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STUDY OF RISK FACTORS FOR *CANDIDA* SPECIES COLONISATION OF NEONATAL INTENSIVE CARE UNIT PATIENT

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ABSTRACT

Purpose :
Candida spp. are increasingly important pathogens in neonatal intensive care units (NICU). Prior colonisation is a major risk factor for candidemia, but few studies have focused on risk factors for colonisation, particularly in NICU patients

Methods:
A prospective study was performed in NICU to determine risk factors for *Candida* colonisation. Sample of blood was taken and swabs from various body sites were collected on day 0 (within 24 hrs. of birth), 48 ± 12 hrs., 72 ± 12 hrs., postnatal day 5, postnatal day 7 and once weekly thereafter till and were cultured .

Results: The prevalence of *Candida* spp. colonisation was 27.5% (302 of 1098 neonates). Of 639 isolates, 50.23% were of *C. albicans* followed by 31.14% of *C. tropicalis*, 6.4% of *C. glabrata* and 12.2% of *C. parapsilosis*. Use of central venous catheters, multiple antibiotics, endotracheal intubation were risk factors for development of colonisation whereas delivery by caesarean section was protective.

Conclusions: We speculate that in NICU patients, use of multiple antibiotics, steroids and IV catheters alter ecology of all body systems, thereby facilitating colonisation of *Candida* spp.

KEYWORDS

Colonisation, *Candida*, risk factors

INTRODUCTION

The incidence of fungal infections has increased drastically in the past 20 years. A consequence of our success with the discovery of antimicrobial agents and improved medical care is the increase in the number of fungal infections. Advances in neonatal management have led to considerable improvement in newborn survival. *Candida* spp., though saprophytic, have risen in importance as opportunistic pathogens as a consequence of modern therapeutic modalities.[1] Most neonatal fungal infections are due to *Candida* spp., particularly *C. albicans*. *C. parapsilosis* and *C. tropicalis* are other species getting notorious in NICU outbreaks.[2] Colonisation of neonatal skin and gastrointestinal tract is the first step in pathogenesis of invasive candidiasis.[3] This has been particularly well demonstrated for patients at high risk to develop invasive candidiasis.[3,4] Colonisation of the infant occurs early in life and this is affected by a variety of common practices in neonatal intensive care unit.[5] In a one year study conducted by Baley et al, fungal colonisation rate in very low birth weight (<1500 g) infants was found to be 26.7%.[6] Borderon et al demonstrated colonisation with *Candida* spp. in 23 out of 791 newborns admitted over a period of one year.[7] Heljic et al in 2005 reported the rate of colonisation in neonates in NICU as 14.8% and the patients colonised or infected by *Candida* spp. in their study were very preterm newborns or compromised term newborns, with congenital abnormalities or gastrointestinal surgical interventions.[8] Colonisation of neonates in NICU by *Candida* is a problem and needs attention, especially in the patients with identified risk factors to avoid their dissemination and causation of life threatening infections. Therefore, the present prospective study was undertaken to identify the time and site of colonisation by *Candida* spp in neonates admitted to the NICU and, to establish the possible risk factors and to estimate the development of candidaemia in colonised neonates.

MATERIALS AND METHODS

Study center

This study was conducted in the Department of Microbiology, in association with Department of Paediatrics in a teaching tertiary care hospital for a period of one year (May 2009 to April 2010).

Patients

This prospective study included all the neonates who were admitted to the NICU for atleast 7 days. Detailed clinical history was noted which included risk factors like low birth weight, using multiple antibiotics, with central/peripheral catheter, prematurity, parental hyperalimentation, intravenous fat emulsion, with endotracheal tube, congenital malformation and any GI disease etc.

