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Original Research Paper

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THE ROLE OF SERUM INTERLEUKIN-6 AS AN EARLY MARKER IN ESTABLISHING THE DIAGNOSIS OF NEONATAL SEPSIS IN COMPARISON WITH CONVENTIONAL BLOOD CULTURE METHOD

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ABSTRACT

Background: Neonatal sepsis (NS) is a common and life-threatening disorder, especially in preterm Infants. Up to 10% of infants have an infection in the first month of life [2]. The rate of mortality and morbidity due to NS is very high. The prognosis and outcome of NS depend on early diagnosis and on-time and efficient antibiotic therapy. Diagnosis of neonatal infection may be the greatest and most difficult challenge for a neonatologist.A diagnostic marker with high diagnostic sensitivity and specificity would be a valuable tool for decreasing the burden of neonatal sepsis. **Objectives:** The objective of this study is to investigate the role of Serum Interleukin-6 as an early marker in establishing the diagnosis of neonatal sepsis and to compare the efficacy of Interleukin-6 with conventional blood culture method. **Methods:** This cross sectional study was conducted in Neonates who were admitted in Neonatal Intensive Care Unit with signs suggestive of sepsis, or those who developed signs of sepsis while they were in the ward for the period of three months from January 2021 to March 2021 at Madurai Medical College hospital in TamilNadu. Blood samples collected from 90 newborn babies were tested for blood culture and Interleukin-6 determination by ELISA.The cut-off values recommended by the respective manufacturers were used to determine the sensitivity and specificity. The correlation of Interleukin-6 level with blood culture for neonatal sepsis was compared statistically and results were analyzed by IBM SPSS Statistics 20. **Results:** Out of the blood samples collected from 90 participants, positive blood culture was found in 28 neonates(31%). Sensitivity of detection of IL-6 by ELISA method was 100% when evaluated against culture , a reference test. Specificity of this test was 41.94% compared to culture and positive and negative predictive value were 40.63% and 100% respectively. **Conclusions:** This study validated the diagnostic capability of Interleukin-6 for the early diagnosis of Neonatal sepsis and may provide a new diagnostic strategy for Neonatal sepsis babies. Neonatal sepsis can be prevented by proper hand washing, proper antenatal care, proper aseptic clean labour ward and proper aseptic clean NICU.

KEYWORDS : Neonatal sepsis,Interleukin-6,Blood culture,ELISA.Sensitivity, Specificity, Positive predictive value, Negative predictive value

INTRODUCTION

Neonatal sepsis is along with high neonatal morbidity (1-10 per 1000 live birth) and mortality (15-50%), especially in preterm babies.[1,2] Neonatal sepsis is the third leading cause of neonatal mortality and a major public health problem, especially in developing countries. Although recent medical advances have improved neonatal care, many challenges remain in the diagnosis and management of neonatal infections. The diagnosis of neonatal sepsis is complicated by the frequent presence of non-infectious conditions that resemble sepsis, especially in preterm infants, and by the absence of optimal diagnostic tests.[3]

Since neonatal sepsis is a high-risk disease, especially in preterm infants, clinicians are compelled to empirically administer antibiotics to infants with risk factors and/or signs of suspected sepsis. Unfortunately, both broad-spectrum antibiotics and prolonged treatment with empirical antibiotics are associated with adverse outcomes and increase antimicrobial resistance rates.

[3] In addition, it increases the duration of hospitalization and costs and creates and expands the cases resistance to different types of bacteria.[4]The amount of successful treatment depends on early initiation of appropriate antibiotic therapy in all infants with clinical signs of sepsis.

Early detection of neonatal sepsis is an essential prerequisite for improving survival and treatment outcomes.[5] Conventional haematological and microbiological methods which are routinely used for diagnosis of neonatal sepsis cannot reduce deaths and serious complications of neonatal sepsis.

