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CANDIDURIA: A RELIABLE INDICATOR OF NEONATAL CANDIDEMIA ? AN EXPERIENCE IN A TERTIARY CARE HOSPITAL.

Microbiology

Vandana Sardana MD, Assistant Professor, Department of Microbiology, Subharti Medical College and associated Chhatrapati Shivaji Subharti Hospital, Meerut- 250005

Anita Pandey* MD, Professor & Head, Department of Microbiology, Subharti Medical College and associated Chhatrapati Shivaji Subharti Hospital, Meerut-250005. *Corresponding author

Archana Dubey MD, Professor, Department of Pediatrics, Subharti Medical College and associated Chhatrapati Shivaji Subharti Hospital, Meerut-250005

ABSTRACT

Background & Objectives: Candidemia is a significant cause of nosocomial mortality and morbidity in neonates. Prompt diagnosis and treatment is crucial. Non-*albicans Candida* (NAC) species are assuming an increasing role in nosocomial infections in neonates. The present study was carried out i) to identify and speciate the *Candida* isolates obtained from cases of neonatal candidemia and to assess the risk factors and clinical manifestations associated with it (ii) to evaluate if candiduria, can be considered as a reliable indicator of candidemia.

Methods: The prospective cohort study included the blood samples from 527 clinically suspected cases of neonatal septicaemia which were subjected to automated culture and processed as per standard microbiological techniques. The urine samples from all the 527 neonates were processed by conventional method.

Results: Frequency of neonatal candidemia in culture proven cases of septicemia was 30.1%. Low birth weight was the commonest risk factor, followed by prematurity. Non-*albicans Candida* (NAC) species were isolated from blood in 86.4% cases compared to *Candida albicans* in 13.6%. *Candida glabrata* was the predominant species. Overall, candiduria was found in 54.5% cases of neonatal candidemia with same species of *Candida* isolated both from blood and urine samples. Among the cases of candiduria, NAC species were isolated in 81.7% compared to *C. albicans* in 18.3%. Correlation between candiduria and candidemia was maximum (73.33%) in *C. albicans* followed by *C. tropicalis* (72.41%) and *C. glabrata* (55.81%).

Conclusions: The present study highlights the emergence of NAC species as an important cause of neonatal candidemia from Meerut city: definitely a changing trend. Further, our study also highlights that candiduria may be considered as a reliable indicator of candidemia in neonates.

KEYWORDS

Neonates, Non - albicans *Candida*, candidemia, candiduria

Introduction

Reporting of fungal blood-stream infection and the spectrum of species involved are essential measures in any intensive care unit in order to implement appropriate preventive and therapeutic strategies. Isolation of *Candida* from blood is the gold standard for the diagnosis of candidemia. Candiduria, the presence of *Candida* species in urine, is a common clinical finding, particularly in hospitalized patients with indwelling bladder catheters.[1,2] Candiduria and candidemia occur commonly in neonatal and pediatric ICUs and particularly in premature infants.[3-5] Risk factors for neonatal candidemia include prematurity, use of central venous lines, endotracheal tubes, parenteral nutrition, broad-spectrum antibiotic administration, prolonged hospitalization, abdominal surgery, exposure to H2 blockers, and *Candida* colonization.[6,7,8]) Isolation of Candida from the urine of newborns can be indicative of contamination or of urinary tract infection. Candidal UTI usually develop in an antegrade fashion from sources such as candidemia and renal candidiasis. [9]

Material and Methods

This prospective cohort study was carried out in the Departments of Microbiology and Pediatrics, Subharti Medical College and associated Chhatrapati Shivaji Subharti Hospital, Meerut. The approval from the Institutional Ethical and Research Committee was obtained before conducting the study. Written informed consent was taken from the parents/guardians of the neonates enrolled in this study.

Study population: A total of 527 clinically suspected cases of neonatal septicemia admitted in neonatal intensive care unit (NICU) over a period of one year (October 2016-September 2017), were studied. The study group included neonates: (i) With clinical presentations suggestive of septicemia such as feed intolerance, abdominal distension, failure to thrive, respiratory distress, bleeding tendency, lethargy, convulsions etc. (ii) With risk factors such as low birth weight, use of broad spectrum and/or multiple antibiotics, central venous catheters, prolonged urinary catheterization, parenteral alimentation and intravenous fat emulsion, colonization with *Candida* and/or previous episode of mucocutaneous candidiasis and immunosuppressive therapy.