Samples and mycological investigations

Swabs from various sites (including oral, rectal, umbilical, axillary and groin), sample of urine and endotracheal aspirates were collected from all the neonates admitted to NICU on day 0 (within 24 hrs. of birth), 48 ± 12 hrs., 72 ± 12 hrs., postnatal day 5, postnatal day 7 and once weekly thereafter till discharge. Samples from those neonates were included in study who had stay of more than 7 days. Two mL of venous blood was collected for culture under aseptic condition. After taking the sample of blood, it was inoculated immediately into two sets of biphasic media containing brain heart infusion agar and broth. Out of the two biphasic media containing brain-heart infusion agar and broth inoculated with blood of neonates, one was incubated at 37°C and other at 25°C. Subcultures were performed after 24 hrs., 48 hrs. and 72 hrs. upto seven days for any yeast growth. All the samples except blood were inoculated in duplicate on to 5% sheep blood agar, Sabouraud's dextrose agar (SDA), SDA with antibiotics gentamicin and chloramphenicol. The inoculated blood agar plates were incubated at 37°C for 48 hours and

inoculated SDA were incubated at 25°C for 7 days. All the cultures were examined daily for growth of any yeast. Isolates of *candida* were identified on the basis of germ tube test, morphology on corn meal agar , color on Hi Crome *Candida* agar (Hi media), carbohydrate fermentation test and carbohydrate assimilation test.[9] Urine of those neonates was included which showed budding yest cells on direct microscopy. Isolation from each site was considered once only, even if repeatedly positive.

RESULTS

Demographic characterstics

A total of 1098 neonates who had stay of atleast 7 days in NICU were included in the study group. Mean age of neonates admitted was 13.3 days. Mean gestational age was found to be 36.7 weeks. Out of 1098 neonates, 660 (60.1%) neonates were premature and 680 (61.1%) neonates were born by vaginal delivery.

Colonisation of candida spp.

Out of the total 1098 neonates *Candida* spp. was isolated from various sites like oral, rectal, umbilical, axillary and groin, urine sample and endotracheal aspirates (if neonate was intubated) in 302 (27.50%) neonates. Maximum number of colonised neonates 148 (49%) were of age group 3-6 days followed by 72 (23.84%) in the age group of 11-20 days, 55(18.21%) in the age group of 7-10 days and 27 (8.9%) of the age group 21-30 days. Male to female ratio among colonised neonates was found to be 1.04:1. Among the 660 preterm neonates, colonisation was observed among 220 (33.3%) neonates whereas in 438 term neonates 82(18.71%) had colonisation at various sites and the difference was significant.

Time and site of colonisation

Among 302 neonates 78 (25.8%) were found to be colonised in <24 hrs. period, 162 (53.64%) neonates by day 3, 193 (63.91%) by day 5 and 302 (100%) neonates were colonised in period of >7 days.(Table1) Rectum was the most commonly involved site (90.06%) followed by groin (54.96%) , oral site(39.73%), axillary (19.2%) and umbilical site were involved in (3.31%) of neonates. Among the babies who got colonised within 24 hrs. of birth, at day 3 & 5, rectum (49.09%) was the most common site involved. But at day 7, groin (33.9%) was commonly involved site followed by rectum (27.82%). Maximum isolation from urine was observed in neonates with >7 days of admission. (Table2)

TABLE 1

DISTRIBUTION OF COLONISED NEONATES WITH RESPECT TO TIME OF COLONISATION

Time of Colonisation	No. of neonates colonised	Cumulative colonisation
< 24 hours	78 (25.82%)	78(25.82%)
Day 3	84 (27.81%)	162 (53.64%)
Day 5	31 (10.2%)	193(63.91%)
Day 7	32 (10.5%)	225 (74.5%)
> 7 days	77 (25.49%)	302 (100%)
Total	302	302

