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ORIGINAL ARTICLE

Clinical Factors Associated with Postnatal Urinary Titin N-Fragment in Neonates

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SUMMARY

Background: **Titin is a large sarcomeric protein (~ 3,800 kDa) essential for muscle function. The urinary N-terminal fragment of titin (N-titin) has emerged as a noninvasive biomarker for muscle injury in adults, but its clinical significance in neonates remains unclear.**
Methods: **A retrospective cohort study involving 523 neonates admitted to the NICU/GCU at Nihon University Itabashi Hospital between October 2021 and December 2023 was conducted. Urinary N-titin, collected within 24 hours of birth, was measured using enzyme-linked immunosorbent assay (ELISA) and normalized to creatinine (N-titin/Cr). Associations among neonatal, maternal, and delivery factors were analyzed. A subgroup analysis was performed in neonates with asphyxia. Reference percentiles of N-titin/Cr were separately established for neonates with and without asphyxia. Clinical courses were reviewed for neonates whose N-titin/Cr ratio exceeded the 95th percentile as well as for those with neuromuscular diseases or chromosomal abnormalities.**
Results: **Urinary N-titin levels negatively correlated with gestational age ($p = 0.0035$) and Apgar score ($p < 0.0001$). Positive correlations were found among AST, ALT, LDH, creatine kinase (CK), and lactate levels (all $p < 0.0001$). Stronger correlations with muscle-derived enzymes were observed in neonates with asphyxia. Higher N-titin levels were associated with non-reassuring fetal status, placental abruption, and emergency cesarean delivery. Six neonates with asphyxia exceeded the 95th percentile; three died and two had mild developmental delays. No neuromuscular disease was identified. Seven patients with Down syndrome were identified in this study.**
Conclusions: **Urinary N-titin levels reflect acute perinatal stress, particularly neonatal asphyxia. The establishment of reference values may support its use as an early biomarker in neonates.**
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KEYWORDS

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INTRODUCTION

Titin, also known as connectin, is an enormous protein with a molecular mass of approximately 3,800 kDa that is specifically located in the sarcomeres of skeletal and cardiac muscles. Titin is essential for generating tension during muscle contraction and relaxation, maintaining muscle elasticity, and plays a critical role in muscle homeostasis [1]. Owing to its large and specialized molecular structure, titin is particularly susceptible to proteol-

ysis by enzymes such as calpains and matrix metalloproteinases during muscle injury, and its degradation products, including the urinary N-terminal fragment of titin (N-titin), can be noninvasively detected in urine [2].

In recent years, urinary N-titin has gained attention as an early and sensitive biomarker of muscle damage and catabolic states. In adult populations, urinary N-titin rapidly increases following exercise-induced muscle injury, showing quicker responsiveness than traditional muscle injury markers such as serum creatine kinase (CK) [3]. Moreover, patients undergoing cardiac surgery exhibit significantly elevated urinary N-titin levels postoperatively, suggesting its potential as a monitoring marker for myocardial injury following cardiac procedures [4].

In critical care settings, elevated urinary N-titin levels have been reported in patients with intensive care unit-acquired weakness, in which sustained high levels correlate closely with decreased skeletal muscle mass and impaired physical function [5]. Additionally, urinary N-titin has demonstrated potential utility in evaluating nutritional status and sarcopenia in patients with nonalcoholic fatty liver disease (NAFLD) and gastrointestinal malignancies, suggesting its broad applicability in various clinical contexts [6,7].

In pediatric patients, particularly in those with Duchenne muscular dystrophy (DMD), significantly elevated urinary N-titin levels have been reported [8]. Even in young patients aged three years, urinary N-titin values are notably higher than those in healthy peers, highlighting its promise as a diagnostic and monitoring tool for neuromuscular diseases during childhood [9]. Although these findings have expanded the clinical utility of urinary N-titin in adults and children, studies focusing on neonates remain limited. In neonatal care, especially among extremely preterm infants, a marked elevation in urinary N-titin levels has been observed immediately after birth. This phenomenon is speculated to reflect muscular immaturity and enhanced catabolism associated with the abrupt environmental transition from intrauterine to extrauterine life [10]. However, studies on urinary N-titin levels in neonates remain limited, and detailed associations with neonatal factors, maternal conditions, and perinatal circumstances are yet to be fully elucidated.

