STUDY OF EARLY-ONSET NEUTROPENIA IN VERY LOW-BIRTH WEIGHT NEONATES AS A RISK FACTOR FOR CANDIDA COLONIZATION

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ABSTRACT

Introduction: Candidal colonization and subsequent invasive infection are increasingly frequent features in preterm neonates in neonatal intensive care units (NICUs), Candida infection accounts for 1.6–9% of late-onset sepsis in very low-birth-weight neonates, and up to16% in extremely low-birth-weight .Many risk factors, mainly related to the aggressive, intensive, and invasive care received during long stay in NICU, are associated with Candidal colonization and invasive candidal infection**.**

Aim of the work: To evaluate the role of early onset neutropenia as a risk factor for candida colonization in preterm VLBW neonates in NICU and The current role of granulocyte colony-stimulating factor in treatment of neonatal neutropenia &candidiasis .

Patients and methods: This case control study was conducted on forty four very low birth weight (VLBW) neonates during the period from April 2010 to March 2011.This study comprised two main groups of VLBW neonates (<1500gm).

Group I: twenty two VLBW neonates with early onset neutropenia (EON) in the 1st week of life and this group subdivided into two equal sub groups Group IA: received recombinant granulocyte colony stimulating factor(G CSF) in a dose of 10 µg /kg daily SC for 3 consecutive days Group IB: received routine treatment.

All neonates were subjected to the following Full history taking ,clinical examination, Complete blood picture ,C Reactive protein ,Liver and kidney functions, Candida isolation and identification from cultures and differentiation of candida species using CHROMagar candida

Results: Our case-control study demonstrates that there is significant increase in overall candida colonization in the 2nd week in neutropenic group and this means that early onset neutropenia is a risk factor for candida colonization in all VLBW infants in NICU and we found that rhG-CSF increase ANC, The timing of the changes in the ANC in the rhG-CSF-treated neonates occurs sooner and remains longer than in the conventionally-treated control neonates. And rhGCSF failed to clear candida colonization . our study showed that there is a significant difference in risk factors in colonized and non colonized as regard to gender increase in female ($p<0.05$) normal vaginal delivery, steroids prenatal or postnatal,3rd generation cephalosporine ,decreased body weight ,gestational age,endotracheal intubation and H2 blocker ($p<0.05$). no significant difference in duration of hospital stay and survival between group treated with rhG-CSF and group receive routine treatment. Colonization occurred in 12 patient which represent (28%) C.albicans the most commonly isolated species in colonized preterm and rectum is the most common site

***Conclusion*:**Early onset neutropenia is independent risk factor for candida colonization and rhG-CSF correct neutropenia sooner and remains longer

***Key words** : Early onset neutropenia, very low birth weight, recombinant granulocyte colony stimulating factor*

INTRODUCTION

Preterm neonates in NICUs are at high risk for sever fungal infections due to the immaturity of their immune system; invasive management techniques; the need for central vascular catheters (CVC) and endotracheal tubes; the use of broad-spectrum antibiotics, parenteral nutrition, H2-blockers and steroids; as well as the immaturity of skin and gut barriers that allow dissemination of *Candida* spp from sites of colonization **(1)**. Systemic fungal infections (SFIs), mainly by Candida spp, are the third most frequent cause of late-onset sepsis in very low birth weight (VLBW) preterm neonates in neonatal intensive care units (NICUs). Their estimated incidence is 1.6 to 3% in VLBW infants and up to 15 to 20% in extremely low birth weight (ELBW) neonates **(2).**

Invasive infections caused by *Candida* spp. are a major healthcare problem in modern medicine. These yeasts rank as the fourth most common bloodstream pathogen in hospitals in the Western world (**3)** . and associated with the highest mortality amongst bloodstream infections. In healthy subjects *Candida* spp., especially *C. albicans*, are present as a commensal. Colonisation by *Candida* spp. is almost always the first step in the development of invasive candidiasis. **(4)**. Many exogenous and endogenous factors may lead to impaired host defence against Candida spp. and, as a consequence, Candid*a* may disseminate into the bloodstream and infect multiple organs **(5)** Candidal colonization (CC) and subsequent invasive infection are increasingly frequent features in preterm neonates in neonatal intensive care units (NICUs) **(6)**

AIM OF THE WORK

To evaluate the role of early onset neutropenia as a risk factor for candida colonization in preterm very low birth weight neonates in NICU and The current role of granulocyte colony-stimulating factor in treatment of neutropenia and candidiasis .

PATIENTS AND METHODS

Study design:

This case control study was conducted in Neonatal intensive care unit (NICU) Pediatrics Department and Microbiology and Immunology Department of Zagazig University Hospital during the period from April 2010 to March 2011.and conducted on forty four very low birth weight (VLBW) neonates admitted to This study comprised two main groups of VLBW neonates .

