Research Article

Apelin and Procalcitonin in Neonatal Sepsis

Lamia H. Ali, Ahmed A. Saedii, Mohammed A. Abdel Hakeem and Enas M. Mostafa Kamal
Department of Clinical Pathology, El-Minia Faculty of Medicine

Abstract
Introduction: The identification and treatment of sepsis continues to be a major health issue. The incidence of sepsis is particularly high in the neonatal population. **Aim of the work:** The aim of the present study is to evaluate the role of apelin in neonatal sepsis and its relation with other biomarkers as procalcitonin and blood culture results. **Subjects and Methods:** This study included 90 neonates divided into two groups: **Group I:** included 60 neonates with clinically sepsis diagnoised according to (Thaver and Zaidi, 2009) who were subgrouped into group I a (EOS) (early onset ≤ 72h) included 36 neonates and group I b (LOS) (late onset > 72) included 24 neonates. **Results:** The study was carried out on 90 neonates divided into two groups: **Group I :** included 60 neonates with clinically sepsis and subgrouped according to onset of sepsis into group I a (early onset ≤ 72h) included 36 neonates and group I b (late onset > 72) included 24 neonates. They were selected from NICU (neonatal intensive care unit) of El-Minia Obstetric and Pediatric University Hospital from February to September 2015. **Conclusion:** Although blood culture is a gold standerd for diagnosis of neonatal sepsis, but we cannot depend on it only. Apelin and procalcitonin are reliable diagnostic markers of neonatal sepsis which have the same diagnostic accuracy. Apelin and procalcitonin are a good marker for early diagnosis of neonatal sepsis but Apelin is more perfect marker than procalcitonin in diagnosis of early neonatal sepsis. The use of apelin and other markers (procalcitonin, CRP, TLC, platlets and blood culture) collectively yeild the best results for diagnosis of neonatal sepsis.
Keywords: Apelin, Procalcitonin, Neonatal Sepsis

Introduction
The identification and treatment of sepsis continues to be a major health issue. The incidence of sepsis is particularly high in the neonatal population. The most reliable diagnostic test of neonatal sepsis, often referred to as the gold standard, is a blood culture test for bacteria. While this test is the most reliable available, it can take 48 hours to obtain the results. As a result, treatment must often begin before the results are known. An additional complication is the fact that the blood culture test can be negative for one in five subjects with sepsis. Thus, it is of critical importance to identify new biomarkers that will enable fast and reliable hematological scoring systems for sepsis in its earliest stages (Kun Wang, et al., 2013).

Neonatal sepsis is one of the most common causes of neonatal morbidity and mortality especially in developing countries. It may be early onset or late onset according to the neonatal age at the onset of the infection (Stoll BJ. et al., 2011). Early onset of neonatal sepsis is often caused by organism acquired during delivery while late onset type is due to organism acquired from nosocomial or community source (Robinson et al., 2008).

Procalcitonin (PCT) is a 116 amino-acid peptide precursor of the hormone calcitonin, which has been proposed as a reliable diagnostic and prognostic marker of sepsis, better differentiating inflammatory responses from bacterial infections. In healthy individuals, PCT is secreted only in neuroendocrine cells of the thyroid; however, during an infection, PCT is released up to a thousand fold increase from nearly all tissues and cell types in the host in response to cytokines and bacterial products (Ravi S. Samraj, et al., 2013).

Apelin is a proinflammatory adipocyte derived factor that participate in vascular wall inflammation (G.I. Gad, et al., 2014). Apelin expression is induced by inflammatory mediators, such as tumor necrosis factor, interleukin-6 and interferon and plasma apelin levels correlate with markers of inflammation (Han et

al.., 2008) and anti inflammatory end inhibitory effect on release of inflammatory mediators which has recently been linked toneonatal sepsis (ShimingXu, et al., 2012).

Aim of the work

The aim of the present study is to evaluate the role of apelin in neonatal sepsis and its relation with other biomarkers as procalcitonin and blood culture results.

