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EVALUATION OF INDIRECT IMMUNOFLUORESCENT TEST AND CD4/CD8 RATIO IN CANDIDIASIS

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ABSTRACT

In order to study and evaluate the indirect immunofluorescent test and the ratio of CD4 lymphocytes to CD8 lymphocytes, 97 cases of positive candida cultures were selected from among 400 patients referring to the hospital and suspected of candidiasis. In this study 39% of the patients had an antibody titer less than 1: 160. No significant correlation was seen between candidiasis and the results of the indirect immunofluorescent test. But, considering the site of isolation, the antibody titer was above 1: 160 in all cases in which candida was isolated from blood, whereas only 48% of samples in which candida was isolated from urine had an antibody titer above 1:160; 7% of cases in which candida was isolated from the vagina had an antibody titer above 1:160. Patients suffering from systemic candidiasis had a decreased CD4/CD8 lymphocyte ratio compared to normal subjects, whereas in vaginal and skin candidiasis, the ratio had not differed significantly.
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INTRODUCTION

Candida organisms are fungi yeasts that exist predominantly in a unicellular form. There are more than 150 species of candida, but only 10 are regarded as important pathogens for humans. Technical advances have made definitive specification possible within two days. The incidence of disease has increased in frequency over the past 20 years, and this may have been due to diagnostic methods, immunosuppressives and the appearance of AIDS, thus highlighting the role of lymphocytes in host defense against this opportunistic infection.

Considering the importance of the role of cellular and humoral immunity in the prevention and eradication of infection, we have undertaken the study of the humoral response and CD4/CD8 ratio in patients infected with candida.

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MATERIAL AND METHODS

Selection of patients and culture

From among 400 patients refering to hospitals affiliated to the Iran University of Medical Sciences, and suspected of having candidiasis, a total of 97 candida-positive samples were isolated from blood, urine, vagina and skin. Direct and culture sample tests on two separate media, Sabouraud's dextrose agar and Sabouraud's dextrose agar with cyclohexamide and chloramphenicol were performed for all patients. If yeast growth was seen, it was transferred to cornmeal agar media with 1% tween 80 and supplementary tests including assimilation of carbohydrates and nitrates, carbohydrate fermentation, urea hydrolysis and germ tube production test for species diagnosis were performed under sterile conditions.

Indirect immunofluorescent test

In this method suspensions of candida isolated from each patient's culture were coated on slides as antigen and a

Table I. Frequencies of different species relative to the area of clinical infection.

Site of isolation	*Candida albicans*		Other candida species		Yeast		Total	
	No.	%	No.	%	No.	%	No.	%
Urinary tract infection	18	58	11	36	2	6	31	32
Vulvovaginitis	21	75	5	18	2	7	28	29
Septicemia	13	100	0	0	0	0	13	13
Esophagitis	4	50	3	37	1	12	8	8
Onychomycosis	5	71	2	29	0	0	7	7
Pneumonia	3	100	0	0	0	0	3	3
Skin infection	3	75	1	25	0	0	4	4
Gastritis	3	100	0	0	0	0	3	3
Total No.	70		22		5		97	
Total %	72		23		5		100	

Table II. Antibody titers relative to site of isolation.

Antibody titer / Site of isolation	$<\frac{1}{160}$		$\geq\frac{1}{160}$	
	No.	%	No.	%
Urinary tract infection	16	52	15	48
Vulvovaginitis	26	93	2	7
Septicemia	0	0	13	100
Esophagitis	6	75	2	25
Onychomycosis	7	100	0	0
Pneumonia	0	0	3	100
Skin infection	4	100	0	0
Gastritis	0	0	3	100
Total No.	59		38	
%	61		39	

serial dilution of 1:10 to 1:1280 of the patient's serum was added. After 30 minutes of incubation in a wet container, slides were washed three times with PBS and conjugated antihuman globulin with FITC was added. 30 minutes later the slides were washed and results evaluated using a fluorescent microscope.

In order to study the CD4/CD8 ratio we used an immunochemical method, namely the alkaline phosphatase anti-alkaline phosphatase antibody technique (Dako Corp., Carpinterial).[4,5] Briefly lymphocytes were treated with Ficoll-Hypaque (Histopaque-1077, Sigma) and 3 smears prepared using a cytospiner (Cytospin 3, Shandon) for each patient. Each slide was incubated with mouse monoclonal antibody against CD3, CD4 and CD8 and then incubated in rabbit antiserum to mouse immunoglobulins, and finally with alkaline phosphatase anti-alkaline phosphatase immune complexes. Each incubation step lasted 30 min. Slides were washed in tris buffer (pH= 7.6) for 5 min and the alkaline phosphatase reaction was developed with napthol AS-MX and fast red TR salt in tris buffer. They were then counter-stained in Mayer's hematoxylin and mounted in glycerol gelatin while still wet for microscopic study. Foreach smear 200 cells were counted and the percentage of lymphocytes positive and negative for CD3, CD4 and CD8 antigens were recorded.

