Research Article

Proadrenomedullin in neonatal sepsis

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Abstract
Introduction: Neonatal sepsis continues to be a global problem with significant morbidity and mortality. The diagnosis of neonatal sepsis is complicated by nonspecific clinical symptomatology, a high-false negative rate, and a delay in obtaining blood culture results. **Subjects and Methods:** The prospective cohort study was conducted in the Department of Clinical pathology and Neonatal Intensive Care Unit (NICU) at Minia University hospitals during the period from August 2014 to October 2015. Neonates were classified into two groups: **group I**: included 60 neonates with sepsis divided into 2 subgroup, 36 EOS (subgroup Ia) and 24 LOS (subgroup Ib), 27 female, 33 male, their ages ranged from 1 day to 27 days. **group II**: included 30 apparently healthy neonates 8 female, 22 male their ages ranged from 1 day to 24 days who served as a control group of matched age and sex. **Results:** The present study included ninty neonates, sixty with sepsis (group I) in addition into thirty apparent healthy neonates who served as a control group (group II) of matched age and sex. **Recommendation:** Proadrenomedullin may be used as a routine investigation for early diagnosis of neonatal sepsis besides blood culture and procalcitonin. Further studies on larger population are recommended to investigate the prognostic value of proadrenomedullin.
Keywords: Neonatal sepsis, Proadrenomedullin

Introduction
Neonatal sepsis continues to be a global problem with significant morbidity and mortality. The diagnosis of neonatal sepsis is complicated by nonspecific clinical symptomatology, a high-false negative rate, and a delay in obtaining blood culture results[(1)].

Procalcitonin (PCT), a precursor of calcitonin is a 116 amino acid protein secreted by the C cells of thyroid gland in normal situation but its levels may increase during septicemia, meningitis, pneumonia and urinary tract infection. This marker also is produced by macrophage, and monocyte cells of various organs in severe bacterial infection and sepsis[(2)].

Adrenomedullin (ADM), a peptide produced by various tissues during physiological stress, has anti-inflammatory, antimicrobial, and vasoregulatory activities. Although promising, this innovative biomarker is rapidly metabolized in the circulation, complicating its measurement. Thus, its precursor, proadrenomedullin (pro ADM or the similar midregional-proADM [MR-proADM]), has received more attention as a biomarker because it is more stable and easier to measure[(3)].

Aim of the work
Aim of the present study is to evalute the diagnostic charactrstics of proadrenomedullin in neonatal sepsis and its relation with other biomarkers such as pro-calcitonin and blood culture results.

Subjects and Methods
The prospective cohort study was conducted in the Department of Clinical pathology and Neonatal Intensive Care Unit (NICU) at Minia University hospitals during the period from August 2014 to October 2015. Neonates were classified into the following two groups:

group I: included 60 neonates with sepsis divided into 2 subgroup, 36 EOS (subgroup Ia) and 24 LOS (subgroup Ib), 27 female, 33 male, their ages ranged from 1 day to 27 days.

group II: included 30 apparently healthy neonates 8 female, 22 male their ages ranged from 1 day to 24 days who served as a control group of matched age and sex.

All neonates were subjected to the following:
1- **Careful history taking from their parents.**
2- **Physical examination.**
3- **Laboratory diagnosis:**

A- ***Routine investigations:***

B- Complete blood count (CBC).

2- Blood culture.

3- C-reactive protein.

B- Special investigations:

1- Serum procalcitonin by Enzyme Linked Immuno Sorbent Assay (ELISA).

2- Serum proadrenomedullin by ELISA.

Sampling Protocol:

Under complete aseptic precautions, 3 ml of peripheral venous blood samples were withdrawn by sterile needle tap in group II, and before initiating antibiotic therapy in group I. This sample was divided as following:

1 ml of venous blood on nutrient broth bottle for blood culture, 0.5 on EDTA tube for (CBC) and the remaining 1.5 ml in plane tube was left 15 minutes at room temperature, cent-rifugation at 3000 rpm then the separated serum was used for CRP determination and the remaining serum was stored at -20^{0}C until the assay procalcitonin and proadrenomedullin by ELISA.

Routine Investigations:

1- CBC was done using automated cell counter (sysmex KX.21-N, Japan).