Sample collection and transportation

Blood Culture: Venous blood approximately 1-2ml was collected with aseptic precautions and transferred to an automated BacT/ALERT pediatric blood culture bottle (BioMerieux).

Urine: Sample was collected by freshly inserted "in and out" catheter with aseptic precautions and was transferred to a sterile universal container.

Samples were transported to laboratory immediately.

Processing

Blood culture: The blood sample inoculated in BacT/ALERT pediatric culture bottle was incubated in an automated microbial detection system (BioMerieux) for up to five days at 37^{0}C. Any growth indicated by colorimetric signal, generated by exciter wavelength, was subcultured on 5% sheep blood agar and Sabouraud dextrose agar slant with antibiotics but without cycloheximide, which was identified later.
Urine: Urine was centrifuged at 2000 rpm for 10-15 minutes. The supernatant fluid was discarded and the following tests were done from the deposit.

(A) Direct examination of urine sample:

i) Wet mount with 10% Potassium hydroxide (KOH): Fungus was identified by morphological characteristics.
ii) Gram stain was done to identify pus cells, bacteria, Gram-positive budding yeast cells with or without pseudohyphae.

(B) Urine culture

Deposit of centrifuged urine (0.01 ml) was inoculated on Saboraud dextrose agar (SDA) with antibiotics but without cycloheximide and incubated at 25°C and 37°C. The culture tubes were examined at 3-4 days interval for growth of yeast colonies.

Identification

Identification of yeast isolate was done by the following methods: colony morphology on SDA, germ tube test (GTT), colony morphology and colour on CHROMagar Candida medium,

microscopic characteristics on cornmeal agar (CMA), pellicle in Sabouraud dextrose broth and sugar fermentation tests.

The various *Candida* species isolated from blood and urine culture were tabulated and correlated and the results were statistically analysed.

Results

Our data suggests the changing trend of *Candida* species in neonatal candidemia in our NICU. NAC species were the predominant isolate from blood in 86.4% (95/110) cases compared to *Candida albicans* in 13.6% (15/110) cases (Table-1)

Table -1: Distribution of non-*albicans Candida* species (NAC) versus *Candida albicans* in neonatal candidemia (n= 110)

Species isolated from blood	No. of cases	Percentage %
Non-*albicans Candida* (NAC)	95	86.4
Candida albicans	15	13.6

Among the isolates, *Candida glabrata* (39%) was the predominant species, followed by *C. tropicalis* (26%), *C. parapsilosis* (14.5%), *C. albicans* (13.6%), *C. guilliermondii* (2.7%), *C. krusei* (1.8%), *C. dubliniensis* (0.9%) and *C. lusitaniae* (0.9%) as shown in table-2

Table-2: Distribution of various *Candida* species isolated from blood in cases of neonatal candidemia(n=110)

Species		No. of Cases	Percentage (%)
Non-*albicans Candida* (NAC) species	*C. glabrata*	43	39.00
	C. tropicalis	29	26.00
	C. parapsilosis	16	14.50
	C. guilliermondii	3	2.70
	C. krusei	2	1.80
	C. dubliniensis	1	0.90
	C. lusitaniae	1	0.90
Candida albicans		15	13.60

All the culture proven cases of neonatal candidemia were investigated for the presence of candiduria by urine direct microscopy and culture. Out of the total 110 cases of neonatal candidemia, candiduria was found in 60 (54.5%) cases. All these cases were positive for yeast like growth on urine culture as compared to only 37 (33.6%) cases which were positive for fungal elements on direct microscopy. (Table-3)

Table-3: Detection of candiduria in cases of neonatal candidemia (n=110)

Urine	Investigations				Z-value	P-value
	Direct Microscopy		Culture			
	No. of cases	Percentage (%)	No. of cases	Percentage (%)		
Positive for yeast	37	33.6	60	54.5	-3.122	0.0018*
Negative for yeast	73	66.4	50	45.5		

*P<0.05

Using Z-test, there was statistically highly significant difference (P<0.01) in identification of yeasts by urine culture compared to direct microscopy of urine sample at 5% level of significance.

