**Original Article**

Lactate and S100 protein as early biochemical indicators of birth neonatal asphyxia caused by intrauterine umbilical cord strangulation: a medicolegal view

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**ABSTRACT**

**Background:** From a forensic pathologist’s perspective, there are several aspects of the perinatal postmortem that are particularly important if the baby was born alive or dead. If the infant is delivered alive and dies a few hours or days later, access to the obstetric and neonatal notes is required to achieve a correct interpretation and conclusion in situations of perinatal morbidities occurring in hospitals. After prenatal hypoxia, hypoxic ischemic encephalopathy (HIE) is a common cause of neonatal morbidity and long-term neurological disability. It has many causes including intrauterine strangulation by umbilical cord (nuchal cord). Failure of early diagnosis of neonatal asphyxia and its treatment is considered a medicolegal negligence against the doctors. **Aim:** The present study aimed to use cord blood lactate & S100 protein levels as early markers of neonatal hypoxia caused by nuchal cord to minimize the risk of medicolegal liabilities against the doctors. **Methods:** This is a comparative cross-sectional study carried out 30 hypoxic neonates due to intrauterine cord strangulation. Lactate & S100 protein levels in the cord blood were evaluated. As a control group, 30 apparently healthy neonates were compared in age, sex, and body weight. **Results:** Lactate & S100 protein levels in cord blood were a higher significant difference in HIE neonates than control group. **In conclusion:** lactate & S100 protein levels in cord blood could be used as an early marker for diagnosis of neonatal HIE.

**Key words:** Hypoxic, ischemic, encephalopathy, Nuchal cord, Lactate, S100 protein.
I. INTRODUCTION

With a reported prevalence of 100-250/1000 live births in the poor countries, birth asphyxia is a major cause of infant illness and mortality (Lawn et al., 2009). Birth asphyxia is the world's main cause of premature neonatal death, accounting for 23% of all neonatal deaths. Every year, between 4 and 9 million babies are born, of which 1.2 million die and a similar number acquire serious neuro-disabilities such as cerebral palsy, epilepsy, and developmental delay (Manandhar and Basnet, 2019).

A typical umbilical anomaly in men is the umbilical cord around the neck, known as the nuchal cord. It has been found in 23-37 percent of human pregnancies, with the rate increasing as the pregnancy progresses. (Clapp et al., 2003). Nuchal cords can be single or multiple in number, as well as tight or loose. The placental end of the [Type A] cord crosses over the umbilical end, entangling the neck in an unlocked pattern, whereas the cord in the [Type B] pattern cannot be undone and ends up as a real knot. The placental end crosses under the umbilical end, entangling the neck in a locked pattern (Collins, 2002).

Multi-organ damage and cardiovascular failure were major outcomes of perinatal hypoxia. Postnatal neurological impairment may be caused by myocardial injury, right ventricular dysfunction, abnormal circulatory transition, and defective autoregulation. As a result, adequate monitoring and specific therapy are required following an asphyxial insult (Kluckow, 2011).

When tissue perfusion is poor and hypoxia is present, lactate is produced invariably. If the clinical reduction of oxygen and substrate delivery occurs, the aerobic metabolism through Krebs cycle cannot be persisted and tissues must rely on anaerobic metabolism to supply their energy needs. This causes an increase in blood lactate results in its accumulation and reflects tissue hypoxia (Jin et al., 2013). Once produced prenatally, the placenta excretes it. During neonatal period liver and kidneys control its excretion. Lactate analysis, when compared to pH, offers equivalent or greater predictive characteristics in identifying short-term newborn morbidity (Borruto et al., 2008).

The S-100 protein is a calcium binding protein detected in astroglial cells in excessive quantities. In neuronal development, outgrowth, and death, it regulates calcium-dependent signalling. In many CNS disorders, it is thought to be an indication of glial activation and/or death (Sedaghat and Notopoulos, 2008).

According to studies, there is a link between the severity of neuronal injury and the concentration of S100, which has sparked a renewed interest in S100 in asphyxiated babies. However, just a few papers have examined brain injury biomarkers in umbilical cord blood samples taken at birth (Wirds et al., 2003; Gazzolo et al., 2009; Michetti et al., 2012).

