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Role of G-CSF in Preterm Neonates With Sepsis

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ABSTRACT: OBJECTIVE:To study effect of supplementing G-CSF to premature neonates with Septicemia withANC<5000/cmm along with appropriate antibiotics in terms of
1.Improvement in clinical symptoms of sepsis and increase in Absolute Neutrophil Count (ANC)
2.Effect on outcomeespeciallymortality and hospital stay
MATERIAL AND METHOD: Prospective observational study in division of neonatology of pediatricsDepartment, PacificMedical College and Hospital,Udaipur from September 2022 to September 2023.
INCLUSION CRITERIA:
1.Low birth weight neonates with birth weight<2000gm
2.ANC<5000/cmm
3.Evidence of sepsis i.e.at least one positive blood culture in first28 days of life
METHODOLOGY: Written consent from guardians of neonateswere taken.Ethical clearance was taken,Necessary laboratory investigations were done and blood culture was taken by proper method.Antibiotic and appropriate treatment was initiated as per NICU unit protocol.Standard hematological techniques were employed for estimation of hematological parameters.
RESULT:The study was carried out in 40 LBW septic neutropenic preterm neonates with ANC<5000/cmm.They were randomly assigned to treatment group(n=20) and control group (n=20). There was male preponderance with 60% of babies were male.Age of neonate at study entry was comparable between G-CSF and control group [6.5 ± 2.98 days Vs6.0 ± 5.38 days (p>0.05)].
Mortality was significantly higher in control group as compared to G-CSF treated group(3.5% Vs 5%).13 out of 20 patientsincontrol group and 11 out of 20 patientsof G-CSF treated group survived.
With administration of G-CSF, ANC rose to significantly higher level with G-CSF group as compared to control groupon day 3(4786±1089/cmm Vs 4212±754/cmm,PC<0.05).Therise was sustained till day 5(5008±1028/cmm Vs 4055±522/cmm, p<0.01).Clinical symptoms of sepsis like gastrointestinal symptoms and hypoglycemiawere resolved earlier in G-CSF group as compared to control group(4.71±2.3 Vs 8.0±4.2 days,p<0.05) and(4.0±1.0Vs 7.0±1.78 days,p<0.01 respectively).Hospital stay was significantly longer in control group as compared to G-CSF study group (mean 29.59±5.40 Vs 22.94±4.29 days,p,0.001)
CONCLUSION:G-CSF use in preterm septic neonates with Birth weight(900-1900 gm,mean 1345±289 gm) and Gestational age (28-36 weeks, mean 31.5±2.68 weeks) increased absolute neutrophil count and resulted in decreasein mortality and hospital stay duration. However,studies with large number of patients is warrantedbefore use of G-CSF can be recommendedasstandard therapy for preterm neutropenic septic neonates.
KEYWORDS:G-CSF,ANC,Sepsis, Preterm neutropenic neonate

I. INTRODUCTION:

Unique susceptibility of neonates to sepsis associated with neutropenia is due to smaller neutrophil storage,reduced capacity for neutrophil mobilization from bone marrow reserves anda slower regeneration.Mortality rate due to sepsis has been reported torange from 30% to 50% but as high as 80% to 90% in the presence of bone marrow neutrophil storage have depletion.[2]Colony StimulatingFactors (CSF) increase neutrophil production ,improve their function and enhanceIL-8 binding to cell surface.[3]Neonatal infection rate is inversely related to low birth weight and gestational age .[4]Colony Stimulating Factors (CSF) appears to be major regulators of peripheral blood cell production during state of sepsisand demand.The study aims to find the relationship between G-CSF use in septic preterm neonate and outcome in terms of increase in ANC levels,decrease mortality and hospital stay.

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II. MATERILA AND METHOD:

Study wasconducted in NICU, Department of Pediatrics,Pacific Medical College andHospital, Udaipur.

A total of 40 babies were studied.These were randomlyassigned to treatment group(n=20) and control group(n=20).the period of study was twelve month from September 2022 to September 2023.