To address this gap, the present study aimed to measure urinary N-titin concentrations within 24 hours after birth and perform a comprehensive analysis of their associations with neonatal clinical characteristics, maternal factors, and delivery-related variables. In addition, this study sought to establish reference values for urinary N-titin levels in neonates, with a view toward its potential application in early neonatal disease screening.

MATERIALS AND METHODS

Study design

This retrospective cohort study was conducted between October 2021 and December 2023 and included 523 neonates admitted to the NICU/GCU of Nihon University Itabashi Hospital. Urine samples were collected within 24 hours of birth and stored at –20°C until measurement. This study was approved by the Ethics Committee of the Nihon University Itabashi Hospital (approval no. RK-190910-3).

Measurement method

Urinary N-titin was quantified using an enzyme-linked immunosorbent assay (ELISA) and corrected for urinary creatinine (Cr), yielding N-titin/Cr (pmol/mg Cr). All samples were measured in duplicate, and the average values were used for the analysis. For measuring urinary N-titin, we used “29501; N-titin Measurement Kit Immuno-Biological Laboratories Co.,” and for urinary Cr, we used “500701; Creatinine (urinary) Colorimetric Assay Kit, Cayman Chemical.”

Study procedure

First, we retrospectively analyzed the association between urinary N-titin levels and neonatal and maternal clinical factors in the entire study population. A potential association between urinary N-titin levels and neonatal asphyxia has been previously suggested. Therefore, we conducted an exploratory subgroup analysis limited to neonates diagnosed with asphyxia to further examine the relationship between urinary N-titin levels and clinical variables. Asphyxia was defined as an Apgar score (1 minute) < 7.

Additionally, we calculated the 2.5th, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 97.5th percentiles of the urinary N-titin/Cr levels in neonates with and without asphyxia. To explore the clinical implications of elevated N-titin levels, we extracted the clinical courses of neonates with asphyxia whose urinary N-titin/Cr levels exceeded the 95th percentile. Furthermore, cases of neuromuscular diseases and chromosomal abnormalities were selected, and their clinical courses were reviewed.

Factors considered for analysis

We examined the following clinical factors in neonates and their mothers: gestational age, birth weight (gram), birth weight standard deviation score (SDS), gender, Apgar scores at 1 and 5 minutes, and AST, ALT, LDH, CK, Cr, and venous blood gas lactate levels within 24 hours of birth. Among the 523 participants, AST, ALT, LDH, CK, and Cr levels were tested in 500 patients, and lactate was tested in 521 patients.

Maternal and delivery factors included hypertensive disorders of pregnancy (HDP), gestational diabetes mellitus (GDM), premature rupture of membranes (PROM), clinical chorioamnionitis, placental abruption, non-reassuring fetal status (NRFS), emergency cesarean section, and induced labor. HDP was defined as new-onset hy-

pertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg) after 20 weeks of gestation [11]. GDM was considered if any of the following values was abnormal, using a 75 g oral glucose tolerance test by International Association of Diabetes and Pregnancy Study Groups criteria: fasting blood glucose ≥ 92 mg/dL, 1-hour post-load ≥ 180 mg/dL, or 2-hour post-load ≥ 153 mg/dL [12]. PROM was defined as the rupture of amniotic sac membranes before the onset of labor [13]. Clinical chorioamnionitis was diagnosed based on fever (> 37.8°C) plus at least two of the following: maternal tachycardia (> 100 bpm), maternal leukocytosis (> 15,000 /mm^3), uterine tenderness, fetal tachycardia (> 160 bpm), and foul-smelling amniotic fluid [14]. Placental abruption was diagnosed based on clinical symptoms, including vaginal bleeding accompanied by severe abdominal pain, uterine tenderness, and tetanic contractions [15]. Emergency cesarean sections require immediate delivery because of the threat to the life of the mother or fetus [16].

