ISSN- O: 2458 - 868X, ISSN–P: 2458 – 8687
National Library of Medicine - ID: 101731606

International Journal of Medical Science and Innovative Research (IJMSIR)
IJMSIR : A Medical Publication Hub
Available Online at: www.ijmsir.com
Volume – 8, Issue – 2, March – 2023 , Page No. : 15 – 21

Immune-Turbidimetry versus Latex Agglutination for Estimation of CRP Levels among Clinically Suspected Early-onset and Late-onset Neonatal Sepsis

[1]Bineeta Kashyap, MD, Professor, Department of Microbiology, University College of Medical Sciences & Guru Teg Bahadur Hospital, Delhi.

[2]Rituparna Saha, MD, Assistant Professor, Department of Microbiology, Faculty of Medicine & Health Sciences, Shree Guru Gobind Singh Tricentenary University, Gurugram, Haryana

Corresponding Author: Bineeta Kashyap, MD, Professor, Department of Microbiology, University College of Medical Sciences & Guru Teg Bahadur Hospital, Delhi.

Citation this Article: Bineeta Kashyap, Rituparna Saha, "Immune-Turbidimetry versus Latex Agglutination for Estimation of CRP Levels among Clinically Suspected Early-onset and Late-onset Neonatal Sepsis", IJMSIR- March - 2023, Vol – 8, Issue - 2, P. No. 15 – 21.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Background: The diagnosis of neonatal sepsis using CRP as a biomarker is widely practiced. The establishment of clinical cut offs especially in the early hours of life has always eluded the diagnosis of neonatal sepsis. We aimed to compare the ability of latex agglutination and immune-turbidimetry to estimate C-reactive Protein(CRP) levels in clinically suspected early and late onset neonatal sepsis.

Methods: CRP levels were estimated in 80 consecutive suspected neonatal sepsis patients, sent for routine screening by latex agglutination and immune-turbidimetry. Two age-groups (viz. < 72 hours and 72 hours-3 months) of 40 neonates each, were formulated by including 20 neonates with CRP levels < 6 mg/L and 20 with CRP≥ 6 mg/L, by latex agglutination.

Results: Overall percentage agreement of 93.75% was observed between latex agglutination and immune-turbidimetry. The two methods showed a percentage agreement of 90% and 97.5% for determining CRP in neonates < 72 hours and above 72 hours respectively.

CONCLUSION: A lower percentage agreement between the two modalities in neonates suspected with early sepsis makes it an attractive modality for rapid estimation of CRP levels, especially during the initial 72 hours after birth.

Keywords: Diagnosis, Neonatal sepsis, early, late, C-reactive Protein

Introduction

The C-reactive protein (CRP)is an established component of acute-phase response. It issecreted in response to tissue injury arising from both infectious and non-infectious etiology; simultaneously attempting to neutralize the inflammatory response and consequently augment tissue healing.[1] Since the first discovery in 1930 by Tillet and Francis at Rockefeller, the biomarker has been detected in a spectrum of disorders encompassing but not limited to infections, rheumatic disorders and malignancies.[2, 3]

A microbial insult or trauma, elicits an acute inflammatory response ushered by local inflammatory cells, which in turn trigger the cascade of recruitment and activation of inflammatory cells like fibroblasts, leukocytes and endothelial cells. Pro-inflammatory cytokines viz. IL-1, TNF-α and IL-6, released in response to immune-activation, induce hepatocytes to produce an array of acute phase response proteins like: components of the complement system, coagulation factors, protease inhibitors, metal-binding proteins and CRP.[1, 4]Essentially binding to the phosphocholine present in most biological membranes as well as microbial lipopolysachharide, CRP acts by promoting complement mediated phagocytosis in addition to the production of pro-inflammmatory cytokines. [4, 5] Hepatic synthesis of CRP rapidly escalates within hours during acute phase response, primarily induced by IL-6 or in synergy with IL-1.[5, 6] With a half life of 19 hours, the levels of CRP continue to remain high during the course of inflammation or tissue injury and rapidly subside thereafter.[7]Since the sole determinant of plasma levels of CRP, is the rate of production, the biomarker has been widely deployed clinically, in monitoring the severity of diseases.[8]