Group I: twenty two VLBW neonates(<1500gm) with early onset neutropenia (EON) in the 1st week of life and this group subdivided into two equal sub groups

Group IA: Eleven neonates received recombinant granulcyte colony stimulating factor(G CSF) in adose of 10 µg /kg daily SC for 3 consecutive days plus routine therapy

Group IB: Eleven neonates received routine therapy

Group II: twenty two matched cases of VLBW neonates without EON as a control group.

Informed consent was obtained from parents before they included in the study ,clinical data were registered excluding neonates with congenital anomalies ,neonates with incomplete data and neonates died or transferred to another hospital before seven days of life.

All VLBW neonates were subjected to the following

1-Full history taking :including

a-Prenatal history : hypertension (pre eclampsia), diabetic mother, antenatal steroids, Infections, Irradiation and antenatal antibiotics.

b-Natal history: premature rupture of membrane and mode of delivery.

c-Postnatal history :

-APGAR score, demographic data, gestational age, birth weight.

-Invasive procedure (central venous catheters , intubation &mechanical ventilation).

-Medications(broad-spectrum antibiotics, steroids ,H2 blockers , parenteral nutrition, antifungal and surfactant

-Duration of hospital stay and outcome will be recorded

From the history we can determine risk factors for sepsis and candida colonization

2-Thorough clinical examination to determine Criteria of sepsis e.g. temperature instability, , respiratory , cardiovascular and neurological manifestation. and complication of prematurity e.g. necrotizing enterocolitis (NEC).

3- Laboratory investigations.:

*Complete blood picture(by automated blood count) to determine absolute neutrophilic count(ANC)

Neutropenia was defined as neutrophils < 500 neutrophils per milliliter in neonates 0–

6 h of life, < 1500 at 6–12 h of life, < 1800 at 12–30 h of life, < 1500 at 30–48 h of life, < 1300 at 48–72 h of life, and < 1100/mL in neonates more than 72 h of life,. A neonate was defined neutropenic if having at least 1 hemogram with values below the above mentioned reference levels (7)and we do CBC at three times in the 1st day of neutropenia and in the 3rd day and in the 7th day to evaluate the effect of rhG -CSF

*C Reactive protein

we consider it positive if the level > 6mg/dl (8)

*Liver functions including

ALT and total billirubin

*Kidney functions including

Serum creatinine level

*Candida isolation and identification from cultures, and laboratory methods

The following cultures were obtained and evaluated for the study:

Surveillance cultures: ear canal swab swab from oropharyngeal secretions ,perianal area and rectum and urine and blood Cultures

1-Swabs was taken from oropharynx by gentle rubbing the swab to the tongue and oral secretions

2- Swabs was taken by gentle rubbing napkin area , perianal area and rectum after adding sterile distilled water to moisture the swab

3- Swabs was taken from ear canal by rotating and gentle rubbing the swab in ear canal after adding sterile distilled water

4-Urine culture:

Morning urine samples were collected in sterile containers after good genital hygiene, about 5ml urine were collected

5- Blood culture

In children ,0.5-1.5mlof blood was added to the ready made bottle for blood culture

Identification of candida growth (suspected colonies) after culture on Sabouraud dextrose agar (9)by

1-Colonal morphology.

2-Microscopic examination**.**

Storage.

Differentiation candida species using CHROMagar candida.

***Lot No. 0000065427**

Himedia:500gm M 12g 7A-500g Each candida species have different morphology and colors on this medium

1. C.albicans appears as light green colonies.
2. C.tropicalis appears as blue to purple colonies.
3. C.glabrata appears as cream to white

RESULTS

Table (1): Absolute neutrophilic count (ANC) in studied groups

	groupI	groupII	t	p
1st day				
Mean ± SD	1.43 ± 0.23	4.335 ± 1.18	11.3	< 0.001HS
Range	0.704-1.75	2.5-6.75		
3rd day				
Mean ± SD	3.025 ± 1.014	5.068 ± 1.352	5.67	< 0.001HS
Range	1.2-5.1	2.75-7.5		
7th day				
Mean ± SD	5.036 ± 2.075	4.975 ± 1.325	0.11	0.9 NS
Range	2.5-9.855	2.6-8.22		

This table shows significant decrease in absolute neutrophilic count in neutropenic group in the 1st day of neutropenia and the 3rd day after rhG- CSF and no significant diference at the 7th day

Table (2): Incidence of overall Candida colonization among studied groups

Candida	groupI (n = 22)		groupII (n = 22)		X^2	p
	No	%	No	%		
-ve	13	59.1	19	86.4	4.13	0.04*S
+ve	9	40.9	3	13.6		
Cumulative colonization						
Colonization in the 1st week	5	22.5	2	9	0. 23	0.69 NS
Colonization in the 2nd week	9	40,9	3	13.6	4.13	0.04 S
Total number of cultured obtained	220		220			
Total number of +ve culture at various sites	26		10		7.74	0.05 S

