prognostic markers in the diagnosis of sepsis is always at the forefront of researchers' and clinicians' attention. In recent years, research has been conducted on influencing various phases of metabolism to manage diseases. Sepsis leads to sepsis-related dysfunctions and mitochondrial damage, which constitute the basis of cellular metabolism disruption during sepsis. These disruptions occur in all members of the matter metabolism. Glycolysis intensifies, and the conversion of pyruvates to the Krebs cycle is disrupted, leading to increased lactate. Regulation of lipolysis processes is disrupted, resulting in increased levels of fatty acids and triglycerides in the blood. However, the utilization of these substances is impaired, leading to their accumulation, along with toxic lipid products. Disruptions in ketone bodies and amino acid exchange are also observed during sepsis [9]. Understanding metabolism during sepsis has become a fundamental area of research in modern times. In diabetes, inflammation, sepsis, and oncological diseases, the central role of adenosine monophosphate-activated protein kinase (AMPK) in metabolism is being studied, and the disruption of its regulation in pathological processes makes it a target for pharmacological interventions during diseases [15]. In recent years, the role of high-mobility group box1 (HMGB1) in the pathophysiology of SIRS and sepsis has also been studied. HMGB1 acts as a complex nuclear and cytoplasmic protein and is detected in systemic circulation during severe injuries. It tends to bind to inflammatory mediators such as protein lipopolysaccharide and pro-inflammatory cytokines [11]. HMGB–1 acts as a molecular substance associated with damage and serves as an endogenous ligand of the innate immune system during inflammation. Blocking the high expression of the HMGB1 protein has been shown to extinguish the inflammatory process in rheumatoid arthritis, myositis, and systemic lupus erythematosus in some model studies. Kornblit et al. first demonstrated the effect of the HMGB1 gene on the risk of developing SIRS and sepsis. It has been noted that carriers of gene polymorphisms have a higher risk of early death from infection [10]. The early synthesis of HMGB-1 before inflammatory cytokines, as well as the presence of gene polymorphism, may affect the risk and course of sepsis, making the study of the role of this protein in the early diagnosis of sepsis in newborns plausible [9].

The purpose of the study was to investigate the role of adenosine monophosphate-activated protein kinase and high-mobility group box1 proteins in the early diagnosis of sepsis in newborns.

Materials and methods. In the current research, an examination of 143 newborns suspected of sepsis has been conducted. The infants had a mean gestational age of 36.8±1.7 (range 34–42) weeks, a mean weight of 2955±479.3 (range 2000–4200) grams and a mean length of 49.2±2.69 (range 41–56) centimeters. Based on the information provided in the discharge summaries of the infants, Apgar scores were evaluated at 1 and 5 minutes, yielding scores of 6.6±1.2 (mean ±SD) (min 2-max 9) and 7.3±0.9 (mean±SD) (min 5-max 9), respectively. The infants were transferred from level I and II maternity centres to the neonatal intensive care unit (NICU) within the first and second days of life. Signs of sepsis, specifically respiratory distress symptoms, were noted in all infants. In addition to the initial clinical examination of the infants, radiographic and ultrasonographic examinations of the chest and abdomen, respectively, were performed within the first 24 hours of life, and an ultrasonographic examination of the brain was performed on the third day of life, if indicated, or upon admission. Upon admission, all infants underwent complete blood cell count analysis, fibrinogen, albumin, CRP, and procalcitonin determination. Furthermore, after centrifugation of the blood samples collected from the infants for biochemical analysis, 40 µL of plasma were separated and stored at -20℃. Determination of AMPK and HMGB1 proteins in plasma was performed with ELISA kits (Shanghai Coon Koon Biotech Co., Ltd) based on the enzyme immunoassay method.

Statistical analysis of the results of these analyses was performed using the SPSS statistical package on the Windows operating system.

The initial assessment of the infants' condition was made using a scale determined according to the guidelines of the European Medicines Agency for the management of sepsis, which requires at least two clinical and two laboratory criteria for the diagnosis of sepsis to be confirmed clinically and by laboratory testing. As a result of the evaluation, these infants were divided into two groups, sepsis (n=98) and non-

sepsis (n=45), and the analysis results were compared between the two groups. Since signs of sepsis were noted within the first 72 hours, they were classified as early sepsis.

Results of the study and their discussion. Increased demand for mechanical ventilation was observed in infants with sepsis, and infants with hemodynamic instability, dopamine, dobutamine, and noradrenaline infusion were administered, and the determination of fresh frozen plasma, erythrocyte mass, platelet mass, and immunological preparations were based on clinical indications. The characteristics of the newborns by group are provided in Table 1.

