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Study of Neutrophil Gelatinase Associated Lipocalin in Neonatal Sepsis

Mrs.Vaishali S. Bhandare[1], Dr.P.E.Jagtap[2], Dr. S.P. Dhonde[3], Dr.G.J.Belwalkar[4], Dr. N.S. Nagane[5], Dr.V.V. Shiralkar[6],Mr.K.Suryawanshi[7], Mr.V.Mane[8]

[1.]Tutor,[2,3,5] Professor,[4].Asso. Prof, [6.]Asst. Prof [7,8.] Tutor

Department of Biochemistry, Bharati Vidyapeeth (Deemed to be University), Medical College and Hospital, Sangli

Abstract: **Neonatal sepsis is one of the most important causes of morbidity and mortality throughout the world. The diagnosis of neonatal sepsis is notoriously difficult due to non-specific signs and symptoms. Currently, no specific biomarker can be considered as an ideal for diagnosis of sepsis in neonates. Therefore, this study has undertaken with aims to determine the utility of plasma NGAL among new biomarkers of sepsis. Out of 140 neonates included in this study, 70 were with suspected sepsis (as study group) and 70 were healthy controls. Mean level of NGAL in study group is 158 ± 79.35 pg/mL, which was statistically highly significant (p value=0.000) than control 77± 64.77 pg/mL. Study group further categorised as early onset (n=27) and late onset (n=43) of disease course. Mean level of NGAL in late onset sepsis group was noted high (170± 79.29 pg/mL) than that of early onset sepsis (141.8 ± 142.25 pg/mL). Blood culture report data was obtained and showed positive blood culture reports in 29 patients while negative blood culture reports in 41 patients. Raised values of NGAL seen in sepsis with positive blood culture (177± 85.05 pg/mL) than sepsis with negative blood culture (150 ± 76.71 pg/mL). Our findings support NGAL as a new promising biomarker helping paediatrician for diagnosis of the neonatal sepsis.**

Keywords: **NGAL-Neutrophil Gelatinase Associated Lipocalin, Neonatal sepsis**.

1. INTRODUCTION

Despite advances in child care, neonatal sepsis is still a major cause of neonatal morbidity and mortality in the world. [1]Neonatal sepsis is defined as a clinical syndrome characterized by signs and symptoms of infection with or without accompanying bacteremia in the first month of life. In spite of ongoing efforts in early diagnosis, treatment and prevention, neonatal sepsis still remains an enigmatic area for neonatologists due to changes in epidemiology and lack of ideal diagnostic markers.

The gold standard for detecting bacterial sepsis is blood culture.[2] However, pathogens in blood cultures are only detected in approximately 30 % patients, means sensitivity of blood culture is suspected to be low. This leads to unnecessary exposure to antibiotic before the presence of sepsis has been proven with potentially poor outcomes. Beside this, confirmation by blood culture is a time consuming process and has restricted availability. Sometimes clinical features of sepsis are non specific and here combining blood culture and other laboratory markers in appropriate clinical background may help to screen sepsis in newborns. These neonates pose a diagnostic and therapeutic dilemma because fatal infections have been reported in the presence of negative blood culture.[3] Therefore, though blood culture reports are negative, the presence of clinical signs of sepsis suggested and treated as clinical or probable sepsis.

According to the onset of age, neonatal sepsis is divided into early-onset sepsis (EOS) and late-onset sepsis (LOS).Early Onset Sepsis reflects trans-placental or, more frequently, ascending infections from the maternal genital tract, whereas, Late Onset Sepsis is associated with the postnatal nosocomial or community environment. Late onset sepsis, by definition, occurs at >72 hours of life and affect as many as 20 to 30 percent of infants hospitalised in NICU. [4]The diagnosis of late onset sepsis is often difficult because of non specific clinical signs and symptoms.[1] Thus diagnosis of neonatal sepsis always remains challenging.