Even in neonates, hepatic synthesis of CRP commences rapidly upon stimulus, rising well above 5 mg/l within 6 hours and subsequently peaking at 48 hours.[9, 10] Considering a remarkably low placental passage, any elevation of CRP in neonatal plasma samples is indicative of endogenous production.[10]Depending upon, study setting, case definition, the timing of sample collection as well as the cut-offs and testing modality used, studies have reported an unacceptably wide range of sensitivities and specificities of CRP varying from 29 to 100 % and 6 to 100% respectively, in diagnosing early onset sepsis in neonates. Considering the low sensitivity of CRP in early stages of infection, the testing modalities, timing and the cut-offs of CRP used in the diagnosis of neonatal sepsis merit reconsideration. [1, 11, 12] Thus the present study aims to elucidate and compare two methods of determination of CRP in two different sub-populations of neonates clinically suspected with sepsis.

Materials and Method

A prospective study was conducted over a period of six months in the Immunology laboratory ofthe Department of Microbiology, University College of Medical Sciences & Guru Teg Bahadur Hospital, to compare the adequacies of Immune-turbidimetry and latex agglutination assays in determining the levels of CRP in neonates of various age groups. Eighty consecutive clinically suspected neonatal sepsis patients, whose serum samples were sent to the Department of Microbiology for routine screening, were included in the study and processed further.

For estimation of CRP levels, Blood samples obtained by venepuncture and transported in non-anticoagulated vacutainers were centrifuged at 2000 rpm for 10 minutes to separate serum. All grossly haemolyzed and lipemic serum samples were excluded from the study.

Latex Agglutination Test

The levels of the acute phase reactant, C Reactive Protein was estimated by the ReCombigen C-Reactive Protein (CRP) Latex (Recombigen laboratories Pvt Ltd, New Delhi, India) as per manufacturer's instructions.Serum samples yielding CRP levels higher than 6 mg/L by qualitative method were further estimated semi quantitatively using the same kit as per manufacturer's instructions. As per kit literature, specimens showing no agglutination were reported to have CRP below 6 mg / L. Positive and negative controls were put up for each batch of tests.

For comparability, forty serum specimens each, from neonatal age groups: less than 72 hours and 72 hours to 3 months, were sequentially selected. These two age groups comprising of: less than 72 hours and 72 hours to 3 months, were individually formulated by including 20 serum samples that yielded CRP levels less than 6 mg/L and 20 serum samples yieldingCRP levels ≥ 6 mg/L, by latex agglutination.

Immune-Turbidimetry

CRP levels were estimated by immune-turbidimetry in all Eighty neonatal serum samples. The SPECTRUM C-Reactive Protein (CRP) Test Kit (Spectrum Medical Industries, Delhi, India) in conjunction with the SPECTRUM Automatic Analyzer (Spectrum Medical Industries, Delhi, India) was used to determine the titre of CRP in the same neonatal patients' sera that were tested for CRP by latex agglutination by the above method.

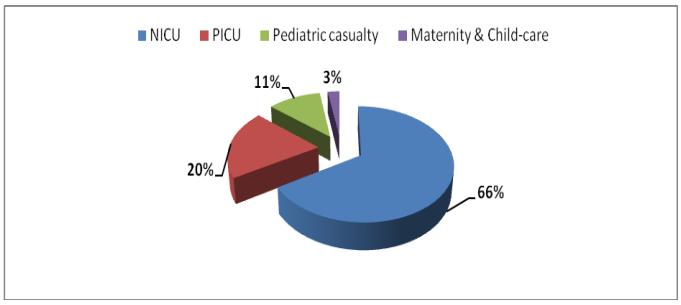
Statistical Analysis

Percentage agreement between the two testing modalities was calculated and the correlation between the results of latex agglutination and immune-turbidimetric analysis was ascertained by Spearman coefficient. The SPSS 20.0 software was used for all statistical analysis.