Isolation and culture of microorganisms from body fluids including blood, cerebrospinal fluid and urine are methods of gold standard for diagnosis of neonatal infection. But, microbiological culture is not available before at least 36-48 hrs.[6] So accurate laboratory tests are required to rule out infection and reduce unnecessary antibiotic treatment.[7]

Pro-inflammatory cytokines, acute phase proteins, adhesion molecules, cell surface markers, and chemokines are being used to identify neonatal sepsis in recent years.[8] Interleukin-6 (IL-6) and C-reactive protein (CRP) are the two most commonly used markers for early detection of neonatal sepsis.[9,10]

IL-6 level increases in early disease stages of bacterial infections and this may be useful for the early identification of neonatal sepsis [11].IL6 is created by monocytes, endothelial cells, fibroblasts and lymphocytes T and B and is much more sensitive than CRPThe results of the studies showed that the average of serum level of IL6 in infants with sepsis is higher than healthy infants.[12]

Despite that blood culture is a gold standard for diagnosis of neonatal sepsis, but, it is not actually helpful in early diagnosis of neonatal sepsis. Interleukin assessment is useful to reduce the time of diagnosis and increase the accuracy of diagnostic tests in the early stage of infection. So, Interleukin-6 can be applied as an important marker for early

neonatal sepsis in neonates care sections.[13] Hence the present study was undertaken to detect the role of IL-6 in early detection of infection and effective management of neonatal sepsis in comparison with Blood culture at a tertiary care hospital.

Objectives:

- To investigate the role of Serum Interleukin-6 as an early marker in establishing the diagnosis of neonatal sepsis.
- To compare the efficacy of Interleukin-6 with conventional blood culture method for the diagnosis of neonatal sepsis.

MATERIALS AND METHODS

This cross sectional study was conducted in Neonates who were admitted in Neonatal Intensive Care Unit with signs suggestive of sepsis, or those who developed signs of sepsis while they were in the ward for the period of three months from January 2021 to March 2021. In Institute of Microbiology, Madurai Medical College, Madurai, Tamilnadu. The permission for conducting this study was obtained from the Institutional Ethical Committee. Informed consent was obtained from reliable informants of neonates who participated in the study. Only the information needed for this study was extracted and coded as required for answering the research question. A total of 90 clinically suspected sepsis cases in neonates (0 day to 28 days)

Inclusion criteria

- Neonates who were admitted with signs suggestive of sepsis such as poor feeding, poor activity, respiratory distress, apnoea, seizure, lethargy, bulging anterior fontanel, fever, hypothermia, jaundice, vomiting, loose stools, abdominal distension, or those who developed signs of sepsis like cyanosis, bleeding, mottling, tachycardia, weak pulse, grunting, retractions, nasal flaring etc. while they were in the ward.

Exclusion criteria:

- Neonates who were on antibiotics,
- Neonates who had birth asphyxia and aspiration syndromes,
- Neonates who had congenital anomalies and inborn errors of metabolism.

Sample collection:

Blood samples were taken from 90 clinically suspected neonatal sepsis cases and were tested for Blood culture and detection of serum level of IL-6 by ELISA.

Blood collection method:

Ideal blood sample collection should be done before initiation of anti-microbial agents. Amount of blood needed for cultures for neonates is significantly lower than that needed for adults because neonates tend to have a higher concentration of bacteria in their bloodstream than adults.[14]Hence 2ml of blood was usually considered as the standard volume of blood adequate to detect bacteraemia in neonates. Proper aseptic precautions were undertaken during blood specimen collection to avoid sample contamination.

Blood culture:

From sepsis-suspected neonate, 2 ml of blood was collected, inoculated into the top of the blood culture bottle that contains 20ml brain heart infusion broth. (HiMedia, India) Bottles were incubated up to 7 days at 37°C.Subculture was routinely performed using solid agar plates such as Blood agar plate, MacConkey agar plate and Nutrient agar plate after 24 hours and 72 hours with last subculture being done after seven days. The isolates were routinely identified by standard bacteriological techniques. Morphology of colonies on Nutrient agar plate, Mac Conkey agar plate and blood agar plate were studied. Using Gram stain, gram positive and gram negative organisms were differentiated.(Figure-1) Biochemical tests such as Indole test, Methyl red test, Voges-Proskaeur test, Triple Sugar Iron test,Urease test, citrate utilization test, various sugar fermentation test and Some specific tests like catalase test, coagulase test were done. All blood samples were subjected to the above said various tests and causative organisms for neonatal sepsis were identified.