Subjects and Methods

This study included 90 neonates divided into two groups:

Group I: included 60 neonates with clinically sepsis diagnoised according to (Thaver and Zaidi, 2009) who were subgrouped into group I a (EOS) (early onset ≤ 72h) included 36 neonates and group I b (LOS) (late onset > 72) included 24 neonates.

They were selected from NICU (neonatal intensive care unit) of El-Minia Obstetric and Pediatric University Hospital from February to September 2015.

Group II: included 30 appearantly healthy neonates as a control group.

All neonates were subjected to:

1- Complete history taking and clinical examination.
2- Laboratory investigations including :
   A) Routine investigations :
      a- Complete blood count.
      b- C-reactive protein.
      c- Blood culture for neonates with sepsis
   B) Special investigation:
      a- Assessment of procalcitonin by enzyme linked immunoassay (EIA).
      b- Assessment of aplien by EIA.

Sampling Protocol:

Under complete sterile conditions, 4ml of venous blood was drawn from each neonate, one ml was used in the blood culture firstly then one ml was used for complete blood count in a tube containing (K-EDTA) and two ml in plane tube was left until clotting then centrifuged , the separated serum was used for CRP and the remaining serum was stored at -70C till the time of assessment of procalcitonin and aplien by EIA .

Laboratory investigations include:

A- Routine investigations:

1- **Complete blood count** was done using automated cell counter (Sysmex KX-21-N - Japan) and blood smear stained by Leishman stain.

2- **C-reactive protein** (TECO DIAGNOSTICS U.S.A.) (Fischel, et al., 1967).

3- **Blood culture** (conventional method):

One ml of withdrawn venous blood was inoculated into blood culture bottle, mixed well then incubated at 370C for 24 hours; subculture on blood agar and Mac-Conkeys agar plates every other day was done for ten days.

Further identification for the growing organism by Gram stained smear and biochemical reactions were done e.g catalase, coagulase, triple sugar iron (TSI), citrate and Lysine iron agar (LIA) then antibiogram were done to complete sensitivity.

B- Special investigation:

1- Quantitative assay of procalcitonin by Humareader plus, model 3700, Germany and kits was supplied by (Ray Bio, U.S.A.):

I- Principle of assay:

The kit was depended upon binding of an antigen specific for human procalcitonin coated on a 96-well plate. Standards and samples were pipetted into the wells and procalcitonin presented in a sample was bound to the wells by the immobilized antibody. The wells were washed and biotinylated anti-human procalcitonin antibody was added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin was pipetted to the wells. The wells were again washed, a TMB substrate solution was added into the wells and color was developed in proportion to the amount of Procalcitonin bound. The stop solution changed the color from blue to yellow, and the intensity of the color was measured at 450 nm which directly proportional to procalcitonin concentration. The given standerd concentrations were assayed and a curve was drawn for determination of each sample level.

Results

The study was carried out on 90 neonates divided into two groups:

Group I: included 60 neonates with clinically sepsis and sub grouped according to onset of sepsis into group I a (early onset ≤ 72h) included 36 neonates and group I b(late onset > 72) included 24 neonates.

They were selected from NICU (neonatal intensive care unit) of El-Minia Obstetric and Pediatric University Hospital from February to September 2015.

Group II: included 30 neonates without apparently healthy without of sepsis.
All different data in studied groups will be summarized in the following tables:

Table I: Comparison of demographic data in both groups

	Group I Neonatal sepsis (n=60)	Group II Control (n=30)	P value
Age (days)			
Range	(1-27)	(1-24)	0.854
Mean ± SD	7.96±5.82	8.33±4.52	
Sex			
Male	33(55%)	22(73.3%)	0.197
Female	27(45%)	8(26.7%)	
Gestational age			
Preterm	36(60%)	14(46.7%)	0.350
Fullterm	24(40%)	16(53.3%)	
Mode of delivery			
SVD	23(38.3%)	14(46.7%)	0.556
CS	37(61.7%)	16(53.3%)	

- ***: significant difference at p value < 0.05***

As shown in table (I) the age in group I was ranged from 1day to 27 days with a mean ± SD of 7.96±5.82. While the age ranged from 1 day to 24 days with a mean ± SD 8.33±4.52 in group II . There was no significant difference between both groups regarding the age (P value = 0.854).
Sex: group I included 33 males (55%) and 27 females (45%) while group II included 22 males (73.3%) and 8 females (26.7%) with no significant difference between both groups regarding to the gender(P value = 0.197).
Gestational age: group I included 36 preterm neonates (60%) and 24 full term neonates (40%) while group II included 14 preterm neonates (46.7%) and 16 full term neonates (53.3%) with no significant difference between both group regarding to the gestational age (P value = 0.350).

