Plasma Proteins and DNA Toxicity Profile Assessment Post Infection of Swiss Albino Rats with Different Pathogenic Species of Candida

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Abstract: Very little studies were conducted to evaluate the toxicity of pathogenic Candida deteriorative activity on murine serum proteins profile and DNA. In the present study protein analysis showed a drastic deteriorative effect of test 5 Candida species on the total IgG, IgM, lipoprotein, and haptoglobins. Results indicated that the protein fractions post-albumin (77.233KD, 96.925 KD) could not be detected post infection with *Candida Alb., C. guilliermondii and C. tropicalis*. Also, ceruloplasmin could not be detected post infection using *C.parapsilosis* and *C. glabrata*. In the same way transferrin could not be detected post infection using *C. glabrata* and *C. tropicalis*. SDS_PAGE of murine DNA indicated that DNA of infected animals showed a slight degenerative activity and a lower MW bands than the control were detected post infection with *C.glabrata* and *C. albicans*.

Keywords: Protein, DNA, Electrophoresis, Candida species, Pathogenicity.

INTRODUCTION

The opportunistic pathogen *Candida albicans* is able to cause disseminated infections in immuno compromised patients. To reduce mortality in patients with invasive candidiasis, early initiation of antifungal drug therapy is critical Anaissie (1992). The clinical diagnosis is complicated because *C. albicans* commonly colonizes or causes mild infection of the mucous membranes or skin. It is, therefore, often difficult to judge the clinical significance of the finding of *C. albicans* in secretions or on the body surfaces when systemic infection is suspected in the case of occult fever or for other reasons. Microbiological evidence for the diagnosis of invasive candidiasis requires the demonstration of *C. albicans* in sterile body fluids, biopsies, or multiple consecutive blood cultures Jones (1990). There are, however, many problems inherent in these tests Murray (1991). Human plasma consists of mainly large number of proteins, which vary in terms of both composition and concentration with the physiological state of the individual. Alterations in protein concentrations reflect the current state of the individual's health and thus may be utilized as valuable biomarkers for a specific biological process or disease Mi-Ryung Kim. and Chan-Wha Kim (2006). Murine injections of the insoluble portion of disrupted yeast cells of *Blastomyces dermatitidis* animals exhibited a biphasic pyrogenic response, and a decrease in total serum proteins Marshall *et al.*, (2004).Protein electrophoresis is a routine method used for diagnosing dysproteinaemia which is considered a condition involving quantitative and qualitative changes in serum proteins and is associated with a large number of disease conditions. It is also a tool used for identifying and monitoring changes in malignant turnouts, indicators of acute and chronic inflammation, liver diseases, antibody deficiencies, and monoclonal gammopathies. There is a dynamic balance between protein biosynthesis, metabolization, and excretion through the gastrointestinal tract. All serum proteins are synthesized in the liver, with the exception of the gamma globulins, which are produced by the beta lymphocytes. Serum proteins have the shortest half-life of all the proteins in the body and are therefore particularly sensitive to acute or chronic changes in amino acid and protein metabolism Wedler *et al.*, (1998). So the present work aims to monitor the effect of Candida albicans in addition other pathogenic Candida strains post infection secreted metabolites on serum protein profile and related cellular DNA of infected murine model.

MATERIALS AND METHODS

Seven groups of white albino rats (6 each) were intraperitoneally injected with 0.5×10^5 cells/ml of the tested yeast suspension; the seventh group was injected with phosphate buffered saline as a negative control group. The infected animals were kept in separate cages and were fed according to Edmund (1950).The animals were examined daily for 8 weeks. Body weight was monitored daily using Sartorious-France balance Blood samples were collected from infected animals via the retro orbital sinus in EDTA containing tubes, plasma of infected animals were collected and processed for SDS-electrophoresis and DNA was extracted according to Clarke (1965) and Buchman *et al.*, (1990) respectively, where blood samples (5 ml each) were collected on EDTA containing tubes. Blood samples were centrifuged for 15 minutes using cold centrifuge (Jouan-France). Plasma of infected rat was separated in clean dry tube. Blood cells were mixed with 50 ml lysis buffer for 10 minutes. White blood cells were precipitated by centrifugation for 10 minutes at 2500 rpm. Pellets were vigorously resuspended in 1-2 ml white blood cells lysis buffer to extract the cellular DNA. The DNA profile was monitored using SDS-PAGE

