IMPACT OF EARLY DETECTION OF BIOFILM PRODUCING *CANDIDA KRUSEI* IN VLBW PREMATURE TRIPLETS

Pooja Nareda [1],Eshank Gupta[2], Vishakha Ashopa [3],Usha Verma[4],PrabhuPrakash[5]

1 Second Year Resident ,Microbiology,Dr SNMC,Jodhpur
2,3,4 Senior Demonstrator, Microbiology,Dr SNMC,Jodhpur
5 Professor , Microbiology,Dr SNMC,Jodhpur
Corresponding Author: Dr. Prabhu Prakash email:dr.prabhuprakash@gmail.com mobile no.- 9413322110

ABSTRACT

Candida is leading cause of Blood Stream Infection(BSI) in premature and very low birth weight (VLBW) neonates and is associated with very high mortality rate. Amongst Non albicans candida(NAC) ,*Candida krusei* infection is difficult to treat due to their inherent resistance to azoles. In biofilm producing Candida isolates this challenge can be overcome by removing intravenous catheters only. In present case very low birth weight (VLBW) premature triplet babies 2 female children and one male child birth weight 1.1kg, 980gms, 920gms were referred after 48 hours of birth with history of respiratory distress, septic shock and Necrotising Enterocolitis (NEC). CRP was positive and PCT was 96.83 ng/ml,16.63 ng/ml and 12.61ng/ml in triplets respectively. Biofilm producing Candida *krusei* was isolated from blood ,urine and central line tip culture by using SDA, Candichrome ,0.1% Glucose Agar and Congo Red Agar medium. Isolate were sensitive with Amphotericin B .All babies responded to treatment and discharged healthy after 45 days of hospitalization with body weight i.e 1.370Kg , 1.360 kg and 1.195 Kg respectively .

Keywords :- *Candida krusei*, Necrotising enterocolitis ,Very low birth weight, Blood stream infections

CASE REPORT : Very low birth weight (VLBW) premature triplet babies (2 female and one male) ,after

48 hours of their birth by LSCS in a private hospital were reffered to tertiary level hospital with complaints of respiratory distress, Necrotising Enterocolitis (NEC) and septic shock . Their mother was a case of primary infertility and conceived after 9 years of her married life. She was regular in ANC follow up and fully immunized . There was no past history of diabetics, tuberculosis orisiosion and her HIV, HbsAg and VDRL were nonreactive. At 28 weeks of gestation the patient complaint of leaking pervagina and abdominal pain for 1 month not responding to treatment hence LSCS was done at 28 weeks of gestation and premature triplets were delivered.

First female child was diagnosed with respiratory distress syndrome with septic

shock. On investigation total bilirubin and direct bilirubin were 6.34mg/dl and 0.65mg/dl respectively and Alkaline phosphatase 370 U/L , SGOT-22U/L , SGPT-10 U/L. Urea was 40 mg/dl, Creatinine was 0.94 mg/dl ,Calcium 8.2 mg/dl , Na+ 143 , K+ 4.6, sepsis markers CRP and PCT were positive(>6mg/L) and 96.83ng/ml respectively which showed sepsis and antibiotics Ampicillin (200 mg/kg/day) and Amikacin (4 mg/kg/day) were given .

Blood culture was done on 8th day of admission in Bactac Peds Plus culture vials [Figure 1] and blood sample was collected from central line taking all aseptic precaution. After 24 hours of incubation blood culture flagged positive, on direct gram staining yeast bodies were identified ,sub culture was done on SDA and Candichrome Agar [Figure 2,3] and preliminary reporting was done to clinician with a request to start empirical anti fungal drugs.

In urine culture done on day 8 th of admission , direct wet microscopic examination yeast bodies with pseudo hyphae were seen ,fungus culture was done on SDA and Candichrome Agar .Empirically Fluconazole was started as antifungal drug .After 24 hours of incubation white 2-3 mm colonies were grown on SDA , by Grams stain yeast bodies were seen .Phenotypic identification of yeast was done by 0.1% Glucose Agar and Candichrome agar [11,12,13]and antifungal sensitivity test was done by disc diffusion method using CLSI guidelines. it was identified as *Candida krusei* which was resistant to azole drugs and sensitive with AmphoB. Liposomal amphotericin B (5 mg/kg/day) was given as antifungal treatment.

