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The Effect of Granulocyte-Macrophage Colony-Stimulating Factor on Neutropenia and Mortality in Neonatal Sepsis

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ABSTRACT

Objective: To evaluate the impact of adjuvant Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) therapy in neonatal sepsis.

Study Design: Quasi-experimental study.

Place and Duration of Study: Combined Military Hospital, Lahore Pakistan, Mar 2019 to Mar 2020.

Methodology: Thirty neonates (15 cases and 15 controls) were randomly selected for this study. The Interventional Group was given GM-CSF and standard-of-care treatment; the Control Group only received standard care. Both groups were followed for the primary outcome (i.e., discharge or death). Secondary outcomes included the effects of GM-CSF on haematological parameters of neonatal sepsis.

Results: The mean gestational age of the children was 33.46±2.47 weeks. In the Interventional-Group, 56.7% of the neonates were discharged with the resolution of the sepsis. There was no significant difference concerning the secondary outcome, but the mortality was higher in the neonates among the Control-Group (p-value>0.05).

Conclusion: Our study showed no difference in mortality between the Interventional and the Control Groups. The GM-CSF therapy could be used as an adjuvant therapy in neonates with neonatal sepsis to increase the total leukocyte and absolute neutrophil counts. Further studies are needed to holistically chart the clinical benefit of GM-CSF adjuvant therapy.

Keyword: Absolute neutrophil count, GM-CSF, Neonates, Platelet count, Sepsis, Total leukocyte count.

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INTRODUCTION

Neonatal sepsis is one of the major causes of mortality among neonates. According to the World Health Organization, in 2020, 47% of the under-five-year-old deaths were due to neonatal sepsis.[1,2] Other major causes include preterm birth, intrapartum-related complications (birth asphyxia or inability to breathe at birth), infections and birth defects.[3] Diagnosis of neonatal sepsis is based upon clinical observation with nonspecific markers, including C-reactive protein, procalcitonin, blood culture and PCR tests. Many causative agents are identified as the cause of neonatal sepsis, which includes Escherichia coli (E coli), Klebsiella, Listeria and Streptococcus strains.[4] Group B Streptococcus (GBS) is also a recognized cause of sepsis, which, however, has been reduced due to screening and intrapartum antibiotic prophylaxis during pregnancy.[5] Despite the advances in neonatal care resulting in improved survival and decreased complications in preterm infants, sepsis still has a major share of mortality and morbidity in very low-birth-weight (<1500 g) infants.[6]

Neutropenia in neonates suffering from sepsis is associated with increased mortality and morbidity. The neonatal susceptibility to sepsis associated with neutropenia is related to the smaller neutrophil storage pool, the decreased capacity of neutrophils to be mobilized and a slower regeneration of neutrophils from the bone marrow.[7] Furthermore, there are functional deficiencies in neonates who fail to have a robust immune response during sepsis. In addition to antibiotic therapy and supportive care, immunotherapies such as granulocyte transfusions and intravenous immunoglobulin have been used to decrease mortality inconsistently.[8] A few studies have shown that granulocyte colony-stimulating factor (GM-CSF) can prime neutrophils for increased respiratory burst and chemotactic responses.[9,10] Initially labelled as hematopoietic-cell growth factors, they might also have additional functions by acting directly on mature myeloid cells. Neonatal sepsis is an extremely grave and prevalent condition worldwide, having risen from 40% in 1990 to 47% in 2020, despite multiple efforts to nip this in the bud.[3] Efforts have been made to control it, but still, there is a long way to go. With rising

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antibiotic resistance, using granulocyte stimulating factors to combat neonatal sepsis seems a promising strategy to treat neonatal sepsis. Therefore, we sought to study its role in neonatal sepsis.

METHODOLOGY

The quasi-experimental study was conducted at Combined Military Hospital, Lahore, from March 2019 to March 2020. The Ethical Committee of the Hospital approved the study.

