Original research article

Usefulness Of Estimation Of CRP With Technologically Better Method Called High Sensitivity C-Reactive Protein (Hs CRP) Estimation, In The Spectrum Of Infection, Ranging From Infection To Septic Shock.

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Abstract

Aim: The present study is carried to ascertain usefulness of estimation of CRP with technologically better method called High Sensitivity C-Reactive Protein (Hs CRP) estimation, in the spectrum of infection, ranging from infection to septic shock. The age group included is from birth to 2 months of age. An effort has been made to correlate values of Hs CRP with respect to severity of infection.

Material and methods: This prospective observational study was carried out in the Department of Pediatrics, Anugrah Narayan Magadh Medical College and Hospital Gaya, Bihar, India for 1 year. Each paediatric patient who came to our Hospital was initially categorized according to age till 2 months of age. Neonates were graded according to the signs and symptoms of FIMNCI. FIMNCI considers bacterial infections in young infants when signs or symptoms of sepsis, pneumonia or meningitis are present. 2 ml of blood was again collected from the corresponding patient in a plain bulb under all aseptic precautions. The sample was then sent laboratory for CRP testing.

Results: Out of 80, 70 cases were successfully followed up to 48 hours as was aimed at the beginning of the study and 10 cases failed to complete a 48 hour study period due to various reasons. Out of 70 newborns studied, 45 were male and 25 were female babies. Clinical improvement was assessed by hemodynamic profile, absence of presenting complaint/s and ability to tolerate feed and absence of blood culture positivity of first culture. 20 babies improved clinically, whereas 27 babies had almost similar clinical profile. 23 babies showed clinical downward status in spite of starting empirical antibiotics and supportive treatment. Surprisingly, majority of babies had shown positivity in HS-CRP at admission (60/70) as well as after 48 hours (62/70).Mean and median of all babies as shown in Table 3 was not conclusive about severity of infection and CRP values. It seems that Hs CRP is very sensitive indicator for neonatal sepsis. Neonates were further categorised in 3 groups depending on clinical improvement and fall or rise of Hs CRP level was studied. It was seen that Hs CRP levels have decreased in group which had shown clinical improvement, however in both other groups it was showing rise.We have calculated independent t-value also for each group and it was seen that change in Hs CRP values after 48 hours was significant.

Conclusion: The study revealed that HS CRP levels were increased in all cases of suspected neonatal sepsis. They remained high in neonates who had deteriorated or remained same clinically at 48 hours of follow-up. However, they significantly reduced in neonates showing clinical improvement.

Keywords: C-reactive protein (CRP), Hs CRP, IMNCI, Neonatal sepsis

Introduction

Neonatal sepsis is a generalized bacterial infection of the neonate's blood during the first month of life. It is either early-onset (7 days)[1] and it is considered one of the important leading factors for neonatal mortality and morbidity, particularly in developing countries. Consequently, early diagnosis is very important as it helps in beginning antibiotic therapy early, reducing neonatal mortality.[2] the use of conventional blood culture for the isolation of microorganisms is still the gold standard method for diagnosis. However, blood culture can detect the pathogen in only 25% of cases, so it has low sensitivity. It gives false negative results, especially due to the use of antibiotics. Also, it may give false-positive results due to contamination. In addition, these cultures have a time delay for 2-3 days, and it is difficult to get serial blood samples from neonates.[2,3]In addition to blood culture, polymerase chain reaction (PCR) amplification of highly conserved DNA sequences found in all bacteria would permit rapid and sensitive detection of bacteria in blood specimens. But its results do not depend on the bacterial viability and so it may still be positive even after antibiotic treatment.[4]Suspected neonates are subjected to broad-spectrum antibiotic treatment empirically until sepsis can be excluded.)is antibiotic overuse favors the development of resistance.[5]So, improving the accuracy of the diagnostic tests may decrease the indiscriminative use of antibiotics in cases without sepsis.[6] Recent several studies have included many sepsis markers, like C-reactive protein (CRP), procalcitonin (PCT), and interleukin-6 (IL-6) to improve sepsis diagnosis.[3,6]C-reactive protein (CRP) is an acute-phase protein synthesized in the liver in response to infection or inflammation. It may be more useful in excluding infections in suspected cases and enable earlier discontinuation of antibiotics.)is may be cost-effective, decreasing the development of resistance and hospital stay duration.[7] Moreover, neonatal sepsis is a challenging problem indeed, and physicians are always in need of methods of prediction and early diagnosis of sepsis to initiate therapy as rapidly as possible to decrease the negative impact on the patient's health and therefore decrease the duration of hospital stay and costs. The present study is carried to ascertain usefulness of estimation of CRP with technologically better method called High Sensitivity C-Reactive Protein (Hs CRP) estimation, in the spectrum of infection, ranging from infection to septic shock. The age group included is from birth to 2 months of age. An effort has been made to correlate values of Hs CRP with respect to severity of infection.