RESULTS

Plasma protein electrophoresis:

Plasma protein gel electrophoresis of negative control group processed showed the existence of 11 bands with their specific molecular weight and related optical density. The plasma of rats infected with *C. albicans, C. tropicalis,* and *C. guilliermondii* showed that the post albumin bands were not detected. While,

ceruloplasmin and transferrin could not be detected post infection with *C.parapsilosis*, *C. glabrata*, *C.tropicalis*, and *C.glabrata respectively*. Total IgG, IgM, lipoprotein, and haptoglobins could not be detected too post rats infection with all tested Candida species table (1).

The deteriorative effect was followed by the occurrence on new bands not detected in the negative control electrophoretic pattern. These bands were 3 bands of MW range of 18.29, 22.657 and 40.908 KD post infection with *C.parapsilosis*, 3 bands of MW range of 30.562, 34.037, and 47.353 KD detected post infection with *C.glabrata* and 1 band detected post infection with *C. tropicalis*, *C. albicans* and *C. guilliermondii* of MW of 55.874, 25.840, and 28.4 KD respectively.

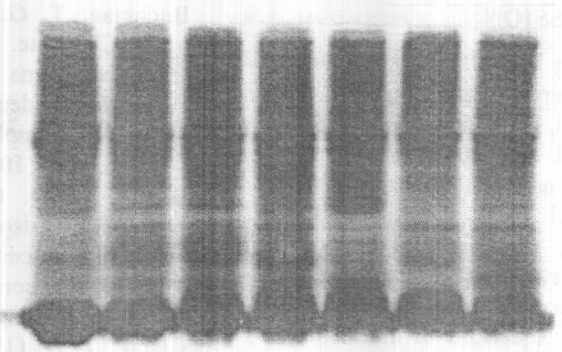
Genomic DNA analysis:

Also, the genomic DNA pattern of control group was separated in the first lane of the gel with molecular weight of 2638 K.D and the genomic DNA of rats infected with *C.tropicalis*, *C.pseudotropicals*,*C. guilliermondii* and *C. parapsilosis* showed no change compared to the control group. While, the *C.albicans* and *C.glabrata* infected rats showed a difference in the genomic DNA pattern where a expanded smear of the DNA was detected figure 8.

Table (1): Candida infected rats' plasma proteins distribution post electrophoretic analysis.

B.T.N	M.WT.	M.	Cont	C .par	C. gla.	C.P.T.	C.T.	C.alb.	C.guil
1-albumin	156.513	.	54.717	49.255	52.860	57.929	58.888	55.676	57.177
	116	206.713	.	.	.	.	.	.	.
	97.4	188.360	.	.	.	.	.	.	.
	79.427	.	.	.	.	.	.	59.414	.
2 –post albumin	77.233	.	50.249	51.879	49.304	51.072	56.589	.	.
	74.468	.	.	.	.	.	.	.	58.029
	66.2	175.197	.	.	.	.	.	.	.
	61.538	.	.	.	.	.	.	58.464	.
3- post albumin	56.925	.	47.838	47.740	48.204	49.754	.	.	54.083
	55.874	.	.	.	.	.	55.320	.	.
	49.32	.	.	.	.	.	.	60.668	.
	48.685	.	.	48.092	.	49.266	56.680	.	.
	47.335	.	.	.	48.775	.	.	.	.
	46.492	.	.	.	.	.	.	.	55.842
4- ceruloplasmin	41.413	.	52.985	.	.	49.378	64.224	74.088	61.418
	40.908	.	.	56.120	.	.	.	.	.
	39.36	.	.	.	52.800	.	.	.	.
	37.5	192.716	.	.	.	.	.	.	.
	36.503	.	.	.	.	.	64.035	.	.
	36.078	.	.	.	55.844	.	.	.	.
5- tranferrin	34.754	.	69.503	63.730	.	50.479	.	78.548	71.562
	34.037	.	.	.	69.358	.	.	.	.
	33.429	.	.	80.688	.	61.468	66.778	93.549	85.647
6- 2-2-haptoglobins	32.756	.	76.700	.	.	.	.	.	.
	32.019	.	.	.	73.560	.	.	.	.
	30.939	.	.	71.493	.	59.774	94.850	83.825	75.728
	30.562	.	.	.	56.856	.	.	.	.
7- haptoglobins	29.244	.	87.919	62.386	53.022	60.367	86.488	80.831	
	28.4	181.700	.	.	.	.	.	.	78.680
8 - haptoglobins	27.751	.	74.970	60.789	55.417	53.326	.	.	.
	25.84	.	.	70.940	.	.	.	79.458	.
9- IgG	25.22	.	81.203	.	.	.	.	.	.
	24.754	.	.	.	63.437	58.595	75.885	.	64.340
	22.657	.	.	69.972	.	.	.	.	.
10- IgM	22.113	.	81.495	.	.	.	.	.	.
	19.557	.	.	.	49.719	52.382	60.107	61.336	57.278
	18.29	146.572	.	48.384	.	.	.	.	.
11-lipoprotein	17.892	.	49.715	.	.	.	.	.	.
	14	237.978	.	.	.	.	.	.	.
	12.244	.	.	214.63	205.577	197.40	208.15	212.72	206.47
		7	**11**	**14**	**14**	**13**	**12**	**12**	**12**