Inclusion Criteria: Neonates of either gender with confirmed Diagnosis of neonatal sepsis, having an absolute neutrophil count of <1500 cells/mm were included.

Exclusion Criteria: All neonates who had major congenital anomalies or those who were not septic were excluded.

Neonates were enrolled after the confirmation of neonatal sepsis (Figure). Neonatal sepsis was confirmed based on clinical and laboratory parameters, which included complete blood count (total leukocyte count less than 5,000 or more than 25,000 µl), absolute neutrophil count less than 1000/mm3, CRP >10 mg/L, fever (temperature >100.5 F), prolonged capillary refill time >3 seconds, tachypnea (respiratory rate >60 breaths/min), tachycardia (heart rate >160 bpm).[11] The clinical parameters that were checked included poor feeding, lethargy, sluggish neonatal reflexes, poor peripheral perfusion, temperature instability, and increased C-reactive protein (CRP).Definitive sepsis was defined as presumed sepsis with a positive blood culture. Neutropenia was defined as an Absolute Neutrophil Count (ANC) of ≤1500/µL.[12] Confidentiality of the data and the study subjects was maintained. Informed written consent was obtained from the parents of the neonate. The intervention of adjuvant GM-CSF was given in the neonates after 24 hours, and the counts were monitored via complete blood count after every 48 hours. The primary measures of the study included an absolute increase in the neutrophil count (ANC) and outcome of the therapy (i.e., either expired or discharged).

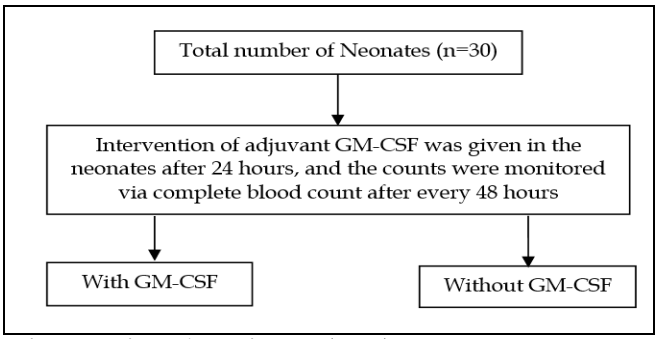

Figure: Patient Flow Diagram (n=30)

Statistical Package for Social Sciences (SPSS) version 23.0 was used for the data analysis. Quantitative variables were expressed as Mean±SD and qualitative variables were expressed as frequency and percentages. Chi-square test was applied to explore the inferential statistics. One-way analysis of variance (ANOVA) was applied to gauge the mean differences. The *p*-value of 0.05 or less was taken as significant.

Table-I: Biochemical Profile Changes in the Neonates from Baseline to End Treatment (n=30)

Parameters	At Baseline	1st Follow-up	2nd Follow-up
Total Leucocyte Count	8.52±5.07/µl	6.37±4.42/µl	12.01±9.49/µl
Platelet Count (per microliter)	201.43±83.48	133.37±88.78	166.93±98.59
Absolute Neutrophil Count (per microliter)	2388.06±1439.81	1131.96±359.29	2740.03±1978.68

RESULTS

A total of 30 neonates were enrolled in this study. The mean gestational age of the neonates was 33.46±-2.47 weeks. Majority of the neonates were born with caesarean section, 29(96.7%), and 1(3.3%) was born via normal vaginal delivery. The mean birth weight of the neonates was 1797±554.81 gm. The mean hematological profile parameters are mentioned in Table-I.

