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Original Research Article

Comparative study of routine diagnostic test, C-reactive protein with antithrombin III levels in diagnosis and prognosis of neonatal sepsis

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ABSTRACT

Background: Antithrombin III is a potent inhibitor of thrombin mediated vascular injury in the micro-circulation in severe sepsis. This endogenous anti-coagulant is rapidly depleted in the early phases of sepsis as a result of decreased synthesis, increased destruction and enhanced clearance by thrombin-antithrombin complexes. This study was conducted to evaluate the role of antithrombin III level in diagnosis and prognosis of culture proven neonatal sepsis as compared to conventional C-reactive protein.
Methods: This prospective study was conducted at a tertiary care hospital in 30 full term, appropriate for gestational age neonates who were admitted in neonatal intensive care unit for suspected sepsis.
Results: Out of 30 neonates suspected of sepsis in NICU, 22 turned out to be culture positive. Keeping antithrombin III cut off level ≤150 mg/L, 18 of those 22 culture positive neonates, had antithrombin III level ≤150 mg/L; sensitivity at ≤150mg/L was 81.82%. This was way higher than the 50% sensitivity of CRP, that was found in the study. (Only 11out of 22 culture positive neonates had positive CRP).
Conclusions: Antithrombin III level ≤ 150 mg/L is a good indicator for neonatal septicemia and helps to detect neonatal sepsis earlier and more accurately as compared to other conventional laboratory tests like CRP. It also predicts the prognosis of neonatal sepsis more precisely as compared to CRP.

Keywords: Antithrombin III, Neonate, Sepsis

INTRODUCTION

Sepsis is the commonest cause of neonatal mortality. It is responsible for about 30-50% of the total neonatal deaths in developing countries.[1,2] Sepsis related mortality is largely preventable with prevention of sepsis itself, timely recognition, rational antimicrobial therapy and aggressive supportive care. In neonates, early warning signs and symptoms are often minimal, subtle, non-specific, can easily misinterpreted as being due to non-infective causes. Although the onset of illness is inconspicuous, the clinical course may be fulminate, leading to septicemic shock, DIC and death within hours of onset of clinical manifestations. Gold standard test for diagnosis of sepsis is a positive microbial growth on culture media from blood, CSF or urine, but it requires days. A wide variety of haematological and biochemical markers have been investigated for the evaluation of clinical sepsis. None of the diagnostic test is able to identify a septic neonate with reasonable accuracy. So, authors have to resort to combinations of tests to enhance the accuracy of diagnosis of sepsis.

A single test that enables to identify septic neonate accurately and rapidly, while awaiting culture results and simultaneously help to give the prognosis, is the need of the day. Preliminary data from early clinical studies shows, antithrombin III deficiency is invariably present in severe sepsis and the level is predictive of outcome.[3,4]

Antithrombin III is a natural anticoagulant that plays a pivotal role in coagulation. Recent data suggest that this glycoprotein also has potent anti-inflammatory properties, and it is protective in sepsis. It probably relates to the local inhibition of the pro-inflammatory properties of thrombin and FXa by endothelial AT and the increased release of prostacyclin from endothelial cells.[5]

METHODS

This study was conducted at the Department of Pediatrics, Smt SCL General Hospital, a tertiary care hospital of Ahmedabad, Gujarat from 1st May 2009 to 30th April 2010.

30 full term, appropriate for gestational age neonates were enrolled in study, who were admitted in neonatal intensive care unit for suspected sepsis.

Manifestation of 2 or more of following physical signs, were used to suspect sepsis.

1. Temperature instability <35C or >38.5C
2. Lethargy, poor cry, refusal to suck
3. Poor perfusion
4. Brady/tachycardia
5. Respiratory distress or apnea
6. Hypo/hyperglycemia
7. Metabolic acidosis.

Complete blood count, immature neutrophil count, C-reactive protein, AT III level, blood culture and other relevant investigations were sent.

CSF analysis, urine culture and X ray chest were done, when required. Blood samples were obtained before starting antibiotics.

Quantitative CRP was measured by latex agglutination and value ≥0.6mg/dl was considered positive.

Measurement of AT III level was done by Nephelometry method and cut off value ≤150mg/L was considered significantly low.

