Role of Serum Apelin in the Diagnosis of Early-Onset Neonatal Sepsis

Safaa Abd ELHamid Nasr ELMeneza, Iman Mohamed Said El Bagoury, Khadiga El Sayed Mohamed

1Department of Pediatrics, Al Azhar University Faculty of Medicine for Girls, Cairo, Egypt
2Department of Clinical Pathology, Al Azhar University Faculty of Medicine for Girls, Cairo, Egypt

ABSTRACT

Objective: Apelin is a proinflammatory adipocyte-derived factor. The aim of this study was to evaluate the role and significance of serum apelin as a new sepsis marker in the identification of full-term and preterm new-born infants with early-onset sepsis.

Material and Methods: This was a case-control study. We included 80 neonates. The cases were divided into 2 groups; neonates with early-onset sepsis and control group with neonates non-sepsis. Apelin was quantified by enzyme-linked immunosorbent assay.

Results: There was a significant elevation in the mean values of serum apelin in the early-onset sepsis group (1214.7 ± 273.06 pg/mmol) than in the non-septic neonates 116.27 ± 21.96 pg/mmol (P < .0001). Apelin values were correlated to clinical sepsis and hematological scores as well as C-reactive protein. Serum apelin concentration was significantly higher among culture-positive cases than the culture-negative cases (mean ± SD was 1239.52 ± 268.47 and 929.42 ± 136.97 pg/mmol, respectively, P < .0001). Moreover, the apelin level was higher in non-survivor neonates than in the survivors in the early-onset sepsis group. No significant difference was found between preterm and full-term new-born infants with regard to the apelin values. The best cut-off estimate of apelin to diagnose early sepsis was >178.33 pg/mmol.

Conclusion: Apelin may be useful in the diagnosis and prognostic prediction of neonates with early-onset sepsis.

Key words: Neonatal sepsis, apelin, early-onset sepsis, new-born infants.

INTRODUCTION

Neonatal infection is one of the major causes of morbidity and mortality among neonates. Each year, about one million new-borns in developing countries die of infection, with a risk of neurological damage seen in survivors.¹

A positive blood culture is considered the "gold standard" to diagnose neonatal sepsis,² but it provides an inadequate sensitivity to start antibiotic therapy.³ When blood and other sterile site cultures are negative but the infant manifests a sign consistent with infection, they may be considered to have "clinical" sepsis.⁴,⁵

The initial treatment is to regularly use antibiotics when sepsis is suspected, but the diagnosis is delayed due to suspicion and then diagnosed with increasing danger. Prompt, precise, and fast identification of neonatal sepsis still an analytical dispute, highlighting the demand for consistent and appropriate analytic markers to provide effective antibiotic management.

Apelin is the endogenous ligand for the G-protein-coupled APJ receptor. This is expressed at the surface of some cell types. It is widely expressed in various organs such as the heart, lungs, kidney, liver, adipose tissue, gastrointestinal tract, brain, adrenal glands, endothelium,
and human plasma. Both mouse and human adipocytes express and secrete apelin, which is a proinflammatory adipocyte-derived factor that participates in vascular wall inflammation. Apelin has been reported to mediate a variety of physiological actions. It showed alteration in adults with severe sepsis. However, there is evidence for its diagnostic and prognostic benefits. Apelin amends heart damage in sepsis by tempering inflammatory reactions and could hopefully be a target to manage severe sepsis.

Research question: Can we use apelin as an early indicator for the identification of EOS in new-born infants?

The aim of this study was to evaluate the role and significance of serum apelin as a new sepsis marker in identifying the full-term and preterm new-born infants with EOS.

METHODS

Materials

Type of Study

This case-control study included 80 new-born infants. The sample size was determined by the electronic calculator from https://epitools.ausvet.com.au/casecontrolssequation. The confidence level was 0.95, power was 0.8, and assumed odds ratio was 4, for the data used. Accordingly, we determined a minimum of 39 cases for each group.

