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RESEARCH ARTICLE

The Evaluation of Serum Candida mannan antigen as a Marker in Neonatal Sepsis

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Abstract

The present study was designed to evaluate the serum concentration of Candida mannan antigen for detection of neonatal fungal sepsis.

Neonates were recruited from neonatal intensive care unit (NICU) of Ain Shams University in the period from October 2011 till April 2012.

In this study, Candida mannan Ag was measured in 36 neonates males 16 (44.4%) and females 20 (55.6) diagnosed as having sepsis, and 36 healthy neonates males 19 (53.3%) and females 17 (46.7%) with no clinical signs or laboratory evidence for sepsis serving as a control group. All neonates were subjected to full history taking, thorough clinical examination and measurement of Candida mannan antigen level using ELISA technique.

This study showed that there is a high incidence of fungal infection in neonates admitted in NICU as 47.2% of cases had positive Candida mannan Ag.

To conclude, this study demonstrated that fungal infection is very common in NICU, long use of antibiotics, long stay in NICU, total parenteral nutrition, mechanical ventilation, and major surgery are risk factors for candidemia. Thrombocytopenia value is the major significant markers of systemic candidiasis.

Negative result of the test for the presence of antigen does not exclude IC. A low concentration of rapidly eliminated antigen during the course of infection may show false-negative because of these antigens are present in the blood stream only transiently, and they are eliminated by forming immune complexes as well as via endocytosis by Kupffer cells in the liver.

So Regular sampling and monitoring for the presence of mannan in the serum is vital for the patients.

Also Parallel monitoring of circulating mannan and antimannan antibodies provided rapid results with high sensitivity (80%) and specificity (93%).

Introduction

The incidence of sepsis is higher in neonates than in adult patients, and the risk of mortality is higher, in particular, neonates with low birth weight show relatively high morbidity and mortality **(Jordan and Durso, 2006).**

Candida mannan Ag is a polysaccharide non-covalently bound to the yeast cell-wall and represents a major component of the C. albicans cell wall, composing up to 7% of the cell dry weight, and it is a highly immunogenic polysaccharide antigen with immunomodulating properties.

Recent epidemiologic studies have shown increasing numbers of invasive fungal infections (IFIs), main pathogens responsible for these infections are yeast-like fungi from Candida genus and Aspergillus spp. moulds **(Pagano et al., 2006) (Enoch et al., 2006)**.

IC is associated with high mortality rates reaching up to 40–50%. Early and precise diagnosis of deep mycosis is difficult because of lack of specific symptoms. **(Yera et al., 2001) (Ostrosky-Zeichner and Pappas, 2006)**.

Serological methods used in fungal diagnostics are generally based on detection of cell-wall components of selected pathogenic fungal species, i.e. mannoproteins, functioning as soluble antigenic markers. During the course of a systemic fungal infection these antigens are present in the bloodstream only transiently, and they are eliminated by forming immune complexes as well as via endocytosis by Kupffer cells in the liver **(Yeo and Wong, 2002)**.

Immunodiagnostics of IC rely on the detection of circulating mannan antigen in the serum, being the main component of the fungal cell wall of Candida spp. Mannan is a highly immunogenic polysaccharide antigen with immuno-modulating properties, and its oligomannose epitopes may induce humoral response **(Sendid et al., 2003)**.

Invasive candidiasis is common, often fatal, and frequently leads to poor neurodevelopmental outcomes. Invasive candidiasis is the second most common cause of infectious disease-related death in infant. Despite antifungal treatment, 20% of infants who develop invasive candidiasis die, and neurodevelopmental impairment occurs in nearly 60% of survivors **(Benjamin et al., 2006).**

The strategy mentioned above is useful for observation of the decrease in anti-mannan antibodies with concurrently high level of mannan antigen, and therefore improves IC diagnosis. Sendid et al. **(Sendid et al., 2003).**

Owing to the fact that the peak of antigenaemia coexists with positive culture results, it seems to be beneficial to collect blood samples for culture when having positive results of antigen present in the circulation **(Sendid et al., 2002).**

Aim of the work: The present study was designed to evaluate the serum concentration of Candida mannan antigen for detection of neonatal fungal sepsis.

