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Research Article

Early Diagnosis of Neonatal Sepsis Caused by Yeast Infection

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Abstract

Early diagnosis of neonatal sepsis is lifesaving to the neonates. The present study was conducted to clarify the rate of incidence of neonatal early and late onset sepsis caused by yeast infection. Conventional methods, Buffy coat examination and molecular (PCR) methods were adopted and compared for the isolation of etiologic agents. In addition, other tests were also carried out to complete our data. One hundred and twenty neonates suspected of having sepsis were identified. One hundred and ten cases were found positive using blood cultures and PCR, and 20 neonates were negative. Torch antibodies were detected for the negative cases confirming viral septicemia. The remaining 100 positive cases were classified as bacterial (88%) and yeast infections (12%). *Candida albicans* was isolated from 11 cases (91.7%) while *Debaryomyces hansenii* was isolated from one case only, representing 8.3% of the positive yeast isolates. The analysis of neonatal cases showed that there is a perfect correlation between molecular and microbiological data. PCR for the 12 cases having yeast infection gave positive results at 615 bp, and that for *C. albicans* was observed at 156 bp. In conclusion PCR is the best and rapid method for early detection of neonatal yeast infection.

Keywords: Neonatal sepsis; Candidemia; Laboratory diagnosis

Introduction

Neonatal infections remain a major cause of morbidity and mortality and death in newborn infants [1]. Septicemia is a systemic illness caused by spread of microbes or their toxins via the blood stream [2]. The reported incidence of neonatal sepsis varies from 7.1 to 38 per 1000 live births in Asia [3,4], from 6.5 to 23 per 1000 live births in Africa [5,6] and from 3.5 to 8.4 per 1000 live births in South America and the Caribbean [7,8]. By comparison, rates reported in the United States and Australia range from 6-9 per 1000 [9,10]. Neonatal sepsis classified according to the time of onset of the disease: early onset (EOS) and late onset (LOS). The distinction has clinical relevance, as "EOS" disease is mainly due to pathogens acquired before and during delivery, and "LOS" disease to pathogen acquired after delivery (nosocomial or community source). Some reports distinguish between early onset (within 24 hours), "EOS" (24 to 6 days), and "LOS" (more than 6 days) sepsis [4,11]. Clinical diagnosis of sepsis is not easy, because, symptoms and signs are not specific and dramatic deterioration of clinical conditions can supervene rapidly long before blood cultures results are available even in asymptomatic newborn infants [12]. Predisposing risk factors associated with neonatal sepsis include neonatal group B streptococcal infection (GBS) colonization, premature rupture of membrane (PROM), and chorioamionitis12. Neonatal candidiasis can be subdivided into two categories, catheter related candidemia and disseminated or invasive candidiasis [13]. *Candida* spp are the common cause of nosocomial infections in neonatal intensive care units (ICUs). Although *C. albicans* has historically been the most frequently species isolated, infections caused by other species of *Candida* have been diagnosed with increased frequency [14,15]. Diagnosis of sepsis is difficult and there are no laboratory tests with 100% specificity and sensitivity, (with the exception of blood cultures which needs at least 48-72 hours). PCR methodology has been used to diagnose different infections, and the possibility of amplifying the DNA region common for all microorganisms could represent an optimal method for the diagnosis of sepsis [9,10]. PCR also allows rapid onset of treatment [1,15].

Materials and Methods

Patients

This study was carried out at the neonatal intensive care unit (during 2004) of Al-Azhar University Hospital, Cairo, Egypt on 120 neonates of an age group ranging from one day to four weeks suspected to have neonatal sepsis.

Sample collection and pre-analytical preparation

Two ml. Of venous blood were drawn in sterile EDTA-treated tubes (Bekton Dickinson Vaccutainer system, Europe, UK). One ml. of venous blood was used for blood culture and one ml. was used for molecular analysis. The blood for molecular analysis was stored at –20^{O}C until use. A complete blood count was carried out using Hemat 8 (Radium Group) and the detection of C-reactive protein level. A Gram stained smear of the plasma buffy coat layer, obtained by centrifuging anti-coagulated capillary was examined and a rapid diagnosis of bacteremia in neonates was carried out [16].

Identification of the isolates

Venous blood samples were inoculated on Sabouraud dextrose agar (SDA) and Sabouraud-Brain Heart Infusion (BHI) broth and incubated for 2 weeks at 25^{O}C prior to the identification of fungal isolates. The isolates were then identified using two techniques, ApI 20C Aux (Bio Mérieux SA, Marcy-L'Eliale, France) and the

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Micro-scan 4-Hour Rapid Yeast Identification panel (YIP, Baxter-Microscan, W. Sacramento, Calif.).