Table shows significant diference in overall Candida colonization and No.of +ve cultured sites among neutropenic and non netropenic groups(P*Significant)

Table(3): Demographic data of colonized and non colonized neonates

	Colonized (n = 12)		Non-colonized (n = 32)		X^2	p
	No	%	No	%		
Gender						
Male	4	33.3	23	71.9	3.96	0.04 S
Female	8	66.7	9	28.1		
Mode of delivery						
NVD	10	83.3	15	46.9	4.73	0.02 S
CS	2	16.7	17	53.1		
Gestation/week						
Mean ± SD	30.4 ± 1.1		31.3 ± 1.1		2.43	0.019 S
Range	29-31		29-33			
Weight /kg						
Mean ± SD	1.154 ± 0.147		1.297 ± 0.15		2.78	0.007 S
Range	0.95-1.4		1.05-1.5			

This table shows significant increase in female patient, normal vaginal delivery ,decreased gestational age and small weight(p* Significant).

Table (4): Intensity of colonization in group treated with rhG-CSF

Intensity of colonization	No	Percentage per week
1st week		
Low grade colonization (1 to 2 sites colonized)	2	40%
High grade colonization (3-4 sites colonized)	3	60%
2nd week		
Low grade colonization (1 to 2 sites colonized)	8	100%
High grade colonization (3-4 sites colonized)	0	0%

This table shows the effect of G CSF on intensity of colonization three cases with high grade colonization improved to low grade of colonization**.**

Table(5):Comparison between colonized and non colonized neonates in all studied groups as regard to risk factors.

	Colonized (n = 12)		Non-colonized (n = 32)		X^2	p
	No	%	No	%		
Preeclampsia	5	41.7	6	18.8	1.38	0.24 NS
Prenatal steroids	11	91.7	13	40.6	9.17	0. 02* S
Maternal diabetes	0	0	4	12.5	0.48	0.4 NS
PROM	6	50	8	25	1.49	0.22 NS
Prenatal antibiotics	6	50	10	31.3	0.64	0.42 NS
Surfactant	8	66.7	14	43.8	1.83	0.17 NS
Intubation	11	91.7	17	53.1	4.06	0.04* S
Sepsis	7	58.3	15	46.9	0.46	0.49 NS
NEC	1	8.3	5	15.6	0.02	0.89 NS
Antibiotics						
Vancomycin	2	16.7	4	12.5	0.02	0.89 NS
3rd generation cephalosp.	9	75	13	40.6	4.13	0.04*S
CVC	10	83.3	18	56.3	1.72	0.18
Steroids	7	58.3	3	9.4	9.29	0.002*S
H_2 blockers	10	83.3	5	15.6	14.9	< 0.001HS
APGAR						
< 5	6	50	24	75	1.49	0.22NS
5-10	6	50	8	25		

This table shows significant increase as regard to risk factors of candida colonization in colonized and non colonized neonates including prenatal steroids ,intubation,3rd generation cephalosporin,steroids and H2 blockers(P* Significant)

Table(6):Comparison between colonized and non colonized in all studied groups as regard to laboratory investigation

	Colonized (n = 12)		Non-colonized (n = 32)		X^2	p
	No	%	No	%		
CRP	7	38.3	16	50	0.24	0.62NS
ANC/cm						
1st day	2.39 ± 1.8 (1.05-6.65)		3.1 ± 1.6 (0.704-6.75)		1.19	0.23NS
2nd day	4.1 ± 1.4 (1.7-7.25)		4 ± 1.6 (1.2-7.5)		0.25	0.8NS
3rd day	6.2 ± 1.66 (3.2-8.6)		4.55 ± 1.5 (2.5-9.85)		3.14	0.003*S

Bilirubin Mean ± SD Range	12.1 ± 2.4 9-18	10.97 ± 2.8 6-16	t = 1.2	0.23NS
ALT(IU/L) Mean ± SD Range	34.9 ± 11.5 15-60	38.9 ± 12 20-60	t = 0.99	0.32NS
Creatinine(mg/dl) Mean ± SD Range	1 ± 0.3 0.6-1.5	0.9 ± 0.3 0.5-1.5	t = 0.95	0.34NS

This table shows significant diference in ANC in the 7th day in colonized and non colonized groups and no significant difference in other parameter

Table (7): Results of ANC in group IA (rhGCSF) and group IB(No rhGCSF)

