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Research Article

C - Reactive protein, Immature to total Neutrophil Ratio and Micro ESR in early diagnosis of Neonatal Sepsis

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Abstract

Neonatal Sepsis is a clinical syndrome characterized by signs and symptoms of infection, identified & confirmed by positive blood cultures. As per National Neonatal Perinatal Database (NNPD) 2002-2003, the incidence of neonatal sepsis in India was 30 per 1000 live birth. Systemic bacterial infection is known by the term neonatal sepsis which incorporates septicemia, pneumonia and meningitis. C-reactive protein (CRP), an acute phase reactant has advantages of low serum levels in normal infants, a rapid rise after 12 to 24 hours of sepsis and a massive rise thereafter as long as inflammatory stimuli persist and followed by immediate fall of serum level as soon as inflammation subside. Micro ESR can be compared to wintrobe's method of doing ESR and can be used in neonatal sepsis. Rates do not vary considerably with gestational age, birth weight, but are inversely related to hematocrit level, Particularly when it is less than 40 mm/dl. The micro ESR is generally mildly elevated in non infectious conditions. In most of the patients with infection the micro ESR is elevated within 24 hours of infection and it is not influenced by antibiotic. It is well-known fact that understanding hematology of neonatal sepsis helps in early identification of suspected cases of neonatal sepsis. Amongst Hematological parameters Immature to total neutrophil (I:T) ratio has a reasonably good predictive value for early diagnosis of neonatal septicemia. This study is done as an endeavor to add to something about our preexisting knowledge of 'diagnosis of neonatal sepsis' early for better management of this group of patients

Keywords: C-Reactive Protein, I:T Ratio, Micro ESR, Neonatal sepsis

1. Introduction

Neonatal Sepsis is a clinical syndrome characterized by signs and symptoms of infection, identified & confirmed by positive blood cultures.[1] As per National Neonatal Perinatal Database (NNPD) 2002-2003, the incidence of neonatal sepsis in India was 30 per 1000 live birth[3] Neonatal sepsis is frequent and important cause of morbidity and mortality particularly in the developing countries, like India. Neonatal sepsis accounts early half of all neonatal death in our countries.[2] the term 'SEPSIS NEONATORUM' refers to the generalized bacterial infection of infants during the first month of life. Systemic bacterial infection is known by the generic term neonatal sepsis which incorporates septicemia, pneumonia and meningitis.[1]

Larger numbers of infants gets admitted for "ruling out sepsis", despite sepsis still continues to be missed. Many studied have investigated a variety of laboratory tests to enhance the early detection of neonatal sepsis. Definitive diagnosis of neonatal sepsis is made by demonstration of the organisms in blood culture, but it takes long time 2-8 days for positive culture[7] and hence various other investigations are necessary for early diagnosis or rule out sepsis. In addition, it is also important to limit inappropriate antibiotic exposure leading to antibiotic resistance and lowering the cost of therapy. Therefore, the need is for a test that is cheap, easily performed with quick availability of reports. C-reactive protein (CRP) has been used as an acute phase reactant to diagnose and follow the course of infection in neonates. Its advantages include its very low serum levels in normal infants, a rapid rise after 12 to 24 hours of sepsis and a massive rise thereafter as long as inflammatory stimuli persist and followed by immediate fall of serum level as soon as inflammation subside make it suitable for diagnosis and follow up of neonatal sepsis.[2,4] Still it has some limitation like rise of level is evident after 12-24hr of infection and there is some false positive results in case of intraventricular haemmorhage, meconium aspiration, NEC (necrotizing enterocolitis), pneumothorax, surgery, immunization etc[2,4] .It is well-known fact that understanding hematology of neonatal sepsis helps in early identification of suspected cases of neonatal sepsis, predicting the prognosis and also helps in decision of line of treatment to these neonates as to have better outcome[6]. Various hematological parameters have already been studied like ANC (absolute neutrophil count), I: T ratio (immature to total granulocyte ratio), Micro ESR, Band cell count, Thrombocytopenia etc. but test are either subjective or become positive in late stage.

1.1 Aims and objectives:

To evaluate the diagnostic efficacy of the following parameters alone or in combination in the early diagnosis of neonatal septicemia, so that immediate treatment can be started before results of bacterial culture (The gold standard confirmatory test) become available.

a) Immature to total neutrophil ratio(I:T Ratio)
b) C-Reactive protein
c) Micro ESR

This study is done as an endeavor to find a suitable parameter for early diagnosis of neonatal sepsis before the results of culture become available; for better management of this group of patients.

2. Material and Methods

The present study was performed on neonates admitted to Neonatal I.C.U., S.S.G. Hospital, Baroda, during the period of November 2012 to September 2013 and all tests were performed at the department of Pathology, Medical College, Baroda. Written informed consent was taken from parents of the included neonates. Total 120 cases were studied. *Three groups of neonates were taken for the study*:

(A) ***Proven sepsis (total 60 cases):*** Patients with obvious clinical course of neonatal septicemia that were blood culture positive.
(B) ***Most probable sepsis (total 40 cases):*** Patients with obvious clinical course of neonatal septicemia but were blood culture negative.
(C) ***No sepsis (total 20 cases):*** Healthy infants without any signs and symptoms of septicemia.

Immediately on admission a detailed clinical history was taken and thorough physical examination was done.

2.1 Collection

Using all aseptic precautions blood was collected from peripheral vein prior to starting antibiotic treatment. Two C.C. blood was collected in plain bulb as well as in EDTA bulb. Plain bulb for CRP estimation and EDTA bulb for leucocyte count. Heel prick method was used to obtained blood for preparation of peripheral smears for estimation of Immature: Total neutrophil (I: T) ratio, to see the morphology of neutrophils and for measurement of micro ESR.