Polymerase chain reaction (PCR)

DNA Extraction from whole blood was carried out using "QIAamp DNA blood kit" (Quiagen, Inc.), this was followed by detection of yeast DNA using the universal primers "F-5'-GCETATCAATAAGCGGAGGAAAA-'3" and "R-5'-GGTCCGTGTTTCAGAAG-'3" that amplifies the V3 region of the large subunit of the ribosomal DNA [17]. For identification of *Candida albicans*, the following specific PCR primers were used "F-5'-TTGGAGCGGCAGGATAATCG-'3" and "R-5'GGTCCGTGTTTCAAGACG-'3" [18]. Detection of the amplified

PCR product was carried out using gel electrophoresis. The fragments were separated in 2% agarose gel stained with ethidium bromide and visualized using ultraviolet transillumination.

Statistical analysis

The data of the study were analyzed using "SPSS" version 12 and this data was represented in a descriptive and analytical form. Appropriate statistical tests were chosen for each table and figure, depending on the type of data present, either Chi square (X^2) and Fisher exact test (if cells are less than 5). The accepted levels of significance were 0.05 or less.

Ethical issues

The Ethics Committees of Al-Azhar University Hospital Cairo, Egypt approved the study. All patient information and test results were kept confidential.

Results

In the present study, one hundred neonates were positive for blood cultures and PCR, Table 1 shows the number of cases and percentage of each etiologic agent.

Two yeast species were isolated from blood of 12 neonates. *Candida albicans* was the predominant species, and isolated from 11 neonates representing 91.7% of the total yeast cases, while *D.hansenii* (*C. famata*) was isolated from one case only, representing 8.3% of the total yeast isolates (Table 2).

Table 1: Number and percentage of the etiologic agents causing sepsis.

Causal agent	No.	%Total cases	% Positive cases
Bacteria	88	73.33	88.00
Viruses	20	16.67	–
Yeasts	12	10.00	12.00
Total	130	100	100

Table 2: The isolated yeast species from blood of neonates.

Yeast isolates	No = 12	%
Candida albicans	11	91.7
Debaryomyces hansenii	1	8.3
Total	12	100

Table 3: Sensitivity comparison between the different methods used in the identification of yeast isolates.

Method	No = 12	%
Blood culture	12	100
PCR	12	100
Buffy Coat	8	66.7

Table 4: Relation between different risk factors and the isolated yeast species.

Risk factors		*C. albicans*	*D. hansenii*	Total	X^2	P
Onset sepsis	Early	8	–	8	0.14	0.71
	Late	3	1	4		
Birth weight	≤ 2.5	7	1	8	0.14	0.71
	≥ 2.5	4	–	4		
Gestational ages (weeks)	Preterm(37)	3	1	4	0.14	0.71
	Term >37	8	–	8		
Gender	Males	10	–	11	2.48	0.11
	Females	1	1	1		

The different methods adopted for the identification of yeast infections were compared. There was no difference between the sensitivity of blood cultures and PCR results (Table 3). It was observed that male neonates weighing less than 2.5kg are highly affected as in Table 4.

Complete blood count revealed that, 80% of the positive neonates had normal HB level, platelets, HCT and MCHC, 50% had normal WBCs count, 70% normal RBCs while 40% suffered from leukocytosis, 70% had high MCH, 60% had high MCV and most of the studied neonates had thrombocytopenia.

The multiplex PCR performed for universal yeast DNA showed positive results of the test sampler with the positive control at 615 bp. Also PCR for *C. albicans* showed positive result of the test samples with positive control at 156 bp (Figure 1 & 2).

Discussion

The number of neonatal deaths according to WHO [19] was3.3 million in 2009, but 98% occurred in less developed countries [20]. Sepsis remains the main cause of morbidity and mortality in neonatal intensive care units [21,22]. Host immunodeficiency, increasing microbial resistance to antibiotics, and late detection of early onset of infection are the major factors altering the course of infections [23,24]. Neonates suffering from candidiasis usually have serious morbid conditions. The epidemiology of candidemia is a marker for with other serious medical problems [25].

In the present study, systemic candidiasis occurred in 12% of the total positive test samples. *C. albicans* was isolated in11 cases (91.7%). These results are in agreement with other investigators who reported that candidemia accounted for 10.9% [26] and 15% [27] among infants in the neonatal intensive care units. *C. albicans* and *C. parapsilosis*, were isolated in 93% of cases [26] while Feja *et al.*, [27] recorded that *C. albicans*, caused 62% of candidemia, *C. parapsilosis* (31%), *C. glabrata*, *C. stellatoidea* and *C. lusitaniae* caused 2% each [27]. *D. hansenii* (*C. famata*) was isolated from one case representing 8.3% of the yeast infection.