Material and methods

This prospective observational study was carried out in the Department of Pediatrics, Anugrah Narayan Magadh Medical College and Hospital Gaya, Bihar, India for 1 year.

Inclusion criteria:

- Newborns and children upto 2 months of completed age and admitted in paediatric department.
- Suspicion of possible infections in newborns
- Admission to NICU/PICU/WARD for further evaluation.

Exclusion criteria:

- Newborns and children with definitive viral and fungal infections at admission.
- Newborns and children with MODS
- Children with known immunodeficiency,
- Autoimmune disorders

Each paediatric patient who came to our Hospital was initially categorized according to age till 2 months of age. Neonates were graded according to the signs and symptoms of FIMNCI.

FIMNCI considers bacterial infections in young infants when signs or symptoms of sepsis, pneumonia or meningitis are present.

For our study, following signs and symptoms were considered for possible infections in neonates to enrol patients: unable to feed, fast breathing (RR >60/min) severe retractions, lethargic or unconsciousness, bulging fontanelle, convulsions, nasal flaring, Grunting, less than normal movements, axillary temperatre 37.5 C or above; or less than 35.5 C, Painful joints, joint swelling, reduced movements around a particular joint and irritability, many skin pustules/ big boils, Umbilical redness extending to the periumbilical skin or umbilicus draining pus, Meningitis is considered in neonates if one or more of the following signs are present: drowsiness, lethargy or unconsciousness, persistent irritability and high pitched cry.

The high sensitivity application was used for hsCRP testing. Highly Sensitive Application – Beckman Coulter AU400/400e /480, AU600/640/640e /680 (0.2 – 160 mg/L), AU2700/5400 (0.2 – 80 mg/L): The main ingredients of the machine were the 2 reagents (R1-Glycine buffer 100 mmol/L & R2-Latex coated with anti-CRP Antibodies < 0.5 %) and the other ingredients being preservative and normal saline. The R1 and R2 reagents used in the analyzer increase the accuracy of the system and helps in detecting extremely low quantities of CRP making it high sensitive CRP (Hs CRP) and making it superior to the conventional CRP. The CRP antibodies present in the serum of the patient's blood react with the antiserum-antibodies present in the reagents R1 and R2 to form immune complexes. These Immune complexes formed in solution scatter light in proportion to their size, shape, and concentration. Turbidimeters in the system measure the reduction of incidence light due to reflection, absorption, or scatter. In this procedure, the measurement of the rate of decrease in light intensity transmitted (increase in absorbance) through particles suspended in solution is the result of complexes formed during the immunological reaction between the CRP of the patient serum and rabbit anti-CRP-antibodies coated on latex particles.

C-reactive protein specimens are stable for 11 days at 20 – 25°C and 2 months at 4 – 8°C in serum and plasma. For longer storage, freeze serum to -20°C. A total of 80 samples could be run simultaneously with a run-time of 30 minutes making it as competitive as the conventional CRP but being more sensitive at the same time. The dynamic range of the beckman coulter helped us attain broad and more specific values of Hs CRP. This value was noted.