All neonates were started with the same antibiotics and were changed according to culture and sensitivity report. Same supportive care and treatment continued.

To confirm the diagnosis of septicemia, neonates were followed up till discharge and investigations were repeated as per requirement.

Data were collected and analyzed by EPI INFO 7.0 software. Unpaired t-test and chi-square test were used to test significance.

RESULTS

In the present study CRP had 50% sensitivity, 37.5% specificity, 68.7% positive predictive value and 21.4% negative predictive value to diagnose neonatal sepsis (Table 1).

Table 1: Relationship between CRP and blood culture.

CRP	Blood culture		Total
	Positive	Negative	
Positive	11	5	16
Negative	11	3	14
	22	8	30

Sensitivity: 11/22=50%, Specificity: 3/8=37.5%, PPV: 11/16=68.7 %, NPV: 3/14=21.4%

In the present study, sensitivity, specificity, positive predictive value and negative predictive value of antithrombin III level at ≤150 mg/L was 81.62%, 62.5%, 85.71% and 55.56% respectively (Table 2).

Table 2: Relationship between antithrombin III and blood culture.

Antithrombin-III (mg/L)	Blood culture		Total	P-value
	Positive	Negative		
0-150	18	3	21	<0.05
>150	4	5	9	
Total	22	8	30	

Sensitivity: 81.62%, Specificity:62.5%, PPV:85.7168%, NPV: 55.56%

The antithrombin III levels were significantly low ($p<0.05$) in neonates with sepsis (culture positive) compared to those without sepsis (culture negative), both by student t test as well as chi square test at antithrombin III level ≤150 mg/L. With the high sensitivity and the high positive predictive value, antithrombin III is a better and earlier test in the diagnosis of sepsis (Table 3).

Table 3: Antithrombin III levels in blood culture positive and negative neonates.

Blood culture	Antithrombin III	
	Mean±SD	P-Value
Positive	112.86±36.032	<0.05
Negative	164.75±45.49	

In the present study, out of 22 culture positive patients, 10 patients had positive CRP and low antithrombin III levels. But there were 8 patients, whose antithrombin III were low (≤150 mg/L) but CRP was negative.

Table 4: Outcome of neonates based on antithrombin III level.

AT III (mg/L)	Outcome		Total	P-value
	Non-survivors	Survivors		
0 –150	11	10	21	<0.05
>150	0	9	9	
Total	11	19	30	

As antithrombin III level falls even before the CRP becomes positive, antithrombin III is a better diagnostic test for diagnosis of neonatal sepsis, when blood culture is awaited (Table 5).

Table 5: Mean antithrombin III value among non-survivors and survivors.

	Non-survivors (n=11)	Survivors (n=19)	P-value
Antithrombin III (mg/L) Mean±SD	93.9±22.78	145.38±43.255	<0.05

In the present study there was a significant association (p<0.05) between antithrombin III levels ≤150 mg/L and neonatal mortality due to sepsis (Table 4).

Table 6: Relationship between blood culture, CRP and antithrombin III in Neonates with suspected sepsis.

Blood culture	CRP	Anti-thrombin III (mg/L)		Total
		0-150	>150	
Positive (n=22)	Positive	10	1	11
	Negative	8	3	11
	Total	18	4	22
Negative (n=8)	Positive	2	1	3
	Negative	1	4	5
	Total	3	5	8

Moreover, initial antithrombin III levels were significantly low (p<0.05) in neonates who expired as compared to those who were discharged (Table 6).

Thus, lower initial antithrombin III levels in neonatal sepsis are associated with severe disease and increased mortality. It may be useful in predicting clinical outcome in neonatal sepsis.

A significant association (p<0.05) was existed between septic neonates (whose blood cultures was positive), antithrombin III levels ≤150 mg/L and the clinical outcomes (Table 7).

Table 7: Relationship between blood culture and Antithrombin III levels among non-survivors and survivors.

Blood culture	Antithrombin III (mg/L)	Outcome			P-value
		Non-survivors	Survivors	Total	
Positive (n=22)	0-150	11	7	18	<0.05
	>150	0	4	4	
	Total	11	11	22	
Negative (n=8)	0-150	0	3	3	NA
	>150	0	5	5	
	Total	0	8	8	

Thus, septic neonates with low antithrombin III level had poor outcomes as compared to septic neonates with normal antithrombin III levels (>150mg/L).