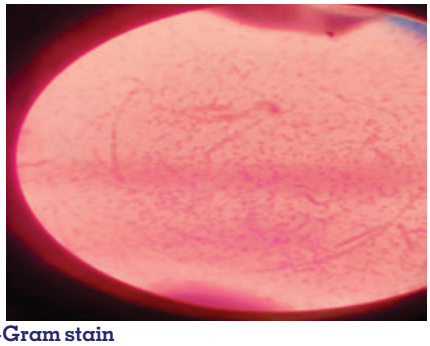

Figure-1-Gram stain

Antibiotic susceptibility was tested manually with antimicrobials like Gentamicin, Amikacin, Cefotaxime, Ceftazidime, Ceftriaxone, Ciprofloxacin ,Co-trimoxazole, Imipenem,Piperacillin and Tazobactam from Hi-media laboratories Ltd, Mumbai in all isolates by Kirby Bauer disc diffusion method (Figure-2) according to the CLSI guidelines.[15]

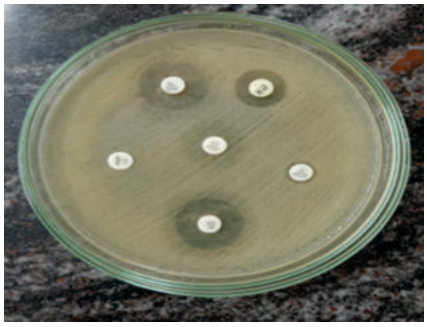

Figure-2-Kirby Bauer disc diffusion method

Serum IL-6 level detection by ELISA:

Blood samples were transported from neonatology department to laboratory immediately after collection. Blood samples were centrifuged to separate the serum and processed for the test as soon as they arrived at the laboratory. All the 90 samples were tested for IL-6 detection by ELISA with the help of Diaclone SAS, France ELISA test kit following the manufacturer's instructions.[16]

Interpretation of results:

As per manufacturer's instructions, Highly Positive–100-200pg/ml,Moderately Positive - 50-100pg/ml,Weakly Positive - < 50- 6.25pg/ml,Negative- < 6.25pg/ml. Sonawane et al.,in a prospective study evaluated the efficiency of IL6 as a primary diagnostic marker of sepsis.[17]

Statistical Analysis:

All the results obtained were analyzed statistically for their completeness, consistency and accuracy by the parameters like mean and percentages. The correlation of Interleukin-6 level with blood culture for neonatal sepsis was compared statistically and results were analyzed by IBM SPSS Statistics 20. Chi-square test was used in calculating the P-value. The P-Values of less than 0.05 were considered as statistically significant ($P<0.05$).

RESULTS:

A total of 90 neonates (0 to 28 days) who fulfilled the criteria of

clinically suspected sepsis were analyzed. The selected 90 study subjects were analyzed based on age and sex, The results of the analysis are tabulated in Table 1.

Table:1 Age and sex wise distribution

Age in Days	Male		Female		Total	
	No	%	No	%	No	%
EONS (0-3days)	9	29.03%	16	27.12%	25	27.78%
LONS (4-28days)	22	70.97%	43	72.88%	65	72.22%
Total	31	100%	59	100%	90	100%

Blood culture results in study group:

Out of the blood samples collected from 90 participants, positive blood culture was found in 28 neonates(31%). The organisms isolated were Escherichia coli in 21 neonates (75%) and Acinetobacter in 7 neonates(25%). Escherichia coli was found to be a most common organism in both early and late onset sepsis.(Table-2)Among 28 blood culture positive cases, 10 (35.7%) cases were male neonates and it contributes 35.7% of total blood culture positive cases. Another 18 cases were female neonates and it contributes 64.3% of total blood culture positive cases.

Out of 28 blood culture positive cases, 7 cases were in the age group of 0-3 days and it contributes 25% of total culture positive cases. Remaining 21 cases were in the age group of 4-28 days and it contributes 75% of total blood culture positive cases.

Table-2:Distribution of Organisms isolated in Blood culture

Organisms isolated	EONS(0-3days)		LONS(4-28days)	
	M	F	M	F
Escherichia coli	2	5	4	10
Acinetobacter	0	0	4	3
Total	2	5	8	13

Antimicrobial sensitivity pattern of the isolated organism was depicted in Table-3

Table-3:Sensitivity pattern of bacterial isolates

Antibiotics	E.coli		Acinetobacter	
	N=21	%	N=7	%
Amikacin	12	57	5	71
Gentamicin	11	52	4	57
Ciprofloxacin	11	52	4	57
Ceftazidime	9	43	2	29
Cefotaxime	9	43	2	29
Ceftriaxone	9	43	2	29
Co trimoxazole	13	62	0	0
Imipenem	21	100	7	100
Piperacillin and Tazobactam	21	100	7	100

Serum Interleukin-6 results in study group:

Out of 90 clinically suspected neonatal cases, 64 neonates were Interleukin-6 positive. Out of 64 positive cases, 18 (28.13%) neonates were EONS and 46 (71.87%) cases were of LONS. Sensitivity of detection of IL-6 by ELISA method was 100% when evaluated against culture, a reference test. Specificity of this test was 41.94% compared to culture and positive and negative predictive value were 40.63% and 100% respectively.(Table:4)