2 ml of blood was again collected from the corresponding patient in a plain bulb under all aseptic precautions. The sample was then sent laboratory for CRP testing. This sample was labelled as the 2 nd Hs CRP sample. It was run on the same machine to avoid technological bias. The 2nd Hs CRP value was noted too.

Results

A total 80 cases were enrolled in the study in which infection was suspected at the time of admission based on the category in IMNCI/FIMNCI for suspicion of infection. Out of these, 70 cases were successfully followed up to 48 hours as was aimed at the beginning of the study and 10 cases failed to complete a 48 hour study period due to various reasons. Out of 70 newborns studied, 45 were male and 25 were female babies. Clinical improvement was assessed by hemodynamic profile, absence of presenting complaint/s and ability to tolerate feed and absence of blood culture positivity of first culture. 20 babies improved clinically, whereas 27 babies had almost similar clinical profile. 23 babies showed clinical downward status in spite of starting empirical antibiotics and supportive treatment.

Surprisingly, majority of babies had shown positivity in HS-CRP at admission (60/70) as well as after 48 hours (62/70).Mean and median of all babies as shown in Table 3 was not conclusive about severity of infection and CRP values. It seems that Hs CRP is very sensitive indicator for neonatal sepsis.

Neonates were further categorised in 3 groups depending on clinical improvement and fall or rise of Hs CRP level was studied. It was seen that Hs CRP levels have decreased in group which had shown clinical improvement, however in both other groups it was showing rise.
We have calculated independent t-value also for each group and it was seen that change in Hs CRP values after 48 hours was significant.

Table 1: Gender distribution of patients

Gender	**No. of patients =70**	**Percentage**
Male	45	64.29
Female	25	35.71

Table 2 Clinical condition after 48 hr

Improved	20	28.57
Same	27	38.57
Deteriorated	23	32.86

Table 3: Clinical characteristics and values of HSCRP

Positive on admission	60
POSITIVE after 48 hr	62
At admission / 48hrs(n=70)	
Mean	60.4/65.2
Mode	38.2/74.2
median	55.4/61.7
Standard deviation (admission /48hours)	37.12/24.88
hsCRP comparison against age	1.387
Paired t-test value (p-value)	(0.181)

Table 4: Comparison of HSCRP in Same, Deteriorated &Improved group

	Mean At admission /48hours	**Median At admission /48hours**	
Same	28.87/44.21	26.12/41.62	
Deteriorated	50.87/74.52	49.11/73.11	
Improved	79.31/53.41	74.9/50.21	
Standard deviation (admission /48hours)	30.02/39.06	28.11/34.12	
Independent t value			
	Same	Deteriorated	Improved
Mean (rise/ fall)	Rise	Rise	Fall
Median (rise/ fall)	Rise	Rise	Fall
Standard deviation (rise/ fall)	Rise	Rise	Fall
Average fall /rise	Rise	Rise	
change in hsCRP values after 48 hours	p- value 0.001	Significant	

Discussion

In our study, total 80 cases were enrolled in the study in which infection was suspected at the time of admission based on the category in IMNCI/FIMNCI for suspicion of infection. Out of these, 70 cases were successfully followed up to 48 hours to find the correlation of Hs CRP

with clinical condition of the patient.Prior studies have associated acute increase in CRP (on the order of 10–200 mg/dL) as a marker of sepsis illness severity as well as predicted of sepsis outcomes.[8-10]For example, in a series of 50 critically ill sepsis patients, Schmit, et al. observed that CRP on admission was 16.7±10.6 mg/dL and that the magnitude of CRP decrease was associated with response to antimicrobial therapy.[11]