Table-II: Comparison of Hematological Profile changes in Treatment Groups (n=30)

Parameters	Groups	At Baseline	1st Follow-up	2nd Follow-up
TL Count	With GM-CSF	9.77±5.63	6.74±4.90	14.12±12.10
	Without GM-CSF	7.27±4.26	5.99±4.02	9.90±5.54
p-value		0.18	0.64	0.22
Platelet Count	With GM-CSF	216.06±84.16	113.13±96.64	143.20±100.72
	Without GM-CSF	186.80±83.02	155.07±77.07	190.67±93.72
p-value		0.34	0.20	0.18
ANC	With GM-CSF	2349.80±1590.11	1135.53±425.76	2671.00±2489.38
	Without GM-CSF	2426.34±1326.74	1128.40±293.44	2809.06±1379.40
p-value		0.88	0.95	0.85

Overall, in neonates who received GM-CSF, total leucocyte count (TLC) increased from an average of 9.79±5.63/µl at baseline (admission) to 14.12±12.10/µl on follow-up. (follow-up was two weeks after discharge). The Control Group had an increase from 7.27±4.26/µl at admission to 9.90±5.54/µl on the second follow-up. There was a difference between the biochemical profiles of the Interventional and Control Groups described in Table-II. Most neonates (i.e., 56.7%) in the Interventional Group were discharged with sepsis resolution. The absolute mortality was higher in the Control Group neonates (Table-III). Overall, 56.7% of neonates were discharged after GM-CSF therapy, with 43.33% expired, 46.7% discharged, and 53.3% expired in the Control Group.

Table-III: Comparison of the Outcome in the Treatment Groups

Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)	Outcome		*p*-value
	Discharged	Expired	0.46
Yes	10	5	
	66.7%	33.3%	
No	7	8	
	46.7%	53.3%	

DISCUSSION

Neonatal sepsis is a serious concern that can lead to increased mortality and morbidity. Despite strong antimicrobial agents against disease-causing organisms, the prognosis remains poor due to the immature neonatal immune system.[8] This calls for the need to develop better therapeutic measures that can cause rapid maturation of the neonatal immune system to combat infection and decrease overall morbidity and mortality.[9] The colony-stimulating factors (CSFs) like granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) are the cytokines that stimulate the production of neutrophils and monocytes.[10])

Although GM-CSF causes an increase in the ANC, the treatment did not yield any clinically significant results (i.e., a decrease in morbidity or mortality) throughout multiple trials.[11–13] Nevertheless, a recent meta-analysis has shown that GM-CSF leads to lesser odds of acquiring sepsis among patients with cancer treatment.[14] In a Cochrane systematic review of seven studies, it was concluded that the addition of G-CSF or GM-CSF to conventional antibiotics in preterm infants with suspected sepsis did not reduce all-cause mortality over two weeks. However, the subsection of neonates with significantly decreased neutrophils (<1.7 x 10(9)/l) did show a decreased risk of mortality when treated with GM-CSF [RR=0.34 (95% CI 0.12, 0.92)] over two weeks.[15]

The concerns with the use of GM-CSF have often been cited as an increased risk for developing chronic lung injury and necrotizing enterocolitis. Notwithstanding, it was observed that none of the interventional studies in the Cochrane systematic review had affirmed the theoretic risks.[16]

Although GM-CSF leads to an increase in ANC and TLC, the clinical benefit remains uncertain. Further prospective studies with better randomization and a larger number of participants will be needed to conclude using adjuvant GM-CSF with standard-of-care treatment to decrease overall mortality and morbidity.[17,18] Moreover, it is important to identify patient-specific parameters that predict better outcomes for the adjuvant GM-CSF therapy (including but not limited to ANC and severity of sepsis).

LIMITATIONS OF STUDY

Our study was limited due to the small number of participants, which has rendered low statistical power to discern any difference in mortality between interventional and control groups. Moreover, our study could be limited due to any unaccountable confounder during the selection and statistical phases.

CONCLUSION

The adjunctive treatment with GM-CSF increased the absolute neutrophil count compared to the Control Group. Further studies are required to characterize its absolute role in decreasing mortality and sustaining quality of life.

Conflict of Interest: None.

Authors Contribution

Following authors have made substantial contributions to the manuscript as under:

SAH & ZA: Conception, study design, drafting the manuscript, approval of the final version to be published.

US & MTN: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

AK: Data acquisition, drafting the manuscript, critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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