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Detection of *Candida albicans* anti-mannan antibodies by enzyme linked immunosorbent assay (ELISA) for diagnosis of invasive candidiasis in human and cattle

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Abstract

Invasive candidiasis (IC) is an important cause of morbidity and mortality in human and animals, early diagnosis and management are a challenge. Therefore, this study was carried out to determine the usefulness of *Candida albicans* anti-mannan antibodies testing by using ELISA in diagnosis of invasive candidiasis in human and cattle. Sixty-nine serum samples (45 from immunocompromised patients and 24 from diseased cattle suspected to suffer from systemic candidiasis) were examined by indirect ELISA to detect anti-mannan IgG and compared with the routine culture techniques. Mycological examination of different human and cattle biological samples (n=177) was performed while, *C. albicans* was detected in 69 % and 83 % of human and cattle respectively. The results of ELISA were 10 (22.2%) positive, 5 (11%) equivocal cases in human patients and 15 (62.5%) positive in diseased cattle. A positive serum IgG response for mannan antigens discriminated IC from exclusively candida positive cultures. In addition, the sequential observation of anti-mannan antibodies could contribute to early diagnosis of invasive candidiasis in human and cattle. In this way, more efficient management of IC and earlier initiation of antifungal therapy can be achieved.

Keywords: Anti-mannan antibodies, *Candida albicans,* Cows, ELISA, Invasive candidiasis.

Introduction

Invasive candidiasis is an important infectious complication in immunocompromised patients and is associated with severe morbidity and high mortality[16]. Although many *Candida* species can produce invasive infection, *C. albicans* continues to be identified as a leading pathogen[14]. Early diagnosis of IC remains difficult as the clinical symptoms are often vague and fungal cultures have low sensitivity and a long turn-around time[12,13]. Therefore problems with clinical and microbiological diagnosis of IC have prompted the development of non-culture based laboratory methods. Immunological methods for diagnosis of IC by antibodies detection techniques include latex agglutination, counter immune- electrophoresis and indirect immunofluorescence[17].These methods lacked sensitivity and specificity and were of limited diagnostic value. A useful test

must combine improvements in both sensitivity and specificity, the specificity of the tests can be improved by selecting the appropriate antigens and sensitivity of the tests can be increased by using sensitive and standardized commercial techniques such as ELISA[10]. Antigen detection has the advantage of high specificity but often lack the desired level of sensitivity for a definitive diagnosis[7]. Previous reports suggest that serological detection of antibodies might be useful for diagnosing systemic candidiasis. Mannan is an abundant antigen located on the candida cell wall surface, this antigen is highly immunogenic and as consequence a rise in the anti-mannan antibody titers when candida enters the bloodstream are generated against it. The detection of anti-mannan antibodies is taken advantage in the candida antibodies assay kits[18]. Although, many studies are published about the detection of anti-mannan antibodies for diagnosis of human invasive candidiasis[22, 6, 2], very little is known about the usefulness of anti-mannan antibodies testing by using ELISA in diagnosis of systemic candidiasis in cattle [20] Therefore, this study was carried out to elucidate the usefulness of *Candida albicans* anti-mannan antibodies testing by using ELISA in diagnosis of systemic candidiasis in human and cattle.

Material and Methods

Collection of samples:

Human samples: Forty-five patients, of different age and sex presenting risk factors for IC hospitalized at Mansoura University Hospitals during 2013-2014 were selected. One hundred and two clinical samples from them including 32 oral swab, 13 skin scrapings, 19 urine, 16 sputum, bronchial lavage and 22 blood samples were collected for mycological examination. Serum samples from all patients were collected for serological examination. Patients and samples were classified into four groups according to risk factor for IC, clinical case and Department from which the samples were obtained as in table 1.

Cattle samples: From twenty four diseased cows of different age and sex reared in dairy farms at El-Daqahlia and Damietta Governorate during 2013 suspected to suffering from different risk factors to candidiasis were subjected for this study. Sera as well as 75 clinical samples including 16 oral , 15 nasal, 13 rectal, 13 vaginal swab, 10 urine and 8 milk samples, were collected and classified into three groups as in table 2, according to clinical cases (risk factor for IC). Sera of healthy control subjects were collected from five healthy calf (<6 months old) who did not have any clinical or microbiological evidence of infection.

The study protocol was approved by the Ethics Committee of Zagazig University, Egypt. Permission to collect samples was obtained from Mansoura University Hospital and informed consent was obtained from patients included in the study. Consent to collect animal samples was obtained from farms owners and veterinarians.