	GroupI A (G CSF) (N. 11)	**Group I B (no GCSF) (N. 11)**	**X^2**	**P**
ANC				
1st day	1.47 ± 0.11	1.39 ± 0.31	t = 0.92	0.36NS
3rd day	2.7 ± 0.7	3.3 ± 1.2	t = 1.5	0.14NS
7th day	3.9 ± 1.17	6.14 ± 2.2	t = 2.91	0.008*S

This table shows significant difference as regard to ANC between group IA receiving rhGCSF and groupIB (receiving routine treatment) in neutropenic patient

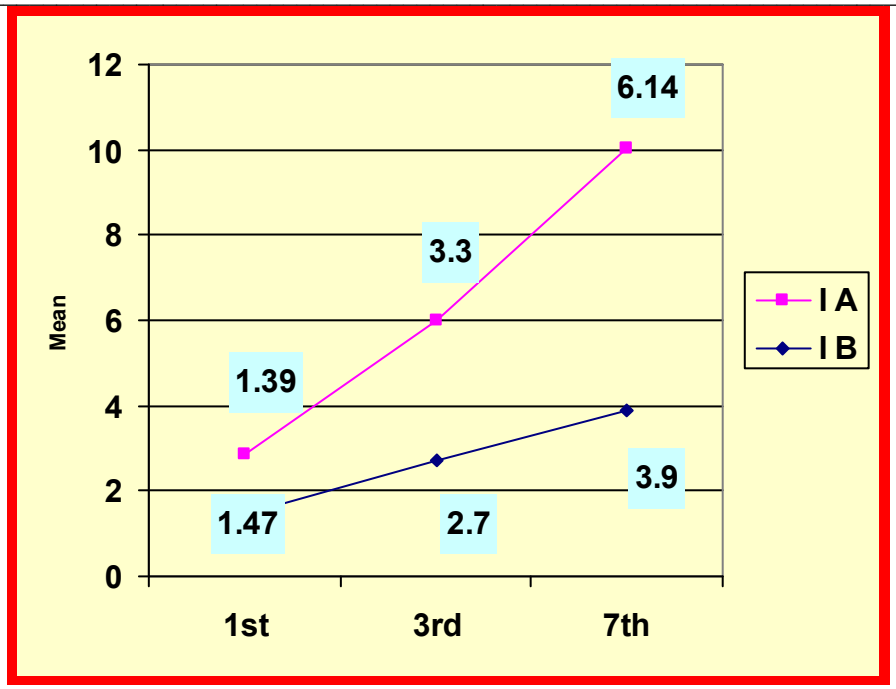

Figure (1): Changes in ANC at day 1, day 3 and day 7 of treatment among neutropenic patient The timing of the changes in the ANC in the rhG-CSF-treated neonates(groupIA) occurs sooner and remains longer than in the conventionally-treated neutropenic neonates.(groupI B)

DISSCUSION

Our case-control study showed that there is significant increase in overall candida colonization in the first two weeks (P <0.04) and significant increase in No. of +ve cultured sites (P <0.05) in neutropenic group and this means that early onset neutropenia (neutropnia in the first week) is a risk factor for candida colonization in all very low birth weight(VLBW) infants in NICU. this in agreement with (**Manzoni** et al**(7),**they reported that fungal Colonization rate in the 2nd week of life was significantly higher in previously neutropenic patients, who had also a significantly higher number of sites involved (p<0.003).and **(Mahieu et al**.(10), found also that early neutropenia increases the risk of candida colonization and candidemia.

We found that Colonization occurred in 12 preterm from 44preterm which represent (28%) these results are in agreement with **Manzoni et al (11)**
Who found Colonization in (32.1%) and It was also clear that invasive fungal infection(IFI) was usually preceded by colonization by the same species, and colonization itself is a major IFI risk factor ,Infants who develop candidemia more likely to have been previously colonized by fungi than matched control . **Catherine (12)** reported that early Candida colonization was highly prevalent (23.5%)
And we found three cases of candidemia(+ve blood culture) in the 2nd week representing (6.8%)and no candidemia in the 1st week, these results are in agreement with (**Baley et al(6**) who found a (26.7%) incidence of fungal colonization and a (7.7%) incidence of systemic fungal disease in a total of 146 infants investigated, One of the most important risk factors for invasive candidiasis is previous colonization by Candida. From our results we can recommend antifungal treatment in the 2nd week .

our findings also shed an important light on the exact timing of neutropenia (early onset neutropenia) acting as an independent risk factor for candida colonization. The condition of neutropenia in group I infants was transient and

lasted only a few days, as all neonates recovered from early onset neotropenia (EON) before day of life (DOL) 7 but neutropenia in the first days of life of our preterm not associated with increased colonization at the same time of neutropenia i.e in the first week no significance difference but there is a significant increase in overall Candida colonization among neutropenic group in the 2nd week .i.e EON infants progressed more frequently to intense degrees of colonization in the 2nd week after correction of neutropenia and They were evidently unable to clear initial colonization. EON, in fact, did not significantly affect the rates of colonization at the 1st week, but increased the risk as from the 2nd week. this in agreement with **Manzoni** et al(7),they reported that absolute neutrophil count in neutropenic group became normal at the day of life 8 after G CSF or spontaneously Absolute neutrophil number was obviously lower in group I but became normal at the end of G-CSF treatment, thus detecting no significant differences between the two groups at day 8 in ANC,and fungal Colonization rate in the 2nd week of life was significantly higher in previously neutropenic patients.