TABLE 2

TIMEWISE DISTRIBUTION OF CANDIDA ISOLATES FROM VARIOUS SITES

Time	Rectal	Oral	Groin	Axillary	Umbilical	Urine	Endotracheal tube
<24 hrs.	54 (49.0%)	20 (18.18%)	25 (22.72%)	10 (9%)	1 (0.9%)	0	0
Day 3	65 (47.10%)	28 (20.2%)	30 (21.7%)	12 (8.6%)	1 (0.7%)	2 (1.4%)	0
Day 5	59 (45.38%)	19 (14.6%)	38 (29.2%)	9 (6.9%)	2 (1.5%)	3 (2.3%)	0
Day 7	32 (27.82%)	25 (21.7%)	39 (33.9%)	3 (11.3%)	3 (2.6%)	1 (0.8%)	2 (1.7%)
>7 days	62 (42.46%)	28 (19.1%)	34 (23.28%)	14 (9.5%)	3 (2%)	4 (2.7%)	1 (0.6%)

Species isolated

A total of 639 isolates were recovered from colonised neonates from various sites. Maximum number of isolates 321 (50.23%) were that of *C. albicans* followed by 199 (31.14%) of *C. tropicalis*, *C. glabrata* 41 (6.4%),

and 78 (12.2%) of *C. parapsilosis*. *Candida albicans* was isolated maximally from rectal site i.e in 52.02% neonates followed by groin (21.8%), oral (17.4%), axillary (4.36%), umbilical and urine (1.86% each) . *C. tropicalis* was isolated most commonly from groin (34.6%) followed by rectal (30.65%) oral site (24.12%).(Table3)

Table 3
SITEWISE DISTRIBUTION OF DIFFERENT SPECIES OF CANDIDA IN COLONISED NEONATES

Species	No. of Isolates	Rectal	Oral	Groin	Axillary	Umbilical	Endotracheal aspirate	Urine
C.albicans	321	167	56	70	14	6	2	6
C.tropicalis	199	61	48	69	15	3	1	2
C. glabrata	41	12	6	11	12	0	0	0
C.parapsilosis	78	32	10	16	17	1	0	2
Total	639	272	120	166	58	10	3	10

Risk factors

On studying the risk factors among 302 colonised neonates, it was observed that 220 (72.84%) were premature and parentral hyperalimentation was seen in 185 (61.25%) neonates. History of multiple antibiotics was recorded in 243 (80.46%) neonates and intubation was done in 74 (24.5%) neonates.

Comparison of risk factors among preterm colonised and preterm non colonized

(Table 4)

When the factors influencing colonisation among 660 preterms were studied, it was found that among 338 male preterms, 111 (32.84%) get colonised whereas 109 (33.85%) of female preterms get colonised but the difference was not significant. Significant difference in rate of colonisation was observed among the neonates borne by vaginal and caesarean section. Use of multiple antibiotics was observed among 328 preterm neonates and of which 61.87% colonised whereas 38.10% were not colonised and the relative risk between two was 1.624 which suggests positive association of use of multiple antibiotics in development of colonisation. On comparing the two groups p-value was <0.05 which is statistically significant. Combination of antibiotics commonly used in preterm colonised were β lactams with aminoglycosides, β lactam + β lactamase inhibitors and aminoglycosides, monobactam with quinolones and one cephalosporin. In preterms who were not colonised, the type of antibiotics used were combination of β lactam (usually cephalosporins) and aminoglycosides. Relative risk for the use of steroid in development of colonisation was found to be 1.66 which indicates positive association. Also comparison was done between two groups who get colonised and who did not develop colonisation after steroid therapy and p-value was found to be <0.05 which is statistically significant. Of the 34 episodes of candidemia in preterm, 30 (88.23%) were found to be colonised. Relative risk in neonates who were intubated and developed colonisation was 0.703 which indicates no positive association and also the diifference was not statistically significant.