Study Groups

The neonates in the study were assigned into 2 groups:

Group I: Early-onset sepsis (EOS). It included 50 neonates (25 full-term neonates and 25 preterm infants [32–34 weeks]) with early-onset neonatal sepsis, diagnosed by clinical examination and laboratory investigation.

Group II: Non-sepsis group. It included 30 neonates (15 full-term neonates and 15 preterm infants). They were healthy new-born infants with no signs of infection, negative C-reactive protein (CRP), and non-suggestive hematological results.

Inclusion Criteria

The inclusion criteria for the neonates with sepsis included the presence of at least 2 clinical symptoms and at least 2 laboratory signs in the presence of, or as a result of, suspected or proven infection (positive culture or microscopy polymerase chain reaction) within the first 72 hours. In the EOS group (30%), followed by hypothermia (28.3%), and the least common clinical presentation among the cases.

Metabolic acidosis, and positive culture. Abnormal radiological findings of pneumonia and necrotizing enterocolitis were also considered. A case with a Töllner score > 3 and hematological score > 5 was diagnosed as sepsis. Measurement of serum apelin was achieved using enzyme-linked immunosorbent assay (MyBioSource, Inc.; catalog number MBS756419_competitive). The research was approved by the Ethics Committee of the Faculty of Medicine for girls, AL-Azhar University. The approval number is 202103752.

Statistical Analysis

Data were collected, coded, revised, and entered into the Statistical Package for the Social Sciences (SPSS) version 25.0 (IBM SPSS Corp.; Armonk, NY, USA). The data were presented as numbers and percentages for qualitative data, mean and standard deviations (SD) and ranges for the quantitative data with parametric distribution, and median with interquartile range (IQR) for the quantitative data with non-parametric distribution.

Student’s t-test was used in the comparison between the mean of 2 groups with regard to quantitative data and parametric distribution. For non-parametric data, the Mann–Whitney U-test was used to compare the nonparametric results that were not normally distributed.

Pearson’s linear correlation coefficient (r) was estimated in order to show the relationship between quantitative parameters.

The receiver-operating characteristic curve was used to assess the best cut-off point between the 2 groups with its sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under curve (AUC). The AUC was interpreted as follows: 0.9–1, excellent biomarker; 0.8–0.9, good; 0.70–0.80, fair; and 0.60–0.70, poor.

The confidence interval was set to 95% and the margin of error accepted was set to 5%. Therefore, the P-value was considered significant if < .05.

RESULTS

The results are shown in Tables 1, 2, 3, 4 and 5. Table 1 shows the distribution of demographic data among the studied groups, while Tables 2–5 show the laboratory findings.

Description of the study population

The study group included 50 neonates with EOS (27 females [54.0%] and 23 males [46.0%]). The mean age of the participants was 42.86 ± 11.9 hours.

There were no statistically significant differences in gestational age, birth weight, postnatal age, and gender between the sepsis and non-sepsis group. The Apgar score at 1 and 5 minutes decreased in the sepsis group compared to the non-sepsis group.

The Töllner score was significantly elevated in the sepsis group (P < .001).

Clinical Presentation

Respiratory distress was the most common clinical presentation in the EOS group (30%), followed by hypothermia (28.3%), and the least common clinical presentation among the cases.
studied was seizure (5%). Hypothermia was significantly increased among preterm infants.

Risk Factors
The number of cases with PROM (>18 hours) was significantly higher in the sepsis than in the non-sepsis groups.

Laboratory Findings
Forty-six newborns (92.0%) were proved to have sepsis by positive blood cultures. The bacteria identified as included Gram negative in 71.7% of the cases and Gram positive in 28.3%.

Blood culture was negative in all the cases of the control group. The sepsis group presented with significant increase in total leukocyte, neutrophil counts, immature neutrophil, immature to mature, and immature to total neutrophil ratios.