Table 1

Frequency distribution of Gestational age, sex, type of delivery, mechanical ventilation, inotropic support, and morbidity across groups

Parameters		Control	Sepsis	Nonseptic	Pearson chi-square
GA	34–36weeks	2 (6.5 %)	20 (31.7 %)	10 (34.5 %)	χ^2=8.3 p=0.016
	37–42 weeks	29 (93.5 %)	43 (68.3 %)	19 (65.5 %)	
sex	girls	13 (41.9 %)	29 (45.3 %)	14 (46.7 %)	χ^2=0.152 p=0.927
	boys	18 (58.1 %)	35 (54.7 %)	16 (53.3 %)	
type of delivery	vaginal delivery	5 (15.5 %)	51 (53.7 %)	23 (53.5 %)	χ^2=15.07 p=0.001
	cesarean section	27 (84.4 %)	44 (46.3 %)	20 (46.5 %)	
mechanical ventilation		0	26 (28.3 %)	1 (2.3 %)	χ^2=22.18 p=0.001
inotropic support (dopamine infusion)		0	22 (23.9 %)	0	χ^2=20.65 p=0.001
morbidity		0	15 (16 %)	0	χ^2=13.3 p=0.001

Particularly in the complete blood count analysis, attention was paid to the counts of white blood cells, lymphocytes, monocytes, neutrophils, and immature granulocytes. When calculated using the Mann-Whitney U test, no statistically significant difference was observed between the sepsis and non-sepsis groups. Although there was no difference in procalcitonin levels between the groups in 65 infants (mean – 12.38, SD-17.56 in the sepsis group (n=51) vs mean – 4.95, SD – 6.57 in the non-septic group (n=14)), the CRP level was higher in the sepsis group (mean – 33.56, SD – 50.3 in the sepsis group (n=88) vs mean – 9.05, SD – 5.27 in the non-septic group (n=38)). Fibrinogen levels were also increased in the sepsis group (mean –223.73, SD – 90.08 in the sepsis group (n=60) vs mean – 191.5, SD – 101.11 in the non-septic group (n=28), $p<0.001$).

The increase in fibrinogen, known as an acute phase reactant was observed in the early stages of sepsis and decreased as the condition worsened. Studies have shown the prognostic importance of fibrinogen in pediatric patients, where levels below 2g/L increase the risk of death. Our research also reflects the prognostic and diagnostic importance of increased fibrinogen levels in infants with sepsis. Lactate levels were higher in the sepsis group (mean –3.18, SD – 2.25 in the sepsis group (n=73) vs mean – 1.77, SD – 0.87 in the non –septic group (n=22), $p<0.001$), while HCO3 (mean – 20.2, SD – 4.37 in the sepsis group (n=72) vs mean –22.18, SD – 3.26 in the non-septic group (n=22)) and BE levels were lower (mean – (-5.16), SD – 4.29 in the sepsis group (n=72) vs mean-(-3.08), SD – 3.27 in the non-septic group (n=23), $p<0.001$), indicating accelerated catabolic processes and oxygen deficiency due to infection.

Procalcitonin levels were higher in infants requiring inotropic support ($p<0.05$). In these infants, the mean±SD was 20.4±25, compared to 6.9±11 in infants not receiving dopamine. Infants receiving dopamine had higher levels of PCT compared to the median level in 10 (83.3 %) neonates (p=0.001). Levels of AMPK and HMGB1 were higher in the sepsis group compared to healthy children but lower compared to non-sepsis children (p=0.001). When examining the correlation relationships of the markers, a direct correlation was observed between hospital days and PCT (r=0.320, p=0.022), an inverse correlation between AMPK (r=-0.226, p=0.028), and a direct correlation between AMPK and HMGB1 (r=0.336, p=0.001), Table 2.

Maximum activation of AMPK occurs with phosphorylation of the subunit within the 172nd threonine. Additionally, allosteric activation of AMP occurs with the dephosphorylation of ADP in the γ subunit. When AMPK is activated, it reduces ATF uptake, weakens the anabolic pathway, and inhibits ATF action and the ketogenic pathway. Thus, AMPK becomes a potential therapeutic target in processes

such as type 2 diabetes mellitus, cancer, and inflammation associated with disrupted energy homeostasis [7, 8]. AMPK activation occurs in response to pathological stresses such as glucose depletion and hypoxia. Some studies have observed AMPK activation in neurological disorders. However, the implications of increased AMPK in neurons are not entirely clear [5]. Experimental research has shown that hypoxia activates the AMPK α1 complex with CaMKKβ in rat models. Increased AMPK during stroke exacerbates damage, while its inhibition reduces cell death. However, until damage occurs, AMPK inhibition sensitizes neurons to stress [7]. Experimental research has demonstrated AMPK inhibition with lipopolysaccharides in vitro.