DISCUSSION

Considering the high mortality and serious morbidity associated with neonatal sepsis, a diagnostic marker with a very high sensitivity and negative predictive value approaching 100% is desirable because all septic neonates with life threatening infection should be identified and treated.

Present results are consistent with Hisammuddin E et al who showed that CRP had sensitivity 76.92%, specificity 53.49%, positive predictive value 80%, negative predictive value 48.94% to diagnose neonatal sepsis. So, the test is not specific enough to be relied upon as the only indicator of sepsis.[6]

Benitz WE et al showed that sensitivity of normal CRP at the initial evaluation is not sufficient to justify withholding antibiotic therapy. Serial CRP levels are useful in the diagnostic evaluation of neonates with suspected sepsis.[7]

AT III deficiency happened in early stages of sepsis with no relation to DIC status.[8] Pharmacokinetic studies on septic patients demonstrated a reduced recovery and shortened half-life, that probably reflects, increased consumption of AT by elevated levels of thrombin: antithrombin complexes (TAT complex).

IL-6 and IL-1b have a synergistic effect in inhibiting AT production. AT also appears to function as a negative acute-phase protein.[9,10] An additional and perhaps preeminent mechanism in sepsis might be related to a specific activation of serine proteases and AT III by elastase release from activated neutrophils.[11,12] Therefore, the pathophysiological mechanism responsible for acquired AT deficiency in sepsis is probably multifactorial, including decreased synthesis, increased consumption and possibly capillary leak owing to increased vascular permeability.

Inhibition of thrombin by AT III results in the formation of a stable TAT complex. TAT is a sensitive and specific marker of thrombin generation. Pelzer H reported that plasma levels of TAT were significantly higher in new-borns with suspected sepsis.[12] Roman et al also showed that, severe neonatal infections were associated with increased levels of TAT.[13]

Present findings were supported by Lauterbach et al, when examining prognostic value of AT III and protein C in 150 neonates with late onset sepsis. The study concluded that both AT III protein C were significantly lower in neonates with sepsis either confirmed or not confirmed by blood culture.[14] A study conducted by Hanan FM et al showed that, there was a significant decrease in AT and RANTES in very low birth weight infants with septicemia and in those who progressed to DIC.[15] Beshlaway et al conducted a study to clarify the effect of sepsis on physiologic inhibition system of coagulation including protein S, protein C and AT III and their effect on thromboembolic accidents in septic new-borns. Study showed marked decrease in the level of physiologic inhibition system of coagulation including protein S, protein C and AT III in 100% of cases, compared to control group.[16]

AT III deficiency relates to mortality in sepsis. These findings were confirmed by Phillipe et al, who also reported, signs of more marked activation of coagulation in non-survivors of septic shock as compared with survivors on the day of hospital admission.[17] Muntaha ST et al showed that non-survivors had a more marked activation of the coagulation system expressed by reduced AT III levels. Survivors showed a pattern of progressive normalization of AT III.[18] Present results are in agreement with Hagag AA et al who found that initial AT levels were significantly lower in neonates with mild and severe sepsis versus control and significantly lower in non-survivors in contrast to survivor cases.[19] Hesselvich et al also showed that on the day following hospital admission patients with septic shock had lower antithrombin III and protein C levels than those with infection but without shock.[20] Several studies have shown that administration of antithrombin concentrate in patients with severe sepsis and DIC is effective in preventing mortality. Use of antithrombin concentrate in severe sepsis may form a new horizon for further evaluation of this compound in therapeutic trials.[13,21]

CONCLUSION

From present study, authors would like to conclude that antithrombin III is a single reliable investigation for diagnosis of neonatal sepsis. Antithrombin III level ≤150 mg/l correlates with culture results and it is a far superior indicator for neonatal sepsis as compared to more conventional CRP. Antithrombin III is a potential predictor of clinical outcome of neonatal sepsis. While present results are encouraging, larger studies are required to define the role of antithrombin III level as a single diagnostic and prognostic marker of neonatal sepsis.

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