Results

Among eighty consecutive neonates, clinically suspected with sepsis and whose serum samples were sent to the Department of Microbiology of University College of Medical Sciences & Guru Teg Bahadur Hospital, 46 (57.5%) were males and 34 (42.5%) were females. Majority (66%) of the participants were admitted at the neonatal ICU (NICU). Figure 1 depicts the departmental distribution of neonates included in the study. The age of the study population ranged between 1 day to 28 days with a median age of 3.5 days (mean 10.8 days ± 10.43 days).

Figure 1: Departmental distribution of the study population



The SPECTRUM C-Reactive Protein (CRP) Test Kit established CRP titres above the normal range (>6 IU/ml) in 44 patients. Whereas 36 patients' sera displayed CRP titres below the normal range (<6 mg/L) by immune-turbidimetry. The CRP levels observed in the neonatal study group below 72 hours ranged from 1.76 mg/ L to 20.11 mg/ L median CRP titre of 7.35 mg / L (mean 7.691 ± 3.771 mg/L). While CRP titres in the age group 72 hours to 3 months ranged between 2.12 mg/ L to 22.72 mg/ L with a median titre of 6.85 mg / L (mean 8.072 ± 5.116 mg/L).

Upon comparing latex agglutination test for determining CRP levels with the help of ReCombigen C-Reactive Protein (CRP) Latex (Recombigen laboratories Pvt Ltd, New Delhi, India) kit and the SPECTRUM C-Reactive Protein (CRP) Test Kit (Spectrum Medical Industries, Delhi, India); an overall percentage agreement of 93.75%. was observed (Cohen's $\kappa = 0.875$). Furthermore, a percentage agreement of 90% (Cohen's $\kappa = 0.8$) was observed between the two testing modalities for determining CRP in neonates less than 72 hours. While among study samples from neonates above 72 hours, the percentage agreement for the above two methods in determining CRP levels was 97.5% (Cohen's $\kappa = 0.95$). Table 1 illustrates the performance statistics of the two methods in the study subpopulations.

Table 1: Performance statistics of latex and immunoturbidimetry for CRP estimation in the two study subpopulations

	neonates < 72 hours	neonates ≥ 72 hours
CRP levels >6 mg/L by latex agglutination	20	20
CRP levels >6 mg/ L by immunoturbidimetry	24	20
Percentage agreement between the above methods in determining CRP	90% (κ= 0.875)	97.5% (κ= 0.95)
Correlation between the two methods in determining CRP	Positive (r=0.6968)	Positive (r=0.7635)

Discordance in the levels of CRP when measured by latex agglutination and immune-turbidimetry, was observed in 4 study samples. Data analysis revealed an overall positive correlation between latex agglutination and immune-turbidimetry assay for CRP, with a Spearman coefficient of r= 0.6968. A comparative overview of the CRP levels determined by latex agglutination and immune-turbidimetry are depicted in Figure 2 and Figure 3.

Figure 2: CRP levels determined by the two methods in < 72 hours study population