Statistical analysis

Continuous data are expressed as medians (minimum – maximum), and categorical data are expressed as numbers (%). Statistical analyses were performed using JMP Pro 17 software (SAS Institute Inc., Cary, NC, USA). Correlations between N-titin levels and clinical characteristics were analyzed using 95% confidence intervals for all patients or for those with asphyxia. Titin levels were compared using the Mann-Whitney U test based on the presence or absence of clinical factors in either the mother or neonate. Statistical significance was set at $p < 0.05$.

RESULTS

Neonatal and maternal characteristics of the participants are presented in Table 1.

Table 2 shows the correlations with neonatal factors. Urinary N-titin demonstrated a significant negative correlation with gestational age ($r = -0.13$, $p = 0.0035$), indicating higher N-titin levels in neonates with lower gestational age. There was no significant correlation between birth weight (g) or birth weight SDS ($r = -0.05$, $p = 0.25$; $r = 0.02$, $p = 0.58$, respectively), and no significant difference was found between males and females ($p = 0.65$). N-titin showed a significant negative correlation with Apgar scores at 1 and 5 minutes ($r = -0.33$, $p < 0.0001$; $r = -0.45$, $p < 0.0001$), indicating higher levels in neonates with more severe asphyxia (Figure 1). Regarding blood test results, N-titin showed a significant positive correlation with AST, ALT, LDH, CK, and lactate (all $p < 0.0001$).

Table 3 shows the associations between maternal and delivery factors and urinary N-titin levels. Urinary N-titin levels were significantly higher in patients with NRFS, placental abruption, and emergency cesarean delivery ($p < 0.0001$, $p = 0.0125$, and $p = 0.0279$, respectively) (Figure 2). In contrast, no significant differences in urinary N-titin levels were observed in patients with HDP, gestational diabetes mellitus, PROM, clinical chorioamnionitis, or induced labor ($p = 0.496$, $p = 0.127$, $p = 0.686$, $p = 0.615$, and $p = 0.165$, respectively).

These findings suggest that urinary N-titin levels increase in response to neonatal asphyxia, so we conducted a subgroup analysis of neonates with asphyxia. Table 4 presents the clinical characteristics of the neonates. Table 5 shows the correlations between urinary N-titin levels and neonatal factors in neonates with asphyxia. There was no significant correlation between N-titin and gestational age ($r = -0.14$, $p = 0.151$), birth weight ($r = -0.04$, $p = 0.653$), or birth weight SDS ($r = -0.07$, $p = 0.471$), and no significant difference was observed between genders ($p = 0.685$). Within the asphyxia subgroup, urinary N-titin retained a significant negative correlation with Apgar scores at 1 and 5 minutes ($r = -0.21$, $p = 0.0276$; $r = -0.42$, $p < 0.0001$, respectively), similar to the overall findings. Furthermore, the correlation between N-titin levels and muscle-derived enzymes was stronger than that observed in the total population. Specifically, N-titin significantly and positively correlated with AST ($r = 0.46$, $p < 0.0001$), ALT ($r = 0.68$, $p < 0.0001$), LDH ($r = 0.58$, $p < 0.0001$), CK ($r = 0.72$, $p < 0.0001$), and lactate ($r = 0.38$, $p < 0.0001$).