Table: 4 Evaluation of Interleukin-6 ELISA with blood culture

IL-6 ELISA	Blood Culture	Total	
	Positive	Negative	
Positive	28	36	64
Negative	0	26	26
Total	28	62	90

Sensitivity $= TP/TP+FN = 28/28 \times 100 = 100\%$
Specificity $= TN/TN+FP = 26/62 \times 100 = 41.94\%$
Positive predictive value $= TP/TP+FP = 28/64 \times 100 = 40.63\%$
Negative predictive value $= TN/TN+FN = 26/26 \times 100 = 100\%$

From the above Table-4, sensitivity of detection of IL-6 by ELISA method was 100% when evaluated against culture, a reference test. Specificity of this test was 41.94% compared to culture and positive and negative predictive value were 40.63% and 100% respectively. According to Chi-square test, it was found to be statistically significant($P<0.05$).

IL-6 Quantitative Assay:

Absorbance values of standards, controls and samples were calculated. A linear standard curve was generated by blotting the average absorbance of each standard on the vertical axis versus the corresponding IL-6 standard concentration on the horizontal axis. (Figure-3) The amount of IL-6 in each sample was determined by extrapolating OD values against IL-6 standard concentrations using the standard curve.

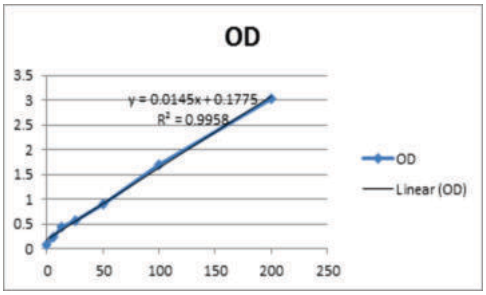

Figure-3 Linear Standard Curve

Out of 64, IL-6 positive cases, 57 cases were under highly positive category (IL-6 level 100-200pg/ml, OD value 1.7-3.038), 2 cases were under moderately positive category (IL-6 level 50-100pg/ml, OD value 0.912-1.6) and 5 cases were weakly positive category (IL-6 level 6.25 -50 pg/ml, OD value 0.236-0.911).Among 64 IL-6 positive cases, 28 cases were culture positive. All 28 neonatal cases have high titre of IL-6 level 100-200pg/ml.

DISCUSSION

Neonatal septicaemia is one of the commonest causes of neonatal mortality and morbidity in India. Accurate and timely diagnosis of neonatal sepsis still remains a major challenge to the paediatricians and neonatologists. Mortality due to neonatal sepsis is preventable and if diagnosed early the outcome is better. Interleukin-6 Levels appears to be one of the most promising candidate cytokine for early diagnosis of neonatal septicaemia. The objectives of this study was to study the role of IL-6 levels as an early marker for diagnosis of neonatal sepsis and to compare IL-6 levels with Blood culture.

The present study shows that out of 90 clinically suspected neonatal sepsis cases, Female neonates were found to be predominant than Male neonates. Male predominance was observed in studies conducted by YR Khinchi AK et al and Bambala Puthattayil Zakariya et al.[18,19]

In the present study, out of 90 cases, 27.78% of cases were under the age group of 0 – 3 days (<72 hours) and remaining 72.22 % of cases were under the age group of 4-28 days.

It was similar to the study conducted by Neema Kayange et al in Tanzania in which a high prevalence of LONS (60%) was reported among 300 clinically suspected neonatal sepsis cases.[20] but in contrast to Flora Chacha et al study in which out of 305 neonates, 224(73.4%) were ≤72 hours of age and in Sucilathangam et al study where Early onset sepsis was confirmed in 29 (58 %) and late onset sepsis in 21 (42 %).[21,22]

The current study shows that among 90 suspected sepsis cases, blood culture was positive in 28 cases (31%).Similar

positivity percentage of blood culture had been reported in the study by Shrestha R K et al in Nepal medical college, Kathmandu, during the period of July 2011 to January 2012 reported similar range results of 30.8% of positive blood culture.[23]

Out of 90 clinically suspected neonatal cases, 62 neonates were Interleukin-6 positive. Out of 64 positive cases, 18 (28.13%) neonates were EONS and 46 (71.87%) cases were of LONS.Out of 64, IL-6 positive cases, 57 cases were under highly positive category (IL-6 level 100-200pg/ml, OD value 1.7-3.038), 2 cases were under moderately positive category (IL-6 level 50-100pg/ml, OD value 0.912-1.6) and 5 cases were weakly positive category (IL-6 level 6.25 -50 pg/ml, OD value 0.236-0.911).