B.T.N= band type number, M.W= Molecular weight (K.D), CONT=Control, M= Marker, C. Prap = *Candida parapsilosis*, C. gla=*Candida glabrata*, C. alb= *Candida albicans*, C.P.T=*Candida pseudotropicalis*, C.T= *Candida tropicalis*, C.guil= *Candida guilliermondii*.



a b c d e f g h

Fig. (1): Plasma proteins gel electrophoresis

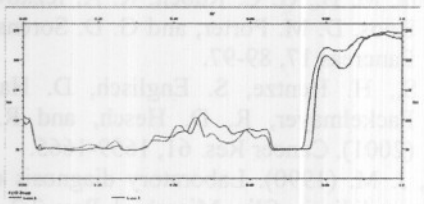


Fig. (2): Control+ *Candida parapsilosis.*

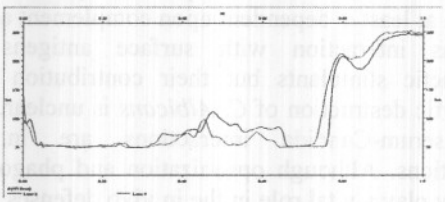


Fig. (3): Control+ *Candida glabrata*

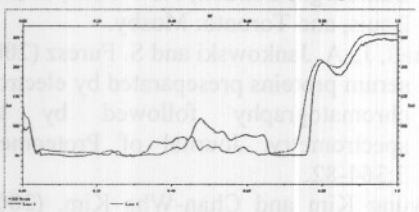


Fig. (4): Control+ *Candida pseudotropicalis*

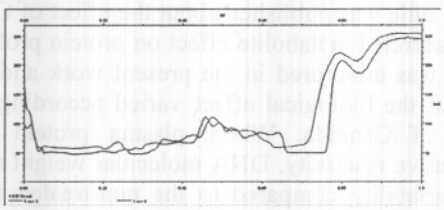


Fig. (5): Control+ *Candida tropicalis.*

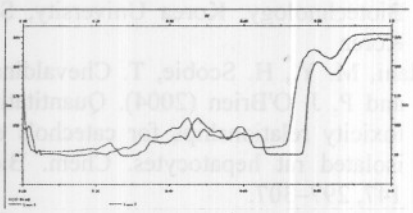


Fig. (6): Control+ *Candida albicans*

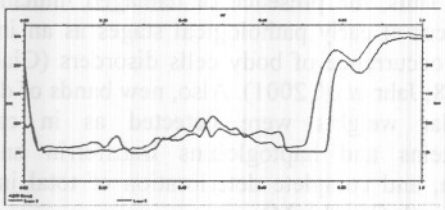


Fig. (7): Control+ *Candida guilliermondii*

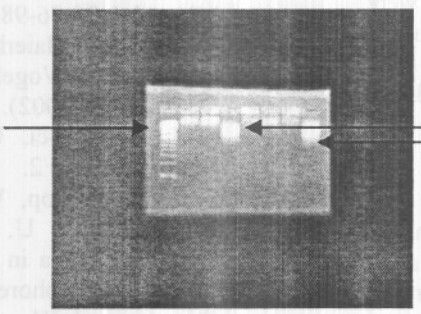


Fig. (8): DNA electrophoretic pattern

DISCUSSION

Toxicity to a wide variety of biological systems was investigated, including bacteria, algae, *Daphnia*, fish, fungi, plants, and mammals Bundy *et al.*, (2001). A large number of traditional statistical methods were used for evaluation of acute toxicity (QSARs) (regression analysis, partial least squares analysis) Moridani *et al.*, (2004), Toxicity of microbial metabolites on biological systems was a clear fact despite the mechanism is not completely investigated Wang *et al.*, (2002c). Since injections of the insoluble portion of disrupted yeast cells of *Blastomyces dermatitidis* made animals exhibited a biphasic pyrogenic response,decreased total serum proteins, and there was no release of interferon Marshall *et al.*, (2004). Also, it was reported that the murine opsonins seem to be a part of the complement system or their function at least is dependent upon complement activity. Opsonins interaction with surface antigens form chemotactic stimulants but their contribution to the phagocytic destruction of *C. Albicans* is unclear. All of these serum-Candida interactions are in vitro observations. Although opsonization and phagocytosis probably play a vital role in the in vivo defenses against invading Candida, the contribution of the other interactions to host resistance remain unknown. Despite the very little