Table 4
FACTORS INFLUENCING COLONISATION IN 660 PRETERM NEONATES

	Factors	Yes (220)	No (440)
1.	**Sex**		
	- Male (338)	111 (32.84%)	227 (67.15%)
	- Female (322)	109 (33.85%)	213 (66.14%)
2.	**Type of delivery**		
	- Vaginal (365)	135 (36.9%)	230 (63%)
	- Caesarean (295)	85 (28.81%)	210 (71.18%)
3.	**H/o Multiple antibiotics (328)**	203 (61.87%)	125 (38.10%)

4.	**Average duration of antibiotics**	7 days	2 days
5.	**Average number of antibiotics administered**	3	2
6.	**Average duration of antibiotic after which colonisation developed**	4 days	--
7.	**H/o Steroid administration (16)**	10 (62.5%)	6 (37.5%)
8.	**Endotracheal intubation (109)**	45 (41.28%)	64 (58.71%)
9.	**Average duration of Intubation**	6 days	3 days

Table 5
COMPARISON OF FACTORS INFLUENCING COLONISATION IN PRETERM AND TERM NEONATES

	Factors	Preterms (n=220)	Terms (n=82)
1.	**Sex**		
	- Male	111	43
	- Female	109	39
2.	**Birth weight (Mean)**	1213.6 gms	1971.34 gms
3.	**Gestational age (Mean)**	32.18 weeks	39.70 weeks
4.	**Type of delivery**		
	- Vaginal	135 (61.36%)	66 (80.48%)
	- Caesarean	85 (38.63%)	16 (19.51%)
5.	**IV Line use**	220 (100%)	82 (100%)
6.	**H/o Multiple antibiotics**	203 (92.27%)	40 (48.78%)
7.	**Average duration of antibiotics administered**	7 days	5 days
8.	**Average number of antibiotics administered**	3	3
9.	**Average duration of antibiotics intake after which colonisation appeared**	4 days	2 days
10.	**H/o steroid intake**	10 (4.5%)	0
11.	**Endotracheal intubation**	45 (20.45%)	29 (35.36%)

Comparison of risk factors among preterm and term colonised neonates

When the factors influencing colonisation in preterm and term neonates were analysed it was found that relative risk of getting colonised after multiple antibiotic use among preterms is 5.075 than term neonates which shows positive association. Use of endotracheal intubation among preterm colonised neonates was significantly (p-value <0.05) higher than term colonised neonates. Also relative risk of neonates getting colonised after intubation among preterm is 1.55 than term neonates which indicates a positive association. Out of the total 1098 neonates, candidaemia developed in 50 (4.5%) cases. Among the neonates who were colonised (302), progression to candidaemia was seen in 37 (4.55%) neonates whereas among the 796 non colonised neonates 13 (1.63%) led to candidaemia and difference was statistically significant.

DISCUSSION

Candida infections are frequent and major cause of septicemia in neonatal ICUs and they are associated with high morbidity and mortality rates. A number of risk factors are associated with *Candida* infections in neonates, of which colonisation is very important. The gastrointestinal tract is the first to become colonised though multiple site may be involved. All species of candida are known to colonise at different sites. A total of 1098 neonates admitted to NICU over a period of one year were included in our study and of which 302 (27.5%) showed colonisation at various sites. Baley et al[6] and El Mohandes & coworkers[10] reported similar results. Baley et al[6] in one year study period, reported fungal colonisation

