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Original Article

Investigation of Urinary Beta-2 Microglobulin Level in Neonates with Asphyxia Admitted in Alzahra Hospitals in Isfahan, 1396-1397

Mehryar Mehrkash[1], Alaleh Gheissari[1], Behzad Barekatain[2*], Fatemeh Ziabi[3], Sayyed Mohammad Hosein Tabatabaei[3]

1. Department of Pediatrics, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
2. Department of Pediatrics, Division of Neonatology, Child Growth and Development Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
3. School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

ABSTRACT

Background: Despite advances in perinatal care, perinatal asphyxia (PA) remains one of the most important causes of mortality and morbidity at birth. Asphyxia is associated with the dysfunction of different organs of the body. Therefore, this study aimed to investigate the urinary biomarker of beta-2 microglobulin in neonates with asphyxia.

Methods: This case-control study was performed on neonates admitted to the Neonatal Intensive Care Unit of AL Zahra and Shahid Beheshti hospitals affiliated to Isfahan University of Medical Sciences, Isfahan, Iran, during 2017-18. On the second day of birth, beta-2 microglobulin was measured in urine samples using the enzyme-linked immunosorbent assay technique.

Results: The mean level of beta-2 microglobulin in the group with asphyxia (9.91±6.16) was significantly higher than that in the control group (3.83±4.03) (P=0.001). Moreover, analysis of beta-2 microglobulin level in the group with asphyxia showed that the mean serum level of neonates with acute renal failure (13.14±6.27) was significantly higher than that in newborns without acute renal failure (6.68±4.24) (P=0.02).

Conclusion: The results of our study suggest that the beta-2 microglobulin level can be evaluated as a marker of neonatal asphyxia. Furthermore, its level was significantly associated with acute kidney injury. It is suggested that further studies be conducted with a larger sample size.

Keywords: Acute kidney injury, Asphyxia, Creatinine, Neonate

Introduction

According to the American Academy of Pediatrics, Hypoxic-ischemic encephalopathy is characterized by the presence of two of the following items (1-3):

1) Apgar score ≤3 at 1 min or ≤ 5 at 5 min
2) Umbilical cord arterial (pH<7.00) with base deficit >10 mmol/L
3) The presence of postnatal clinical complications attributed to perinatal asphyxia (PA), such as abnormal neurological signs (seizure, coma, and hypotonia)
4) Multiple organs involvement (kidney, lungs, liver, heart, and intestine)

In addition to severe brain damage, this condition can be associated with the failure of other organs. Despite advances in asphyxia treatment, this complication is still mortal (3, 4). Asphyxia can cause dysfunction in various organs of the body, which is due to the alteration of the cardiac output to the brain, heart, and adrenal glands. Moreover, it reduces blood flow to organs, such as the kidneys, and causes acute kidney injury (3, 5). Recently, hypothermia therapy has become one of the neuroprotective therapies used in PA, which is associated with side effects, such as arrhythmias, thrombocytopenia, and necrosis of

* *Corresponding author:* Behzad Barekatain, Department of Pediatrics, Division of Neonatology, Child Growth and Development Research Center, Isfahan University of Medical Sciences, Isfahan, Iran. Tel: 00989133158797; Email: b_barekatain@med.mui.ac.ir

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the visceral or subcutaneous fat (6, 7).

Beta-2-microglobulin is a small transmembrane protein (11,800 Daltons) with heavy chains of major histocompatibility complex. Therefore, it is observed on the surface of all nucleated cells. Due to its small size, beta-2 microglobulin passes through the glomerular membrane; however, it is completely reabsorbed in proximal tubules (8-10). Elevated serum beta-2 microglobulin levels are also found in diseases associated with increased cell circulation. It has also been shown to increase levels in several benign conditions, such as chronic inflammation, liver disease, renal dysfunction, some acute viral infections, and several malignancies, especially leukocyte B blood cancer.

In multiple myeloma, beta-2 microglobulin is a potent prognostic factor, and less than 4 μg/ml is considered a good prognostic factor (9-11). Beta-2 microglobulin is secreted by the glomerulus, and a small amount is reabsorbed into the bloodstream by the renal tubule. In patients with kidney diseases, the glomerular disease can be distinguished from tubular disease by simultaneous measurement of blood and urinary levels of beta-2 microglobulin. Furthermore, in the glomerular disease, blood levels are high and urinary levels are low; however, serum and urinary levels are low and high in renal tubular disease, respectively.