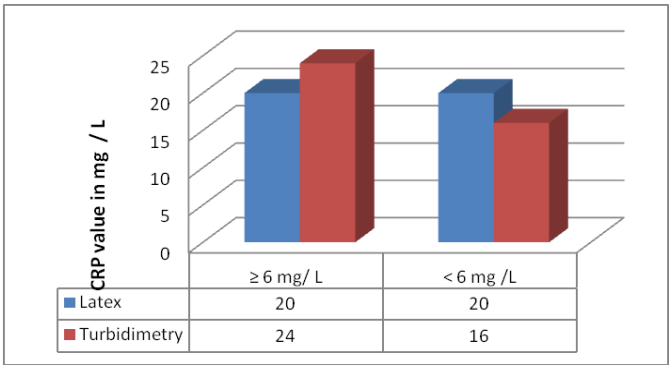
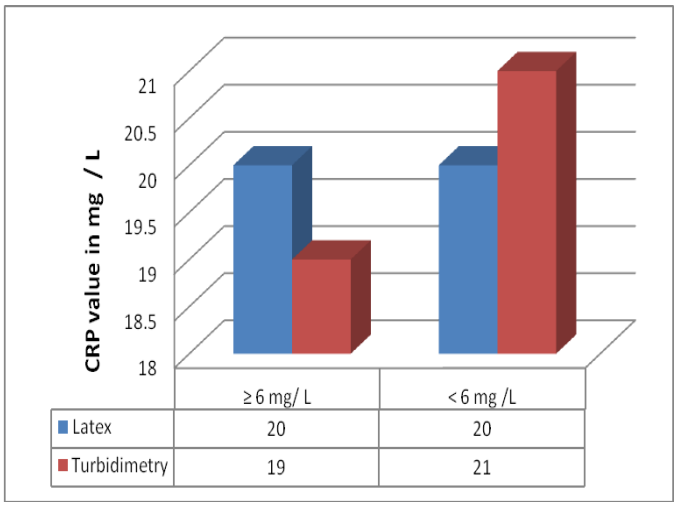


Figure 3: CRP levels determined by the two methods in ≥72 hours study population



Discussion

Neonatal sepsis is the second most common cause of neonatal mortalityworldwide and India reports the highest incidence of clinical sepsis.[13]Neonatal case fatality rates due to sepsis (25%-65%), is grossly under estimatedand less likely to be reflect the true picture.[14]High incidence and mortality in early-onset sepsis as compared to late onset neonatal sepsis, highlight the "time critical clinical course" of neonatal sepsis.[15]Though blood culture is considered gold standard, high turnaround time and low culture positivity (25%-56%) often hinder diagnosis.[16-19]

Over the years several biomarkers like C-reactive protein (CRP), procalcitonin (PCT), and tumor necrosis factor alpha (TNF-α), Interleukin-6 (IL-6) have shown promising results in diagnosing and predicting the course of neonatal sepsis. Several studies have delineated CRP as the single best marker for neonatal sepsis between 24 to 48 hours.[18] However, the positive predictive value of an elevated CRP in proven culture positive early-onset infection is low and makes for difficult prognostication.[12]Nevertheless, the clinical use of CRP as a biomarker for sepsis in neonates is well validated and widely practiced.[19] CRP levels can be determined

by a range of available testing modalities in varying formats encompassing laser nephelometry, turbidimetric immunoassay, enzyme immunoassay and latex agglutination.[20]Owing to its rapid turn around time, high sensitivity and specificity, the quantitative methods have been widely deployed for estimating CRP levels in critical settings.

The present study attempted to compare the efficiency of two widely used rapid CRP test formats viz. latex agglutination and immune-turbidimetry, in clinically suspected neonatal sepsis patients in the age groups: below 72 hours and above 72 hours.The majority of the study population belonged were male (42.5%) which grossly corresponds to the "male disadvantage hypothesis". In addition to higher premature birth rates, several studies have accounted an increased sensitivity of male neonates to adverse perinatal events and increased requirement of ventilator support, to collectively attribute to an increased risk of neonatal sepsis.[21-23]

As depicted by other studies, an overall high percentage agreement was observed between latex agglutination and immune-turbidimetry in determining CRP in the entire study population (Cohen's $\kappa = 0.875$).[24, 25]However, the two testing modalities showed a higher concordance with a percentage agreement of 97.5% (Cohen's $\kappa = 0.95$) in determining CRP values in neonates above 72 hours. Corollary to the above, a lower agreement of 90% (Cohen's $\kappa = 0.8$) was observed between the two modalities in neonates below 72 hours with four study samples yielding CRP levels ≥ 6 mg/L by immune-turbidimetry and < 6 mg/L by latex agglutination test. Thus, discordance was observed in serum CRP valuesof suspected early neonatal sepsis, byimmunoturbidometryassay. Similar to the findings from other studies comparing the two testingformats for CRP in human sera, the immunoturbidometry assay yielded a higher positivity despite a high positive correlation between the two testing modalities.[24, 26]

Upon extensive search, we could not find any research comparing the efficacy of latex and immune-turbidimetry in determining CRP levels in neonates ≤ 72 hours and above 72 hours. The time sensitive dynamics of CRP synthesis, especially in the early neonatal phase, may possibly give erroneous false negative results using latex agglutination test, as observed in our study as well as reinforced by others.[25, 27]However, unlike the present study, the above research subjects did not include neonates. Considering the opportunity for automation, reduced observer bias as well as low turnaround time, immune-turbidimetry presents an attractive alternative for accurate determination of serum CRP levels in critically ill newborns, especially below 72 hours.