An ideal marker must have high sensitivity and specificity. It must be easy to estimates also. Most significantly, it should be able to reflect the course of disease and guide the use of antibiotic treatment. Neutrophil Gelatinase Associated Lipocalin (NGAL) is a member of lipocalin superfamily of extracellular transport proteins.[5]It exists in the disulphide-

bond form of a 25 KDa homodimer, lipocalin-2 and a neutrophil gelatinase. The secretion of NGAL is activated by lactoferrin and vitamin B_{12} and its expression increases in epithelial cells in the inflammatory state.[6] It is secreted mainly from neutrophil as well as various organs including in the kidney after ischemic or nephrotoxic injury.

Recent studies demonstrate that NGAL levels measured in both plasma and urine of patients, represent a novel specific biomarkers for detection of acute kidney injury, after cardiac surgery.[7] It is also found in other tissues in response to various conditions like infection and ischemia also. In case of neonates, higher NGAL concentration in urine determined with high probability of sepsis.[6] Similarly, close relationship is found between urinary NGAL and sepsis in VLBW infants.[8]

Due to these extreme applications of NGAL in sepsis, this research study is undertaken to estimate utility of plasma NGAL in diagnosis of neonatal sepsis comparing with conventional blood culture reports.

2. MATERIALS AND METHODS

After Institutional Ethical Committee (IEC) approval, this research study was conducted at Department of Biochemistry, in association with Department of Pediatric, Bharati Vidyapeeth (Deemed to be University) Medical College & Hospital, Sangli. Out of total 140 neonates, 70 were included as cases with clinical signs and symptoms of sepsis suggested by Pediatrician and 70 were as control with no clinical signs of sepsis. From sepsis group, 27 cases were further categorized as early onset while 43 as late onset sepsis patients. Blood culture reports of these patients were obtained from patient data and found 29 cases were with positive blood culture reports and 41 cases has negative blood culture reports.

Before starting antibiotic treatment, blood sample was collected from all cases of neonatal sepsis by pediatrician for routine laboratory investigations. Simultaneously 2 ml blood was collected in plain container. Also 2 ml blood was collected from all 70 controls. Serum was separated and used for the determination of NGAL concentration.

Measurement of NGAL was performed by commercial available ELISA kit (Ray Bio ELISA Kit-Cat#: ELH-Lipocalin2-001). The mean absorbance was calculated for each set of duplicate standards, controls and samples, and was subtracted the average zero standard optical density. The standard curve was plotted on log-log graph paper with standard concentration on the x-axis and absorbance on the y-axis. The best-fit straight line was drawn through the standard points.

Written consent was taken from parents or guardians of all these neonates for involving them in the study. SPSS software was used for the data analysis. Values of NGAL compared between controls and neonatal sepsis. Data of blood culture reports was recorded during the study period and compared with NGAL levels.

3. OBSERVATIONS AND RESULT

Table-1:- Concentration of Serum NGAL in the study and control group

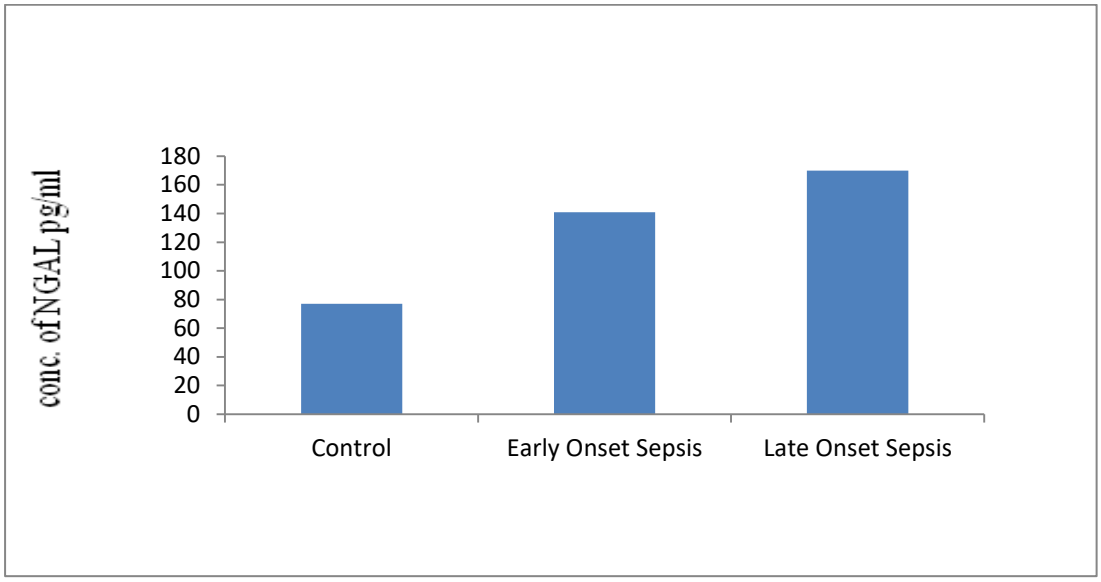
Groups	**Conc. Of NGAL (pg/mL) (Mean ± S.D.)**
Study Group (N=70)	158 ±79.35
Control Group(N=70)	77 ± 64.77
*P value	0.000