Inclusion Criteria:

1.Low birth weight neonates <2000gm
2.ANC<5000/cmm
3.Evidence of sepsis i.e positive blood culture in first 28 days of life

Exclusion Criteria:

1.Babies with congenital malformation
2.Babies with intrauterine infection
3.Babies with serum creatinine >2mg/dl
4, Babies with SGOT/SGPT >four times normal value were excluded

Those without consent for study wereexcluded from study.Basic maternal and neonatal data was recorded including basic neonatal characteristics like Birth weight,Birth length,Period of gestation,Apgar score etc. All babies were treated as per unit protocol. Initially on suspicionof sepsis injectable cefotaxime and amikacin was started. On receiving blood culture, antibioticswere revised accordingly. The babies in treatment groupin addition received injection G-CSF 10 microgram/kg as slowintravenous infusion over a period of 2 hour once daily for a period of five consecutivedays. All babies had hematological evaluation for Total Leucocyte Count,Absolute Neutrophil Count,AbsolutePlatelet Counton day1,3,5,7 and 14 of study entry.Blood culture was repeated 48-72till reported sterile.Neonatal problems,duration of hospital stay and mortality were recorded for all cases.At the end of study, the data was collected and analyzed statistically by using studentst-test,z-test of proportion.

III. RESULT:

A total of 40 babies were included who fulfilled the criteria of inclusion as per provision of study protocol.These were randomly assigned to the treatment group (n=20) and the control group(n=20). All babies had hematological evaluation on day 0,1,3,5,7 and 14.Blood culture were repeated every 48-72 hours till reported sterile. Various observations were as below

TABLE 1: PATIENT CHARATERSTICS

Characterstics	G-CSF (n=20)	Control(n=20)	p-value
Male/Female	11/9 1.22:1	13/7 1.95:1	
Birth weight (gm) Mean±SD Range <1000 1000-1499 1500-2000	 1305±289 900-1900 1(5%) 14(70%) 5(25%)	 1500±231 1100-1900 _ 12(60%) 8(45%)	>0.05
Gestationalage(weeks) Mean±SD Range	 31.5+2.68 28-36	 32.6+2.23 30-36	>0.05(NS)
AGA/SGA	15/5 3:1	15/5 3:1	

There was no significant difference between G-CSF and control group with reference to Birth weight,Gestational age and sex.Males outnumbered females in both the groups in ratio of 1.2:1 and 1.95:1 in G-CSF and control group respectively.Most babies in both groups were appropriate for Gestational age and for late onset sepsis.

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TABLE 2:PREDOMIDANT NEONATAL PROBLEMS

Neonatal problems	G-CSF (N=20)	Control (N=20)	p – value
Respiratory distress	12(60%)	12(60%)	>0.05(NS)
Feed intolerance	14(70%)	17(85%)	>0.05(NS)
NEC	2	---	>0.05(NS)
Hypoglycemia	10(50%)	9(45%)	>0.05(NS)
Thrombocytopenia	16(80%)	13(65%)	>0.05(NS)
Seizures	4(20%)	5(25%)	>0.05(NS)
Shock	3(15%)	5(25%)	>0.05(NS)
Assisted ventilation	3(15%)	6(30%)	>0.05(NS)
Meningitis	7	13	>0.05(NS)

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Neonatal problemswere almost similar in both G-CSF and control group.There waas no significant difference.

TABLE:3NEONATAL SEPSIS

Neonatal sepsis	G-CSF (N=20)	Control group (N=20)
Thrombocytopenia	16(80%)	13(65%)
CRP Positive	16(80%)	14(70%)
Pseudomonas Sepsis	17	14
Acinobacter Sepsis	1	4
Streptococcus Sepsis	---	6
Enterobacter Sepsis	2	2
Enterococcus Sepsis	1	---
Candida Sepsis	---	1
Citrobacter Sepsis	---	4
Polymicrobial Sepsis	1	11

Incidence of thrombocytopenia and rate of CRP positivitywere comparable in both G-CSF treated group and control group.Predominant primary organism wasPseudomonas in native study and control group.Staphylococcus was predominantly secondary organism in control group.Citrobacter and candida sepsis was present only in control group.