On follow-up investigation on day 15th CRP was negative (<6mg/L) and PCT came down to 75.28ng/ml, on day 30 th there was decrease in PCT 17.20 ng/ml and leucocytic counts came in normal range. Patient was discharged healthy on 45 days of admission with improvement in birth weight (1.370kg)

Second male child was diagnosed with respiratory distress syndrome with NEC (Necrotising enterocolitis) and had very low birth weight i.e.980 gm. All routine investigations were done, CRP was positive (> 6 mg/L) and PCT was 16.63ng/dl but blood and urine culture were sterile.

On day 14th baby had an apnoeic episode in the morning and PCT was 6.31ng/dl. Stool examination show presence of occult blood. 2D ECHO show (S,D,S) Laevocardia , moderate PDA (1.5mm) ,L>R , PPG/ED 40/21mmhg.LA/W dilated. LA/A0 1.5, No PAH with good biventricular function. On day 19th central line tip culture showed positive for *C. krusei*, on further testing for biofilm testing on Congo Red medium it was biofilm producing , clinician were advised to change intravenous canula and patient was treated with Liposomal Amphotericin B.

On follow-up investigation on day 15th CRP was negative (<6mg/L) and PCT came down to 6.13ng/ml. Patient was discharged healthy on 45 days of admission with improvement in birth weight (1.360kg)

Third female child was diagnosed with respiratory distress syndrome and extremely low birth weight i.e. 920gm. On day 14th child had apnoeic episode in morning. On routine investigation CRP (>6mg/L) and PCT (12.61 ng/ml) were positive but blood and urine culture were sterile. *Candida krusei* was isolated in central tip culture ,patient was treated with Liposomal Amphotericin B . Follow up investigation on day 15,30 and 45 all culture were sterile and on day 25 Child was shifted to her mother from PICU. After 45 days of hospital stay body weight increased 1.195 kg and was discharged healthy.

Observations:-

S.N.	Observation	Baby1(female)	Baby2 (male)	*Baby 3 (female)*
1.	**At time of admission**			
	Birth weight	1.1 kg	0.980 kg	*0.920 kg*
	CRP(mg/L)	Positive	Positive	Positive
	PCT(ng/ml)	96.83 ng/ml	16.63 ng/ml	12.61 ng/ml
	Urine culture	*C. krusei*	Sterile	Sterile
	Blood culture	*C. krusei*	Sterile	Sterile
	Central line tip	Sterile	Not received	*C. krusei*
2.	**After 15 days**			
	Birth weight	1.150 kg	1.12 kg	1.1 kg
	CRP(mg/L)	Negative	Negative	Negative
	PCT(ng/ml)	75.28 ng/ml	6.13 ng/ml	2.62 ng/ml
	Urine culture	*C. krusei*	Sterile	Sterile
	Blood culture	*C. krusei*	Sterile	Sterile
	Central line tip	Sterile	*C.krusei*	Sterile
3.	**After 1 month**			
	Birth weight	1.320kg	1.360kg	1.195kg
	CRP(mg/L)	Negative	Negative	Negative
	PCT(ng/ml)	17.20 ng/ml	1.26 ng/ml	0.61 ng/ml
	Urine culture	Sterile	Sterile	Sterile
	Blood culture	Sterile	Sterile	Sterile
	Central line tip	Sterile	Sterile	Sterile
4	**After 45 days**			
	Birth weight	1.320kg	1.360kg	*1.195kg*
	CRP(mg/L)	Negative	Negative	Negative
	PCT(ng/ml)	Negative	Negative	Negative
	Urine culture	Sterile	Sterile	Sterile
	Blood culture	Sterile	Sterile	Sterile
	Central line	Sterile	Sterile	Sterile