We explained that by neutropenia in the first days of life decreases the ability of preterm neonates to withstand the adhesion and initial proliferation of colonies in the peripheral sites where fungi are first contacted, and this might explain why attainment of normal absolute neutrophil count values before day of llife 7spontaneously as well as by means of treatment with rhGCSF, did not prevent the onset of candida colonization,and the preterm is liable to risk factors of colonization like antibiotics, CVC, endotracheal intubation , parenteral nutrition and H2blockers with long stay in NICU so colonization increase in the 2nd week.

We give rhGCSF to Eleven neutropenic preterm to determine the effect of rhGCSF on ANC and candida colonization and we found that correction of neutropenia in rhGCSF treated neonates occurs sooner and remains longer than neutropenic group didn't receive rhGCSF. this in agreement with **Carr et al (13**) do multicentre, randomised controlled trial in 26 centres between June, 2000, and June, 2006, 280 neonates of below or equal to 31 weeks' gestation and below the 10th centile for birthweight were randomised within 72 h of birth to receive GM-CSF 10 μg/kg per day subcutaneously for 5 days or standard management.The findings of the trial indicate that prophylactic GM-CSF significantly raises neutrophil counts in a population at high risk of postnatal neutropenia but does not result in a reduction in systemic sepsis, mortality

We found that rhGCSF failed to clear candida colonization this in agreement with **Manzoni et al(7)** we explain failure of rhGCSF to clear candida colonization completly because there is many risk factors responsible for colonization other than neutropenia and these factors must be prevented or corrected, so preventive measures other than correction of the neutrophil count are required .

But there is rhGCSF effect on intensity of colonization by changing high grade colonization in three cases in the first week to low grade colonization in the second week by rapid correction of neutropenia this explained by neutrophils appear to be a very important component of the host's defense against Candida invasion into deep tissues. These cells are capable of ingesting and killing C. albicans yeast cells in vitro; however, unkilled yeast cells have also been observed to germinate within neutrophils and perforate through the cell membrane to the outside of the cell. More virulent strains of C. albicans have been demonstrated in one study to be relatively resistant to intracellular killing by human or mouse neutrophils. **David and Peter (14)** .and **Yoshimasa et al (15**). results

show that G-CSF can promote the antimicrobial activity of peripheral blood PMNs against C. albicans.

Our results showed a significant difference in risk factors in colonized and non colonized as regard to gender increase in female ($p<0.05$) normal vaginal delivery,steroids prenatal or postnatal, 3rd generation cephalosporine ,decreased body weight ,gestational age, endotracheal intubation and H2 blocker ($p<0.05$).

and These results are in agreement with **(María** et al.(16) they reported that there are risk factors associated with candidiasis include immunocompromized hosts, early fungal gastrointestinal tract colonization, predisposition to invasive fungal dermatitis, and use of parenteral antibiotics ,corticosteroids ,parenteral nutrition and central venous line and **(Manzoni** et al.(11) they found that low birth weight, low gestational age, use of third-generation cephalosporins, steroids and H2 blockers, intubation and mechanical ventilation, use of intravenous nutrition and central venous catheter are risk factors for candida colonization . **(Mendiratta et al(17)** found that Male sex, longer duration of rupture of membrane, administration of steroids and antibiotics were found to be significant risk factors in preterms with colonization when compared to those without colonization. But our results showed that candida colonization was common in female because we found that colonization more common in napkin and genital organs and in these sites infection is more common in female and we found that no significant diference in premature rupture of membrane .

(**Mahieu et al.(10)** reported that low birth weight and vaginal birth are risk factors for neonatal colonization. the number of sites colonized with Candida at birth contributes to neonatal nosocomial candidemia.

Saiman et al.(18) found that colonization of the gastrointestinal tract was associated with low gestational ages, use of antibiotics - especially third-generation cephalosporin -, presence of central venous catheter, and use of intravenous lipids and H2 blockers . Suppression of the normal gastrointestinal flora due to treatment with cephalosporins, especially third-generation ones, and delayed enteral nutrition were associated with an increased colonization by Candida parapsilosis Early colonization is associated with Candida albicans and, although horizontal transmission can occur, vertical transmission seems to be a more common route of infection Late colonization is associated with Candida parapsilosis, and horizontal transmission seems to be more common.