Mode of delivery: group I included 23 neonates were delivered by spontanous vaginal delivery (38.3%) and 37 neonates were delivered by cesserian section (61.7%) while group II included 14 neonates were delivered by spontanous vaginal delivery (46.7%) and 16 neonates were delivered by cesserian section (53.3%) with no significant difference between both group regarding to mode of delivery (P value = 0.556).

Table II: Comparison between both groups regarding TLC, Hb, Platelets count and CRP

	Group I Neonatal sepsis (n=60)	Group II Control (n=30)	P value
TLC (c/mm^3) Range Mean ± SD	(1600-21900) 11540±4932.84	(4100-6500) 4926.66±698.43	**< 0.001***
Hb(g/dl) Range Mean ± SD	(8.8-19.9) 14.22±2.58	(14-18.6) 16.14±1.38	**< 0.001***
Platelets (/mm^3) Range Mean ± SD	(18.000-350.000) 196.380±94.230	(198.000-365.000) 267.330±53.170	**< 0.001***
CRP -Ve	17(28.3%)	30(100%)	**< 0.001***

- ***: significant difference at p value < 0.05***

Table (II) showed the results of total leucocytic count (TLC), haemoglobin, platelets and C-reactive protein . TLC count in group I ranged from 1600 to 21900/mm3 and the mean ±SD was 11540± 4932.84 while in group II it ranged from 4100 to 6500/mm^3 and the mean ±SD was 4926.66± 698.43. There was a high statistically significant increase in TLC in group I comparing with group II (P value <0.001).

Haemoglobin in group I ranged from 8.8 to 19.9 gm/dl and the mean ±SD was 14.22 ± 2.58 while in group II it ranged from 14 to 18.6 gm/dl and the mean ±SD was 16.14 ±1.38. There was a high statistically significant decrease in Hb in group I when compared with group II (P value <0.001).

Platelets in group I ranged from 18.000 to 350.000/mm^3 and the mean ±SD was 196.380± 94.230 while in group II ranged from 198.000 to 365.000/mm^3 and the mean ±SD was 267.330± 53.170. There was high statistically significant decrease between two groups (P value =0.001).

CRP: Group I included 17 neonates with negative CRP (28.3%) while group II included 30 neonates with negative CRP (100%). There was a high statistically significant difference between two groups (P value <0.001).

Table III: Frequency of microorganisms in blood cultures of neonatal sepsis group

Blood culture	Group I Neonatal sepsis (n=60)
Blood culture **-Ve (no growth)** **+Ve**	28(46.7%) 32(53.3%)
Staphylococcus aureous	7(11.7%)
Staphylococcus epidermidis	5(8.3%)
E. Coli	5(8.3%)
Enterobacter	4(6.7%)
E.Coli Kelebsiella	3(5%)
Streptococcus pyogens	2(3.3%)
NH streptococci	2(3.3%)
Staphylococcus saprophyticus	1(1.7%)
Pseudomonous	1(1.7%)
Proteous	1(1.7%)
Candida	1(1.7%)

Discussion

Neonatal sepsis is one of the most common causes of neonatal morbidity and mortality especially in developing countries. It may be early onset or late onset according to the neonatal age at the onset of the infection (Stoll BJ. et al., 2011). Early onset of neonatal sepsis is often caused by organism acquired during delivery while late onset type is due to organism acquired from nosocomial or community source (Robinson et al., 2008).