TABLE 4:HEMATOLOGICAL VALUES

Days after G-CSF administration	Total Leucocyte Count (Cells/cmm)		p-Value
Day 0	5950±1465	6750±824	<0.05 (NS)
Day 1	7135±1357	6625±1320	<0.05 (NS)
Day 3	7665±1427	6995±737	<0.05 (NS)
Day 5	8184±1570	6936±1128	<0.05 (NS)
Day 7	8400±1133	7147±951	<0.05 (NS)
Day14	7677±727	7957±815	<0.05 (NS)

Although initial total leucocyte was higher in control group by day 5 and day 7 after administration of G-CSF,Total leucocyte count weresignificantly higher in G-CSF group than in control group.

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TABLE 5

Absolute neutrophil count (cells/cmm)			
Day after administration of G-CSF	G-CSF (n=20)	Control group (n=20)	p-Value
Day 0	3037±7.9 Range (1200-4060)	3761±402 Range (2660-4385)	<0.001(HS)
Day 1	4052±922	3755±676	>0.05(NS)
Day 3	4756±1089	4213±354	<0.05(S)
Day 5	5008±1028	4055±522	<0.05(S)
Day 7	5077±657	4652±779	>0.05(NS)
Day 14	4780±554	5067±733	>0.05(NS)

The initial absolute neutrophil count was significantly lower in G-CSF group as compared to control group0.001).However, with administration of G-CSF the ANL rose to 4.52±922 cmm by day 1,4256±1084 cmm by day 3,5008± 1028 cmm by day 5 and 5077±657 cmm by day 7 when peak values were documented. These ANC values were significantly higher than in control group by day 3 through day 5.

TABLE 6:TIME TAKEN TO RESOLVE SYMPTOMS

Clinical Symptoms	Days taken to resolve symptoms after G-CSF treatment		P-Value
	G- CSF	Control	
1.Respiratory Distress Mean±SD Range	3.8±2.69 2-11	5.0±2.36 2-8	>0.05(NS)
2.Feed Intolerance Mean±SD Range	4.71±2.30 3-11	8.0±4.20 4-21	<0.05(S)
3.Hypoglycemia Mean±SD Range	4.0±1.0 3-5	7.0±1.78 4-21	<0.01(S)

In the G-CSF group feed intolerance and hypoglycemia resolved earlier than in control group.Mean duration to resolve feed intolerance was 4.71±2.30 days(Range 3-11 days) in G-CSF group as compared to 8,0±4.20 days (Range 4-20)in control group.Hypoglycemia resolved in 4.0±1.0 days (Range 3-5 days) in G-CSF group compared to 7.0±1.78 days(4.21 days) incontrol group

TABLE 7: DURATION OF ANTIBIOTIC REQUIRED

	Duration of antibiotic(in days)		p-Value
	G-CSF (n=17)	Control (n=13)	
In patients with sepsis alone	15.75±1.70	19.8±1.92	<0.01(S)
In patients with sepsis+meningitis	23.4±2.50	27.12±3.04	<0.05(S)
TotalMean±SD Range	17.88±4±11 14-27	24.35±4.34 19-33	<0.001

Antibiotics were given for significantly longer period in the control group compared to G-CSF group.This was probably because polymicrobial sepsis and incidence of meningitis were more in control group as compared to G-CSF group.