In addition, its blood level rises rapidly when rejecting a renal transplant. In aminoglycoside poisoning, beta-2 microglobulin increases even before creatinine. Urinary levels of beta-2 microglobulin increase in patients with renal disease due to abundant exposure to heavy metals, such as cadmium or mercury (8, 9). In the case of workers with high exposure to these metals, periodic tests are performed to diagnose kidney disease at its earliest stage. The synthesis rate of beta-2 microglobulin varies from 2 to 4 mg/kg/day with a half-life of 2.5 h, and plasma concentrations range from 1-3 micrograms per milliliter. About 90% of the beta-2 microglobulin is removed by glomerular filtration and is almost completely reabsorbed by proximal tubules; accordingly, in people with chronic kidney disease, especially end-stage renal disease, beta-2 microglobulin can accumulate in the blood (9, 10).

Under laboratory conditions, the synthesis and release of beta-2 microglobulin can be induced by acidosis, endotoxins, or inflammatory cytokines. Beta-2 microglobulin may act as an acute phase reactor. Serum beta-2 microglobulin has been identified as an important prognostic marker in some hematological and non-hematological disorders. In renal tubular disorders, despite normal plasma levels, urinary beta-2 microglobulin levels are high reflecting a reabsorption disorder by proximal tubules (8, 9). Serum and plasma levels of beta-2 microglobulin have been identified as indicators of cellular immune activation and a tumor marker in some specific hematological malignancies. Urinary beta-2 microglobulin levels indicate renal filtration disorders. Measurement of both serum and urinary levels can help differentiate the activation of the cellular immune system from renal impairment (9, 10).

Given the importance of beta-2 microglobulin as an indicator of renal function as well as markers that can be used to predict the prognosis of various diseases, this study aimed to investigate the level of beta-2 urinary microglobulin in neonates with asphyxia hospitalized in Al Zahra and Shahid Beheshti hospitals in Isfahan, Iran, during 2017-18.

Methods

This case-control study was performed on 37 neonates admitted to the Neonatal Intensive Care Unit of Al Zahra Hospital affiliated to Isfahan University of Medical Sciences, Isfahan, Iran, from September 2017 to April 2019. The study protocol was approved by the Ethics Committee of Isfahan University of Medical Sciences, Isfahan, Iran. Subsequently, the research procedure and objectives were entirely explained to the legal guardians of the patients, and written consent was obtained from them.

The inclusion criteria were: 1) gestational age of 32 weeks and older, 2) perinatal asphyxia with acute renal failure, 3) parents' willingness regarding their neonates' participation in the study, 4) lack of known congenital renal abnormalities, 5) absence of sepsis, 6) lack of kidney disease in the mother, and 7) normality of the maternal creatinine based on the prenatal test results. On the other hand, the neonates who missed the follow-up and those whose mothers had a high level of creatinine were excluded from the study.

Urine samples of all infants with asphyxia were taken on the second day of birth to analyze the level of beta-2 microglobulin using a special Human Beta-2 Microglobulin ELISA Kit (ASCO company, Germany). The results were then recorded in the study.

Neonates with similar demographic characteristics, such as age, gender, and weight, without asphyxia but other criteria for entering the study were included in a control group. On the second

day of birth, their urine sample was taken to measure beta-2 microglobulin levels. It should be mentioned that the newborns in the control group had no medications and diseases that made them susceptible to renal failure.

The urine samples were collected using urine bags, and the measurement of beta-2 microglobulin was performed immediately following transmission to the laboratory. If it was not possible, the samples were preserved at -20°C, and the measurements were performed within the next day. In order to minimize the bias, all of the assessments were conducted in the laboratory of AL Zahra Hospital affiliated to Isfahan University of Medical Sciences, Isfahan, Iran. The beta-2 microglobulin and creatinine of the control group were also measured in this study. The level of creatinine of the case and control groups was measured on the second day. Moreover, the daily measurement of the creatinine in the case group continued until the level returned to a normal range. Renal failure in this study was defined as serum creatinine greater than 1.5 mg/dl or an increase in creatinine more than 0/2 to 0/3 mg/dl/day. According to this definition, renal failure occurred in 50% of the patients in this study.