Povoa ,Schmit and colleagues continued their longstanding work on C-reactive protein (CRP) kinetics by evaluating the patterns of evolution of CRP in patients with severe community-acquired pneumonia (CAP).[12,13]In a study conducted by LOBO SM et al., CRP is an acute-phase protein synthesized by the liver after stimulus by cytokines and its serum levels increase markedly within hours after the onset of infection, inflammation or tissue injury. Decreasing plasma concentrations of this biomarker have been used as an indicator for resolution of infection or sepsis.[14] Coelho et al. studied 891 intensive care unit patients with community-acquired sepsis, observing a mean hospital admission CRP level of 20.1±13.9 mg/dL and finding association between rates of CRP decline and hospital survival.[15]A time-dependent analysis was performed and CRP ratios were calculated daily in relation to the CRP concentration on day 0, considered equal to 1. They showed that survivors of CAP had a continuous decrease of the CRP ratio during the first week of antibiotic therapy.[13]Along with cases showing a clinical improvement in our study, 20 cases showed a fall in Hs CRP values after 48 hours. Hence a decreasing trend of Hs CRP was seen in majority of cases from admission to 48 hours of admission. Only patients with severe sepsis failed to show a significant change in Hs CRP values i.e. 28.57%.

The secretion of CRP begins within 4–6 h of the stimulus, doubling every 8 h and peaking at 36–50 h. With a very intense stimulus, the CRP concentration can rise above 500 mg/l, i.e. more than 1000 times the reference value.[16,17]After disappearance or removal of the stimulus, CRP falls rapidly, as it has a half-life of 19 h . However, CRP can remain elevated, even for very long periods, if the underlying cause of the elevation persists. With the exception of severe hepatic failure, CRP rises whenever an inflammatory process is present; its serum concentration only depends on the intensity of the stimulus and on the rate of synthesis. The CRP level is independent of the underlying pathology and is not modified by any therapy or intervention such as renal replacement therapy . Only those interventions affecting the inflammatory process responsible for the acute phase reaction can change the CRP level.

In a study by Suprin et. al., mean values were 70 mg/l in systemic inflammatory response syndrome (SIRS) patients, 98 mg/l in sepsis, 145 mg/l in severe sepsis and 173 mg/l in septic shock, probably reflecting different degrees of inflammatory response .[16]

Our study also is similar values at admission, but as shown in above discussion it can be deduced that fall or rise documentation was not important in all groups and clinical acumen is more important. In fact, it is not worthy to prick the child frequently to show that child has deteriorated or improved.

Recent study by Nagwan I. Rashwan et al states that CRP could be a helpful prognostic marker in late onset neonatal sepsis. Hs CRP and PCT have higher diagnostic accuracy in neonatal sepsis in comparison to other studied markers. Both IL-6 and presepsin have equal diagnostic utility in neonatal sepsis, but presepsin could be helpful diagnostic marker in early onset neonatal sepsis.[18]In a study for Evaluation of IL-6, CRP and Hs-CRP as Early Markers of Neonatal Sepsis by Purushothaman Ganesan et al in India it was concluded that IL-6 is a highly sensitive marker and CRP is a more specific marker for the diagnosis of neonatal sepsis. Hs-CRP is a less reliable marker. So, the combination of IL-6 and CRP are the better predictors of neonatal sepsis. However, the sample size of this study was only 40, so may have biased reporting. However, it is noteworthy that in this study, Hs CRP showed sensitivity of 92% and specificity of 34.24%.[19]In extensive work done by William E. Benitz et al Serial Serum C-Reactive Protein Levels in the Diagnosis of Neonatal Infection, it was

observed that Serial CRP levels are useful in the diagnostic evaluation of neonates with suspected infection. Two CRP levels < 1 mg/dL obtained 24 hours apart, 8 to 48 hours after presentation, indicate that bacterial infection is unlikely. The sensitivity of a normal CRP at the initial evaluation is not sufficient to justify withholding antibiotic therapy. The positive predictive value of elevated CRP levels is low, especially for culture-proven early-onset infections. This was very old study of 1998 and since now Hs CRP has altered the values but principle remains same.[20]

In our study also similar results are found and it can be concluded that serial Hs CRP may not be helpful if it is already high initially.

Conclusion

The study revealed that HS CRP levels were increased in all cases of suspected neonatal sepsis. They remained high in neonates who had deteriorated or remained same clinically at 48 hours of follow-up. However, they significantly reduced in neonates showing clinical improvement.

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