*P value =0.000 – Highly significant

Table-2 :- Concentration of Serum NGAL in early onset and late onset neonatal sepsis, compared with control

Groups	**Conc. Of NGAL (pg/mL) (Mean± S.D.)**
Early onset (N= 27)	141.8 ± 142.25
Late onset (N= 43)	170 ± 79.29
*P value of control against Early onset	0.000
**P value of control against Late onset	0.0005
***P value of Early onset against Late onset	0.17

*P value= highly significant, **P = significant, ***P value < 0.005 =non significant

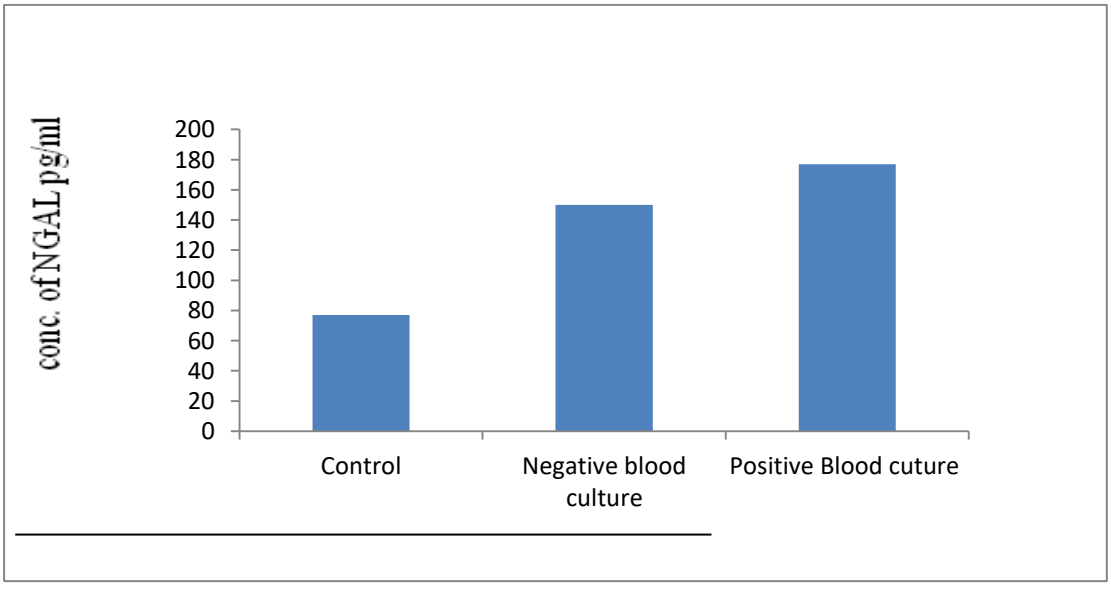


Graph 1:- Concentration of Serum NGAL in Control, Early onset and Late onset neonatal sepsis.