Pregnant women are concerned about the possibility of developing a nuchal chord. The level of concern varies depending on their education, but in general, moms believe that the nuchal cord is to blame for the newborn's poor growth. As a result, moms who have a newborn with a nuchal cord and delayed development blame midwives and obstetricians and seek legal dispute, claiming everything from compensation to an occupational ban (Kong et al., 2015).

For this medicolegal aspect, the current...
S100 protein levels as early markers of neonatal hypoxia caused by nuchal cord to reduce the risk of medicolegal negligence & liability against the doctors.

II. PATIENTS AND METHODS:

2.1 Type of study:

The current study is a cross-sectional study that was carried out after receiving ethical approval from the ethical committee, Faculty of Medicine, Benha University. Before the study began, all participants were given thorough information about the study's goals, and their parents gave their informed consent.

2.2 Patients:

This research involved 30 asphyxiated full-term babies who were delivered vaginally or via caesarean surgery at Gynecology & Obstetric department, Faculty of Medicine, Benha University Hospitals, Egypt. The study was carried out at the period between beginnings of June till the end of September 2021, so we chose the sample size of the study matching the inclusion and exclusion criteria. As a control group, 30 age-matched seemingly healthy full-term babies with no obstetrical problems were compared.

➢ Inclusion criteria

1. Full-term neonates delivered with wrapped umbilical cord around his neck one or more times.

2. When newborns meet the standards put by the American Academy of Pediatrics (AAP), they are considered hypoxic (Blackmon et al., 2006):

   Neonatal neurologic complications if an expanded Apgar score of 0-3 persists and multiple organ involvement (e.g., kidney, lungs, liver, heart, intestines).

➢ Exclusion Criteria:

1. Preterm neonates delivered before 36 weeks gestation.

2. Newborn delivered with major congenital anomalies or chromosomal abnormality.

3. Traumatic brain injuries.

4. Maternal drug addiction.

5. Intrauterine infection, and general anesthesia during birth process.

All patients and controls underwent a comprehensive physical examination by a paediatrician, including a full maternal history, a detailed history of resuscitation, and an Apgar score at 1 and 5 minutes. Systemic evaluation with a focus on neurological examination and the use of Sarnat and Sarnat staging to determine the severity of hypoxia ischemic encephalopathy. For both groups the following data was collected:

- Socio-demographic data of cases: age, gender and residence.
- Medico-legal aspects of cases: cause, pattern, site, type and outcome of injuries. Causative instruments, types of treatment (surgical or conservative) provided, and condition of the case at discharge time.

2.3 Sample collection:

The umbilical cord blood was extracted immediately after birth from
double-clamped segment of the umbilical cord into 3 ml plastic syringes flushed with a heparin solution. Upon arrival of the samples in the laboratory, blood gas, lactate, and glucose levels were measured. From all neonates included in the study, 1mL of umbilical cord blood samples was collected then centrifuged at 2500 rpm for 5 min and frozen at -80°C. S100 protein level was measured in these samples.

2.4 Determination of umbilical cord blood lactate:

Lactate oxidase converts lactate to pyruvate and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide combines with chromogen precursors to produce a purple molecule. The color intensity is proportional to the amount of lactate in the sample being analysed. The DXC-800 Automated Chemistry Analyser (Beckman Coulter) was used for lactate assays.

2.5 Determination of S100 protein in umbilical cord blood:

The umbilical cord blood’ S100 protein was measured by ELISA kit. The purified anti-S100 protein antibody was precoated onto 96-well plates, and Anti-S100 antibodies coupled to horseradish peroxidase were employed as detecting antibodies. The wells were filled with the standards, test samples, and HRP-conjugated detection antibodies, which were mixed and incubated; unbound conjugates were then washed away with wash buffer. Tetramethyl benzidine (TMB) substrates (A and B), The HRP enzymatic process was

TMB was catalysed by HRP to create a blue-colored product that became yellow when acidic stop was added.