DISCUSSION
This study illustrated that there was an increase in the level of serum apelin in the sepsis group compared to the non-sepsis group. Gad et al. found an 8-fold increase of serum apelin in neonates with sepsis compared to the controls. Apelin is upregulated following sepsis, and plays a protective role in sepsis–induced cardiac impairment. It is possible that the upregulation of apelin in response to inflammation is a compensatory process to regulate the onset of metabolic disturbances. The release of apelin is controlled by inflammatory mediators, such as TNF-α, IL-6, and IFN-γ.

In this study, gender has no effect on apelin values in the sepsis and in the non-sepsis groups. This finding is similar to Malamitsi-Puchner et al. Moreover, gestational age had no effect on serum apelin values, as there was non-significant

| Table 1. Distribution of Demographic Data According to Patient Groups |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| **Gestational age (weeks)**     | **Range**       | **Mean ± SD**   | **Significant Test** | **P**          |
| Sepsis Group                    | 32-39           | 35.32 ± 2.71    | 0.627^            | .532           |
| Non-sepsis Group                | 32-39           | 35.7 ± 2.48     |                 |                |
| **Postnatal Age (hours)**       | **Range**       | **Mean ± SD**   | **Significant Test** | **P**          |
| Sepsis Group                    | 25-64           | 42.88 ± 11.9    | 0.843^            | .180           |
| Non-sepsis Group                | 27-62           | 35.73 ± 12.5    |                 |                |
| **Gender**                      |                 |                 |                  |                |
| Male, N (%)                     | 23 (46.0)       | 27 (54.0)       | 0.054^            | .816           |
| Female, N (%)                   | 13 (43.3)       | 17 (56.7)       |                 |                |
| **Birth weight (kg)**           |                 |                 |                  |                |
| Range                           | 1.00-5.200      | 1.330-4.200     | 2.73^             | .257           |
| Mean ± SD                       | 2.370 ± 1.00    | 2.656 ± 0.729   |                 |                |
| **Length (cm)**                 | **Range**       | **Mean ± SD**   | **Significant Test** | **P**          |
| Sepsis Group                    | 30-54           | 44.06 ± 5.85    | 1.575^            | .119           |
| Non-sepsis Group                | 37-54           | 46.02 ± 4.51    |                 |                |
| **Apgar 1 min**                 |                 |                 |                  |                |
| Median                          | 5               | 10              | 108.000^          | .000           |
| IR (25%-75%)                    | 5-6.25          | 7-8             |                 |                |
| **Apgar 5 min**                 |                 |                 |                  |                |
| Median                          | 8               | 10              | 98.500^           | .000           |
| IR (25%-75%)                    | 8-9             | 10-10           |                 |                |
| **Resuscitation**               |                 |                 |                  |                |
| Needed, N (%)                   | 41 (82)         | 0 (0)           | 50.462^           | .000           |
| Not needed, N (%)               | 9 (18)          | 30 (100)        |                 |                |
| **PROM > 18 h**                 |                 |                 |                  |                |
| Yes, N (%)                      | 27 (54.0)       | 5 (16.7)        | 10.889^           | .001           |
| No, N (%)                       | 23 (46.0)       | 25 (83.3)       |                 |                |
| **PROM covered by antibiotics** |                 |                 |                  |                |
| Yes, N (%)                      | 23 (46.0)       | 21 (70.0)       | 6.299^            | .043 S         |
| No, N (%)                       | 5 (10.0)        | 4 (13.3)        |                 |                |
| **Blood transfusion**           |                 |                 |                  |                |
| Yes, N                          | 10              | 0               | 6.857^            | .009           |
| No, %                           | 20.0            | 0.0             |                 |                |
| **Survival**                    |                 |                 |                  |                |
| Live, (%)                       | 32 (64.0)       | 30 (100)        | 13.935^           | .000           |
| Dead, N (%)                     | 18 (36.0)       | 0 (0)           |                 |                |