Early diagnosis of neonatal sepsis and intervention are essential to avoid serious complication (fatal organ failure and death) (Tang et al., 2007). The most reliable diagnostic test of neonatal sepsis, often referred to as the gold standard, is a blood culture test for bacteria. While this test is the most reliable available, it can take 48 hours to obtain the results. As a result, treatment must often begin before the results are known. An additional complication is the fact that the blood culture test can be negative for one in five subjects with sepsis. Thus, it is of critical importance to identify new biomarkers that will enable fast for sepsis in its earliest stages (Kun Wang et al., 2013).

In this work, a total number of this study was 60 septic neonates which were admitted to NICU at Minia University Hospital from the period of February to September 2015 according to clinical mainfestation as in a study done by Wynn J. et al., 2010. Out of this 60 septic neonates, 36 cases were diagnosis as EOS (60%) and 24 cases as LOS (40%). The results were in agreement with Gad GI. Et al., 2014 and Katherine Soreng, and H. Roma Levy, 2011.

The study included 23 cases (38.7%) were delivered by normal vaginal delivery and 37 cases (61.7%) were born by CS showing higher sepsis rate among neonates born by CS and this was in concordance with studies done by Aaron B. Caughey MD. et.al, 2014 reported that CS was more safe and decreased risk of sepsis and mortality rate and this may be explained by roles of infection control done perfectly in U.S.A.

In the present study, total leucocytic count in septic neonates was increase when compared with control group (P value <0.001) that means it a good indicator of sepsis although it was a wide range in count so (range from 1600 to 21900/mm^3), it is of little clinical use in diagnosis of neonatal infection because of wide variation in values. These results were in agreement with Khair et.al., 2010 who reported that TLC was not increased in all cases and its sensitivity was 50%. It remains nonspecific and have a low positive predictive value. In our study there were 38 neonates with normal TLC counts in group I which means we cannot depend on TLC count only. This was in agreement with a study done by Khashu M. et al., 2006reported that normal TLC counts may be initially observed in as many as 50% of cases of culture-proven sepsis.

Haemoglobin level in septic neonates was decreased when compared with control group (P value <0.001) these results were in agreement with that obtained by Ryon M. et al., 2017 who reported that the effects of sepsis on the erythrocyte, including changes in RBC volume, metabolism and hemoglobin's affinity for oxygen, morphology, RBC deformability (an early indicator of sepsis), antioxidant status, intracellular Ca2+ homeostasis, membrane proteins, membrane phospholipid redistribution, clearance and RBC O2-dependent adenosine triphosphate efflux (an RBC hypoxia signaling mechanism involved in micro vascular auto regulation) and also consider the causes of these effects by host mediated oxidant stress and bacterial virulence factors.

As for platelets counts in my study, there were markedly lower in neonates with sepsis than in control group with (P value < 0.001) which means that thrombocytopenia is one of the laboratory markers for sepsis and this was in agreement with a study done by Khair et al., 2010 who found that neonates with sepsis develop thrombocytopenia, possiblly because of the damaging effects of endotoxin on platletes.

Conclusion

1- Although blood culture is a gold standerd for diagnosis of neonatal sepsis, but we cannot depend on it only.
2- Apelin and procalcitonin are reliable diagnostic markers of neonatal sepsis which have the same diagnostic accuracy.
3- Apelin and procalcitonin are a good marker for early diagnosis of neonatal sepsis but Apelin is more perfect marker

than procalcitonin in diagnosis of early neonatal sepsis.

4- The use of apelin and other markers (procalcitonin, CRP, TLC, platlets and blood culture) collectively yeild the best results for diagnosis of neonatal sepsis.