Out of 54.5% cases of candiduria, non-albicans Candida species were isolated from urine in 49/60 (81.7%) cases compared to C. albicans in 11/60 (18.3%) cases as shown in table- 4.

Table-4: Isolation of *non-albicans Candida* species versus C. albicans from urine in cases of neonatal candidemia (n=60)

Species isolated from urine	No. of cases	Percentage %
Non-*albicans Candida* (NAC)	49	81.7
Candida albicans	11	18.3

In our study, candiduria was observed in 54.5% cases of neonatal candidemia and the species of *Candida* isolated both from blood and urine samples in all these cases were the same.

Association between candidemia and candiduria in our neonates is shown in table-5.

Table-5: Percentage correlation between candidemia and candiduria (n=110)

Species isolated		From blood	From urine	Z-value	P-value	Percentage urine to blood (%)
NAC species	*C. glabrata*	43	24	1.0757	0.282	55.81
	C. tropicalis	29	21	3.503	0.0005*	72.41
	C. parapsilosis	16	2	-4.2426	0.0001*	12.50
	C. guilliermondii	3	1	-	-	33.33
	C. krusei	2	0	-	-	0.00
	C. dubliniensis	1	1	-	-	100.00
	C. lusitaniae	1	0	-	-	0.00
C. albicans		15	11	2.552	0.0107*	73.33

*P<0.05

In the present study, *C. albicans* showed highest percentage correlation between candiduria and candidemia (73.33%, 11/15 cases) (P<0.05, statistically moderately significant). Among the NAC species, candiduria was associated with candidemia most commonly in *C. tropicalis* (21/29, 72.41% cases) (P<0.01, statistically highly significant), followed by *C. glabrata* (24/43, 55.81% cases) (P>0.05, statistically not significant) and *C. parapsilosis* (2/16, 12.5% cases) (P<0.01, Type I error). Further, there was no statistically significant difference (P>0.05) in the isolation rates of *C. tropicalis* and *C. albicans* from urine and blood at 5% level ofsignificance.

Pearson product moment correlation coefficient (r) was used to assess the relation between the variables candiduria and candidemia for all the species and was found to be 0.84 suggestive of high positive correlation between the two variables. P value for given instance of r was 0.018, i.e. P<0.05, making positive correlation between candiduria and candidemia in our study statistically significant.

Discussion

Isolation of *Candida* from blood is the gold standard for the diagnosis of candidemia.[10,11] Urinary tract infection due to *Candida* occurs in adults, children and the newborn, either as an ascending infection or by hematogenous dissemination from another organ focus.[12,13](Studies have shown that significant number of high risk neonates with candidemia had candiduria.[3,14-17] The present study was carried out to identify and speciate, the *Candida* isolates obtained from cases of neonatal candidemia. Further, we tried to evaluate if candiduria, can be considered as reliable indicator of candidemia.

The present study emphasizes the changing trend of Candida species in neonatal candidemia with predominance of NAC species (86.4% cases) compared to *Candida albicans* (13.6% cases) (Table-1). Our results are comparable with a studies carried out by various workers who have reported the changing trend of neonatal candidemia.[18-25] Studies conducted in New Delhi by Gupta N *et al.*[21] and Rani R *et al.*[19] have reported NAC species in 78.9% and 96% cases of neonatal candidal septicemia respectively. Agarwal J *et al.*[18] isolated NAC species in 84.4% cases of neonatal candidemia from Lucknow (Uttar Pradesh). Juyal D *et al*.[26] in Uttarakhand, found that NAC species were responsible for 80.30% cases of neonatal candidemia. Caggiano G *et al.*[8] in their study of 41 neonatal candidemia cases done in Italy, found that 65.9% (27 cases) of candidemia episodes were caused by non-*albicans Candida*.

In our study, among the NAC species isolated from the cases of neonatal candidemia, the predominant was *C. glabrata* (39%), followed by *C. tropicalis* (26%), *C. parapsilosis* (14.5%) (Table-2).