N, number; PROM, premature rupture of membrane; ^t-test; ^chi-square test; ^Mann-Whitney rank-sum test.
### Table 2. Comparison Between the Studied Groups Regarding Serum Apelin and C-Reactive Protein

|                        | Serum Apelin, pg/mmol | Independent t-test |         |         |         |         |         |         |         |         |
|------------------------|------------------------|-------------------|---------|---------|---------|---------|---------|---------|---------|---------|
|                        | Mean   | SD     | Minimum | Maximum | Median | IR (25%-75%) | Range | t       | P       |         |
| Sepsis group           | 1214.7 | 273.06 | 683.33  | 2112.33 | 28.29  | .000     |        | 28.29   | .000    |         |
| Non-sepsis group       | 116.27 | 21.96  | 78.5    | 178.33  | -1.128 | .265     |        |         |         |         |
| Gender                 |          |        |         |         |        |         |        |         |         |         |
| Male                   | 1167.6  | 339.12 | 683.33  | 2112.33 | -1.606 | .115     |        |         |         |         |
| Female                 | 1254.8  | 198.96 | 690.66  | 1556.67 |         |         |        |         |         |         |
| Sepsis Group           |          |        |         |         |        |         |        |         |         |         |
| Full-term              | 1153.6  | 245.66 | 683.33  | 1550.67 | -1.560 | .130     |        |         |         |         |
| Preterm                | 1275.7  | 290.03 | 815.33  | 2112.33 |         |         |        |         |         |         |
| Non-sepsis Group       |          |        |         |         |        |         |        |         |         |         |
| Full-term              | 110.17  | 12.41  | 93.33   | 132.5   | -1.560 | .130     |        |         |         |         |
| Preterm                | 122.38  | 27.66  | 87.5    | 178.33  |         |         |        |         |         |         |
| Blood culture          |          |        |         |         |        |         |        |         |         |         |
| Positive blood culture | 1239.5  | 268.47 | 683.33  | 2112.33 | 2.269  | .028     |        |         |         |         |
| No growth              | 929.42  | 136.97 | 817.33  | 1112    |         |         |        |         |         |         |
| Outcome                |          |        |         |         |        |         |        |         |         |         |
| Live                   | 1112.1  | 242.78 | 683.33  | 1779.17 | -4.062 | .001     |        |         |         |         |
| Dead                   | 1397.0  | 229.03 | 901.33  | 2112.33 |         |         |        |         |         |         |
| Postnatal age          |          |        |         |         |        |         |        |         |         |         |
| 2nd day                | 510.06  | 502.04 | 87.5    | 2112.33 | -2.506 | .015     |        |         |         |         |
| 3rd day                | 848.88  | 522.77 | 93.33   | 1556.67 |         |         |        |         |         |         |
| CRP mg/dl              |          |        |         |         |        |         |        |         |         |         |
| Sepsis                 | 24      | 24-48  | 2-96    |         |         |         |        |         |         |         |
| Non-Sepsis             | 4.5     | 3-6    | 2-6     |         |         |         |        |         |         |         |

CRP, C-reactive protein.

### Table 3. Cut-Off Point, Sensitivity, and Specificity of Serum Apelin, CRP, Töllner, and Hematological Score.

| Test                      | AUC     | Cut-Off Value | Sensitivity | Specificity | PPV | 95% CI Lower bound | 95% CI Upper bound | NPV | 95% CI Lower bound | 95% CI Upper bound |
|---------------------------|---------|---------------|-------------|-------------|-----|-------------------|-------------------|-----|-------------------|-------------------|
| Apelin                    | 1.000   | >178.33       | 100.00      | 100.00      | 100.0 | 92.9              | 100.0             | 100.0 | 88.4              | 100.0             |
| CRP                       | 0.950   | >6            | 90.00       | 100.00      | 100.0 | 92.1              | 100.0             | 85.7  | 69.7              | 95.2              |
| Töllner score             | 0.961   | >5            | 82.00       | 100.00      | 100.0 | 91.4              | 100.0             | 76.9  | 60.7              | 88.9              |
| Hematological score       | 0.939   | >2            | 74.00       | 100.00      | 100.0 | 90.5              | 100.0             | 69.8  | 53.9              | 82.8              |