Nevertheless, abundant caution has to be observed while interpreting and inferring prognosis from CRP levels in the first few days of life. The stress of delivery alone, often leads to a nonspecific elevation of neonatal CRP levels. [1,27] In fact studies have depicted significantly higher CRP levels after 24 hours of life compared to immediately after birth. [10] Moreover, a shorter and lower rise of CRP in preterm, compared to term neonates, necessitates an overhaul of the existing static cut-off values, with due consideration of the kinetics of CRP after birth.[28]

References

1. Jaye, David L., and Ken B. Waites. "Clinical applications of C-reactive protein in pediatrics." The Pediatric infectious disease journal, vol. 16, no. 8, 1997, p 735-747.
2. Tillett, William S., and Thomas Francis Jr. "Serological reactions in pneumonia with a non-protein somatic fraction of pneumococcus." The

Journal of experimental medicine, vol. 52, no. 4, 1930, p 561.

3. Pepys, Mark B. "C-reactive protein fifty years on." The Lancet, vol. 317, no. 8221, 1981, p 653-657.
4. Du Clos, Terry W. "Function of C-reactive protein." Annals of medicine, vol. 32, no. 4, 2000, p 274-278.
5. Volanakis, John E. "Human C-reactive protein: expression, structure, and function." Molecular immunology, vol. 38, no. 2-3, 2001, p 189-197.
6. Weinhold, Birgit, and Ulrich Rüther. "Interleukin-6-dependent and-independent regulation of the human C-reactive protein gene." Biochemical Journal, vol. 327, no. 2, 1997, p 425-429.
7. Vigushin, David M., Mark B. Pepys, and Philip N. Hawkins. "Metabolic and scintigraphic studies of radioiodinated human C-reactive protein in health and disease." The Journal of clinical investigation, vol. 91, no. 4, 1993, p 1351-1357.
8. Hofer, Nora, Eva Zacharias, Wilhelm Müller, and Bernhard Resch. "An update on the use of C-reactive protein in early-onset neonatal sepsis: current insights and new tasks." Neonatology, vol. 102, no. 1, 2012, p 25-36.
9. Pepys, Mark B., and Gideon M. Hirschfield. "C-reactive protein: a critical update." The Journal of clinical investigation, vol. 111, no. 12, 2003, p 1805-1812.
10. Kääpä, Pekka, and Eeva Koistinen. "Maternal and neonatal C-reactive protein after interventions during delivery." Acta obstetricia et gynecologica Scandinavica, vol. 72, no. 7, 1993, p 543-546.
11. Hengst, Joan M. "The role of C-reactive protein in the evaluation and management of infants with suspected sepsis." Advances in neonatal care, vol. 3, no. 1, 2003, p 3-13.
12. Benitz, William E., Michael Y. Han, Ashima Madan, and Pramela Ramachandra. "Serial serum C-reactive protein levels in the diagnosis of neonatal infection." Pediatrics, vol. 102, no. 4, 1998, p 41.
13. Fleischmann-Struzek, Carolin, David M. Goldfarb, Peter Schlattmann, Luregn J. Schlapbach, Konrad Reinhart, and Niranjan Kissoon. "The global burden of paediatric and neonatal sepsis: a systematic review." The Lancet Respiratory Medicine, vol. 6, no. 3, 2018, p 223-230.
14. Gordon, Scott M., Lakshmi Srinivasan, and Mary Catherine Harris. "Neonatal meningitis: overcoming challenges in diagnosis, prognosis, and treatment with omics." Frontiers in pediatrics, vol.5, 2017, p 139.
15. Fleischmann, Carolin, Felix Reichert, Alessandro Cassini, Rosa Horner, Thomas Harder, Robby Markwart, Marc Tröndle et al. "Global incidence and mortality of neonatal sepsis: a systematic review and meta-analysis." Archives of Disease in Childhood, vol. 106, no. 8, 2021, p 745-752.
16. Ranjan, R., S. Jerupula, and D. K. Bhagwani. "Procalcitonin and CRP markers in neonatal sepsis." International Journal of Scientific Research, vol. 8, no. 2, 2019.
17. English, Mike, Mwanajuma Ngama, Laura Mwalekwa, and Norbert Peshu. "Signs of illness in Kenyan infants aged less than 60 days." Bulletin of the World Health Organization, vol. 82, no. 5, 2004, p 323-329.
18. Klingenberg, C., R. Olomi, M. Oneko, N. Sam, and N. Langeland. "Neonatal morbidity and mortality in a Tanzanian tertiary care referral hospital." Annals of tropical paediatrics, vol.23, no. 4, 2003, p 293-299.