Among 64 IL-6 positive cases, 28 cases were culture positive. All 28 neonatal cases have high titre of IL-6 level 100-200pg/ml. This finding was similar to the study of Laura LR et al which revealed Serum of gram negative septicemic cases had increased titre of serum IL-6 level when compared with gram positive septicemic cases. A cytokine score using threshold for interleukin (IL)-6 had 100% sensitivity and 69% positive predictive value (PPV) for GNB.IL-6 < 130 pg/ml, had 100% sensitivity and 52% PPV for sepsis ruled out (SRO). Gram negative bacterial endotoxins are potent inducer for cytokines production by leucocytes comparing with gram positive bacteria.[24]

In the current study, the sensitivity of IL-6 for proven sepsis was 100% its specificity was 41.94%, its positive predictive valve was 40.63% and its negative predictive value was 100%.Shalini Tripathi et al investigated the role IL-6 in the diagnosis of neonatal sepsis and its correlation with the CRP. The sensitivity, specificity, positive predictive value and negative predictive value of IL-6 were 98.4%, 81.2%, 63.5% and 93.5% and those of CRP were 41%, 91%, 87% and 78% respectively.[25] The current study confirmed the findings of various authors that serum IL-6 level determination is mainly useful in early detection of neonatal sepsis and also detects the severity of infection and evaluation of the response to antibiotic treatment.

Limitations and future studies

Earlier diagnosis of neonatal sepsis by Interleukin-6 evaluation helps to prevent neonatal mortality and morbidity and avoid unnecessary initiation of empirical antibiotics which in turn helps in preventing drug resistance. It is helpful to conduct extensive studies to identify and standardize the values of Interleukin-6 in early diagnosis of neonatal sepsis.

CONCLUSION

Serum level of IL-6 is also useful in evaluation of response of neonatal sepsis to antibiotic therapy. The benefit of measuring serum IL-6 routinely as an early marker in the diagnosis and follow up of neonatal sepsis, is that it reduces neonatal mortality and morbidity and also reduces the hospital stay and cost of health care. Neonatal sepsis can be prevented by proper hand washing, proper antenatal care, proper aseptic clean labour ward and proper aseptic clean NICU.