This table shows that at the cut-off point of >178.33 pg/mmol serum apelin has 100% sensitivity, 100% specificity, negative predictive value (NPV) 100%, and positive predictive value (PPV) 100% in the diagnosis of EOS. The cut-off point of > 6 mg/dl C-reactive protein has 90% sensitivity, 100% specificity, NPV 100%, and PPV 85.7% in the diagnosis of EOS. The cut-off point of > 5 Töllner score has 82% sensitivity, 100% specificity, NPV100%, and PPV 76.9% in diagnosis of EOS. The cut-off point of >2 hematological score has 74 % sensitivity, 100% specificity, NPV 100%, and PPV 69.8% in the diagnosis of EOS.

### Table 4. Cut-Off Point, Sensitivity, and Specificity of Serum Apelin in Preterm and Full-Term Infants

| GA          | Cut-Off Point | AUC | Sensitivity | Specificity | PPV | 95% CI Lower bound | 95% CI Upper bound | NPV | 95% CI Lower bound | 95% CI Upper bound |
|-------------|---------------|-----|-------------|-------------|-----|-------------------|-------------------|-----|-------------------|-------------------|
| Preterm     | >178.33       | 1.000 | 100.00      | 100.00      | 100.0 | 92.9              | 100.0             | 100.0 | 88.4              | 100.0             |
| Full-term   | >132.5        | 1.000 | 100.00      | 100.00      | 100.0 | 92.9              | 100.0             | 100.0 | 88.4              | 100.0             |

This table shows that at the cut-off point of >178.33 serum apelin has 100% sensitivity, specificity, negative predictive value, and positive predictive value for diagnosis of EOS in preterm neonates. The cut-off point of >132.5 has 100% sensitivity, specificity, negative predictive value and positive predictive value in the diagnosis of EOS in full-term neonates.
which can be useful in the treatment of the sepsis.23 Among the physiological functions of the apelinergic system, Boraey et al.,20 who reported positive blood cultures in 81.2% of 71.7% of these cases. This was slightly higher than the results of culture. The percentage of positive blood culture in the sepsis tive blood culture cases than in those with negative blood

In this study, serum apelin was significantly higher in the posi-
tive blood culture cases than in those with negative blood culture. The percentage of positive blood culture in the sepsis group was 92%. Gram-negative organisms were detected in 71.7% of these cases. This was slightly higher than the results of Borae et al.,26 who reported positive blood cultures in 81.2% of the cases. On the other hand, the study of Arif et al. showed that only 15% of septic cases had positive blood cultures and concluded that more than half of the cases of neonatal sepsis are missed if only blood culture is used as the basis of diagno-
sis. This variation may be due to the differences in the etiology and stage of sepsis as well as start of antibiotic therapy prior to the laboratory diagnosis, which may be the major cause for the low culture results.

In this study, serum apelin was significantly higher in the posi-
tive blood culture cases than in those with negative blood culture. The percentage of positive blood culture in the sepsis group was 92%. Gram-negative organisms were detected in 71.7% of these cases. This was slightly higher than the results of Borae et al.,26 who reported positive blood cultures in 81.2% of the cases. On the other hand, the study of Arif et al. showed that only 15% of septic cases had positive blood cultures and concluded that more than half of the cases of neonatal sepsis are missed if only blood culture is used as the basis of diagno-
sis. This variation may be due to the differences in the etiology and stage of sepsis as well as start of antibiotic therapy prior to the laboratory diagnosis, which may be the major cause for the low culture results.