Based on these results, it is evident that urinary N-titin levels increase strongly under the influence of neonatal asphyxia. Therefore, to exclude the effect of asphyxia, reference values for urinary N-titin were established using only the 409 neonates without asphyxia, defined as a 1-minute Apgar score of ≥ 7 (Table 6). Additionally, we compared the reference values between neonates with and without perinatal asphyxia and identified six neonates in the asphyxia group whose urinary N-titin/Cr levels exceeded the 95th percentile. The clinical characteristics and outcomes are shown in Table 7a. Among the six neonates in the asphyxia group whose urinary N-titin/Cr levels exceeded the 95th percentile, three died, two showed mild developmental delay, and only one exhibited normal development.

We also assessed the presence of neuromuscular disorders and chromosomal abnormalities in our study cohort. No neonates with neuromuscular disorders were identified in this study. However, seven neonates were diagnosed with Down syndrome. The clinical characteristics of the patients are summarized in Table 7b.

DISCUSSION

In this study, urinary N-titin levels measured within 24 hours of birth showed a significant negative correlation with Apgar scores at both 1 and 5 minutes. Urinary N-titin also positively correlated with muscle-derived enzymes, including AST, ALT, CK, and LDH. These findings suggest that urinary N-titin levels reflect the severity of perinatal stress in neonates.

Table 1. Clinical characteristics of the study population.

Characteristic	n = 523
Neonatal factors	
Gestational age (weeks)	36 (22 - 41)
Gender (male)	271 (51.7)
Birth weight (gram)	2,368 (414 - 4,188)
Birth weight (SD)	-0.3 (-5.7 to 3.9)
Apgar score (1 minute)	8 (0 - 9)
Apgar score (5 minutes)	9 (1 - 10)
Titin (pmol/mg Cr)	15.5 (0.05 - 1,409.6)
Maternal and delivery factors	
Pregnancy-induced hypertension	57 (10.9)
Gestational diabetes mellitus	71 (13.6)
Pre-labor rupture of membranes	84 (16.1)
Clinical chorioamnionitis	4 (0.8)
Placental abruption	9 (1.7)
NRFS	96 (18.4)
Emergency cesarean delivery	139 (26.6)
Induction of labor	53 (10.1)

Data are presented as median (range) or number (percentage).
NRFS non-reassuring fetal status.

Table 2. Correlations between urinary N-titin and neonatal variables.

Parameter	Correlation coefficient (r)	p-value
Gestational age and birth measurements		
Gestational age (weeks)	-0.13	0.0035
Birth weight (gram)	-0.05	0.25
Birth weight (SD)	0.02	0.58
Gender	-	0.65
Apgar scores		
Apgar score (1 minute)	-0.33	< 0.0001
Apgar score (5 minutes)	-0.45	< 0.0001
Blood tests		
AST (U/L)	0.42	< 0.0001
ALT (U/L)	0.64	< 0.0001
LDH (U/L)	0.56	< 0.0001
CK (U/L)	0.66	< 0.0001
Creatinine (mg/dL)	0.07	0.13
Lactate (mmol/L)	0.36	< 0.0001

Table 3. Association between maternal/delivery factors and urinary N-titin.

Factor	N-titin (median [range])	p-value
Hypertensive disorder of pregnancy	yes: 20.4 (0.4 - 339.0) no: 15.3 (0.05 - 1,409.6)	0.496
Gestational diabetes mellitus	yes: 15.0 (0.05 - 335.1) no: 15.7 (0.1 - 1,409.6)	0.127
Pre-labor rupture of membranes	yes: 15.3 (0.4 - 430.4) no: 15.7 (0.05 - 1,409.6)	0.686
Clinical chorioamnionitis	yes: 14.7 (2.5 - 36.1) no: 15.5 (0.05 - 1,409.6)	0.615
Placental abruption	yes: 40.6 (4.4 - 1,409.6) no: 15.3 (0.05 - 1,138.5)	0.0125
Non-reassuring fetal status (NRFS)	yes: 27.3 (1.7 - 430.4) no: 14.3 (0.05 - 1,409.6)	< 0.0001
Emergency cesarean delivery	yes: 23.4 (0.1 - 1,409.6) no: 14.8 (0.05 - 1,138.5)	0.0279
Induction of labor	yes: 15.7 (0.4 - 1,138.5) no: 15.3 (0.05 - 1,409.6)	0.165

Data are presented as median (range) for descriptive statistics and correlation coefficients (r) with corresponding p-values for associations with urinary N-titin.

