RESEARCH PAPER | Microbiology | Volume : 3 | Issue : 7 | July 2013 | ISSN - 2249-555X

An evaluation of newly developed C-reactive protein slide Latex agglutination test against Immunoturbidimetric test for diagnosis of neonatal septicaemia

KEYWORDS: Micro particles, Immunoassay, Buffer type and pH

Pranav R. Naik
Shree Ramakrishna Institute of Computer Education and Applied Science, Surat -395 001, Gujarat, India.

Dr. Pratibha B. Desai
Shree Ramakrishna Institute of Computer Education and Applied Science, Surat -395 001, Gujarat, India.

ABSTRACT *CRP, the classic acute-phase protein of human serum is synthesized by Hepatocyte. Normally it is present only in trace amount in serum but it can increase by as much as 1000 fold in response to injury or infection. It falls quickly after efficient elimination of microbial stimulus, due to its short half life of 19 hours. Serial CRP measurement is a good practical guide for discontinuing antibiotic therapy in neonates with suspected sepsis. In present study we have compared newly developed quantitative slide agglutination test with quantitative test for measurement of CRP in sample from suspected cases of Neonatal sepsis. We have observed very high correlation between two methods in normal as well as abnormal samples.*

Introduction
Serum C - reactive protein has been advocated as a reliable indicator of disease activity in variety of Inflammatory conditions (Pepeys and Balt, 1983). Infection is one of the major problem in neonates, it is estimated that sepsis accounts for 26% of all neonatal deaths globally (Lawn, Cousens & Zupan, 2005) and developing countries contributes for 98% of this deaths (Omene,1979).With the scientific advancement effective antibiotics are available for treatment but early diagnosis represents a major challenge due to non-specific nature of signs and symptoms (Mathai *et al*., 2004; Enguix *et al*., 2001; Sluncheva *et al*., 2006) and non-availability of standard microbial culture results in the first 48 hours(Døllner, Vatten & Austgulen., 2001). As a result as many as 30 uninfected neonates get treated unnecessarily for everyone who is eventually diagnosed to be infected (Hammerschlag MR, Klein JO, Herschel M, Chen FC and Fermin R 1977; Philip & Hewitt, 1980; Gerdes & Polin, 1987). Blood culture is the gold standard for definitive diagnosis but it takes at least 48 hours by which time the infection may have progressed with important consequences on the morbidity and mortality of the neonate (Buttery, 2002). There is an abundance of studies evaluating laboratory markers in the diagnosis of neonatal sepsis. Despite the promising results for some diagnostic markers, none of them can consistently diagnose 100% of infected cases. C-Reactive Protein (CRP) is the most extensively studied acute-phase reactant so far, its wide availability and its simple, fast & cost-effective determination make it one of the preferred test in many neonatal intensive care units (NICUs), (Khashabi J, Karamiyar M, Taghinejihad H and Shirazi M, 2007). Within 4 to 6 hours of an inflammatory process CRP increases as much as thousand fold (Weitkamp & Aschner, 2005) which make it useful in the diagnosis of neonatal sepsis. It falls quickly after efficient elimination of microbial stimulus, due to its short half-life of 19 hours (Weitkamp & Aschner, 2005; Clyne & Olshaker, 1999) which help in determining the duration of antibiotic therapy to prevent unnecessary use of Antibiotics. Unlike blood culture, CRP level is not affected by prior antibiotic therapy (Khashabi, Karamiyar, Taghinejihad & Shirazi, 2004) and hence useful in the situation where neonates may have been given antibiotics before presentation at the hospital. Variety of quantitatively or qualitatively test for CRP estimations are available. The quantitative method is widely used in developed countries (Clyne & Olshaker, 1999) because it provides rapid, highly sensitive and specific results (Deodhare, 2001). However quantitative estimation requires costly analyzer and is more complex and expensive to perform (Clyne & Olshaker, 1999) and hence not suitable for point of care testing. The qualitative method provides results within 15 minutes but it is comparatively less specific. It has the advantage of being simple and easier to perform and interpret and being non instrumental visual test it can be performed at the patient bed side or side laboratory (Deodhare, 2001; Mustafa, Farooqui, Waheed & Mahmook, 2005). It is also less expensive and requires less skill. The qualitative method therefore is most suitable for resource poor centers, where there may be no laboratory services for the investigation of neonates with suspected septicaemia. In our study we have compared quantitative slide agglutination test with a quantitative method for measurement of CRP in suspected Neonatal sepsis samples.

Additional key phases: *C reactive protein, Neonatal sepsis, Slide latex agglutination test*

Materials & Methods:
Plain surface polystyrene latex particles with Diameter of 300 nm were used. Particles were obtained from Magsphere, USA. Rabbit anti human CRP (IgG) was used for reagent development; antibody was obtained from Midland Bioproduct, U.S.A. The latex particle-antibody conjugate was produced by the method described by (Litchfield *et al*., 1984).