The neonate is susceptible to neutropenia during bacterial infections, particularly with fulminant sepsis. Physiologically this is due to three factors: the small marrow pool of polymorphonuclear neutrophils (PMNs); the extensively rapid release of the PMN storage pools; and the PMN production in response to sepsis is diminished **Strauss(19**)
and a major reason for the neutropenia may be the deficiency of G-CSF and GM-CSF. Therefore, treatment with G-CSF or GM-CSF seems rational. **Bedford Russell et al(20**).

G-CSF increases circulating neutrophils by increasing the release of immature neutrophils from the bone marrow and by increasing production of new neutrophils from progenitor cells. Early case reports for its use were promising because G-CSF seemed to reliably increase neutrophil counts in neutropenic and septic neonates.(20)

. The study by(**Ahmed et al(21**) was designed to determine whether recombinant human granulocyte colony-stimulating factor (rhG-CSF) affects absolute neutrophil count (ANC), phagocytic function, and oxidative burst in neutropenic VLBW neonates. 14 ventilated VLBW neonates were treated with rhG-CSF (10 microg/kg/day x 3 days i.v.). Phagocytic activity and oxidative burst were assessed before and after treatment

with rhG-CSF These results suggest that other disease-specific factors delay full functional recovery even after rhG-CSF treatment. they speculate that septic neonates may remain susceptible to infection due to deficient neutrophil-killing capacity, even though their ANC returns to normal ranges, Augmenting immune function beyond the immediate period of ANC recovery .

Our results showed no significant difference in duration of hospital stay and survival between groupIA receiving rhGCSF and group IB receiving placebo **Miura,et al(22**) found in their studies, no survival benefits were shown and mortality did not improve . **Ahmed et al(23**) found that Administration of rhG-CSF to premature neonates with the clinical diagnosis of early-onset sepsis was associated with lower incidence of nosocomial infection over the ensuing three weeks period, but it did not change the overall mortality rate.

Ohlsson and Lacy (24) study leads them to suggest that the lack of benefit from prophylactic G-CSF might indicate the critical importance of other aspects of the neonate's immature immune system. Possible cofactors are a deficiency of opsonins, although prophylactic immunoglobulin trials have not had a clinically useful effect on sepsis rate or associated mortality. Alternatively, the almost complete absence of interferon gamma production by preterm neonate peripheral blood lymphocytes might indicate a mechanism underlying their inability to control systemic bacteraemia in the presence of normal neutrophil numbers

Bernstein et al(25)do a recent meta analysis of five studies involving 73 G-CSF recipients and 82 control subjects demonstrated mortality was lower among the G-CSF recipients ($P < 0.05$).. Although routine use of G-CSF cannot be recommended at this time, its use in the neutropenic patient (neutrophil count < 500 μL) that does not respond to IVIG should be considered at a dose of 10 μg/kg/day until neutrophil counts rise above 1,000/mm3. **Gathwala et al(26)** found that Preterm neonates with sepsis and neutropenia treated with rhG-CSF adjunctive therapy have decreased all-cause mortality at discharge and a quicker recovery of their total leucocyte and ANC. **Kocherlakota** et al(27) reported that it seems reasonable to consider rhG-CSF therapy in septic neonates with persistent neutropenia (>24 hours) in an attempt to improve their overall chances for survival The 10% mortality in their rhG-CSF treatment group was lower than in the matched conventionally-treated control patients (55%, $P < .03$).

Our result showed that C.albicans is the most commonly isolated species in colonized or infected infants (66.6%)followed by C.tropicalis (25%)and C .glabrata(8.4%) these results are in agreement with**(17)**who found that C. albicans is the most commonly isolated species in colonized or infected infants C. albicans (45.9%) was the most common species isolated from preterms followed by C. glabrata and C. tropicalis (21.6% each) and C. parapsilosis (8.1%).

Candida albicans accounted for 90% of the positive blood cultures and C. tropicalis accounted for the remaining 10% and mortality rate associated with these infections is 20–50% and occurs among all ages. In their report, the mortality rate was 34%**María** et al(**16**)

Catherine (12) reported that. Candida albicans was the commonest species that colonized these neonates with the rectum and groin being the most frequently colonized sites. In their study candidemia was not found. Although mortality among Candida colonized preterm neonates was high (30%) especially among those with multiple body sites colonized.

Our results in the 1st week showed rectal colonization is the most common 6 site(31%)followed by ear canal colonization 5 sites(26%) followed by 4

sites colonization in the oral and urine culture(21%) and blood culture 0. .