Table 4. Characteristics of neonates with asphyxia.

Characteristic	n = 114
Gestational age (weeks)	36 (22 - 41)
Gender (male)	61 (53.5)
Birth weight (gram)	2269 (414 - 3,870)
Birth weight (SD)	-0.2 (-4.6 to 1.9)
Apgar score (1 minute)	3 (0 - 6)
Apgar score (5 minutes)	8 (1 - 10)
Titin (pmol/mg Cr)	35.4 (0.9 - 1,409.6)

Data are presented as median (range) or number (percentage).

In our overall analysis, urinary N-titin showed a significant negative correlation with gestational age and, additionally, a negative correlation with both 1-minute and 5-minute Apgar scores. In other words, both immaturity (lower gestational age) and asphyxia (lower Apgar scores) may contribute to elevated N-titin levels. Previous studies reported that extremely preterm infants are vulnerable to muscle development and metabolism, leading to higher N-titin levels [8]. Conversely, acute hypoxic stress, such as neonatal asphyxia, may induce muscle cell damage and catabolic enhancement, resulting in elevated N-titin levels.

Indeed, in the subanalysis limited to neonates with asphyxia (Apgar score at 1 minute < 7), the correlation between N-titin and gestational age disappeared, while the negative correlation with Apgar score persisted. This suggests that in neonates experiencing asphyxia, acute stress factors overshadow the influence of immaturity or that the limited sample size in this subgroup reduces the detectability of correlations. Additionally, in cases of severe asphyxia, active resuscitative interventions and other complications likely affect N-titin levels beyond the gestational age.

Regarding maternal and delivery factors, urinary N-titin was significantly elevated in NRFS, placental abruption, and emergency cesarean section, presumably reflecting fetal distress. Conditions such as HDP or clinical chorioamnionitis are generally associated with chronic fetal distress [17,18]. However, in our study, these conditions did not lead to a significant increase in

Table 5. Correlations between urinary N-titin and neonatal factors in neonates with asphyxia (n = 114).

Parameter	Correlation coefficient (r)	p-value
Neonatal factors		
Gestational age (weeks)	-0.14	0.151
Birth weight (gram)	-0.04	0.653
Birth weight (SD)	-0.07	0.471
Apgar score (1 minute)	-0.21	0.0276
Apgar score (5 minutes)	-0.42	< 0.0001
Gender	-	0.685
Blood tests		
AST (U/L)	0.46	< 0.0001
ALT (U/L)	0.68	< 0.0001
LDH (U/L)	0.58	< 0.0001
CK (U/L)	0.72	< 0.0001
Creatinine (mg/dL)	0.06	0.52
Lactate (mmol/L)	0.38	< 0.0001

Correlation coefficients and p-values are shown for continuous variables. N-titin levels are presented as medians (ranges).

Table 6. Reference values of urinary N-titin (pmol/mg Cr) in neonates with and without neonatal asphyxia.

Percentile	Asphyxia (n = 114)	No asphyxia (n = 409)
2.5th	2.5	0.7
5th	4.6	2.0
10th	7.7	3.4
25th	16.8	6.7
50th	35.4	15.5
75th	78.8	37.5
90th	206.1	88.7
95th	316.7	147.0
97.5th	412.1	216.4

Urinary N-titin levels (pmol/mg creatinine) measured within 24 hours after birth are summarized by percentiles (2.5th, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 97.5th) in neonates with and without neonatal asphyxia. Percentiles were calculated separately for the asphyxia (n = 114) and non-asphyxia groups (n = 409).

urinary N-titin levels. This may be because urinary N-titin rises rapidly in response to acute muscle injury and has a relatively short half-life of approximately 12 hours [2]. Therefore, urinary N-titin is more likely to reflect acute fetal distress occurring around the time of delivery, rather than prolonged or chronic stress.