D. hansenii gained the status of emerging pathogen as being implicated in human infections [28,29]. However its incidence during candidemia is low based on data surveillance implemented by the Centers for Disease Control and Prevention, whereas one review on world wide-collected isolates states that *D. hansenii* accounts for 0.08 to 0.5% of isolates recovered during invasive candidiasis [30], But Desons-Olivier *et al.* [31], stated that *D. hansenii* has been repeatedly associated with catheter-related blood stream infection

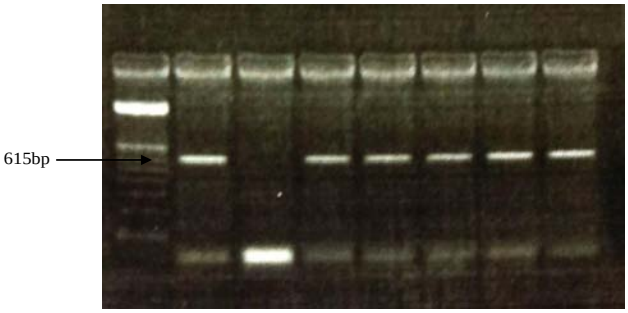

Figure 1: PCR amplification of yeast isolates DNA using universal primers that amplifies the V3 region of the large subunit rDNA. Lane 1: DNA marker (OX Hae III, Promega), lane 2: positive control, lane 3: negative control, lanes 4 – 8 yeast isolates.

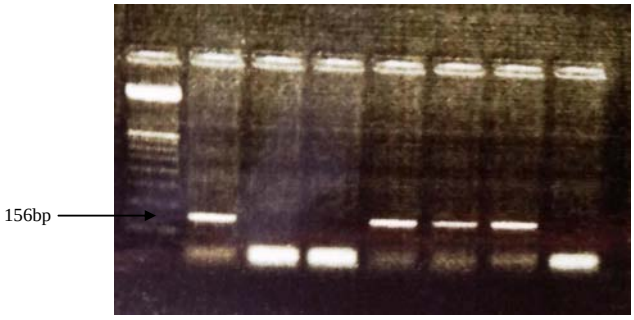

Figure 2: PCR amplification of *Candida albicans* DNA using specific PCR primers. Lane 1: DNA marker (OX Hae III, Promega), lane 2: positive control, lane 3: negative control, lanes 4 – 8 yeast isolates.

and rarely with other infections. In a recent study by Kumar *et* al. [32] on neonatal sepsis caused by *Candida* species, Candidemia was observed in 66 out of 442 neonates representing 14.9%. *C. albicans* was predominant isolate followed by *C. glabrata* (19.7%), *C. tropicalis* (6.1%) [33].

Cahanand Deville [34] reported in a study that lasted for four years that neonatal candidiasis has risen with a mortality rate of 35%. The mortality rate in developing countries among neonates was 23-52% [11]. Concerning the risk factors, yeast infection among neonatal males represented 91.7 (11 cases) and a single female case represented 8.3%. This male predominance is apparent in almost all studies of sepsis in newborn infants [35,36]. Klein and Marcy [37] stated that this might be due to a gene located on the X-chromosome and involved with the function of the thymus, or with synthesis of immunoglobulins. Premature infants represent 33.34% of the test group, which is lower than other studies [38]. Early onset sepsis represented 66.7% of the yeast infection. While late onset sepsis represented 33.3% and *C. albicans* was the predominant species in both cases. In a study performed by Shin *et al.,* [39] the estimated incidence rate of neonatal sepsis was 30.5 per 1000 live births for clinical sepsis and 6.1 per 1000 live births for sepsis with positive culture, with case-fatality rates of 4.7% and 2.2%, respectively. When only early-onset sepsis considered, the incidence and fatality rates were 2.1 per 1000 live births and 6.1% for clinical sepsis, and 4.1 per 1000 live births and 2.5% for culture confirmed sepsis, respectively. The incidence of candidiasis is 3-4% in low birth weight infants [36,40], while Friedman *et al* [41], found that the incidence in very low birth weight infants was 2-9% with mortality rate 37%. In the present study, infection in low birth weight infants was 66.6%. According to published data, 0.004% to 1.5% of all patients in NICU, 2.6 to 3.1% of very low birth weight infants (birth weight < 1500 gm), and 5.5% to 10% of extremely low birth weight infants (birth weight <1000 gm) develop candidemia [36,42,43].

The analysis of neonatal yeast infection in this study showed that there is a perfect correlation between molecular and microbiological data (100% sensitivity). PCR is more sensitive than blood culture, since some of the neonates at risk for invasive yeast infection, whose blood cultures was negative for yeast, tested positive in PCR amplification [37]. C-reactive protein is the most accessible and widely used as a marker for detecting infection [44]. This test (C-RP) is considered of low diagnostic value because of its low specificity (66.7%), where in this study it recorded 66.7% in detecting yeast infection compared with PCR and blood culture (100%). It may be helpful in excluding infections in some cases of sepsis, if normal levels are obtained 24-48 hours after the onset of sepsis.

In conclusion, the incidence of neonatal yeast infection was 10% of the total test group. The identification of the isolated yeast species using PCR method matched the routine blood culture (100% specificity). PCR is a rapid method that saves time and medical cost of treatment and hospital accommodation and prevents the use of antibiotics for nonseptic cases. Preventive strategies for blood stream infection among neonates in intensive care unit should continue to focus on the possible risk factors leading to neonatal species.

Recommendation

It is important to publish this data in order to carry out a future study to find out the changing pattern of fungemia in cases of neonatal sepsis.

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