Apelin was significantly elevated in the non-survivor new-born infants than in the survivors. Severe infection in very ill cases led to the release of more apelin. In elderly patients with sepsis, Wang et al.22 reported a lower level of apelin in the survival group than in the mortality group.22 Body fluid homeostasis is among the physiological functions of the apelinergic system, which can be useful in the treatment of the sepsis.23

APLN-13 significantly increases survival and improves left ven-
tricular performance, better than the continuous infusion of dobutamine. It also reduces inflammation and stress.24

The best value for apelin for diagnosis of EOS was >178.33 pg/mmol. This value has high sensitivity and specific-
ity. Comparative results were reported by Gad et al.14 but with a lower cut-off value.14 The variations in cut-off values can be attributed to different laboratory methods, sampling tech-
iques (plasma, serum, or whole blood), and the characteristics of the studied populations such as postnatal age, birth weight, proportion of positive culture cases, and severity of illness.

The cut-off value of apelin was higher in immature cases, which may be related to modulation of the apelinergic system throughout pregnancy. Apelin expression levels had slightly decreased from the first to the third trimester of gestation due to the accelerated placental metabolism.25

The Töllner score had an AUC of 0.961 with a cut-off value > 5 with 82% sensitivity and 100% specificity, NPV 76.9%, and PPV 100%, for diagnosis of EOS in new-born infants. In addition, the hematological sepsis score had an AUC of 0.939 with a cut-off value > 2, with 74.00% sensitivity and 100% specificity, 69.8% NPV, and 100% PPV. Therefore, apelin had the highest AUC, sensitivity, specificity, NPV, and PPV, which makes the serum apelin level the most accurate parameter in the pre-
diction of EOS. The Töllner and hematological sepsis scores can be used as initial diagnostic tools to predict EOS in the neonatal population, especially in developing countries.26

A negative association was noticed between serum apelin and mean blood pressure. An experimental apelin injection lowered the blood pressure of study animals.27 Apelin has vasodilator effects attributed to nitric oxide.9

In the present study, a non-significant positive association 

was detected between serum apelin and length of hospital stay. Cömert et al.28 reported a significant positive correlation between the presence of sepsis and prolonged NICU stay.

Finally, this study showed that the serum apelin may be a useful and novel biomarker, as it is able to distinguish septic patients from other patients admitted to the NICU and is also able to predict sepsis severity and outcome. Additional information and studies for exploring the new family of apelinergic drugs to replace catecholamines in the treatment of sepsis are required.29

The application of safety standards for the neonatal patient, as well as the strict dedication to measures such as hand hygiene and the proper usage of antibiotics are considered mandatory to reduce infections in the NICU.30

Table 5. Correlation Between Serum Apelin and Different Parameters in the Sepsis Group

| Parameter                          | Serum Apelin |
|------------------------------------|--------------|
|                                   | r    | P   |
| Gestational age                    | −0.209 | .145 |
| Postnatal age                      | −0.054 | .708 |
| Gestational age                    | −0.209 | .145 |
| Duration of premature rupture of membrane | 0.410 | .003 |
| Birth weight                       | −0.316 | .026 |
| Mean blood pressure                | −0.377 | .007 |
| C-reactive protein                 | 0.701  | .000 |
| Töllner score                      | 0.807  | .001 |
| Hematological score                | 0.48   | .001 |
| Hospital stay                      | 0.087  | .549 |

difference between the full-term and preterm neonates in the sepsis group and in the non-sepsis cases.

Although the Apgar score was lower in the EOS group at the first and fifth minutes than in the non-sepsis group, it was within normal range at the fifth minute. This finding excluded perinatal asphyxia. A lower Apgar score in new-borns with EOS is reported by others.18,19 Some isoforms of apelin, such as APLN-13 and APLN-36 inhibit ROS, and improve the oxidative stress.

We have reached the conclusion that a negative blood culture does not exclude sepsis. Apelin was found to be of significant reliable diagnostic and prognostic value in the diagnosis of EOS among preterm and full-term infants. Serum apelin can be used in predicting neonates with EOS.