When evaluating neonatal urinary N-titin levels in relation to maternal and delivery factors, one must consider whether N-titin crosses the placenta, indicating whether maternal stress influences neonatal N-titin levels. Generally, molecules larger than 1 kDa do not readily cross the placenta. Although specific evidence regarding N-titin (21 - 22 kDa) is unavailable, it is likely to be too large to cross, suggesting that neonatal urinary N-titin reflects the neonate's condition, not maternal status.

Urinary N-titin, as explored in this study, offers unique advantages over existing biomarkers for perinatal asphyxia. Compared to neutrophil gelatinase-associated lipocalin (NGAL), serum enzymes (AST, ALT, LDH, and CK), and urinary markers such as malondialdehyde

Table 7a. Neonates with urinary N-titin levels above the 95th percentile among the asphyxia group.

Sample number	N-Titin (pmol/mg Cr)	GA (weeks + days)	Birth weight (gram)	Apgar score (1'/5')	Perinatal complications	Outcome
1	1,409.6	40w6d	3,519	1/2	placental abruption, emergency CS	mild developmental delay at 3 years 0 months
2	1,138.5	22w1d	450	2/3	extreme preterm birth	died on day 8 due to septic shock
3	430.4	25w4d	650	1/3	NRFS, placental abruption, emergency CS	died on day 66 due to GI perforation
4	408.2	25w0d	414	1/6	NRFS, emergency CS	died on day 155 due to sepsis
5	339.0	40w1d	3,024	4/7	HDP, NRFS, vacuum extraction	normal development *
6	335.1	32w5d	820	5/8	oligohydramnios, emergency CS	mild developmental delay at 1 year 8 months

Six neonates in the asphyxia group had urinary N-titin levels above the 95th percentile.
GA gestational age, NRFS non-reassuring fetal status, CS cesarean section, GI gastrointestinal, HDP hypertensive disorder of pregnancy.
*** Sample no. 5 presented with a depressed skull fracture at birth, although subsequent neurodevelopment was normal.**

Table 7b. Clinical characteristics of neonates with Down syndrome.

Sample number	Gestational age (weeks + days)	Birth weight (gram)	Apgar score (1'/5')	Urinary N-titin/Cr (pmol/mg Cr)
1	36w3d	2,976	5/8	33.5
2	37w3d	2,616	8/9	13.4
3	37w4d	2,730	8/8	41.4
4	38w1d	2,585	7/8	39.8
5	38w2d	3,040	8/9	15.0
6	38w3d	2,102	8/9	70.6
7	39w4d	2,716	8/9	52.3

Clinical characteristics of neonates with 21 trisomy.

(MDA) or uric acid, N-titin is a non-invasive, muscle-specific indicator of injury. For example, NGAL levels are significantly elevated in the umbilical cord blood of asphyxiated neonates and may reflect vascular endothelial injury or systemic inflammation [19]. Similarly, AST, ALT, LDH, and CK levels are correlated with the severity of asphyxia and short-term outcomes, such as the duration of mechanical ventilation [20].

Urinary malondialdehyde (MDA), uric acid, and protein-to-creatinine ratios have also been demonstrated to be potential markers of hypoxic stress and brain injury, showing significant correlations with the severity of hypoxic-ischemic encephalopathy (HIE) and mortality [21]. However, these markers typically reflect systemic oxidative stress and may lack tissue specificity.

Urinary N-titin can be measured non-invasively and may serve as a surrogate for hypoxia-induced muscle injury. Its elevation in severely asphyxiated neonates with poor outcomes indicated its potential prognostic value. Nonetheless, similar to the other biomarkers, N-titin alone may not provide sufficient predictive power for long-term outcomes.