rate to be 26.7% whereas El Mohandes & coworkers[10] reported that 19% of VLBW infants were colonised with *Candida* species in NICU. The difference of rate of colonisation may be due to difference in management protocol followed in different centers and rate of yeast carriage in the health personnel of an institute who handle the neonates. Colonisation was found in 25.82% of neonates in first 24 hours. Our results are in accordance with Baley et al[6] and Menderata et al[5] who reported 17.1% and 25.1% colonisation at 24 hours in their studies respectively. However Singh et al[11] observed that acquisition of yeast within 24 hrs. of life occurred in 38% of neonates. This discordance in results might be due to the reason that they did the study on preterm neonates which are more prone to get colonised early in life. In the present study by day seven, 74.5% of neonates were colonised which was similar to Mendirata et al[5] (77.1% by day 7). However it was higher than Baley et al[6] (48.73%) but lower than Singh et al[11] (96%). Baseline fungal colonisation i.e at birth or at day one or two is more influenced by endogenous factors but at day seven or more it is more so affected by exogenous factors like management strategy, use of IV line, use of central venous catheters and nature and intensity of routine antifungal antiseptic measures applied in a particular set-up and thus vary from place to place. Most common site involved among colonised neonates was rectum (90.06%) which was similar to Baley et al[6] and Mendirata et al[5]. Gastrointestinal tract or respiratory tract colonisation are predominant sites of colonisation and furthermore GIT can serve as a reservoir from where fungus can spread, particularly if there is a breach in mucosal integrity. Colonisation of urine was relatively lower than Baley et al[6] (30.8%) and Mahieu et al[12] (15.9%) which is due to the fact that we have considered only those samples in which on direct microscopy, budding yeast cells were seen. Rest of the samples were not considered for further processing. As rectum is the usually the most commonly involved and that also at the earliest. In our study we also observed that among the babies who got colonised within 24 hrs. of birth, rectum (49.09%) was the most common site involved. The most common species of *Candida* isolated in colonised neonates was *C. albicans* (50.23%) followed by *C. tropicalis* (31.14%), *C. parapsilosis* (12.2%) and *C. glabrata* (6.4%). Borderon et al[13], Mendirata et al[5] and Heljic et al[8] also reported the similar results but a shift towads non albicans *candida* is visible from results of present study as well as other authors also. A very important reason for this shift is expanded use of antifungal drugs, not only for therapeutic purpose but prophylactically also.

Risk factors

All the neonates in present study were on IV line and H/O multiple antibiotics was observed among 80.46% of neonates. Use of multiple antibiotics was found in 91.4% and 50% of preterm and term neonates by Mendirata et al.[5] As management strategies in different ICUs vary, so is the frequency and intensity of antibiotic use also. Steroids and antibiotics are known to suppress immune system and their administration to already immune suppressed preterm and low birth weight neonates promote colonisation. On comparison of factors influencing colonisation in preterm neonates, it was found that 36.9% of neonates borne by vaginal delivery get colonised, however 28.81% of neonates borne by caesarean section were colonised and difference between two was statistically significant. Our results were similar to Mendirata et al[5] and Baley et al[6] whereas Singh et al[11] observed highly significant difference of colonisation by two routes of delivery i.e. vaginal (76.9% vs. 76%), caesarean section (23.1 vs. 24%) respectively. In vaginally colonised mothers there are more chances of ascending infection or colonisation in neonates. As vaginal colonisation of mother is an important risk factor for colonisation in neonates, so mandatory screening of all pregnant women for *Candida* irrespective of symptoms should be done and also treatment of all irrespective of vaginal colonisation or infection is recommended to prevent colonisation and subsequent infection in neonates. When the factors influencing colonisation in preterms and terms were compared, a number of them showed significant difference between two. Results of current study were similar to Mendirata et al[5] who also observed significant difference in certain factors like birth weight, gestational age, steroid intake and antibiotics administered among preterm and term neonates. As immune system in preterm neonates is not well developed, so they are more prone to get infections, not only fungal but bacterial also and because of which number and duration of antibiotics administered in these neonates is significantly higher than term neonates. In our study we found that 13.63% of preterm neonates developed candidemia whereas 10.97% of term neonates led to candidemia. However, Mendirata et al[5] observed that 20% of preterm neonates and none of the term neonates developed candidemia.

CONCLUSION

Results of current study shows that a number of risk factors are associated with colonisation and further infection in neonates, prevention of which could have tremendous effect in decreasing the incidence of candidemia cases in NICUs. Despite the evidence for transmission of *Candida* by direct or indirect contact and evidence of cross infection by health care workers, no standard policy of patient isolation measures have been made. Policies regarding patient isolation measures for preventing transmission of *Candida* in neonatal units is suggested to reduce the morbidity and mortality associated with *Candida* infection in NICUs. Also the initiation of antifungal therapy with the first positive blood, urine or CSF culture in critically ill neonates is recommended.

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