Table-3 :- Concentration of Serum NGAL in Neonatal sepsis with positive and negative blood culture, compared with control group

Groups	**Conc. Of NGAL (pg/mL) (Mean ± S.D.)**
Sepsis with negative blood culture (N= 29)	150 ± 76.71
Sepsis with positive blood culture (N= 41)	177± 85.05
Control (N=70)	77 ± 64.77
*P value of control against positive blood culture 0.000	
**P value of control against negative blood culture 0.0004	
***P value of negative blood culture against positive blood culture 0.17	

* P value =0.000 – Highly significant, **P= significant, ***P= non significant



Graph 2:- Concentration of Serum NGAL in neonatal sepsis with Positive blood culture and Negative blood culture reports and control

4. DISCUSSION

Newborns with "suspected sepsis", are often subjected to extensive diagnostic procedures and unguided systemic antibiotic therapy pending further laboratory results.[8] Blood culture, though considered as a gold standard for diagnosis of neonatal sepsis, has limited utility because of delayed reporting, frequent use of maternal antibiotics, and low neonatal sample volume.[9] In this context there is need of new biomarker for diagnosis of neonatal sepsis. Thus in search of new biomarker, present study undertaken to evaluates level of NGAL in neonatal sepsis.

Earlier the utility of urinary NGAL as marker of AKI and sepsis in adults and children has been studied.[10,11,12] In present study serum NGAL in neonatal sepsis was estimated and found the mean level of NGAL in the study group (158 ±79.35 pg/mL) much higher and statistically highly significant (p value=0.000) than that of control group (77 ± 64.77 pg/mL).(Table-1) This was in support with work of Mike 2014[13] in septic newborn and Angeletti who showed significantly raised value of NGAL in sepsis than in non-septic adult patients. [14]

NGAL has ability to bind with bacterial siderophore [15] which is required as supplement of iron for bacterial growth. When NGAL binds with iron-siderophore complex, bacteria are devoid of iron nutrition [5,16] and this may cause to reduce bacterial growth. In response to infection leukocytes multiplied dramatically which releases NGAL. Thus NGAL serve as bacteriostatic component of defense. In present study, raised NGAL level may be as a result of defense mechanism against infection.

Since the early 1980s, epidemiological studies have showed general observation as a low incidence of Early Onset Sepsis than Late Onset, probably due to advances in obstetric care.[4]

Present data indicates highly significant elevated NGAL levels in late onset sepsis while significantly raised values detected in early onset of disease; when both conditions compared with control. (Table-2). Inflated concentration of NGAL observed in late onset than early onset (Graph-1) may be due to more activation of immune system against bacterial overload in neonates with LOS than EOS.

The plan of the study offers us an opportunity to compare the concentration of serum NGAL in between neonatal sepsis with positive blood culture, negative blood culture and control. When compared with control group, concentration of NGAL in positive blood culture group was found elevated with high significant while in negative blood culture group it was significantly raised than control. (Table-3 and Graph-2).This is in agreement with work of Per Venge [17] and Mohammad farouk. [18]

Though blood culture report appears negative, with the presence of clinical signs and symptoms of sepsis, the case is labeled as clinical sepsis and treated. Lower NGAL concentration in negative blood culture group than positive culture group can be explained as it may be due to suppression of active infection. As NGAL is released in response to active bacterial infection [19] as bacteriostatic component; in patients with positive blood culture it may be released at high rate than negative blood culture due to active bacterial load.

NGAL has an ability to bind with iron siderophores; it reduces availability of free iron which has been proved as proxidant and thus role of NGAL as antioxidant can be suggested.

In conclusion along with clinical parameters and in context with non specific blood culture reports, serum NGAL can be considered as additional new biomarker for diagnosis of neonatal sepsis.

5. CONCLUSION

Considering the observations, results and findings of the present study, serum NGAL can be suggested as a biomarker for diagnosis of neonatal sepsis and its efficacy in confirmation of onset of disease. Hence it is recommended that NGAL may be accepted as surrogate marker in correlation with blood culture reports & other conventional markers, helping pediatricians in treating neonatal sepsis.

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