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TABLE 8: DAYS TAKEN TO BECOME CULTURE STERILE

Days after G-CSF treatment when culture was sterile	G-CSF (n=20)		CONTROL (n=20)	
		Second Organism		Second organism
D3	9(45%)	1(5%)	---	4(20%)
D6	5(25%)		2(10%)	4(20%)
D9	3(15%)		6(30%)	3(15%)
D12	---		5(25%)	
D15	---		1(5%)	
Culture did not become sterile uptill death	3(15%)		6(30%)	

In 70% of G-CSF cases, blood culture become sterile by day 6 of treatment.In control group,second organism was grown in 20% of casesby day 3 and 40% cases by day 6, mostly staphylococcus and Citrobacter whereas in G-CSFgroup, only in one patient, second organism Enterobacter was grown on day 3 of treatment.In 15% cases of G-CSF group and in 30% of control cases, blood culture remained positive until death.

TABLE 9: DURATION OF HOSPITAL

	Duration of hospitalization (in days)		p-Value
	G-CSF (n=20)	Control (n=20)	
Hospitalization (days) Mean±SD Range	 22.94± 4.29 20-32		 29.57±5.40 20-42

G-CSF group patients require significantly lessstay in hospital as compared to control group,when patients who died were excludedfrom both groups. Patient mean duration of hospital staywas 22.94± 4.20days (20-29 days)in study group and 29.57±5.40 days (range 20-42 days0in control group($p<0.01$)

TABLE 10: MORTALITY

Cause	G-CSF (n=20)	Control (n=20)	p-Value
NEC (stage III)	1(33.3%)	0	>0.05(NS)
Septic shock	3(100%)	3(43%)	>0.05(NS)
Pulmonary Hemorrhage	---	3(43%)	>0.05(NS)
Intraventricular hemorrhage (Grade III&IV)	1(33.3%)	1(33.3%)	>0.05(NS)
Respiratory Failure	---	1(33.3%)	>0.05(NS)

In G-CSF group one patient died of NEC (stage III),one with refractory shock and one had intraventricular hemorrhage and died on day 4 of treatment of G-CSFi.e. had received 3 doses of G-CSF.In control group ,3 patients died of pulmonary hemorrhage,one with intraventricular hemorrhage and 2 with septic shock and one with recurrent apnea and respiratory failure.

IV. DISCUSSION:

A total of 40 septic blood culture positive neonates with birth weight<2000gm and an absolute neutrophil count <5000/cmm,admitted in NICU, department of Pediatrics,PacificMedical College Hospital, Udaipur formed the subjects of study. They were randomly assigned the treatment group (n=20) and control group(n=20).There was male preponderance with 60% of babies beingmale. There was no significant difference between neonates of G-CSF group and control group with

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regard to mean birth weight [1395±289gm Vs 1500±231gm(p>0.05)], mean gestational age[31.5±2.08 weeksVs32.6±2.32 weeks(p>0.05)],AG Vs SGA [3:1 Vs3:1].On suspicion of sepsis,septic screening was doneand injectable antibiotics Cefotaxim and Amikacin were started.On getting blood culture sensitivity, antibiotics were revised according to blood culture sensitivity and in the study group additionallyG-CSF was given in a dose of 10microgm/kg/day as an intravenous infusion over 0-2 hour for five consecutive days.All babies had late onset sepsis.