The density of the yellow product is proportional to the S100 amount of sample captured in plate. The optical density absorbance was read at 450 nm in a microplate reader, and then the concentration of S100 was calculated (Chongqing Biospes Co. Ltd Chongqing, China).

2.6 Sarnat & Sarnat staging:

All newborn delivered with hypoxia due to nuchal cord will be graded according to Sarnat & Sarnat staging (Gardiner et al., 2009) as shown in table (1).

*Statistical analysis:*

SPSS software, version 22.0 (IBM, Armonk, NY, USA) for Windows was used to analyse the data. Categorical data were presented as number and percentages, Chi square (χ2) test was used to analyse them. Quantitative data were tested for normality using Shapiro-Wilks test assuming normality at P>0.05. Normally distributed variables were expressed as mean ±standard deviation and analyzed by Student “t” test and ANOVA for 2 and 3 independent groups respectively, while non parametric variables were presented as median and range, and analyzed by Mann Whitney U test (ZMWU) for 2 independent groups. Significant To find the significant pairs, ANOVA was followed by post hoc multiple comparisons using Bonferroni adjusted testing. ROC
Curves were constructed to assess the performance of the studied markers as early detection of NA. P ≤ 0.05 was considered significant.

Table (1): Sarnat & Sarnat staging system (Gardiner et al., 2009).

|                | Grade I Mild       | Grade II Moderate | Grade III Severe |
|----------------|--------------------|-------------------|------------------|
| Alertness      | Hyperalert         | Lethargy          | Coma             |
| Muscle tone    | Normal or increased| Hypotonic         | Flaccid          |
| Seizures       | None               | Frequent          | Uncommon         |
| Pupils         | Dilated, reactive  | Small, reactive   | Variable, fixed  |
| Respiration    | Regular            | Periodic          | Apnoea           |
| Duration       | < 24 Hours         | 2 - 14 Days       | Weeks            |

III. RESULTS

All children were examined by a pediatrician immediately after birth. All of the children were born after the 38th week of pregnancy. Of all nuchal cords have been a single entanglement in 27 cases, 3 children presented a double umbilical cord entanglement.

Regarding the perinatal history, in terms of gestational age, mode of delivery, sex, weight, head circumference, and length, there was no statistically significant difference between the study and control groups. However, there was a highly significant difference between studied groups according to Apgar score (1, 5 minutes) as illustrated in Table 2.

Regarding Sarnat & Sarnat stage of cases group, stage I and II represented 36.7% for each one while stage III represented 26.7% as shown in (Figure 1).

Figure (1): Sarnat & Sarnat stage of cases group.
Lactate and S100 protein

Table (3) demonstrated that there was a statistical high significant decrease of the level of umbilical cord PH, base excess among cases group. Moreover, there was statistically significant increase regarding lactate and S100 protein levels.

Analysis of receiver operating characteristics (ROC) curve of the studied markers for neonatal asphyxia caused by intrauterine umbilical cord strangulation was demonstrated in tables (4 & 5) and figure (2). As regards the studied markers in all studied groups, serum S100 protein level had a sensitivity of 100% and specificity of 96.7%, PPV of 96.8%, and NPV of 100%, which is superior to other markers such as lactate.

There were no significant statistical differences between the studied markers umbilical cord PH, base excess, umbilical lactate and umbilical S100 protein regarding the mode of delivery as showed in table 6, while table (7) revealed that S100 protein level was significantly higher in grade III HIE according to Sarnat & Sarnat stage.

Table (2): Comparison between the study and control groups regarding perinatal history.