2- CRP (TECO DIAGNOSTICS, U.S.A)

3- Blood culture (conventional method):

Under complete aseptic conditions, the first 1 ml of withdraw on venous blood was inoculated to an 8 ml broth containing blood culture bottle which they incubated at 37^{0}C for 24 hours and sub-cultured made on MacConkey and Blood agar plates every other day for 10 days, the grown colonies identified using Gram stained smear and biochemical reaction as catalase, coagulase, urase citrate and triple sugar iron (TSI).

Results

The present study included ninty neonates, sixty with sepsis (group I) in addition into thirty apparent healthy neonates who served as a control group (group II) of matched age and sex.

Group I:

Included 60 neonates with sepsis, 33 (55%) were males and 27(45%) were females their age ranged from 1-27 days with mean ± SD of 7.96 ± 5.82. Twenty three neonates (38.3%) were born by simple vaginal delivery (SVD) and 37(61.7%) by caesarean section (CS), 36 (60%) were preterm and 24(40%) were fullterm, 36 (60%) showed early onset sepsis (EOS) and 24 (40%) were late onset sepsis (LOS).

Group II:

Included 30 apparently healthy neonates 22 (73.3%) were males and 8 (26.7%) were females, their age ranged from 1-24 days with mean ± SD of 8.33 ± 5.32. Fourteen (46.7%) were born by SVD and 16(53.3%) by CS, 14 (46.7%) were preterm and 16(53.3%) fullterm, 18(60%) early postnatal age and 12 (40%) late postnatal age.

Table (1): 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)	0.815
Mean ± SD	7.96±5.82	8.33±5.32	
Sex (n%)			
Male	33(55%)	22(73.3%)	0.093
Female	27(45%)	8(26.7%)	
Gestational age(weeks)			
Preterm	36(60%)	14(46.7%)	0.230
Full term	24(40%)	16(53.3%)	
Mode of delivery: (n%)			
SVD	23(38.3%)	14(46.7%)	0.449
CS	37(61.7%)	16(53.3%)	

significant difference at p value < 0.05

There were no significant differences between the studied groups regarding age (P. 0.815), sex (p. 0.093), gestational age (p. 0.230) and mode of delivery (p. 0.449).

Discussion

Sepsis in neonates continues to be a public health problem with significant morbidity and mortality. Timely diagnosis is vital to prevent serious complications in neonates (Licona et al., 2016).

A total number of 60 neonates with sepsis submitted to NICU at Minia University hospitals enrolled in the study from August 2014 to October 2015 and diagnosis was made according to EGNN sepsis screening test (Egyptian Neonatal Network (EGNN), 2010)

In the present study, 23 neonates (38.3%) were born by NVD and 37 neonates (61.7) by CS. The incidence of sepsis was higher in neonates born via CS than in those born via VD. This may be due to an increased risk of infection during C.S more common than during NVD. These results were similar to that obtained by Abdel Hakeem A and Bothina A 2015 who reported that among neonates with sepsis (42.6%) had NVD while (57.4%) had C.S.

In the present work Hb level in septic neonates were lower than those of control group. The low Hb level could be due to increased hemolysis of red blood cell caused by bacterial infection in blood (Mondal et al., 2012).

Also platelet count in septic neonates were lower than those of control group due to increased platelet destruction, increased sequestration secondary to infection and failure in platelet production as a result of reduced megakaryo-cytes or effect of endotoxins (Makkar et al., 2013). But TLC was higher in neonatal septic group than control group. These results were similar to those obtained by Arunachalam & Pammi., 2015. While Mally et al., 2014 found that opposite result and referred this to concomitant viral and fungal infection in his cases. However, TLC, platelet and hemoglobin not ensure accurate diagnosis of neonatal sepsis. This discrepancy in addition to the wide range of TLC explains the low value of those parameter as a markers of neonatal sepsis if used alone as stated by Laurent et al., 2013.

CRP is a peptide synthesized by liver in response to infection and inflammation (Markanday 2015). It was positive in 43 neonates with sepsis while it was negative in 17 neonates with sepsis. Blood culture results obtained from this work, revealed 32 positive cases (53.3%) and 28 negative cases (46.7%) from all neonates with sepsis. These results were similar to those obtained by Chiesa et al., 2015, who reported that 56.5% positive blood culture and 43.5% negative blood culture.

Recommendation

We recommended that:

- Proadrenomedullin may be used as a routine investigation for early diagnosis of neonatal sepsis besides blood culture and procalcitonin.
- Further studies on larger population are recommended to investigate the prognostic value of proadrenomedullin.

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