We had taken relative neutropenia ANC<5000/cmm as selection criteria for study entry because preterm neonates with sepsis anddefined neutropenia(ANC<1500/cmm) are critically ill and have high mortality.Failure to mount a neutrophil response infection and a relative neutrophilia (Defined asANC<5000/cmm)has been associated with high mortality among preterm neonates [5,6,7].In studies enrolling neonates with ANC<1500/cmm,mortality was not significantly affected by G-CSF use[8].While in a study that enrolledseptic preterm neonateswith relative neutropeniai.eANC <5000 /cmm there were significantly fewer deaths in the neonate screening G-CSF when compared to control group[9].In our study mortality was significantly higher in the control group as compared to G-CSF group(35%Vs15%,p<0.05).13 out of 20 patients in control group and 17 out of20 in G-CSF group survived.G-CSF lead to rapid increase in ANC in all babies who received G-CSF. With G-CSF treatment ,ANC was>5000/cmmin 60% patients by day 5 and is 80% patients by day7 of treatment as compared to5% and 35% in control group on day 5, day 7respectively.Baseline ANC was significantly higher in control group than G-CSF(3761±402/cmmVs3037±709/cmm,p<0.05) but on day 3, ANL had risen to significantly higher level in G-CSF group as compared to placebo group(4756±1089/cmm Vs 4212±354/cmm,p<0.05) and rise was sustained till day 5(5008±1028/cmm Vs4055±522/cmm,p,0.01).ANC reached its peak value by day 7 in G-CSF group.17 out of 20 patients (85%) responded to G-CSF administration i.e G-CSF led to an increased in ANC to 7500/cmm in 85% patients.The remaining of patients died and has ANC <5000/cmm till death.In term Baseline mean ANC was2720/cmm.Mean increase in ANCwas 1300/cmm but ANC did not reach 5000/cmm.Mortality was higher in patients in which ANC did not rise to significant levels.In 6 out of 7 case who died in control group,ANC was still <5000/cmm at the time of death.In these neonates mean baseline ANC was 3626,67/cmm and increase in ANC from baseline was478.33/cmm.Therefore, failure of ANC to rise was associated with mortality.In the study by Miura et al[10],44 preterm neonates weight 500gm to 2000gm with gestational age<37 weeks were randomized to treatment group(n=22) to receive 10microgm/kgof iv G-CSF once daily for 3 days and the placebo group(n=20).At 24 and 48 hrs,ANC was significantly higher in the G-CSF recipients than the placebo group(mean9522/cmmVs4526/cmm at 24 hrs.p<0.06 and 16843/cmm Vs 4703/cmm at 48 hours,p<0.00042) although baseline ANC was comparable between two groups.

In the study,mean duration of reliving clinical symptoms of sepsis like feed intolerance,hypoglycaemia were significantly lower in G-CSF group as compared to control group(feed intolerance (4.71±3.30 days Vs8.0±4.2 days,p<0.05,Hypoglycemia 4.0±1 days Vs7.0±1.78 days p<01).In the study by Barek et al[8] 14 neonates with presumed or confirmed sepsis are neutropenic (ANC <2000 /cmm) with gestational age of 26-35 weeks were selected.Favourable clinical response in terms of disappearance of clinical manifestation of sepsis,such as poor feeding,apnoea,temperature instability,bradycardia etc were seen in 86%cases as compared to60% control cases.In our study duration of antibioticsand hospital stay were significantly longer in control group as compared to G-CSF group(17.88±4.11 days Vs 24.38±4.34 days,p<0.001 and 22.9±4.29 days Vs 29.57±5.4 days,p<0.001 respectively). In study Rumel et al[8] neonates in intensive care unit with birthweight 500-1000 gm ANC <5000/cmm with clinical evidence of sepsis were randomly assigned to receive G-CSF(10 microgm/kg/day) intravenously (n=13) or placebo (n=15) for amaximum of 14 days in addition to standard treatment and antibiotics.In this study babies treated with G-CSF spent fewer days in mechanical ventilation(average 5 days Vs 12 days,p<0,23),fewer days in intensive care(average 8 days Vs 12 days) and fewer days receiving antibiotics(average 8 days Vs 16 days,p=0.14).Although these results did not reach statistical significance but there was trend towards benefits in their short term end points,This study supports our results.

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V. CONCLUSION:

It has been well established that neonates fail to produce and deliver adequate number of phagocytes at site of infection.Colony stimulating factors through their stimulation of granulopoiesis and phagocyte formation,have the potential to enhance cellular defence against maternal infection.it can be concluded from this study that G-CSF use in preterm septic neutropenic neonates with birth weight(900-1900gm,maen1395$\pm$289gm) and gestational age(28-36 weeks,mean 31.5$\pm$2.68 weeks) increase ANC and results in decreased mortality,decreased hospital stay and decreased antibiotics duration.However, studies with large number of patients are needed before the use of G-CSF can be recommended as adjunct therapy for preterm neonates with sepsis.

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