Table 2

Comparable levels of HMGB1 and AMPK levels in groups

Proteins	Groups	N	Mean	Std. Deviation	Std. Error	95 % Confidence Interval for		Minimum	Maximum
						Mean Lower Bound	Upper Bound		
HMGB, pq/ml	control	12	1151.28	472.14	136.30	851.29	1451.26	620.10	2332.00
	sepsis	56	1509.73*^	410.82	54.90	1399.71	1619.75	425.30	2974.00
	non-septic	28	1709.71*	422.45	79.84	1545.91	1873.52	1064.00	2647.00
AMPK, pq/ml	control	24	873.00	167.62	34.21	802.22	943.78	664.40	1231.00
	sepsis	80	1090.06*^	352.92	39.46	1011.52	1168.60	607.30	2095.00
	non-septic	38	1355.18*	465.41	75.50	1202.20	1508.15	645.50	2290.00

Note: * – $p<0.05$ comparison with control,^ – $p<0.05$ comparison with nonsepsis group.

Activation of AMPK during sepsis is considered a new potential therapeutic approach to treat myocardial dysfunction. Research suggests that α1-AMPK is crucial for intercellular endothelial communication. Additionally, decreased AMPK in cardiovascular dysfunction increases vascular permeability and causes myocardial edema. It is believed that AMPK can prevent cardiovascular dysfunction during sepsis. Experimental research has shown that pharmacologically activating AMPK during sepsis reduces mortality and thus demonstrates its therapeutic importance [3].

HMGB1 levels were lower in infants with sepsis compared to non-septic infants, similar to AMPK. HMGB1 is a representative of damage-associated molecular patterns associated with cellular damage. It is present within cells under normal conditions and is actively or passively secreted outside cells. When outside the cell, it binds to various receptors and determines the proliferation, differentiation, and mobilization of hematopoietic stem cells [4]. HMGB1 has been studied in various diseases such as cancer, traumatic shock, etc., and it has been shown that systemic HMGB1 levels significantly increase in rodent sepsis models, and HMGB1 levels increase in rats after exposure to lipopolysaccharides, reaching high levels after 16–32 hours. The delayed secretion of HMGB1 serves as a window for the use of HMGB1 antagonists in septic patients. Studies have shown that reducing HMGB1 secretion effectively increases survival in septic rodents [14].

HMGB1, like pro-inflammatory cytokines, plays a significant role in various inflammatory diseases [2]. Specifically, it has been determined in sepsis models and septic patients that circulating HMGB1 levels significantly increase and are positively correlated with disease severity and inflammation severity. Compared to other early-phase inflammatory markers, such as interleukin-1beta, HMGB1 begins to increase after 8 hours and then significantly increases, serving a broad clinical window for clinical septic patients. Organ dysfunction occurs in the early stages of sepsis due to excessive inflammation, and as inflammation continues, more severe consequences occur, leading to organ failure. Therapeutic window intervention can prevent excessive activation of the immune response. Research shows that blocking HMGB1 expression can alleviate inflammatory diseases such as rheumatoid arthritis, arthritis, myositis, and systemic lupus erythematosus [2, 6]. Targeting HMGB1 has been shown to reduce organ damage associated with sepsis and, especially by reducing neutrophil bacterial clearance ability and increasing continuous inflammation, affects neutrophils and bacteria. In septic mice and in patients recovering from septic shock, HMGB1 has reduced neutrophil bactericidal capacity, such as affecting neutrophil nicotinamide adenine dinucleotide oxidase function. Interestingly, HMGB1 derived from platelets in abdominal sepsis rodent models helps stimulate neutrophil extracellular trap formation and, in septic shock, also leads to a significant increase in extracellular neutrophil traps. These increased networks collect neutrophils to the liver and contribute to the formation of inflammatory components in the liver. In addition

to this effect, neutrophils also stimulate macrophages by stimulating the secretion of massive inflammatory mediators such as tumor necrosis factor-alpha HMGB1, thereby accelerating the systemic inflammatory response [6].

Conclusion

The findings of this study suggest that the determination of serum AMPK and HMGB1 levels may serve as potential biomarkers for the early diagnosis of sepsis in newborns. These biomarkers could aid in the timely initiation of treatment, thereby reducing neonatal mortality and morbidity associated with sepsis. Further studies are warranted to validate these findings and explore the clinical utility of AMPK and HMGB1 as diagnostic markers for neonatal sepsis.

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