The Take-Home Message

Neonatal sepsis is still the major cause of mortality and morbidity in the NICU. Although sepsis markers such as apelin show promising early diagnostic value, adherence to infection control policies—including attention to strict hand hygiene,
antibiotic stewardship, and catheter management—is required to decrease the number of infections in hospitalized neonates.

**Ethical Committee Approval:** Ethics committee approval was received from the Ethics Committee of the Faculty of Medicine for girls, AL-Azhar University (number is 202103752).

**Informed Consent:** Informed consent was obtained from all participants who participated in this study.

**Peer Review:** Externally peer-reviewed.

**Author Contributions:** Concept – S.A.E.N.E.; Design – S.A.E.N.E.; Supervision – S.A.E.N.E.; Resource – K.E.S.M.; Materials – K.E.S.M.; Data Collection and/or Processing – K.E.S.M.; Analysis and/or Interpretation – I.M.S.E.B.; Literature Search – K.E.S.M.; Writing – S.S.A.E.N.E.; Critical Reviews – S.A.E.N.E., I.M.S.E.B.

**Conflict of Interest:** The authors have no conflicts of interest to declare.

**Financial Disclosure:** The authors declared that this study has received no financial support.

**REFERENCES**

1. Pappas A, Kendrick DE, Shankaran S, et al. Chorioamnionitis and early childhood outcomes among extremely low-gestational-age neonates. *JAMA Pediatr*. 2014;168(2):137-147. [CrossRef]

2. Wynn JL, Wong HR, Shanley TP, et al. Time for a neonatal-specific approach: has it come yet? *Pediatr Crit Care Med*. 2014;15(6):523-528. [CrossRef]

3. Lever A, Mackenzie I. Sepsis: definition, epidemiology, and diagnosis. *BMJ*. 2007;335(7625):879-883. [CrossRef]

4. Balk RA. Systemic inflammatory response syndrome (SIRS): where did it come from and is it still relevant today? *Vrulnec*. 2014;5(1):20-26. [CrossRef]

5. Safaa A, Gaber A, Al Refaey AA. Ventilator-associated pneumonia in neonatal intensive care unit: characteristics, risk factors and outcome. *Azhar J Pediatr*. 2010;3:1-14.

6. Kleinz Mj, Davenport AP. Emerging roles of apelin in biology and medicine. *Pharmacol Ther*. 2005;107(2):198-211. [CrossRef]

7. Hu G, Wang Z, Zhang R, Sun W, Chen X. The role of apelin/apelin receptor in energy metabolism and water homeostasis: a comprehensive narrative review. *Front Physiol*. 2021;12:632886. [CrossRef]

8. Kidoa H, Takakura N. Biology of the apelin–AP receptor axis in vascular formation. *J Biochem*. 2012;152(2):125-131. [CrossRef]

9. Xu-Yan C, Xin-Min L, Li-Li F, Chao-Shu T. Changes and clinical significance of serum apelin in patients with severe sepsis and septic shock. *J Acta Academiae Medicinae Sinica*. 2008;30:131-134.

10. Pan CS, Teng X, Zhang J, et al. Apelin antagonizes myocardial impairment in sepsis. *J Card Fail*. 2010;16(7):609-617. [CrossRef]

11. European Medicines Agency (EMA). Report on the expert meeting on neonatal and paediatric sepsis [EMA EMA/477725/2010]. An *Agency of the European Union*. 2021: 1-6. Available at: https://www.ema.europa.eu/en/documents/report/report-expert-meeting-neonatal-paediatric-sepsis_en.pdf.