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REFERENCES

1. Boskabadi H, Omidian M, Tavallai S, Mohammadi S,Parizadeh M, Ghayour Mobarhan M, et al. Serum Hsp70 antigen:early diagnosis marker in perinatal asphyxia. Iran J Pediatr. 2015;25:381
2. Blencowe H, Vos T, Lee AC, Philips R, Lozano R, Alvarado MR,et al. Estimates of neonatal morbidities and disabilities at regional and global levels for 2010: introduction, methods overview, and relevant findings from the Global Burden of Disease study. Pediatr Res 2013;74:4-16.
3. Zea-Vera A and Theresa J. Ochoa.Challenges in the diagnosis and management of neonatal sepsis.J Trop Pediatr. 2015 ; 61(1): 1–13.
4. Ng PC, Cheng SH, Chui KM, Fok TF, Wong MY, Wong W, et al.Diagnosis of late onset neonatal sepsis with cytokines, adhesion molecule, and C-reactive protein in preterm very low birth weight infants. Arch Dis Child Fetal Neonatal Ed 1997;77:221-7.
5. Shah BA, Padbury JF. Neonatal sepsis: an old problem with new insights. Virulence 2014;5:170-178.
6. Martin RJ, Fanaroff AA, Walsh MC. Neonatal-perinatal medicine: diseases of the fetus and infant. Elsevier/Mosby; 2011.
7. Døllner H, Vatten L, Austgulen R. Early diagnostic markers for neonatal sepsis: comparing C-reactive protein, interleukin-6,soluble tumour necrosis factor receptors and soluble adhesion molecules. J Clin Epidemiol 2001;54:1251-7.
8. Hendricks-Munoz, K.; Xu, J.; Mally, P. Biomarkers for neonatal sepsis: Recent developments.
9. Res. Rep. Neonatal. 2014;157.
10. Horns KM. Neoteric physiologic and immunologic methods for assessing early-onset neonatal sepsis. J Perinat neonatal Nurs 2000;13:50-66.
11. 10.Prashant A, Vishwanath P, Kulkarni P, Sathya Narayana P,Gowdara V, Nataraj SM, et al. Comparative assessment of cytokines and other inflammatory markers for the early diagnosis
12. of neonatal sepsiseA case control study. PLoS One 2013;8:e68426.
13. Silveira, R, Procianoy, R. Evaluation of interleukin-6, tumour necrosis factor- and interleukin-1_ for early diagnosis of neonatal sepsis. Acta Paediatr. 2007;88: 647–650.
14. Boskabadi H, Maamouri G, Tavakol Afshari J, Mafinejad S,Hosseini G, Mostafavi-Toroghi H, et al. Evaluation of serum interleukins-6, 8 and 10 levels as diagnostic markers of
15. neonatal infection and possibility of mortality. Iran J basic Med Sci 2013;16:1232-7.
16. 13. Dima M, Ilie C, Boia M, Iacob D, Iacob RE, Manea A, et al. Acute phase reactants and cytokines in the evaluation of neonatal sepsis. Jurnalul Pediatrului 2012;15:27-30.
17. 14. Schelonka RL, Chai MK, Yoder BA, Hensley D, Brockett RM, Ascher DP. Volume
18. of blood required to detect common neonatal pathogens. J Pediatr. 1996;129(2):275-
19. 278.
20. 15.Clinical Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. In: 24th informational supplement (M100-S23) CLSI, Wayne PA, USA; 2014.
21. 16. Lara RS, Ahmed NM, Alzahraa EA , Osama BS.Diagnostic Markers for Early Detection of Neonatal Sepsis. Journal of Scientific Research in Medical and Biological Sciences. 2021; 2(3): 13-25.
22. 17.Sonawane VB, Mehkarkar NS, Jadhav PB, Gaikwad SU,Kadam NN. Study of interleukin-6 levels in early diagnosis of neonatal sepsis. Int J Res Med Sci 2015;3:41e6.
23. 18.Khinchi YR, Anit K, Satish Yadav. Khinchi YR, Kumar A, Yadhav S. Profile of neonatal sepsis. J Coll Med Sci. 2010;6:1–6.
24. 19.Zakariya BP. Vishnu B, Belgode N, Harish T, Noyal MJ. Neonatal Sepsis in a Tertiary
25. Care Hospital in South India: Bacteriological Profile and Antibiotic Sensitivity
26. Pattern. Indian J Pediatr. 2011 ;78(4):413-7.
27. 20.Neema K, Erasmus K, Damas LM, Seni J,Stephen EM. Predictors of positive blood culture and deaths among neonates with suspected neonatal sepsis in a tertiary hospital, Mwanza-Tanzania.BMC Pediatrics. 2010;10:39.
28. 21. Flora C, Mariam MM, Martha FM, Neema K, Antke Z,Benson RK.et al. Utility of qualitative C-reactive protein assay and white blood cells counts in the diagnosis of neonatal septicaemia at Bugando Medical Centre, Tanzania. BMC Pediatrics. 2014;14:248.Flora Chacha1, Mariam M Mirambo, Martha F Mushi, Neema Kayange1, Antke
29. Zuechner, Benson R Kidenya and Stephen E Mshana. Utility of qualitative C-reactive
30. protein assay and white blood cells counts in the diagnosis of neonatal septicaemia at
31. Bugando Medical Centre, Tanzania. BMC Pediatrics. 2014;14:248.
32. 22.Sucilathangam G, Amuthavalli K, Velvizhi G, Ashihabegum MA, Jeyamurugan T, Palaniappan N. Early Diagnostic Markers for Neonatal Sepsis: Comparing 101 Procalcitonin (PCT) and C-Reactive Protein (CRP). Journal of Clinical and Diagnostic Research.2012 ;(Suppl-2), Vol-6(4): 627-631.
33. 23. Shrestha R K, Rai S K and Mandhal P K. Bacteriological study of neo natal sepsis
34. and antibiotic susceptibility pattern of isolates in Kathmandu, Nepal. Nepal Med Coll
35. J 2013; 15(1): 71-73.
36. 24. Laura L. Raynor , Jeffrey J. Saucerman, Modupeola . Akinola, Douglas E et al. Cytokine screening identifies NICU patients with Gram-negative bacteremia. Pediatr Res. 2012 ; 71(3): 261–266.
37. 25.Shalini T, Malik G K. Neonatal Sepsis: past, present and future; a review article. Internet Journal of Medical Update 2010. ;5(2):45-54.