In the 2nd week rectal colonization is the most common 8 sites (44.4%)followed by ear canal colonization ,oral and blood culture 3sites(16.6) for each followed by 1 times colonization in urine culture(5.5%) these results in agreement with **Baley et al(6)** who found that the gastrointestinal tract is one of the first sites of fungal colonization and **Saiman et al(8**) found in a prospective study involving 2,157 infants whose gastrointestinal tracts were cultured on admission and weekly until discharge found the rectum to be the most frequent site of colonization.

Catherine (12) Study evaluating neonatal GI colonization have shown that 4.8% to 10% of infants will harbor a strain of Candida on admission to the nursery Candida albicans is the most prevalent fungal pathogen in neonatal disease, yet the incidence of infection caused by other Candida species, particularly C parapsilosis and C glabrata, has also increased dramatically.

CONCLUSION

We found that there is a significant increase in overall Candida colonization among neutropenic and non netropenic groups in the 2nd week, there is 9 candida positive cases in neutopenic group against 3 cases in non neutropenic group ($P< 0.04$)and a significant increase in No.of colonized sites in neutropenic group ($P<0.05$). Colonization occurred in our study in 12 preterm from 44preterm which represent (28%) ofVLBW.After treatment with G CSF changes in ANC at day 1, day 3 and day 7 of treatment among both study and control The timing of the changes in the ANC in the rhG-CSF-treated neonates occurs sooner and remains longer than in the conventionally-treated control neonates but after correction of neutropenia either with treatment or spontaneously not able to clear candida colonization because there is another risk factors affect colonization . in our study we found that risk factors of candida colonization are significant in colonized group (female sex, Gest . Age ,birth weight,H2 Blockers , intubations ,normal vaginal delivery, antenatal steroids and 3rd generation cephalosporin) so we consider neutropenia is independent risk factor for candida colonization

But we note the effect of G CSF on intensity of colonization 3 cases with high grade colonization improved to low grade of colonization

RECOMMENDATIONS

-Treatment of neutropenia with G CSF correct ANC sooner and remains longer and decreases the intensity of colonization

- Proper antenatal care to decrease the antenatal risk factors for candida colonization and decrease prematurty

- Proper postnatal care to decrease the postnatal risk factors for candida colonization and neutropenia like sepsis.

-Research for another treatment modalities for sepsis and neutropenia like gamma globulins granulocytes transfusions and using anti fungal drugs we recommend antifungal treatment in the 2nd week because there is three cases of candidemia in the 2nd week.

-Further larger studies to determine other risk factors and preventive measures for candida colonization and neutropenia.

REFERRENCE

1-Kaufman D.A and P. Manzoni, Strategies to prevent invasive candidal infection in extremely preterm infants, Clin Perinatol 37 (2010), pp. 611–628.

2-Kaufman D. and K.D. Fairchild, Clinical microbiology of bacterial and fungal sepsis in very-low-birth-weight infants, Clin Microbiol Rev 17 (2004), pp. 638–680.

3-Pfaller M.A. and D.J. Diekema, (2010) Epidemiology of invasive mycoses in North America, Crit Rev Microbiol 36, pp. 1–53.

4-Guery B.P., M.C. Arendrup, G Auzinger, E. Azoulay, M. Borges Sá and E.M. Johnson et al(2009)., Management of invasive candidiasis and candidemia in adult non-neutropenic intensive care unit patients: Part I. Epidemiology and diagnosis, Intensive Care Med 35, pp. 55–62.

5-Lilic D. and K.C. Haynes In: G.D. Brown and M.G. Netea, Editors, (2007) Immunology of fungal infections, Springer, Dordrecht p. 359–80.

6-Baley JE, Kliegman RM, Boxerbaum B, Fanaroff AA (1986) Candida colonization in the very low birth weight infant. Pediatrics 78:225 .

7-Manzoni, P Daniele Farina, Cesare Monetti, Claudio Priolo, MariaLis Leonessa, Chiara Giovannozzi,GiovannaGomirato(2007):Earl y-onset neutropenia is a risk factor for Candida colonization in very low-birth-weight neonates Diagnostic Microbiology and Infectious Disease 57 77 – 83.

8-Chiesa C, Natale F, Pascone R, Osborn JF, Pacifico L, Bonci E, De Curtis M(2011): C reactive protein and procalcitonin: Reference intervals for preterm and term newborns during the early neonatal period.Clin Chim Acta. 12;412(11-12):1053-9. Epub 2011 19.

9-Cheesbrough M. (1991): Biochemical test to identify bacteria. In: Cheesbrough M., ed. District practice in tropical countries. Cambridge University Press, UK Chapter 2:52-60.

10-Mahieu LM, Van Gasse N, Wildemeersch D, Jansens H, Ieven M.(2010) Number of sites of perinatal Candida colonization and neutropenia are associated with nosocomial candidemia in the neonatal intensive care unit patient.Pediatr Crit Care Med.;11(2):240-5.