12. Töllner U. Early diagnosis of septicemia in the newborn: clinical studies and sepsis score. *Eur J Pediatr*. 1962;138(4):331-337. [CrossRef]

13. Narasimha A, Harendra Kumar ML. Significance of Hematological Scoring System (HSS) in early diagnosis of neonatal sepsis. *Indian J Hematol Blood Transfus*. 2011;27(1):14-17. [CrossRef]

14. Gad GI, Ismail RI, El-Masry SA, Gouda HR. Serum apelin in early-onset neonatal sepsis: is it diagnostic? *J Neonatal Perinatal Med*. 2014;7(3):207-212. [CrossRef]

15. Luo Q, Liu G, Chen G, et al. Apelin protects against sepsis-induced cardiomyopathy by inhibiting the TLR4 and NLRP3 signalling pathways. *Int J Mol Med*. 2018;42:1161-1166. [CrossRef]

16. Han S, Wang G, Qi X, Englander EW, Grehley GH Jr. Involvement of a Stat3 binding site in inflammation-induced enteric apelin expression. *Am J Physiol Gastrointest Liver Physiol*. 2008;295(5):G1068–G1078. [CrossRef]

17. Malamitsi-Puchner A, Gourgiotis D, Boutsikou M, et al. Circulating apelin concentrations in mother/infant pairs at term. *Acta Paediatr*. 2007;96(12):1751-1754. [CrossRef]

18. Polciwiatrek LB, Smith PB, Benjamin DK, et al. Early-onset sepsis in term infants admitted to neonatal intensive care units (2011-2016). *J Perinatol*. 2021;41(1):157-163. [CrossRef]

19. Salama K, Gad A, El Tatawy S. Sepsis profile and outcome of pre-term neonates admitted to the neonatal intensive care unit of Cairo University Hospital. *Egypt Pediatric Association Gaz*. 2021;69(1):8-9. [CrossRef]

20. Boraey N, Sheneef A, Mohammad MA, Yousef L. Procalcitonin and C-reactive protein as diagnostic markers of neonatal sepsis. *Aust J Basic Appl Sci*. 2012;6:108-106.

21. Arif S, Ehsan A, Arif M, Hussain J, Bano R. Early diagnosis of neonatal sepsis through hematological and biochemical markers. *Gomal J Med Sci*. 2012;11:178-174.

22. Wang Y, Zheng Y, Yan F, Ding N. The changes and the clinical significance of plasma apelin in elderly patients with sepsis. * Chin J Geriatr*. 2013;8:861-862.

23. Galanth C, Hus-Cilharel A, Li B, Llorens-Cortes C. Apelin in the control of body fluid homeostasis and cardiovascular functions. *Curr Pharm Des*. 2012;18(6):789-798. [CrossRef]

24. Chagnon F, Coquerel D, Salvail D, et al. Apelin compared with dobutamine exerts cardioprotection and extends survival in a rat model of endotoxin-induced myocardial dysfunction. *Crit Care Med*. 2017;45(4):e391-e398. [CrossRef]

25. Aslan M, Celik O, Celik N, et al. Cord blood nesfatin-1 and apelin-36 levels in gestational diabetes mellitus. *Endocrine*. 2012;41(3):424-429. [CrossRef]

26. ELMeneza SA, Elsmail HO, Elbagonery EM, Abd Allah NA. Soluble triggering receptors expressed on myeloid cell-1 and proadrenomedullin for diagnosis and prognosis of early onset neonatal sepsis. *EC Paediatrics*. 2018;7:619-628.

27. Hosoya M, Kawamata Y, Fukusumi S, et al. Molecular and functional characteristics of APJ tissue distribution of mRNA and interaction with the endogenous ligand apelin. *J Biol Chem*. 2000;275(28):21061-21067. [CrossRef]

28. Cömert S, Ağzıkuru T, Akin Y, et al. The cost analysis of preterm infants from a NICU of a state hospital in Istanbul. *Iran J Pediatr*. 2012;22(2):185-190.

29. Coquerel D, Sainsily X, Dumont L, et al. The apelinergic system as an alternative to catecholamines in low-output septic shock. *Crit Care*. 2018;22(1):10. [CrossRef]

30. ELMeneza SA. Egyptian neonatal safety standards. *Egyptian Neonatal Safety Training Network*. 2014. Available at: https://www.researchgate.net/publication/322831357_Egyptian_Neonatal_Safety_Standards.