19. Rashwan, Nagwan I., Mohammed H. Hassan, Zeinab M. Mohey El-Deen, and Ahmed El-Abd Ahmed. "Validity of biomarkers in screening for neonatal sepsis–a single center–hospital based study." Pediatrics & Neonatology, vol. 60, no. 2, 2019, p 149-155.
20. Tosson, Angie, Dina Koptan, Rabab Abdel Aal, and Marwa Abd Elhady. "Evaluation of serum and salivary C-reactive protein for diagnosis of late-onset neonatal sepsis: a single center cross-sectional study." Jornal de Pediatria, vol. 97, 2021, p 623-628.
21. Deodhare, S. "C-reactive protein: Clinical applications." Cleve. Clin. J. Med, vol. 56, 2001, p 126-130.
22. Cortese, Francesca, Pietro Scicchitano, Michele Gesualdo, Antonella Filaninno, Elsa De Giorgi, Federico Schettini, Nicola Laforgia, and Marco Matteo Ciccone. "Early and late infections in newborns: where do we stand? A review." Pediatrics & Neonatology, vol. 57, no. 4 2016, p 265-273.
23. Satar, Mehmet, and Ferda Özlü. "Neonatal sepsis: a continuing disease burden." The Turkish journal of pediatrics, vol. 54, no. 5 (2012): 449.
24. Roy, Priyamvada, Ashwani Kumar, Iqbal Rajinder Kaur, and Mohammad MA Faridi. "Gender differences in outcomes of low birth weight and preterm neonates: the male disadvantage. "Journal of tropical pediatrics, vol. 60, no. 6, 2014, p 480-481.
25. Dhamecha, Manisha N., Mayurika K. Patel, and Urvesh V. Shah. "A Comparative Study of Semi Quantitative Latex Agglutination Test and Quantitative Turbidimetric Immunoassay Method for the Detection of C-Reactive Protein from Human Sera". GCSMC J Med Sci, 2013.
26. Gella, F-J., and M-J. Capdevila. "Determination of β2-microglobulin in human serum and urine by latex turbidimetry." Pure and applied chemistry, vol. 68, no. 10, 1996, p 1881-1885.
27. Pulkki, Kari, and Kerttu Irjala. "Evaluation of a semiquantitative C-reactive protein latex slide test as compared to quantitative turbidimetric measurement in hospital laboratory practice." Scandinavian Journal of Clinical and Laboratory Investigation, vol. 46, no. 6, 1986, p 605-607.
28. Chiesa, Claudio, Fabrizio Signore, Marcello Assumma, Elsa Buffone, Paola Tramontozzi, John F. Osborn, and Lucia Pacifico. "Serial measurements of C-reactive protein and interleukin-6 in the immediate postnatal period: reference intervals and analysis of maternal and perinatal confounders." Clinical chemistry, vol. 47, no. 6, 2001, p 1016-1022.