Subjects and methods: The present case control study took place in NICU, Faculty of medicine, Ain Shams University. Candida mannan Ag was measured in 36 neonates diagnosed as having sepsis, and 36 healthy neonates with no clinical signs or laboratory evidence for sepsis serving as a control group.
The study was approved by Ethical Committee of Pediatrics department, Ain Shams University. An informed consent was taken from parents before enrollment of neonates in the study. All neonates were subjected to full history taking, thorough clinical examination, Laboratory investigations [Complete blood count (CBC) using blood samples collected in EDTA tubes by coulter counter T660 with differential leucocytic count using Leishman-stained blood smear by counting at least 200 cells; c-reactive protein (CRP) estimated by latex agglutination assay; Blood culture using a signal blood culture system (oxoid), Serum Candida mannan antigen level: blood samples was withdrawn from patients with sepsis in group 1 and at any time in the control group, serum was separated for evaluation of Candida mannan antigen level using ELISA technique (The Platelia™ Candida Ag); an immunoenzymatic sandwich microplate assay.

Statistical methodology:

Analysis of data was done by IBM computer using SPSS (statistical program for social science version 12). Quantitative variables are described as mean, SD and range. Qualitative variables expressed as number and percentage. Chi-square test was used to compare qualitative variables between groups. Unpaired t-test used to compare quantitative variables, in parametric data (SD<50% mean). Mann Whitney test used in non parametric data (SD>50%mean). Spearman Correlation co-efficient test used to rank variables versus each other positively or inversely. ROC (receiver operator characteristic curve)

P<0.05 significant,P<0.01 highly significant.

Results

was used to find out the best cut off and validity of certain variables (sensitivity,specificity, PPV and NPV).

The present study included 36 newborns with sepsis (cases); 16males (44.4%) and 20 females (55.6%), and 36 healthy controls [19 males (53.3%) and 17 females (46.7%); p=0.47]. Both groups were statistically difference concerning

gestational age [(14 fullterm 38.9% and 22 preterm 61.1%) and (25 fullterm 73.3% and 11 preterm 26.7%) weeks respectively; p=0.005, and mean birth weight [(2.03±0.1947) and (3.07±0.17) Kg respectively; p=0.00]. Demographic characteristics of studied neonates are shown in ***table 1.***

Neonates with sepsis had significantly higher positive candida mannan Ag number in cases compared to controls [17 (47.2%) and 0 (0.0%) respectively p= 0.0001], as shown in table 1.

9 of our cases (25%) received blood product, 11 (30.5%) had thrombocytobenia, 29(80.6%) had leucopenia and 28(77.8%) had positive CRP.

Table (1): Demographic and candidate mannan Ag of the studied groups:

Descriptive data Items	**Cases group n = 30 (Mean ± SD)** (Range) n(%)*	**Control group n = 30 (Mean ± SD)** (Range) n(%)*	**Statistical test**	**P (Significance)**
Gestational age (Wks)	Ft 14(38.9%) Pt 22(61.1%)	Ft 25(73.3%) Pt 11(26.7%)	*t* = -3.101	0.005 S
Mode of delivery **SVD** **CS**	 16(44.4%) 20(55.6%)	 19(53.3%) 17(46.7%)	X^2 =.606	0.47 NS
Sex **Male** **Female**	 16 (44.4%) 20 (55.6%)	 19(53.3%) 17(46.7%)	X^2 = 0.067	0.47NS
Birth weight (Kg)	2.03±0.86	3.07±0.82	*t* = -1.610	0.00 S
Apgar score at 1 minute	3.50±0.88	6.47±0.78	*t* =-4.694	**<0.0001 HS**
Apgar score at 5 minutes	5.72±0.78	10.00±0.00	*t* =-6.831	**<0.0001 HS**

***Candida* mannan Ag**	Negative19(52.8% Positive 17(47.2%	-ve 36(100%) +ve 0(0.0%)	t = 8.98	**0.0001 HS**

Studied neonates were subdivided according to candida mannan Ag positive or negative among cases. Demographic characteristics of those neonates are shown in ***table 2***

Table (2): Comparison between candida mannan positive and negative cases as regard demographic and laboratory data:

		Candida mannan Negative		*Candida* mannan Positive		P
		N	%	N	%	
GA	Fullterm	10	62.5%	4	20.5%	0.01*
	Preterm	6	37.5%	16	79.5%	
Mode of delivery	NVD	8	42.1%	8	47.1%	0.971
	CS	11	57.9%	9	52.9%	
PROM (18hr)	No	13	68.4%	7	41.2%	0.101
	Yes	6	31.6%	10	58.8%	
TPN	No	7	36.8%	0	.0%	0.008
	Yes	12	63.2%	17	100.0%	
Ventilation	No	9	47.4%	0	.0%	0.001
	Yes	10	52.6%	17	100.0%	
Surgery	No	19	100.0%	10	58.8%	0.002
	Yes	0	.0%	7	41.2%	
Procedures	No	11	57.9%	5	29.4%	0.08
	Yes	8	42.1%	12	70.6%	
Central Line	No	18	94.7%	2	11.8%	0.001
	Yes	1	5.3%	15	88.2%	
Steroids use	No	18	94.7%	8	47.1%	0.002
	Yes	1	5.3%	9	52.9%	
H2 blockers use	No	14	73.7%	6	35.3%	0.021
	Yes	5	26.3%	11	64.7%	
Receiving Blood products	No	8	42.1%	1	5.9%	0.02
	Yes	11	57.9%	16	94.1%	
Thrombocytopenia	No	17	89.5%	8	47.1%	0.006
	Yes	2	10.5%	9	52.9%	
Leucopenia	No	3	15.8%	4	23.5%	0.684
	Yes	16	84.2%	13	76.5%	
CRP	Negative	3	15.8%	5	29.4%	0.434
	Positive	16	84.2%	12	70.6%	
Outcome	Discharged Died	16 3		5 12		0.001**

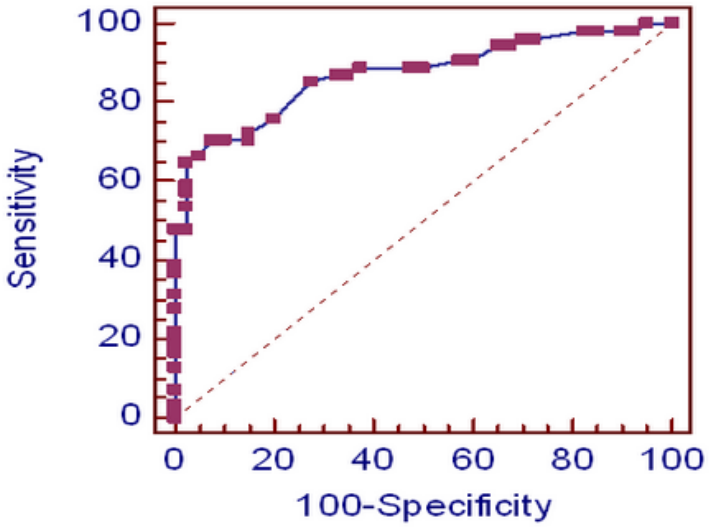
GA: Gestational age, PROM: Premature rupture of membrane, NVD: Normal vaginal delivery, CS: Cesarean section.

tatistically significant correlation between serum *Candida* mannan Ag level in patients group and their GA. But no significant correlation between serum *Candida* mannan Ag level in this patients and their mode of delivery and PROM.

statistically significant correlation between serum *Candida* mannan Ag level in patients group and their management, except for procedures which showed no correlation with serum *Candida* mannan Ag level.

statistically significant correlation between serum *Candida* mannan Ag level in patients group and their thrombocytopenia and no correlation in serum *Candida* mannan Ag level of these patients and their leucopenia and CRP.

The ROC Curve defines the best cutoff value of candida mannan Ag is 5.8 mg/dl with a sensitivity of 89.6% and specificity of 96.4% as shown in figure 1.



AUC = 0.872 95% Confidence Interval = 0.787 – 0.932

Figure (1): Receiver Operating Characteristic Curve (ROC) for identifying best cut-off for candida mannan antigen in case group.

Discussion:

In present study revealed that *Candida* mannan Ag was negative in all controls group as there is no risk factor however 47.2 % of cases group had positive *Candida* mannan Ag during their stay in NICU.

Sendid et al. (1999) showed that it was possible to detect *Candida* mannan Ag in 40% of patients with neonatal sepsis and this goes in agreement with our study.