Isolation and identification of yeasts from clinical samples: Mycological examination for clinical samples was performed by culture on Sabouraud's dextrose agar media with chloramphenicol and blood samples (3 ml from child or 5 ml from adult) were aseptically inoculated into Myco/ F lytic bottles (Becton Dickinson) for detection of fungal infection in blood by BACTIC 9240 system. Identification of yeast isolates into candida species were done by Gram's stain. Micromorphology on rice agar media and macro morphology on CHROMagar Candida Medium (Oxoid)[21].

Indirect ELISA for serodiagnosis of candidiasis in human and cattle:

Human sera were tested for the presence of candida anti-mannan antibodies by Ridascreen Candida IgG ELISA test (R-Biopharm-Germany) and the assay was performed according to the manufacturer's instructions. Table of values and standard curve provided with the kits allowed the determination of anti-mannan antibody concentration in human sera. The absorbance for

the negative control at 450/620 nm must be<0.3.
For cattle, homemade ELISA was prepared, using Anti-bovine IgG conjugate (KPL-USA) (Catalog no.14-12-06), the procedure was done according Jenkins *et.al*[8]. Using the optical density of 5 control healthy calves sera the cut off value was calculated (mean ±2 standard deviation) to evaluate the results of ELISA, so animal sera were regarded as positive when optical density was above 0.2679[19, 10].

Results and Discussion

Mycological examination of biological samples was performed, beside examination of serum samples by indirect ELISA to detect anti-mannan IgG. Out of 104 yeasts, 61 and 43 isolates were obtained from human and diseased cows respectively. The prevalence of *Candida* spp. isolated from human was as follows: *C. albicans* 86.3 % (n= 44), *C. tropicalis* 5.9% (n= 3), *C. glaberata* 3.9% (n= 2) and *C. parapsilosis* 3.9% (n= 2). Out of 43 yeasts obtained from diseased cows, *37Candida* spp. were identified from which *C. albicans* was 86% (n=32) and others were non- *albicans*. The recorded results revealed that *candida* spp. mainly *C. albicans* are incriminated as the most common cause of infection and this is in accordance with *Edelmann et al.*[5] who found that *C. albicans* has been cited as the most common pathogenic *Candida* spp. and it is the predominant cause of human and animal candidiasis.
RIDASCREEN Candida ELISA kit was used in this study for detection of anti-mannan IgG against *C. albicans* as a marker for invasive candidiasis in 45 immuno-compromised patients. The results were; 10 (22.2%) positive, and 30 (66.6%) negative while 5 (11%) were equivocal. It was observed that five positive and 2 equivocal cases by ELISA have negative blood culture; this could be attributed to the initiation of antifungal therapy. It is agreed that blood cultures lack sensitivity, and due to the risk associated with systemic candidiasis, several physicians recommend empirical treatment based on a compendium of clinical signs, risk factors, and assessment of colonization. Therefore, ELISA can serve as a better diagnostic assay for invasive candidiasis than routine microbiological methods and this is of particular importance in situations when culture fails[4]. Nevertheless, the ELISA results for 5 blood culture positive cases (candidemia) were 3 negative and 2 equivocal, this could be due to recent infection and these cases maybe positive if repeated after days. Therefore, the observation of anti-mannan Ab could contribute to early diagnosis of candidiasis more than candida mannan antigen in immunocompetent patients[2].Combined use of mannan/anti-mannan test is useful for supporting the diagnosis of candidemia[12].Use of various markers highlights the difficulties in interpreting discrepancies in their results. Despite the cost associated with these diagnostic methods, the benefit of early diagnosis and targeted, not empirical, treatment is undeniable[13].
Concerning with detection of anti-mannan IgG against *C. albicans* in 24 diseased cows by ELISA, the results revealed 15 (62.5%) positive and 9 negative cases. Although low concentration of anti-mannan was detected in negative and control cases it is not considered positive: these may be because cattle unlike other mammals, possess natural bovine antibodies against mannan and three serum collectins, all of which are capable of binding to the mannan antigen[20]. By conventional mycological examinations, *C. albicans* was detected in 69 % and 83 % of human and cows respectively. But the results of ELISA were 10 (22.2%) positive cases, 5 (11%) equivocal cases in human patients and 15 (62.5%) positive and 9 (37.5%) negative cases in diseased cows. The difference between two results could be explained by the ability of ELISA as diagnostic methods to differentiate Candida colonization of mucous membranes or superficial infection from tissue invasion and candidemia requiring antifungal therapy[7]. Even if the antibody titers can be high in colonized patients,

or antibody response may be delayed, reduced or absent, it is possible to overcome these limitations by using sensitive and standardized commercial techniques, such as the ELISA and the specificity of the tests can be improved by selecting the appropriate antigens (mannan)[3]. Furthermore, Badiee *et al*.[1] proved that the colonization of mucosal surfaces by endogenous *Candida* spp. in immunocompromised patients is often followed by the invasion of the vascular space which carries a high risk of disseminated candidiasis.