Recent studies on neonates undergoing therapeutic hypothermia for HIE have emphasized the importance of combining multiple diagnostic modalities, including neurological assessment, imaging, and electrophysiology, for a more accurate prognostication [22]. In this context, urinary N-titin could play a complementary role as part of a multimodal strategy to improve early risk stratification and outcome prediction in neonatal as-

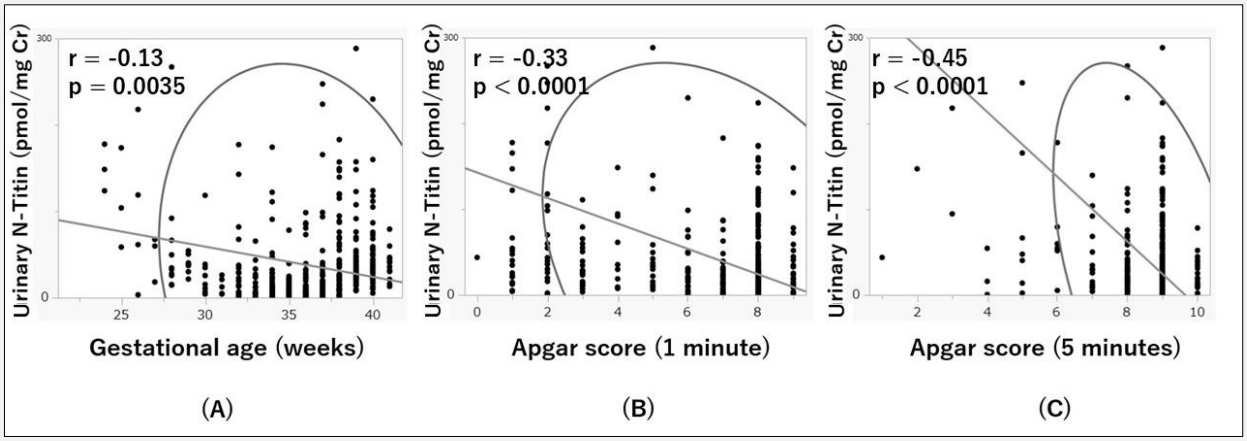

Figure 1. Correlation between urinary N-titin levels and clinical factors in neonates.

Scatter plots showing negative correlations between urinary N-titin/creatinine (Cr) ratios and Apgar scores at 1 minute (B) and 5 minutes (C) after birth in all neonates (n = 523). Each dot indicates a single patient. The black lines represent the regression trends, and the 95% confidence ellipses are shown in gray. Urinary N-titin levels were significantly higher in neonates with lower Apgar scores, indicating a strong association with the severity of perinatal asphyxia (r = -0.33 at 1 minute and r = -0.45 at 5 minutes, both p < 0.0001).