| Variable                      | Cases (n=30) | Controls (n=30) | Test of significance | P    |
|-------------------------------|--------------|----------------|----------------------|------|
| Gestational age (week)        | Mean±SD      |                |                      |      |
|                               | 38.3±1.1     | 38.5±0.7       | St."t"=0.61          | 0.54 (NS) |
| Mode of delivery              |              |                |                      |      |
| CS                            | 15 50.0      | 18 60.0        | χ2=0.61              | 0.43 (NS) |
| NVD                           | 15 50.0      | 12 40.0        |                      |      |
| Sex                           |              |                |                      |      |
| Male                          | 15 50.0      | 15 50.0        | χ2=0.0               | 1.0 (NS) |
| Female                        | 15 50.0      | 15 50.0        |                      |      |
| Weight (gm)                   | Mean±SD      |                |                      |      |
|                               | 3736.7±298.7 | 3689±408.4     | 0.52                 | 0.6 (NS) |
| Head circumference (cm)       | Mean±SD      |                |                      |      |
|                               | 35.4±2.7     | 35.1±0.97      | 0.71                 | 0.47 (NS) |
| Length (cm)                   | Mean±SD      |                |                      |      |
|                               | 48.4±1.18    | 49.0±0.96      | 1.85                 | 0.069 (NS) |
| APGAR 1 min                   | Median (range)|              |                      |      |
|                               | 2.0 (0-4)    | 8.0 (7-9)      | ZMWU=6.7             | <0.001 (HS) |
| APGAR 5 min                   | Median (range)|              |                      |      |
|                               | 4.0 (1-6)    | 9.0 (8-10)     | ZMWU=6.83            | <0.001 (HS) |
section, NVD: normal vaginal delivery, ZMWU= Z value of Mann Whitney U test, NS: non-significant, HS: Highly significant.
Table (3): Comparison between the study and control groups regarding umbilical cord PH, lactate and S100 protein.

| Variables                      | Patients (n=30) | Controls (n=30) | St. "t" | P value  |
|-------------------------------|----------------|----------------|---------|----------|
|                               | Mean ± SD     | Range          | Mean ± SD | Range    |         |
| Umbilical cord PH             | 6.99 ± 0.12   | 6.68-7.2       | 7.24 ± 0.03 | 7.2-7.32 | 10.3    |
|                              |               |                |          | <0.001  |
| Umbilical cord base excess    | -15.16 ± 2.37 | -19-(-11)      | -2.00 ± 3.62 | -8.0 - 4 | 16.6    |
|                              |               |                |          | <0.001  |
| umbilical lactate (mmol/l)    | 7.50 ± 3.12   | 3.1-14.1       | 3.16 ± 1.05 | 1.9-5.1  | 7.19    |
|                              |               |                |          | <0.001  |
| 12-24 hr lactate (mmol/l)     | 3.64 ± 1.90   | 0.8-7.1        | 1.20 ± 0.38 | 0.8-2.1  | 6.9     |
|                              |               |                |          | <0.001  |
| umbilical S100 protein (ug/l) | 4.56 ± 1.06   | 3.01-6.8       | 1.39 ± 0.45 | 1.02-3.24 | 14.9    |
|                              |               |                |          | <0.001  |

SD: standard deviation, n: number, St."t": Student “t” test, hr: hour, HS: Highly significant.

Table (4): The performance of the studied markers as early indicators of neonatal asphyxia caused by intrauterine umbilical cord strangulation.

| Variable                      | Cutoff | Sensitivity % | Specificity % | PPV % | NPV % | AUC  | SE   | 95%CI | P       |
|-------------------------------|--------|---------------|---------------|-------|-------|------|------|-------|---------|
| umbilical lactate (mmol/l)    | ≥4.6   | 83.3%         | 90%           | 89.3% | 84.4% | 0.924| 0.032| 0.86-0.98 | <0.001  |
| 12-24 hr lactate (mmol/l)     | ≥1.45  | 86.7%         | 86.7%         | 86.7% | 86.7% | 0.897| 0.045| 0.81-0.98 | <0.001  |
| umbilical S100 (ug/l)         | ≥2.54  | 100%          | 96.7%         | 96.8% | 100%  | 0.994| 0.006| 0.98-1.0   | <0.001  |