Similar observation has been reported by various workers [21,27,28] showing the predominance of *C. glabrata* in neonatal candidemia. Gupta N *et al*.[21] from New Delhi have reported *C. glabrata* (42.1%) as the most common isolate, followed by *C. tropicalis* (31.6%) in neonatal candidemia. Baradkar VP *et al*.[27] in 2008, have found *C. glabrata* (61.2%) as the most frequent cause of neonatal candidal sepsis, followed by *C. parapsilosis* (20.4%) from Mumbai. Studies by Agarwal J *et al*.,[18] Rani R *et al*.,[19] Kumar V *et al*.[23] and Goel N *et al*.[24] have reported *C. tropicalis* as most frequent isolate in neonatal systemic candidiasis. While Caggiano G *et al.,* [8] from Italy have reported *C. parapsilosis* (58.5%) and *C. albicans* (34.1%) as the most common species recovered, Juyal D *et al.,*[26] from Uttarakhand have reported *C. parapsilosis* (25.0%) and *C. tropicalis* (21.97%) as the predominant species; whereas 19.70% of cases were caused by *C. albicans.*

In the present study, overall candiduria was found in 54.5% (60/110) cases. All of these 60 cases were positive for yeast like growth by urine culture. Direct microscopy for fungal elements were positive in 37 (33.6%) cases out of the culture positive cases (Table-3). showing that urine culture is a bertter marker of candiduria compared to direct microscopy (P<0.01, statistically highly significant).

Among the 54.5% cases of candiduria in our neonates, same species of NAC were isolated from urine in 81.7% compared to *C. albicans* in 18.3% (Table-4).

Chowdhary *et al*.[29] reported candiduria in 87.5% cases of neonatal candidemia from New Delhi. Chakrabarti *et al*.[10] in their study conducted in Chandigarh, reported isolation of *Candida* from blood in 41.8%, from urine in 50.5% and both from blood and urine in 27.5% clinically suspected cases of disseminated candidiasis. In a study by Phillips *et al*.[3] candidemia was associated with 52% cases of candidal UTI in neonates.

In the present study, *C. albicans* showed highest percentage correlation between candiduria and candidemia (73.33%, 11/15 cases) (P<0.05, statistically moderately significant) as compared to *C. tropicalis* (21/29, 72.41% cases) (P<0.01, statistically highly significant), followed by *C. glabrata* (24/43, 55.81% cases) (P>0.05, statistically not significant). There was no statistically significant difference in the isolation rates of *C. tropicalis* and *C. albicans* from urine and blood at 5% level of significance (Table-5).

In a study by Chakrabarti *et al.*[10] *C. tropicalis* was found to be the commonest yeast isolated from blood (55.3%) and urine (58.7%). Similarly, in the same study, it was observed that the isolation of NAC species (59.5%) from urine was better indicator of candidemia compared to isolation of *C. albicans* (33.3%)

Candida is the most frequent cause of urinary tract infection in intensive care nurseries.[14,30] About half of these babies have concomitant candidemia and are predisposed to renal candidiasis.[14,30] Renal insufficiency may be the first clinical manifestation of invasive candidiasis.[30]

A basic problem associated with the diagnosis of candida urinary tract infection is the significance of candiduria in respect of discriminating infection from colonization due to contamination with flora of adjacent anatomic sites.[31] To conclude, the present study emphasizes the mycological shift of *Candida* species in neonatal candidemia with predominance of non-*albicans Candida* (NAC) species from Meerut city: definitely a changing trend. The present study also highlights that candiduria may be considered as a reliable indicator of candidemia in neonates. Urinary tract infection (UTI): This infection is extremely common. Evaluation of late-onset sepsis should include a urine culture obtained via sterile catheterization (or suprapubic bladder aspiration). If the urine culture is positive for fungus, renal ultrasonography should be performed to detect fungus in the collecting system. [32] Candiduria develops in approximately 2.4% of VLBW infants and up to 6% of ELBW infants. [1] Studies have demonstrated similar mortality in infants with Candida UTI alone (26%) compared to Candida BSIs (28%) in ELBWs. [33, 34] These findings emphasize the need for prompt treatment for a duration of a minimum length of 14 days in preterm infants.

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