In our study we did comparative tables between 47.2% of cases who had positive *Candida* mannan Ag with others cases 52.8% who had negative *Candida* mannan Ag according to modes of delivery, gestational age, PROM, long use of antibiotics, central vascular catheterization, major surgeries, mechanical ventilation, steroids use, lab data, and total parenteral nutrition, in trying to detect the risk factors associated with candidemia.

This study found that gestational age is a risk factor (P=0.01) for candidal infection, preterm are more prone (79.5%) than full term (20.5%), studies demonstrated that preterm infants are more frequently colonized with *Candida* species than full term infants which could be contributed to immaturity of the preterm infant's immune system leads to defects in chemo taxis, cytokine production, phagocytosis, and production of type-specific antibodies ***(Chapman and Faix, 2000; Lewis and Wilson, 2001).***

This came in agreement with ***Makhoul et al. (2007)*** study which stated that low birth weight is an important risk factor in neonatal mortality and in various morbidities, because of the immaturity of both the immune system and the barrier functions of the gastrointestinal tract, as well as the invasive diagnostic and therapeutic procedures.

Several risk factors were evaluated in our study and found to be not associated with colonization with *candida* species such as mode of delivery (P =0.971), PROM (P =0.101), procedures (P =0.08). ***Saiman et al. (2001)*** has studied these risk factors and found them not associated with colonization except for caesarian section and the use of H2 blockers.

Which goes in agreement with ***Mathai et al. (2004), Mustafa et al. (2005)*** and ***Al Sarnagawe (2009)***. However, ***Stoll et al. (1996)*** observed that babies born by vaginal delivery were more likely to have early onset sepsis than those delivered by cesarean section may be due to maternal genital tract infection with an increased risk of vertical transmission to the newborn.

However, ***Rowen et al. (1999)*** stated that delayed enteral feeding, parenteral nutrition, vaginal delivery (P=0.03), use of steroids (P=0.01) and small gestational age (P=0.01) are risk factors for candidal infection. ***Saiman et al. (2001)*** stated that a antibiotic therapy and infective events induce rapid changes in the intestinal microflora, whereas hygienic conditions and nutrition induce slow changes.

Our work showed that total parenteral nutrition is a very important risk factor for candidal infection in NICU (P=0.008) as all patients with candidal infection were receiving total parentral nutrition. ***Leibovitz et al. (2006)*** reported that all the babies had candidal infection were receiving total parentral nutrition. And that may be due to the association between the duration of total parenteral nutrition and sepsis or related to its lipids content which decrease phagosetic function or due to central venous access or glucose abnormalities as hyperglycemia ***(La Gamma et al., 1983).***

Our study showed that long use of antibiotics for more than 14 days is an important risk factor for increased incidence of candidal infection (P= 0.0001). ***Samonis et al. (1994)*** stated that antibiotics with the broadest spectrum of cover are more likely to encourage over growth of *candida* species. ***Saiman et al. (2001)***, added that loss of normal GIT flora due to treatment with antibiotics may facilitate candidal infection.

Our study showed that neonates undergoing major surgeries are more liable for candidal infection (P=0.002) mostly due to delayed enteral feeding, gastrointestinal surgery with subsequent use of total parenteral nutrition and use of antibiotics after surgery, and may be due to compromise of the intestinal lumen barrier ***(Fanaroff et al., 1998; Saiman et al., 2000).***

Our study showed that mechanical ventilation and endotracheal intubation is a major risk factor for candidal infection as 75% of our patients were ventilated. Mechanical ventilation was described by many reporters as a risk factor for candidemia (87.8%) ***(Makhoul et al., 2001).***

This study showed that use of steroids in treatment is a risk factor for candidal infection (P=0.002)***, Saiman et al. (2000)*** showed that H2 blockers (P=0.025) and use of steroids (P=0.001) are risk factors for candidemia while maternal antibiotics, mode of delivery are not.

The use of H2 blockers is considered a further risk factor for candidal infection (P=0.021). Gastric acidity is considered to be protective against *Candida* infection. Histamine-2 (H2) blockers (ranitidine) raise the gastric pH and thereby promote bacterial and fungal overgrowth of the upper gastrointestinal tract ***(Driks et al., 1987; Kaufman, 2003).***

Steroids have been implicated as a risk factor in some studies (P=0.0039) ***(Botas et al., 1995),*** while others not.