Table (5): Comparing the performance of the studied markers.
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| Variables | NVD (n=15) | CS (n=15) | St."t" | P value |
|-----------|------------|------------|--------|--------|
| Umbilical cord PH | Mean | ± SD | Mean | ± SD | 1.5 | 0.14 (NS) |
| Umbilical cord base excess | -14.60 | 2.13 | -15.73 | 2.54 | 1.32 | 0.19 (NS) |
| Umbilical lactate (mmol/l) | 7.68 | 3.42 | 7.32 | 2.91 | 0.31 | 0.76 (NS) |
| 12-24 hr lactate (mmol/l) | 3.88 | 1.72 | 3.41 | 2.10 | 0.66 | 0.51(NS) |
| Umbilical S100 protein (ug/l) | 4.76 | 1.00 | 4.36 | 1.12 | 1.03 | 0.31 (NS) |

Figure (2): ROC curve for the performance of the studied markers as early indicators of neonatal asphyxia caused by intrauterine umbilical cord strangulation.

Table (6): Comparing the studied markers according to mode of delivery.

NS: non-significant, hr: hour
studied markers according to Sarnat & Sarnat stage.

*significant in comparison with stage I, †significant in comparison with stage II, n: number, hr: hour, HS: Highly significant.
IV. DISCUSSION

The umbilical cord is the blood vessel that connects the foetus to the placenta, allowing foetal blood to travel to and from the placenta. It runs from the foetal umbilicus to the placenta’s foetal surface. Excessively long cords are linked with cord entanglement. One of the theories for birth asphyxia is cord compression (Verma et al., 2020).

Prenatal asphyxia causes hypoxic ischemic encephalopathy (HIE), which seems to be a cause of neonatal morbidity, neurological damage, and fatality. Because of the short therapeutic window and potential adverse effects of neuroprotective interventions, early detection is critical (Guan et al., 2017; Graham et al., 2018).

Because of the short therapeutic window and potential adverse effects of neuroprotective measures, hypoxic ischemic encephalopathy (HIE) after prenatal hypoxia is a major cause of neonatal morbidity, neurological impairment, and mortality. Its early detection is especially critical (Guan et al., 2017; Graham et al., 2018).

In the present study, there was no statistically significant difference between the HIE group due to strangulation by umbilical cord and control group as regards gestational age, sex, head circumference, weight, length, and mode of delivery. While as regard Apgar score, At 1 and 5 minutes, the median Apgar score was considerably lower than the control group, which had a normal Apgar score (7 - 9 at 1 and 5 minutes), these results were in agreement with Leybovitz-Haleluya et al., 2019 who shown that a low Apgar score was linked to a variety of issues in newborns.

The results of the current study revealed that umbilical cord blood lactate and S100 protein level in HIE group due to umbilical cord strangulation were statistically significantly higher than control group, and the umbilical cord blood lactate level was detected in grade III HIE according to Sarnat & Sarnat staging.

These findings were consistent with previous research, which indicated that serum lactate levels in hypoxic neonates were higher than in healthy neonates (Gasparović et al., 2012; Simovic et al., 2015). Also, the study of Chiang et al. (2016) revealed that higher serum lactate in hypoxic neonates and higher level was found in grade III HIE.

The current work showed that S100 protein levels were significantly higher in hypoxic neonates due to cord strangulation than control. This is in accordance with the findings of Beharier et al. (2012) and Douglas-Escobar et al. (2012).

S100 protein levels were shown to be greater in grade III HIE when compared to the different clinical phases of the Sarnat & Sarnat clinical classification for HIE diagnosis in this investigation. This is in accordance with the findings of Qian et al. (2009).

According to the ROC curve, the present study revealed that cord blood can be used to diagnose neonatal hypoxia caused by intrauterine strangulation by umbilical cord with sensitivity 83.3% and specificity 90%. This is consistent with studies that show that umbilical lactate level can be utilised as a measurement of intrapartum hypoxia in a middle-low resource scenario with reasonable sensitivity and specificity (Haiju et al., 2008; Allanson et al., 2018).

Also, the current study showed that S100 protein can be used for diagnosis of neonatal hypoxia due to intrauterine cord strangulation with higher sensitivity and specificity (100% & 96.7% respectively) than cord blood lactate. These findings were consistent with the research which stated that serum S100 protein level had a sensitivity of 97% and specificity of 91%, with a diagnostic
accuracy of 94%, which is superior to other markers such as lactate, which showed a sensitivity of 94% and specificity of 87% (Beken et al., 2014). Moreover, S100 protein is superior to other markers such as NRBCs, which showed a sensitivity of 83.4% and specificity of 73.5% (Boskabad et al., 2010).