2.2 Total Leucocyte Count

This was performed by using Automated haematology analyzer Abacus based on principle of Electric impedence.

2.3 Peripheral blood smear

The two heel prick smears were stained using Leishman stain. These were used for determination of the differentional leucocyte count by counting 100 cells, Absolute neutrophil count, Band cell count and Immature: Total neutrophil ratio.

2.4 C reactive protein

KIT: CRP Latex, Reckon, Vadodara, India. CRP was estimated by the semi quantitative latex agglutination method.

Principle: The latex slide agglutination test is based on the immunologic reactions between CRP antigen and latex particles coated with monospecific anti human CRP antibody. The kit used was the CRP Latex, agglutination slide test for C- reactive protein manufactured by Reckon Diagnostic Ltd., Vadodara, India. This test detects CRP concentration > 0.6 mg/dl.

2.5 Micro ESR

Blood was collected in preheparinised microhematocrit tubes of 75 mm length with an internal diameter of 1.1 mm & external diameter of 1.5 mm by heel prick technique. One end was sealed using clay/ wax. The tube was then fixed vertically with the help of sticking plaster & left undisturbed for 1 hour. At the end of 1 hour the fall in the red cell column was measured accurately to the nearest millimeter[1].

2.6 Statistical Methods

Data is presented as comparison of I:T Ratio, CRP & Micro ESR in proven and probable sepsis group with control group. 'Gold standard' confirmatory test was blood culture[8].Data is presented as percentages, sensitivity & specificity.

3. Results

The present study was performed on neonates admitted to Neonatal I.C.U., S.S.G. Hospital, Baroda, during the period of November 2012 to September 2013 and all tests were performed at the department of Pathology, Medical College, Baroda. Total 120 cases were studied. *Three groups of neonates were taken for the study*:

(A) ***Proven sepsis (total 60 cases):*** Patients with obvious clinical course of neonatal septicemia that were blood culture positive.
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(C) ***No sepsis (total 20 cases):*** Healthy infants without any signs and symptoms of septicemia.

- Out of 60 total cases of proven neonatal sepsis 62% had early onset (onset of Symptoms <72 hours) sepsis.
- Male: Female ratio was 2:1 in the proven sepsis group.
- Incidence of Proven neonatal sepsis was higher in low birth weight (≤2500gms) infants .(65%)
- Incidence of Proven neonatal sepsis was higher in preterm neonates (< 37 weeks) (58%).
- In our hospital, isolation of *Klebsiella* spp. from blood culture was highest (38%) Followed by *staph aureus*(26%).

3.1 Immature to total Neutrophil Ratio

Amongst 100 neonates with clinical presentation of neonatal sepsis, **70%** cases had I:T Ratio ≥ 0.2 (50% cases were blood culture positive and 20% cases were blood culture negative)
Amongst 20 neonates with no sepsis 5% cases had I: T Ratio ≥ 0.2.

3.2 C-reactive proteins (CRP)

Amongst 100 neonates with clinical presentation of neonatal sepsis**, 80%** cases had CRP value≥1mg/dL (52% cases were blood culture positive and 28% cases were blood culture negative)Amongst 20 neonates with no sepsis 20% cases had CRP value ≥1mg/dL.

3.3 Micro ESR

Amongst 100 neonates with clinical presentation of neonatal sepsis, **67%** cases had micro ESR >15 mm at the end of 1st hour (43% cases were blood culture positive and 24% cases were blood culture negative) Amongst 20 neonates with no sepsis 35% cases had micro ESR >15 mm at the end of 1st hour.

Table I: Sensitivity and specificity of various tests in proven sepsis cases

Sr.	Test	Sensitivity	Specificity
1	I:T Ratio ≥0.2	83.33%	95%
2	CRP positive ≥1mg/dL	86.66%	80%
3	Micro ESR > 15 mm	71.66%	65%

Above table shows sensitivity and specificity of various tests ;out of which CRP shows highest sensitivity and I:T Ratio shows highest specificity amongst proven sepsis cases.

4. Discussion

Early onset sepsis was more common than late onset sepsis. Thus probability of acquiring sepsis is more during early neonatal period. Male neonates are more prone to neonatal sepsis than female neonates. The hypothesis to explain this difference is that factors regulating Immunoglobulin (IgG) synthesis may be on X – chromosomes. Therefore presence of pair of X – chromosomes in females probably confers a greater genetic diversity to female immunological system and accounts for relatively more strength to fight infection.[2]Premature neonates are more prone to acquire sepsis than the full term neonates. The high incidence of neonatal septicemia in premature babies is due to many factors but mainly due to diminished resistance because of fragile skin, poor cilliary action, defective chemotaxis etc. It is also observed that premature

infants are more frequently exposed to resuscitation, inhalation therapy and other life supportive measures leading to higher risk of infection[2] Neonates with low birth weight are more prone to sepsis. Low birth weight & very low birth weight neonates (< 2 kg weight) act as independent risk factor and also they have suboptimal defence to withstand the demands put upon them in a hostile extra uterine environment.[2]

●CRP positivity is the single & most useful parameter for early diagnosis of neonatal sepsis. It has the highest sensitivity in the present study.CRP can be used to predict neonatal sepsis early with reasonable accuracy.

●I: T Ratio also has reasonably good sensitivity. It is more specific than CRP, thus it can more accurately be used for ruling out proven sepsis. Micro ESR has lower sensitivity and specificity, so can't be used alone to confirm or rule out sepsis.

Reasonable clinical judgment with CRP and I:T Ratio provides rational bases for treatment decision in neonatal sepsis. Such strategy significantly reduces unnecessary antimicrobial therapy which can otherwise permit emergence of resistant strains.

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