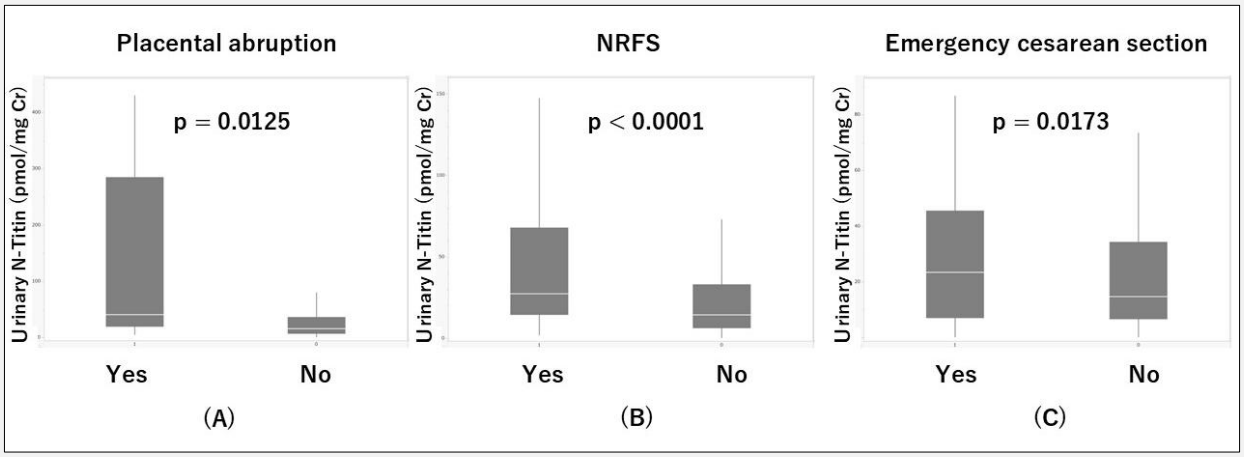

Figure 2. Association between urinary N-titin levels and maternal/delivery factors.

Box-and-whisker plots comparing urinary N-titin/creatinine (Cr) ratios within 24 hours after birth among neonates with or without the following perinatal conditions: non-reassuring fetal status (NRFS), placental abruption, and emergency cesarean section. In each condition, neonates who experienced the risk factors exhibited significantly higher urinary N-titin/Cr levels than those who did not. Horizontal lines indicate medians, and boxes represent interquartile ranges.

phyxia.

As shown in Table 7a, the six neonates with urinary N-titin levels above the 95th percentile in the asphyxia group were all diagnosed with perinatal asphyxia. Although one term infant survived with a mild developmental delay, the other five were either extremely preterm or had severe perinatal complications, with three resulting in in-hospital deaths. These findings suggest that urinary N-titin may be associated not only with perinatal distress but also with adverse long-term outcomes.

Furthermore, previous reports have suggested that urinary N-titin could be useful in screening 3-year-old children diagnosed with Duchenne muscular dystrophy [5]. However, as there were no infants with neuromuscular disorders in our cohort, we could not evaluate whether neonatal urinary N-titin could screen for such conditions. Although not classified as a neuromuscular disorder, 21 trisomy is a representative condition associated with the floppy infant phenotype. In this study, we examined neonates with 21 trisomy to investigate whether congenital disorders influenced early postnatal urinary N-titin levels. All seven neonates with 21 trisomy exhibited urinary N-titin/Cr values below the 95th percentile thresholds established for both the asphyxia and non-asphyxia groups. The clinical characteristics and urinary N-titin/Cr values are summarized in Table 7b. These findings suggest that 21 trisomy, even when accompanied by perinatal asphyxia, may not substantially elevate urinary N-titin levels. Although further studies are needed, our results indicate that urinary N-titin is likely to reflect perinatal stress or muscle injury rather than being influenced by congenital chromosomal abnormalities.

This study has three main limitations. First, it was a retrospective cohort study conducted at a single institution. Second, only one time-point measurement was performed; therefore, we could not examine the time-course trends in N-titin or their prognostic implications. Third, no neonates with neuromuscular disorders were included.

In the future, prospective multi-center studies and detailed longitudinal analyses are needed to quantitatively distinguish the respective influences of immaturity and acute stress factors on N-titin and further explore their relationship with outcomes.

CONCLUSION

Urinary N-titin levels reflect acute perinatal stress, particularly neonatal asphyxia rather than congenital abnormalities. A review of the cases exceeding the reference values established for neonates with asphyxia revealed a high proportion of adverse outcomes. Future studies should assess the time-course changes in urinary N-titin levels and their association with long-term outcomes to evaluate their potential utility as biomarkers.

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Declaration of Generative AI and AI-Assisted Technologies in the Writing Process:

In the course of preparing this work, the authors utilized ChatGPT (GPT-4 and GPT-4o by OpenAI) and Claude (Claude 3 Opus by Anthropic) to improve the readability and perform proofreading of the English text. Following the use of these tools, the authors thoroughly reviewed and revised the content as necessary, taking full responsibility for the final version of the publication.

Declaration of Interest:

The authors have no conflicts of interest to declare.

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