1mg/ml of Goat Anti human CRP antibody and 1% W/V polystyrene latex particle of 300 nm size were mixed in 0.1 M pH 7.4 Phosphate buffer saline for 2 hour at 37^{0}C, after incubation supernatant was removed by centrifugation at 3000 g for 15 minute, re-suspended in same buffer and 0.5% BSA as a blocking agent. We used 1% W/V latex suspension for the assay.

One drop of sample was placed on a microscopic slide and mixed with an equal volume of latex reagent. The mixture spread to a circular area of about 1.5 cm diameter. The slide was then rocked gently and the appearance of agglutination observed with the unaided eye against a dark background. The level of agglutination was scored from 1+ to 4+ depending up on the clumping of latex particle. Equivocal agglutination was denoted by ± (Trace)

Quantitative CRP test is working on the principle of immunoturbidimety in which Latex particles coated with anti-human C reactive protein (CRP) antibodies agglutinate when mixed with sample containing CRP, resulting into insoluble antigen-antibody complexes. These insoluble complexes increase the turbidity, which is measured at 550 nm. Increase in turbidity is directly proportional to concentration of CRP in the sample.

The present study was conducted during December 2012 to February 2013. Total 107 samples from suspected Neonatal

Volume : 3 | Issue : 7 | July 2013 | ISSN - 2249-555X

septicaemia were analysed by both method.

Results:

Table 1: CRP results between qualitative latex slide test vs. quantitative Immunoturbidimetric test

Qualitative test results	Quantitative Immunoturbidimetric test results (mg/dl)				
	<10	10 to 25	25 to 50	50 to 100	100 to 200
Negative	60				
Trace	4				
+1		12			
+2		3	7		
+3			4	13	
+4					4
Total	64	15	11	13	4

As shown in above mentioned Table no. 1 total 64 samples were within normal limit by Quantitative method, out of it 4 have shown "Trace" with Qualitative test. 12 samples were border line positive i.e. between 10-25 mg\l by quantitative method and 1+ by qualitative method. Similarly 10 samples were moderately positive, 2+ by qualitative method, out of that 7 are between 25-50 mg/l by quantitative method and 3 were in the border line positive with quantitative method. 17 samples have shown 3+ in qualitative test, majority of them were between 50 -100 mg/dl in quantitative test, rest 4 samples were between 25-50 mg/dl. 4 samples which were above 100 mg/l by quantitative test were 4+ with qualitative test.

Four sample which were negative in Quantitative test have shown Trace result with Slide agglutination test but being a equivocal results it must get further confirmation before reporting. Few samples were underestimated by slide agglutination test but given clear cut positive. There was no apparent reason for this discrepancy, other than subjective interpretation. Based on above result we can consider that slide agglutination test have very high correlation with Quantitative immunoturbidimetric test results.

Discussion:

Estimation of CRP has been found to be useful in the early diagnosis of neonatal sepsis (Al-Zwaini., 2009). Quantitative CRP assays have become sensitive, precise and accurate and available commercially (Sven & Henning, 1989). Unfortunately quantitative CRP assay are required to be used with automated instruments and hence not suitable for Point of care testing or for remote area in developing countries which result in to high therapeutic turnaround time (Hilary& Gillian, 1991). Though it subjective in interpretation specifically when it is required to score the results, Slide Latex agglutination method is simple to perform and easy to interpret, being a non instrumental test it is ideal for remote area.

In our study we have observed that results of qualitative Slide agglutination test are in concordance with quantitative immunoturbidimetric test. Our study findings are not in line with study carried out by Kari Pulkki and Kerttu Irjala in which they have measured CRP results by Latex agglutination slide test and turbidimetric test in 708 patients with suspected bacterial disease. They have observed that Latex agglutination slide test shown 80 discordant results out of 708. They concluded that CRP latex slide test is not useful in emergency laboratory in hospital material with a high incidence of bacterial infections (Kari Pulkki & Kerttu Irjala, 1986).

Unlike above finding our results are in concordance with findings of study carried out by Hindocha P, Campbell CA, Gould JDM, Wojciechowski A and Wood CBS, they have concluded that Latex slide may be helpful diagnostic tool to detect infection at the bedside and it shows a good correlation with quantitative estimation (Hindocha P, Campbell CA, Gould JDM, Wojciechowski A, Wood CBS (1984). Kwang shin kim (1993) compared CRP results by immunoturbidimetric method & latex agglutination tests in the sera from 20 healthy person & 263 patients. He observed that 37 cases showed low CRP values in latex agglutination test as compared with those in immunoturbidimetric test & 13 cases had relatively high CRP values in latex agglutination test as compared with those in immunoturbidimetric test. Overall they have concluded that two test should be considered to be concordant (Kwang Shin Kim, Dong Euk Byun & Dong Wook Ryang, 1993).

In our study difference observed between two methods is mainly because of the subjective scoring of Qualitative Slide agglutination test results given for comparison with Quantitative method. In fact Qualitative test are made for the Yes/ No type of results, considering this fact value higher or lower than quantitative method does not have much significance because semi quantitative value of CRP using latex reagent provide a practical alternative to quantitative method, when level of CRP are needed urgently. Simplicity in performance and visual result interpretation is the biggest advantage of Qualitative slide test. This test may be less precise as compare to quantitative assay but it is still acceptable for decentralised use where quantitative testing facility and skilled manpower is not available.