Winter (1992) stated that the administration of steroids significantly increase the risk of systemic candidiasis in susceptible preterm infants younger than 35 days of age. Exogenous glucocorticoids have multiple serious side effects including marked retention of water and sodium, hypertension, hyperglycemia, pituitary and adrenal suppression, hypocalcaemia, bone demineralization and suppression of immune system.

Hyperglycemia caused by glucocorticoids may predispose to systemic candidiasis by facilitating fungal nutrition and multiplication and up regulation gene expression that may enhance pathogenicity ***(Gustofson et al., 1991).***

Our study found that 75% of cases were receiving blood products and 94.1% of this cases had candidal infection may be due to contamination, so this is point needs more studies for evaluation.

In addition, this study found that 52.9% of cases with candidal infection had a very low platelets count (P=0.006). It was documented by many authors that thrombocytopenia is most significant laboratory findings of fungal infection ***(Rowen et al., 1999).***

The thrombocytopenia in sepsis was explained previously by ***Guida et al. (2003)*** to be due to direct toxic injury to platelets, megakaryocyte suppression and increased peripheral consumption as in DIC or the presence of an immune component due to increased level of platelet associated immunoglobulins.

In our study found that 77.8 % of cases had a high C-reactive protein (CRP). But there is no statistically significant correlation between serum *Candida* mannan Ag in cases and their CRP (P= 0.434) because of 84.2% of cases had a high CRP but their Ag is negative while 70.6% of cases had negative CRP and Ag positive.

This came in agreement with ***Watson et al. (1999)*** reported that CRP is not a specific marker. It was also demonstrated that serial estimation of CRP is more significant in predicting sepsis especially if there is a localized infection ***(Abdel-Wahed et al., 2001).***

Ottolini et al. (2003) stated that white blood cells count is sometimes useful in evaluating a newborn infant for infection, however they cannot reliably predict the presence or absence of infection.

Our study found that 80.6 % of cases had leucopenia, but there is no statistically significant correlation between serum *Candida* mannan Ag in cases and their leucopenia (P= 0.684).

The risk factors and demographic data of neonates with candidemia in this study paralleled previous studies in some respects; prematurity, use of central venous catheterization, use of prolonged antibiotic treatment, prolonged mechanical ventilation, prolonged parenteral nutrition, and prolonged hospitalization ***(Duran et al., 2005).***

Demonstration of mannan antigen in the early phase of infection depends mainly on the quantity of samples examined for each patient and as to the frequency of their collection in patients suspected of IC. Collection of more than one sample should probably increase detection of IC due to most antigens are often rapidly cleared from the circulation, so that antigen detection tests may lack the desired level of sensitivity required for a definitive diagnosis. A good example is shown in the results of ***Sendid et al. (1999).***

Negative result of the test for the presence of antigen does not exclude IC. A low concentration of rapidly eliminated antigen during the course of infection may show false-negative because of these antigens are present in the blood stream only transiently, and they are eliminated by forming immune complexes as well as via endocytosis by Kupffer cells in the liver ***(Yeo and Wong, 2002)***.

Sendid et al. (1999) showed that it was possible to detect mannan in only 40% of patients with sepsis. Presence of antibodies was shown for 53% of patients. Importantly, parallel detection of both markers provided rapid results with high sensitivity (80%) and specificity (93%).

So the new diagnostic strategy for IC in its early stage and low antigenaemia is the immunoenzymatic Platelia *Candida* Ag (Bio-Rad), done in parallel with the anti-mannan antibody test (Platelia *Candida* Ab/Ac/Ak; Bio-Rad). based on the parallel monitoring of circulating mannan and antimannan antibody titres in patients with risk factors for developing systemic candidiasis.

The strategy mentioned above is useful for observation of the decrease in anti-mannan antibodies with concurrently high level of mannan antigen, and therefore improves IC diagnosis ***(Ruhnke et al., 2003).***

a new enzyme immunoassay (down-flow immuno-assay) has become available commercially. Its application is the early diagnosis of IC (mannan antigens) and it is called the Unimedi *Candida* monotest (Unitika) ***(Fujita et al., 2006)***.

It was reported that the sensitivity and specificity of the Unimedi *Candida* monotest were 82 and 96% respectively. The sensitivity of this assay was significantly ($P<0.01$) higher than that of the Platelia *Candida* Ag for diagnosing candidemia ***(Fujita et al., 2006)***.

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