V. CONCLUSION
This study concluded that both umbilical blood lactate and S100 protein might be used as early predictors in the assessment of hypoxic ischemic encephalopathy caused by strangulation of the intrauterine cord which is very easy, cheap and non-invasive measures.

VI. LIMITATIONS
One of the limitations of the current study could be related to the small sample size as it is being a single-center study. Hence, we suggest future larger multicenter studies.

VII. RECOMMENDATIONS
On the light of the results of the present study, we recommend using S100 protein and lactate in umbilical cord blood as early markers of neonatal asphyxia caused by nuchal cord because they are simple, non-invasive and cheap. Also, further studies on another useful biomarker for early detection of neonatal asphyxia should be done.

VIII. CONFLICTS OF INTEREST:
The authors of the study declared that there are no conflicts of interest.

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اللاكتات وبروتين S100 كمؤشر بيوكيميائي مبكر لاختياع الأطفال حديثي الولادة عن طريق خنق الحبل السري داخل الرحم:

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المقصد البحث: من وجهة نظر اختصاصي الطب الشرعي، هناك العديد من الجوانب ذات أهمية عن الوقاية خاصة في الفترة المحيطة بالولادة إذا ولد الطفل حيًا أو ميتًا. في حالات القلق بشأن أعراض الفترة المحيطة بالولادة التي تحدث في المستشفيات، من الضروري الحصول على ملاحظات التوليد وحديثي الولادة إذا ولد الطفل على قيد الحياة وتوفي بعد بضع ساعات أو أيام للوصول إلى تفسير واستنتاج صحيحين. وقد يعد اعتلال الدماغ الإفرازي بنقص الأكسجين ما قبل الولادة سببًا هاماً لأمراض الأطفال حديثي الولادة والإعاقة العصبية طويلة الأمد. وله العديد من الأسباب بما في ذلك الخنق داخل الرحم بواسطة الحبل السري. ويعتبر عدم التشخيص المبكر لاختياع الوليد وعدم علاجه أشياءًا خطيرة قانونياً تجاه الأطباء.

المؤشر البيوكيميائي المبكر لاختياع الأطفال حديثي الولادة عن طريق خنق الحبل السري في وكالات ونقطة الأكسجين في الأطفال حديثي الولادة الناجح عن الحبل الفقري قبل محاور المسؤوليات الطبية القانونية

طريقة البحث: تم إجراء هذه الدراسة على ثلاثون من الأطفال حديثي الولادة الذين يعانون من نقص الأكسجين بسبب خنق الحبل السري لقياس مستويات بروتين S100 واللاكتات في الحبل السري. تم مقارنة الولادة مع ثلاثين حديثي الولادة الذين يتمتعون بصحة جيدة على ما يبدو من منطقيين في العمر والجنس ووزن الجسم كمجموعة ضابطة. النتائج: وقد وجدت هذه الدراسة أنه يوجد ارتفاع دلالة إحصائية في مستويات بروتين S100 واللاكتات داخل الدم الحبل السري في حديثي الولادة الذين يكونون من نقص الأكسجين نتيجة خنق الحبل السري بالمقارنة مع المجموعة الضابطة. الخلاصة: خلصت هذه الدراسة إلى أنه من الممكن استخدام نسبة بروتين S100 واللاكتات داخل الدم الحبل السري كمؤشر بيوكيميائي مبكر لتشخيص الاعتدال الدماغي لنقص الأكسجين في حديثي الولادة. التوصيات: في ضوء نتائج الدراسة الحالية، نوصي باستخدام بروتين S100 واللاكتات في الحبل السري كعلامات مبكرة لاختياع الوليد الناجم عن الحبل الفقري لأنها سببية وغير جراحية ورخيصة. كذلك، ينبغي إجراء مزيد من الدراسات حول مؤشر جيبي مفيد آخر للكشف المبكر عن الاعتدال الولدي.