Table/Fig 1: Showing comparative reports of triplets

Figure 1-Candida krusei growth on Candichrome Agar

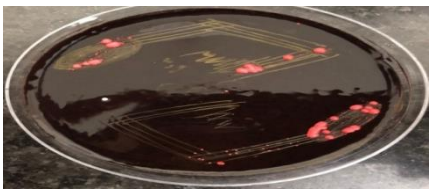

Figure 2 Biofilm production on CongoRed Medium

Figure 3 Triplet in PICU

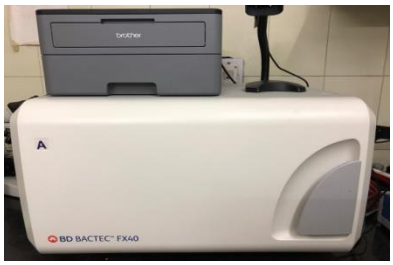

Figure 4 Bactac BD Machine for Blood Culture

Figure 5 Machine for PCT detection

Discussion: - Candida is the fourth leading cause of blood stream infections in hospitalized patients, with an associated mortality of 40–50% [1]. Invasive candidiasis refers to systemic infection with *Candida* of either vital organs or normally sterile body fluid like blood, cerebrospinal fluid (CSF) or urine [2].

Risk factors for Candida blood stream infections (BSI) in neonates may be due to prematurity and very low birth weight (VLBW), use of contaminated milk bottles, parenteral nutrition, glycerin suppositories, long term indwelling vascular devices, contaminated intravenous fluids, poor hand hygiene of health care workers etc. [3,4,5,6].*Candida krusei* has been recognized as a multidrug resistant and difficult to treat fungal pathogen due to inherently resistance

to azoles and decreased susceptibility to flucytosine and amphotericin B [7].

In present case of VLBW preterm triplet, suffering from respiratory distress, septic shock and necrotising enterocolitis (NEC) . C.*krusei* was isolated in blood culture (by Bactac),urine culture and intravenous tip culture .Candida species identification was done by 0.I% Glucose Agar [8,9,10], Candichrome agar[11] and was identified as C.*krusei* which was biofilm producing on Congo Red Agar [12] and sensitive with Amphotericin B and resistant to azoles [7].Triplets were completely cured by systemic Liposomal Amphotericin B therapy and discharged healthy after 45 days of hospitalization.

Preterm neonates are at high-risk of morbidity and mortality from invasive *Candida* disease and earlier treatment of *Candida* sepsis, including central vascular catheter removal, may be associated with an improved out come . Emergence of non albicans candida (NAC) as a common cause of candidemia has been reported by previous Indian studies[4].

Prevalence of non albicans candida (NAC) like Candida parapsilosis and Candida krusei are being increased [4,16].Biofilm-producing yeasts cells acquire an increased resistance to antifungal agents, often leading to therapeutic failure and chronic infection [12]

Levy l *et al* reported 58.3% of VLBW neonatal candidemia cases were having persistent candidemia [17], This observation was in concordance with study reported persistent candidemia in 52% of infants[7].

Fridkin *et al* in decade study reported 1.52% incidence of BSI with various candida spp , majority of cases were seen in VLBW newborn (73.71%) in which only three cases of *C. krusei* were reported and all were azole sensitive[5].

Wanjari et al. reported BSI infection by *Candida krusei* in a 28 weeks, LBW, premature baby suffering from congenital tuberculosis and was completely cured with antifungal therapy as on repeat blood culture reports were negative[16,19].

Selma Amaral Lopes et al reported *Candida krusei* septicemia in neonates and due to early initiation of antifungal therapy by Amphotericin B there was good clinical response [15] .

In present case of premature VLBW triplets as C. *krusei* fungemia was diagnosed and all newborn were managed by Amphotericin B therapy with good clinical outcome without any mortality or morbidity.

Conclusion-To reduce infant mortality rate rapid diagnosis of fungemia ,identification of causative agent and appropriate management plays pivotal role in preventing mortality and morbidity amongst VLBW premature triplet

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