References

1. Aaron B.Caughey, Alison G.Cahill, Jeanne-MarieGuise and J.Rouse (2014). Safe prevention of the primary cesarean delivery. Vol 210. 179-193.
2. Adam SS, Key NS and Greenberg CS (2009). D-dimer antigen: current concepts and future prospects. Blood; 113:2878–2887.
3. Adams-Chapman, Ira Stoll and Barbara J (2006). Neonatal infection and long-term neuro developmental outcome in the preterm infant, Vol 19: 3, 290–297.
4. Adib M, Bakhshiani Z, Navaei F, SahebFosoul F, Fouladi S and Kazemzadeh H (2012). Procalcitonin: a reliable marker for the diagnosis of neonatal sepsis. Iran J Basic Med Sci.; 15: 777–782.
5. Agnieszka M. Tycinska, Anna Lisowska, Wlodzimierz J. Musial and Bozena Sobkowicz (2012). Apelin in acute myocardial infarction and heart failure induced by ischemia; vol 7 (408-412).
6. Alan G. Japp and David E (2008). Newby The apelin–APJ system in heart failure Pathophysiologic relevance and therapeutic potential, 1882 – 1892.
7. Alcaide P, Auerbach S and Luscinskas FW (2009). Neutrophil recruitment under shear flow: It's all about endothelial cell rings and gaps. Microcirculation;16:43–57.
8. Ali AM, Elkhatib WF and Abdelaziz SS (2014). Procalcitonin versus C-Reactive Protein in Neonatal Sepsis. Journal of Immunology and Infectious Diseases. vol 1, 2394-6512.
9. Alison Bedford Russell (2011). Paediatrics and Child Health (6):265–269.
10. Altunhan H, Annagur A, Ors R and Mehmetoglu I (2011). Procalcitonin measurement at 24 hours of age may be helpful in the promptdiagnosis of early-onset neonatal sepsis. International Journal of Infectious Disease;15:e854-e858.
11. Atluri P, Morine KJ, Liao GP, Panlilio CM, Berry MF and Hsu VM (2007). Ischemic heart failure enhances endogenous myocardial apelin and APJ receptor expression. Cellular & molecular biology letters; 12:127–38.
12. Cagno, C.K., Pettit, J.M., and Weiss, B.D (2012). Prevention of perinatal group B streptococcal disease: updated CDC guideline. Am Fam Physician; 86: 59–65
13. Committee on Infectious Diseases, Committee on Fetus and Newborn, Baker, C.J., Byington, C.L., and Polin, R.A (2011). Policy statement—Recommendations for the prevention of perinatal group B streptococcal (GBS) disease. Pediatrics.128: 611–616.
14. Fukushima H, Kobayashi N, Takeshima H, Koguchi W and Ishimitsu T (2010). Effects of olmesartan on Apelin/APJ and Akt/eNOS pathway in Dahl rats with end-stage heart failure. J CardiovascPharmacol. Jan;55(1):83–8.
15. Han S, Wang G, Qi X, Englander EW, and Greeley GH Jr (2008) Involvement of a Stat3 binding site in inflammation-induced enteric apelin expression. Am J Physiol Gastrointest Liver Physiol 295:G1068–78.
16. Jiang, Z. and Ye, G.Y.(2013). 1:4 matched case-control study on influential factor of early onset neonatal sepsis. Eur Rev Med Pharmacol Sci; 17: 2460–2466.
17. Kun Wang, Vineet Bhandari, Sofya Chepustanova, Greg Huber, Stephen O′Hara, Corey S. O′Hern, Mark D. Shattuck, and Michael Kirby (2013). Which Biomarkers Reveal Neonatal Sepsis? PLoS One 8(12): e82700.
18. M. J. Hill, S. Kumar and P.G. McTernan (2009). Adipokines and the clinical laboratory; what to measure, when and how? J.Clin. Pathol., 62: 206- 211.
19. Metsvaht T, Pisarev H, Ilmoja3 M-L, Parm U, Maipuu L, Merila M, Müürsepp P and Lutsar I (2009). Clinical parameters predicting failure of empirical antibacterial therapy in early onset neonatal sepsis, identified by classification and regression tree analysis BMC Pediatrics, 9:72. doi:10.1186/1471-2431-9-72.
20. Nakamura, A., Wada, H., Ikejiri, M., Hatada, T., Sakurai, H., Matsushima Y, et al., (2009). Efficacy of procalcitonin in the early diagnosis of bacterial infections in a critical care unit. Shock.; 31: 586–591.