The level of urinary beta-2 microglobulin in neonates who had asphyxia and renal failure based on serum creatinine levels or urinary output was compared with that of infants with asphyxia who did not have renal impairment. Furthermore, infants of mothers with kidney disease who had high creatinine in prenatal tests were not included in the study to avoid bias in creatinine interpretation. Data were analyzed in SPSS software (version 24). Furthermore, normal distribution of the data was assessed using the Kolmogorov-Smirnov Z-test, and based on the results, parametric or non-parametric tests were used in this study. In addition, independent T-test and Mann-Whitney tests were employed to compare the means between the two groups. A p-value less than 0.05 was considered statistically significant.

Results

This study evaluated 18 neonates with asphyxia and 19 healthy newborns as a control group. According to the Sarnat staging, all of the asphyxiated newborns had moderate to severe signs. The mean ages of the case and control groups were 35.15±2.52 and 35.47±2.49 weeks, respectively, which showed no significant difference between them in this regard (P=0.69). In the case and control groups, 11 (61.1%) and 9 (47.4%) newborns were male, respectively, and there was no significant difference between the two groups in terms of gender (P=0.51).

The results of this study showed that the mean creatinine in the group with asphyxia (1.33±0.89) was significantly higher than that in the control group (0.62±0.20) (P=0.002). Moreover, the mean level of beta-2 microglobulin in the case group (9.91±6.16) was significantly higher than that in the control group (3.83±4.03) (P=0.001) (Diagram1). It is worth mentioning that no newborns in the control group had renal failure or end-organ damages.

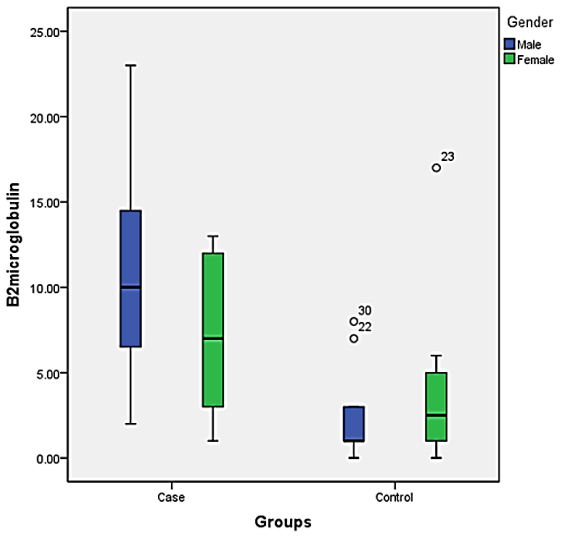

Diagram 1. Mean level of beta-2 microglobulin in the case and control groups regarding gender

Table 1. Demographic and clinical characteristics of the neonates in the case and control group

Variables		Case group (with asphyxia)	Control group	P-value
Mean fetal age		35.15 ± 2.52	35.47±2.49	0.69
Gender	Male Female	11 (61.1%) 7 (38.9%)	9 (47.4%) 10 (52.6%)	0.51
Creatinine on the second day of birth		1.33±0.89	0.62±0.2	0.002
Beta-2 microglobulin		9.91±6.16	4.03±3.83	0.001
Acute renal failure	Yes No	9 (50%) 9 (50%)	0 19(100%)	<0.1

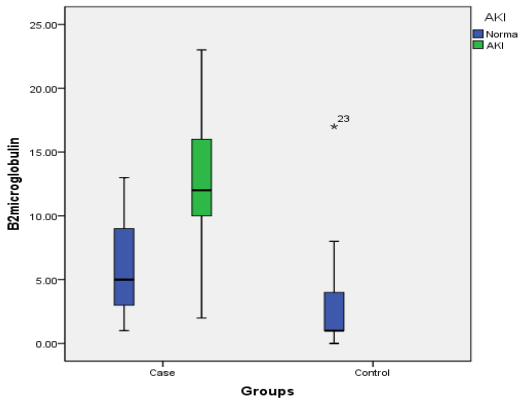

Diagram 2. Mean beta-2 microglobulin levels in the case and control groups regarding acute renal failure