RESULTS

Of the 97 cases with positive cultures, 70 (72%) were *Candida albicans*, 22 (23%) were other types of candida and 5 cases (5%) were yeast. The distribution of the absolute and relative frequency of separated organisms and the site of

Table III. The ratio of CD4 to CD8 lymphocytes in patients afflicted with candidiasis in comparison with the control group.

	Number	CD4/CD8 Ratio Mean±SD
Candidiasis	22	1.65±0.5
Normal control	15	1.95±0.46
Systemic candidiasis	8	1.37±0.3
Skin and mucosal candidiasis	14	1.82±0.5

isolation are shown in Table I. The titer of antibody against candida with the indirect immunofluorescent test is given in Table II. After classifying the patients into three groups with skin, mucous membrane and systemic candidiasis, respectively, we found no humoral immune response against candida organisms in skin involvement, whereas in systemic candidiasis a high antibody titer was detected (Fig. 1).

Concerning the CD4/CD8 lymphocyte ratio, 22 patients out of a total of 97 were studied and compared with the control group and no significant difference was seen. However, following classification of candidiasis into membranous, mucosal and systemic forms, we observed that the ratio of CD4/CD8 cells was reduced significantly in systemic candidiasis when compared to controls (a= 0.005) whereas in membranous and mucosal candidiasis no significant difference existed between the two groups (Table III).

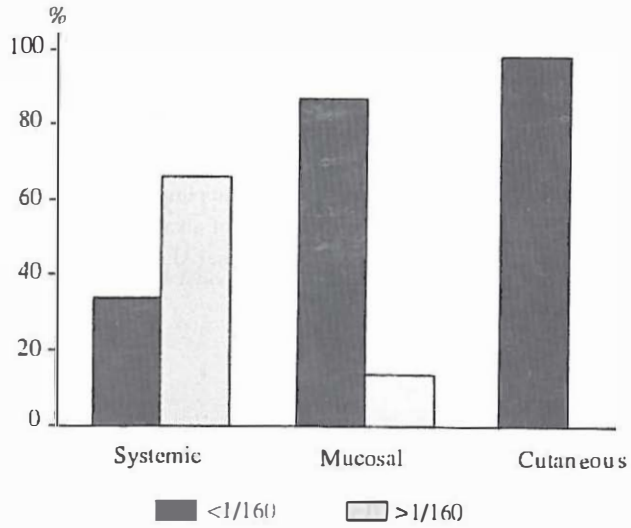

Fig. 1. Antibody titers in patients with cutaneous, mucosal and systemic candidiasis.

DISCUSSION

Candidiasis is of special importance because factors that increase susceptibility to this infection are increasing daily. Antibody determinations were the first tests used for the immunodiagnosis of disseminated candidiasis in the early 1980's.[6] A variety of methods have been studied, but it is difficult to compare published studies because of differences among patient populations, test techniques and study designs.[6] However, antibody tests are not sensitive among immunosuppressed patients with candidiasis who are often unable to produce antibodies, and the prevalence of false-negative results is 30-70% in this population.[7] False-positive results may occur in patients with burns.[8] In our study 60% of the patients had no humoral immune response against candida while 40% had antibody titers above 1:160 against this organism. By classifying candidiasis according to the site of isolation, in those cases in which candida was separated from blood, 100% had an antibody titer above 1:160, whereas in skin and mucous membrane candidiasis, 95% had an antibody titer below 1:160. Therefore, in the process of systemic candidiasis we have a proper humoral immune response, while in other forms of involvement a proper humoral immune response is not observed. It is perhaps for this reason that patients with skin and mucous membrane candidiasis do not recover very easily, whereas patients with systemic candidiasis recover when the immune system is activated. Thus, in mucocutaneous candidiasis, in order to help patients recover quickly, we must think of methods of humoral response modulation or elimination of agents or factors that prevent the response of the immune system against this infection. In order to prove the protective role of antibodies in candidiasis, further studies are necessary. Results of the study on cellular immunity in our survey—which was performed by determination of the CD4/CD8 ratio—confirm the point that in mucocutaneous candidiasis, as the immune system fails to become active, a change is not observed in the CD4/CD8 ratio, whereas in systemic infection in which candida organisms appear in the blood, due to activation of the immune system, the ratio of CD4/CD8 lymphocytes decreases relative to controls.

Our study also showed that concerning laboratory diagnosis, the indirect immunofluorescent test is not valid in mucocutaneous candidiasis but is a valuable diagnostic tool in systemic candidiasis.

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