In the present study we observed that not all oral colonized cases with *C. albicans* were positive for ELISA (don't have systemic candidiasis). Consistent with our results, Krishnan[9] reported that oral candidiasis is generally a localized infection and rarely appears as a systemic fungal disease. A notable feature in this study that, ELISA results in cows showed 12 positive cases from which 9 (75%) were colonized by *C.albicans* in more than one site, 2 (16%) were colonized in one site, and 1 (8.3%) case was none colonized. Similarly, Caggiano, *et al*.[2] proved that multiple-site colonization with Candida spp. is commonly recognized as a risk factor for invasive fungal infection in critically ill patients. This study proved that IgG ELISA test for *C. albicans* mannan has important value in early diagnosis of invasive candidiasis. This is in agreement with Yera, *et al* [22], Persat, *et al*.[15] and Pfaller and Diekema[16].

Conclusion

This study proved that IgG antimannan ELISA test has important value in early diagnosis of invasive candidiasis in human and cattle. In this way, more efficient management of IC and earlier initiation of antifungal therapy can be achieved.

Table (1): Correlation between ELISA results and *C. albicans* colonization in human

Case No.	Age	Sex	Clinical case/risk factor for candidiasis	Type of samples					Optical Density	ELISA Result
				Skin	Urine	Oral	Respiratory	Blood		
1	Child	Female	Renal failure (R)		+ve	+ve		+ve	0. 869	Positive
2	Child	Male	Surgical(S)		+ve		+ve	+ve	0.519	Positive
3	Child	Male	Renal failure(R)		+ve			-ve	0.797	Positive
4	Child	Male	Surgical intensive care(S)			+ve		+ve	0.016	Negative
5	Child	Male	Acute myeloid leukemia(L)	-ve		+ve			0.079	Negative
6	Child	Male	Acute myeloid leukemia(L)	-ve		+ve			0.384	Positive
7	Child	Female	Surgical intensive care(S)			+ve	+ve	+ve	0.360	Equivocal
8	Child	Male	Surgical(S)		-ve	-ve			0.138	Negative
9	Child	Male	Cirrhosis and hepatic carcinoma(G)	+ve		-ve			0.177	Negative
10	Child	Male	Surgical intensive care(S)		+ve		-ve	-ve	0.307	Equivocal
11	Child	Male	Nephritic syndrome(R)			-ve	-ve		0.034	Negative
12	Child	Male	Pancreatitis(O)	+ve		-ve			0.034	Negative
13	Child	Male	Enteritis(G)			+ve			0.040	Negative
14	Child	Female	Ulcerative colitis(G)			+ve	-ve	-ve	0.327	Equivocal
15	Child	Male	Colon surgery(S)		-ve	-ve			0.019	Negative
16	Child	Male	Diabetic(O)	-ve		+ve			0.021	Negative
17	Child	Male	Sickle cell anemia(O)		-ve	-ve			0.021	Negative
18	Child	Female	intensive care(S)		-ve	-ve		-ve	0.017	Negative
19	Adult	Male	Nephritic syndrome(R)		+ve		-ve	-ve	0.903	Positive
20	Adult	Male	Pneumonia(O)			-ve	-ve		0.014	Negative
21	Adult	Female	Rheumatoid(O)			+ve	+ve		0.033	Negative
22	Adult	Male	Gastroenteritis(G)		+ve				0.023	Negative
23	Adult	Male	Diabetes(O)		-ve				0.020	Negative
24	Adult	Male	Open heart surgery(S)			-ve	-ve	-ve	0.057	negative
25	Adult	Female	Hemolytic anemia(O)	-ve	-ve				0.095	Negative
26	Adult	Female	Cardiac intensive care(S)			-ve	-ve		0.038	Negative
27	Adult	Male	Hepatic carcinoma(G)	ve-		-ve			0.037	Negative
28	Adult	Female	Renal failure(R)		ve+				0.458	Positive
29	Adult	Male	Renal failure (R)		ve-			ve+	0.164	Negative
30	Adult	Male	Renal failure(R)	-ve					0.055	Negative
31	Adult	Female	Renal failure(R)		ve+		ve+	-ve	0.171	Negative
32	Adult	Male	Renal failure(R)		ve-	ve-		ve-	0.043	Negative
33	Adult	Female	Thyroid carcinoma(O)	ve-		ve-			0.084	Negative
34	Adult	Female	Acute lymphocytic leukemia(L)			-ve	ve-	ve+	0.117	Negative
35	Adult	Female	Acute anemia(O)	ve+		ve+			0.013	Negative
36	Adult	Female	chronic myeloid leukemia(L)				ve+	-ve	0.779	Positive
37	Child	Female	Acute pharyngitis(O)			ve+	ve+	ve-	0.721	Positive
38	Adult	Male	Acute myeloid leukemia(L)		-ve	ve+			0.240	Equivocal
39	Adult	Female	Acute myeloid leukemia(L)	ve-				ve+	0.313	Positive
40	Child	Male	Acute lymphocytic leukemia(L)		ve-	ve-		ve-	0.099	Negative
41	Adult	Female	Acute myeloid leukemia(L)			ve+	ve+	ve+	0.198	Equivocal
42	Child	Female	Gastro intestinal tumor(G)		ve+	ve+		ve-	0.235	Positive
34	Adult	Female	Acute myeloid leukemia(L)	-ve		ve+		ve-	0.032	Negative
44	Child	Female	Acute lymphocytic leukemia(L)			ve+	ve+	ve-	0.081	Negative
45	Child	Female	Leukemia(L)	ve+		ve-			0.015	Negative