was published about the effect of Candida species secreted metabolite effect on protein profile and DNA it was monitored in the present work and it was clear that the biological effect varied according to the species of Candida. Where plasma protein profile deteriorative reactivity, DNA molecular weight showed some variability compared to the non-treated animals model group, the damage of DNA could be attributed to a specific toxic effect of any chemical or biological agents. Thus, the presence of damaged /mutant DNA may occur at early pathological stages as an indicator for the occurrence of body cells disorders (Giacona *et al.*, 1998; Jahr *et al.*,2001). Also, new bands of different molecular weights were detected as in case of, lipoproteins and haptoglobins transferrin and post albumin, and complete deterioration of total immuno-globulins (IgG and IgM).

Detected post infection with yeast cells of interest body weight loss post infection was detected till animal mortality. These biological disorders induced were almost explained by (Marshall, 2000). So, further more biological and immunological investigations are a must to clarify the pathways of pathological yeast induced toxicity

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تقييم الشكل السمي لأنواع الكانديدا علي بروتينات بلازما الدم والحامض النووي الديوكسيريبوزي للفأر السويسري الأبيض

إيمان أنور عطية

قسم النبات- كلية العلوم- جامعة قناة السويس- ٤١٥٢٢ - الإسماعيلية - مصر

علي الرغم من خطورة تأثير جنس الكانديدا المرضية علي الإنسان و الحيوان إلا أن قليل من الدراسات إهتمت بتقييم سمية جنس الكانديدا. ومن خلال النتائج التي توصل إليها البحث بدراسة تأثير جنس الكانديدا علي بروتينات بلازما الدم و الحامض النووي الديوكسيريبوزي تلاحظ أن هناك خلل واضح في بعض البروتينات الخاصة بالجهاز المناعي مما أدي إلي إختفاء كل من الجلوبيولين المناعي بنوعية (الجي و الإم)وكذلك الهابتوجلوبين و اليبوبروتين كما تلاحظ إختفاء أنواع الألبيومين وذلك نتيجة نواتج أيض الكانديدا من نوع الكانديدا البكانس و جيلارموندى و الكانديدا تروبيكالس التي أصيبت بها حيوانات التجارب معمليا.وكذلك تلاحظ إختفاء بروتين السيروبلازمين و الترانسفيرين نتيجة الإصابة بالكانديدا بارابسيلوسيز و الكانديدا جلابراتا. ومن خلال الدراسة تلاحظ أن هناك تأثيرا علي الحامض الديوكسيريبوزي للفئران المصابة بجنس الكانديدا البكانس و الكانديدا جلابراتا