REFERENCE

• Al-Zwaini E.J. (2009). C-reactive protein: a useful marker for guiding duration of antibiotic therapy in suspected neonatal septicaemia? Eastern Mediterranean Health Journal, 15(2), 269-75. | • Buttery JP, (2002).Blood cultures in newborns and children: optimising an everyday test. Arch Dis Child Fetal Neonatal Ed, 87, 25–28. | • Clyne B, Olshaker JS, (1999).The C-Reactive Protein.J Emerg Med,17, 1019–1025. | • Deodhare SG.(2001).C-Reactive Protein: Clinical Applications, Pathology. Update: Microbiology and Clinical Pathology Series. | • Døllner H, Vatten L, Austgulen R. (2001). Early dignostic markers for neonatal sepsis: comparing C-reactive protein, interleukin-6, soluble tumor necrosis factor receptors and soluble adhesion molecules, J Clin Epidemiol, 54, 1251–57. | • Enguix A, Rey C, Concha A, Medina A, Coto D & Diéguez MA (2001). Comparison of procalcitonin with C-reactive protein and serum amyloid for the early diagnosis of bacterial sepsis in critically ill neonates and children. Intensive Care Med, 27(1), 211–15. | • Hammerschlag MR, Klein JO, Herschel M, Chen FC, Fermin R, (1977). Patterns of use of antibiotics in two newborn nurseries. N Engl J Med, 296, 1268–69. | • Hilary Vallance and Gillian Lockitch, (1991). Rapid, Semi-Quantitative Assay of C - reactive protein Evaluated. Clin. chem., 37, 1981-82. | • Hindocha P, Campbell CA, Gould JDM, Wojciechowski A, Wood CBS (1984). Sequential study of C reactive protein in neonatal septicaemia using a latex agglutination test. J Clin Pathol, 37, 1014-17. | • Kari Pulkki and Kerttu Irjala, (1986). Evaluation of a semi-quantitative C-reactive protein latex slide test as compared to quantitative turbidimetric measurement in hospital laboratory practice. Journal of Clinical & Laboratory Investigation.46, 605-07. | • Khashabi J, Karamiyar M, Taghinejihad H, Shirazi M. (2004). Use of Serial C - reactive protein Measurements for Determination of the Length of Empiric Antibiotic Therapy in Suspected Neonatal Sepsis, Iran J Med Sci, 29, 31–35. | • Khassawneh M, M., Hayajneh W. A., Kofahi H., Khader Y., Amarin Z. & Daoud A.,(2007). Diagnostic Markers for Neonatal Sepsis: Comparing C-reactive Protein, Interleukin-6and Immunoglobulin, Scandinavian Journal of Immunology ,65(2), 171–75 | • Kwang Shin Kim, Dong Euk Byun and Dong Wook Ryang, (1993). Measurement of C-reactive protein by a qualitative latex agglutination and quantitative turbidimetric immunoassay methods. Korean J Clin Pathol,13:653-59. | • Lawn JE, Cousens S, Zupan J, (2005) 4 million neonatal deaths: When? Where? Why? Lancet, 365,891–900. | • Litchfield WJ,Craig AR,Frey WA, Leflar CC, Looney CE, Luddy MA,(1984). Novel Shell/Core particles for Automated Turbidimetric immunoassays.Clin.Chem., 30, 1489-93. | • Mathai E, Christopher U, Mathai M, Kumar Jana A, Rose D, Bergstro¨m S., (2004). Is C - reactive protein level useful in differentiating infected from uninfected neonates among those at risk of infection, Indian Pediatr, 41, 895–900. | • Mustafa S, Farooqui S, Waheed S, Mahmook K,(2005). Evaluation of C - reactive protein as early indicator of blood culture positivity in neonates. Pak J Med Sci, 21, 69–73. | • Omene JA, (1979).Neonatal Septicaemia in Benin city, Nigeria: A Review of 74 cases. Trop Geogr Med, 31, 35–39. | • Pepys, M.B., and Baltz, M.L. 1983. Acute phase proteins with special reference to C-reactive protein and related proteins (pentaxins) and serum amyloid A protein. Adv. Immunol. 34,141–212. | • Philip AG, Hewitt JR, (1980). Early diagnosis of neonatal sepsis. Pediatrics, 65:1036-1041Gerdes JS, Polin RA., (1987). Sepsis screen in neonates with evaluation of plasma fibronectin, Pediatr Infect Dis J, 6,443–446. | • Slncheva B, Vakrilova L, Emilova Z, Iarkova N, Shishkova R & Popivanova A (2006) Inborn bacterial infections during neonatal period. Akush Ginekol (Sofiia), 45, 42–8. | • Sven Grutzmeier and Henning Von Schenck, (1989). Four Immunochemical Methods for Measuring C reactive protein in Plasma Compared. Clin. chem. 35, 461-63. | • Weitkamp J, Aschner J, (2005). Diagnostic Use of C - reactive protein in Assessment of Neonatal Sepsis, Am Acad Pediatre 6,508–515.