L: leukemia group, R: Renal failure Group, G: GIT group, S: Surgical intensive care, O: Other immunocompromised

Table (2): Correlation between ELISA results and *C. albicans* colonization in cattle

Case No.	Age	Sex	Clinical Case/risk factor)group(	Type of samples						Optical Density	ELISA results
				Oral	Nasal	Fecal	urine	Vaginal	Milk		
1	Adult	Female	Diseased(G)	+ve	-ve	-ve		-ve		0.2952	Positive
2	Adult	Female	Diseased (G)	-ve		+ve		+ve		0.3035	Positive
3	Adult	Female	Diseased(M)		-ve		+ve	+ve	+ve	1.700	Positive
4	Adult	Female	Diseased(M)	+ve	+ve				-ve	0.3711	Positive
5	Calf 6 month	Female	Diseased (G)	-ve		+ve	-ve			0.1895	Negative
6	Adult	Male	Diseased (G)	+ve	-ve	+ve				0.3642	Positive
7	Adult	Male	Disease (R)	-ve		-ve				0.2706	Positive
8	Adult	Female	Diseased(M)		-ve			+ve	+ve	1.736	Positive
9	Calf 6 month	Male	Diseased (G)	+ve		+ve				0.1593	Negative
10	Calf 6 month	Male	Diseased (G)	-ve	+ve	-ve				0.1833	Negative
11	Adult	Female	Diseased(M)	+ve			-ve		-ve	0.3742	Positive
12	Calf 6 month	Male	Diseased (G)		-ve	+ve	-ve			0.1845	Negative
13	Adult	Female	Diseased (R)			+ve		-ve		0.2612	Negative
14	Adult	Female	Diseased (G)	-ve	+ve	-ve	+ve			0.5493	Positive
15	Calf 6 month	Female	Diseased (R)		-ve	+ve		-ve		0.1709	Negative
16	Adult	Female	Diseased (G)	+ve			-ve	+ve		2.350	Positive
17	Adult	Female	Diseased(M)	-ve			-ve	-ve	-ve	1.238	Positive
18	Adult	Female	Diseased (G)	-ve	-ve		+ve	-ve		2.006	Positive
19	Adult	Female	Diseased(M)		+ve		+ve	-ve	+ve	2.274	Positive
20	Calf 6 month	Female	Diseased (R)	+ve	+ve	-ve				2.381	Positive
21	Adult	Female	Diseased(M)				-ve	+ve	-ve	0.3391	Positive
22	Adult	Female	Diseased(M)		-ve	-ve		+ve	-ve	0.2249	Negative
23	Adult	Female	Diseased (R)	-ve	-ve					0.1502	Negative
24	Adult	Female	Diseased (R)	-ve	-ve			-ve		0.1754	Negative

G: Gastro intestinal tract disturbance, R: Respiratory manifestation, M: Mastitis, +ve/-ve: positive/ negative for *C. albicans* isolation

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