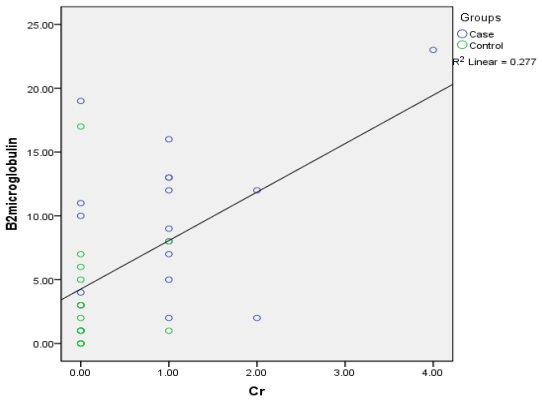

Diagram 3. Relationship between beta-2 microglobulin and creatinine levels in the case and control groups

Analysis of beta-2 microglobulin level in the group with asphyxia showed that the mean serum level in neonates with acute renal failure (13.14±6.27) was significantly higher than that in those who were not suspected of acute renal failure (6.68±4.24) (P=0.02) (Diagram2).

Renal failure in this study was defined as serum creatinine greater than 1.5 mg/dl or creatinine increase of more than 0/2 to 0/3 mg/dl/day. According to this definition, renal failure occurred in 50% of the patients in the present study.

The results also showed that there was no significant relationship between beta-2 microglobulin and creatinine levels (r=+46, P=0.052) (Diagram 3).

Discussion

The results of the present study showed that the mean level of beta-2 microglobulin in the case group with asphyxia was significantly higher than that in the control group. Moreover, the group with asphyxia obtained a higher mean creatinine level, compared to the control group. Comparison of the mean levels of beta-2 microglobulin between neonates with asphyxia who had acute renal failure and those with asphyxia and without this disorder showed a significant difference between the two groups in terms of the level of this marker.

In a study conducted by Kazemi et al., the level of urinary beta-2 microglobulin was assessed as an indicator of kidney damage after extracorporeal shock wave lithotripsy (ESWL) (12). For this purpose, 20 patients underwent ESWL, and 18 cases entered the study as a control group after varicocelectomy. Urinary level of beta-2 microglobulin was examined on the days before surgery, 24 h later, as well as the 3rd ,7th, and 14th day post-surgery.

The results of the aforementioned study showed that beta-2 microglobulin levels increased significantly in the post-ESWL period indicating transient tubular damage; therefore, beta-2 microglobulin was introduced as a marker of renal injury level. Similarly, in our study, the level of beta-2 microglobulin was significantly higher in patients with asphyxia than that in the control group; moreover, this marker could be associated with asphyxia. Further studies have shown that its levels are significantly higher in patients with renal insufficiency, compared to those without renal failure. Accordingly, it can be considered a marker of acute renal failure in neonates with asphyxia.

Willis et al. examined 35 infants with asphyxia and 55 healthy infants as a control group in a prospective study (13). In that case-control study, N-acetyl-glucosamine and beta-2 microglobulin levels were measured 24-48 hours after birth, as well as 4-6 days, and 4-6 weeks after birth. According to the results, N-acetyl-glucosamine and beta-2 microglobulin levels were significantly higher in the 24- to 48-hour period and 4-6 weeks after birth in infants with asphyxia, compared to the control group. Therefore, their results suggested that beta-2 microglobulin levels could be used as a marker for asphyxia, which was similar to the findings in our study; however, they did not assess creatinine levels and the association of beta-2 microglobulin with renal failure.

In a randomized controlled trial conducted by Bhat et al., the effect of theophylline prophylactically was investigated on reducing the incidence and severity of renal failure in infants with asphyxia (14). The results of their study

showed that theophylline consumption could significantly reduce serum creatinine levels and urinary beta-2 microglobulin. It was also found that levels of beta-2 microglobulin were significantly higher in the group of neonates with asphyxia who had renal failure, compared to those who did not have renal failure. The results of our study similarly showed that beta-2 microglobulin levels were higher in patients with asphyxia who had simultaneous renal failure, compared to patients with asphyxia who did not have renal insufficiency.

Regarding the limitations of our study, one can name the small sample size, as well as lack of follow-up and measurement of creatinine along with beta-2 microglobulin levels in consecutive times. Therefore, it is suggested that future studies be conducted on larger sample sizes and consider the level of these markers at longer consecutive times.

Conclusion

The results of this study suggested that beta-2 microglobulin levels could be assessed as a marker of asphyxia in neonates. Its level can also be significantly associated with acute kidney damage. However, it is recommended that future studies include larger sample sizes, utilize a prospective method, and evaluate the level of beta-2 microglobulin marker in patients with asphyxia.

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Conflicts of interest

The authors declare no conflict of interest regarding the publication of the study.

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