11-Manzoni P, Daniele Farina, , MariaLisa Leonessa, , Elisa Antonielli d'Oulx, , Paolo Galletto, , Michael Mostert, , Roberto Miniero, and Giovanna Gomirato, (2006); Risk Factors for Progression to Invasive Fungal Infection in Preterm Neonates With Fungal Colonizationa PEDIATRICS 118. 6, pp. 2359-2364.

12-Catherine M. Bendel (2003)Colonization and epithelial adhesion in the pathogenesis of neonatal candidiasis : 27, 5, , p357-364.

13- Carr R, , , Brocklehurst P Caroline J Doré Neena Modi (2009) Granulocyte-macrophage colony stimulating factor administered as prophylaxis for reduction of sepsis in extremely preterm, small for gestational age neonates (the PROGRAMS trial): a single-blind, multicentre, randomised controlled trial Lancet; 373, 9659Pages 226-233 .

14-David K Wagner and Peter G Sohnle (2004)Candida, Infection and Immunity, Encyclopedia of Immunology (Second Edition)
Pages 409-411.

15-Yoshimasa Yamamoto, Thomas W. Klein, Herman Friedman, Sadao Kimur and Hideyo Yamaguchi 1993: Granulocyte colony-stimulating factor potentiates anti-Candida albicans growth inhibitory activity of polymorphonuclear cell FEMS Immunology and Medical MicrobiologyVolume 7, Issue 1, 15-22

16-María L. Avila-Aguero, Alejandro Canas-CotoRolando Ulloa-GutierrezMarco CaroBraulio Alfaro and María M. Paris (2005) Risk factors for Candida infections in a neonatal intensive care unit in Costa Rica International Journal of Infectious Diseases:9, 2, 90-95.

17-Mendiratta, DK V Rawat1, D Thamke1, P Chaturvedi2, S Chhabra3, P Narang(2006); Candida colonization in preterm babies admitted to neonatal intensive care unit in the rural setting: Indian Journal of Medical Microbiology Volume : 24 | Issue : 4 Page : 263-267.

18-Saiman, L. E. Ludington, J.D. Dawson, J.E. et al. (2001): Risk factors for Candida species colonization of neonatal intensive care unit patients, Pediatr Infect Dis J 20, pp. 1119–1124.

19-Strauss, R. (2000). Blood banking and transfusion issues in perinatal medicine. In: R. Christensen, Editor, Hematologic Problems of the Neonate ((ed 1)), Saunders, Philadelphia, PA.

20-Bedford Russell, A.J. Emmerson and N. Wilkinson et al., A trial of recombinant human granulocyte colony stimulating factorfor the treatment of very low birthweight infants with presumed sepsis and neutropenia, Arch Dis Child Fetal Neonatal Ed 84 (2001), pp. F172–F176

21-Ahmed S.,Mustafa A.S., Khan Z., etal.(2004):PCR-enzyme immunoassay of Rdna in the diagnosis of candidemia and comparison with amplicon detection by agarose gel electrophoresis.Int.J.Medical
Microbiology 294:45-51.

22-Miura E, R.S. Procianoy and C. Bittar et al., (2001): A randomized, double-masked, placebo-controlled trial of recombinant granulocyte colony-stimulating factor

administration to preterm infants with the clinical diagnosis of early-onset sepsis, Pediatrics 107, pp. 30–35.

23-Ahmad A, Laborada G, Bussel J, Nesin M 2002:Comparison of recombinant granulocyte colony-stimulating factor, recombinant human granulocyte-macrophage colony-stimulating factor and placebo for treatment of septic preterm infants. Pediatr Infect Dis J. 2002;21(11):1061-5.

24-Ohlsson A and JB Lacy, (2004) Intravenous immunoglobulin for preventing infection in preterm and/or low birth weight infants, Cochrane Database Syst Rev 1 CD000361.

25- Bernstein, H.M .B.H. Pollock and D.A. Calhoun et al., (2006): Administration of recombinant granulocyte colony-stimulating factor to neonates with septicemia: A meta-analysis, J Pediatr 138 (2001), pp. 917–920.

26-Gathwala G, Walia M, Bala H, Singh S. (2011):Recombinant Human Granulocyte Colony-stimulating Factor in Preterm Neonates with Sepsis and Relative Neutropenia: a Randomized, Single-Blind, Non-placebo-controlled Trial JTrop Pediatr. [Epub ahead of print] abstract pubmed.

27-Kocherlakota P and Edmund F. La Gamma(1997) Human Granulocyte Colony-stimulating Factor May Improve Outcome Attributable to Neonatal Sepsis Complicated by Neutropenia .PEDIATRICS Vol. 100. 1 p. 66.