Available online at http://www.sjomr.org

SCIENTIFIC JOURNAL OF MEDICAL RESEARCH

Vol. 2, Issue 5, pp 42-46, Winter 2018

ISSN: 2520-5234

ORIGINAL ARTICLE

Evaluation of immunization protocol in mice injected with Whole Cell Fraction antigen of *C. albicans* isolated from vaginal infections

Nada Fadil Abass[1] , Wafaa Sadoon Shani[1] and **Inaam Mahmood Najim[1]**

[1] Department of Biology, College of Science, University of Basrah, Basrah, Iraq.

ARTICLE INFORMATIONS

Article History:
Submitted: 21 January 2018
Revised version received:
31 January 2018
Accepted: 2 February 2018
Published online: 1 March 2018

Key words:
C. albicans
Vaginal candidiasis
SDA
IgG
IL-4
IFN-γ

Corresponding author:
Nada Fadil Abass
Email: dewdropaa90@gmail.com
Department of Biology
College of Science
University of Basrah
Basrah
Iraq.

ABSTRACT

Objective: The present study aimed to isolation and identification of *C. albicans* from women infected with vaginal candidiasis, then Preparation of whole cell fraction (WCF) antigen and testing immunization efficiency by evaluating the humoral immune response (by measuring IgG level in serum) and cellular immune response (by measuring IFN-γ and IL-4 concentration).

Methods: Seventy five clinical specimens were collected from women suspected to infect with vaginal candidiasis were cultured on Sabouraud Dextrose Agar (SDA) and CHROMagar Candida media and identified by biochemical test. Three of them identified by molecular diagnosis by using ITS1 and ITS4 primers. BalB/c mice were immunized subcutaneously and intradermaly with *C. albicans* strain M366B whole cell fraction antigen for 21 days to evaluate humoral and cellular immune response represented by IgG, Th1 and Th2 cytokine level by microiterplate ELISA.

Results: Among total positive cases of vaginal infections only 43 (57.3%) was caused by yeast infections, whereas *Candida albicans* was recorded more dominance (30/43) 69.77% among other yeast infections. Humoral and cellular immune response represented by IgG, Th1 (IFN-γ) and Th2 (IL-4) cytokine level were measured respectively. IgG level appeared in high concentration in immunized mice by comparison with control groups in a high significant differences ($P<0.01$), and immune response represented by (IL-4) concentration in immunized group was higher than those in control mice in a significant differences ($P<0.01$); while there was no significant differences in (IFN-γ) concentration between immunized and control groups. So WCF antigen can efficiently stimulate both humoral and cellular immune response by elevation both IgG and IL-4 concentration in sera of immunized mice.

Conclusion: Different species of yeast are causing vaginal candidiasis, *C. albicans* was most prevalence than others in infection in V. candidiasis, . WCF antigen was immunogenic in regarding in inducing IgG and IL-4 secretion.

Citation: Abass N.F., Shani W.S. and Najim I.M. "Evaluation of immunization protocol in mice injected with Whole Cell Fraction antigen of *C. albicans* isolated from vaginal infections". Sci. J. Med. Res. 2018, 2 (5): 42-46.

INTRODUCTION

The endogenous yeast *Candida albicans* is an opportunistic fungal pathogen which can cause disease called (candidiasis or candidiosis) in human niches. It is occupying a part of microflora of oral cavity ,superficial surfaces, the gastrointestinal tract, vaginal canal, and the urinary environment of the host[1,2]. Over growth of these

organisms, however will lead to infection that are ranging from superficial infection of the skin to life threatening systemic infections, and it commonly takes place in immunocompermised individuals[3]. Vulvovaginal candidiasis usually intensified in pregnant women or women who used contraceptives that contain estrogen which increase glycogen levels that increasing lactic acid bacteria which decomposing glycogen that cause increase mannoproteins that helps to increase the probability of yeast adherence to mucous membrane, infection seems to be red ulcers with distinctive edges with thick white or yellow discharge, burning and pain in infected area[4,5]. In recent years, a different publications have focused on the immunogenicity and efficacy of vaccines against candidiasis infections in an animal models, and even have tested the efficacy and safety in clinical trials. Fungal cell-wall polysaccharide, proteins, and live attenuated fungi have been tested as a vaccine targets. Even considering the capital and technical barriers, bringing protective vaccines to the clinic appears promising[6]. *C. albicans* have variable antigens that have the ability to induce humoral immune response and cell mediated immunity for example; cell wall proteins antigens that include (Secreted aspartyl proteinases (Sap), 58-kilodalton fibrinogen binding cell wall mannoprotein (mp58) Heat shock proteins (Hsps)[7,8,9,10]. In addition to glycolytic enzymes such as enolase[11].

MATERIALS AND METHODS

Seventy five of moist vaginal swap specimens were collected by trained physician from women suspected with vaginal candidiasis infection with age range from 15-50 year from the-Child and birth hospital in Al-Basra province from October, 2016 to February, 2017. Swaps were streaked on Sabouraud dextrose agar and chrome agar Candida media and incubated for (24-48) h at 37^{o} C[12], then identified by using API Candid system. In addition by using ITS1 and ITS4 primers[13]; three isolates from *Candida* spp. were identified by sequencing by MEGA.6 program in macrogen company/Korea.

A Loopfall of *C. albicans* strain M366B colony were inoculated in Sabouraud dextrose broth and incubated for 24-48 h at 37^{o} C under aeration with reciprocal shaking at 100 rpm. Cells of *C. albicans* were harvested by centrifugation for 10 min at 4^{o}C at 1400 rpm washed twice with sterile 50mM potassium phosphate buffer (pH 7.5) containing 1M NaCl as a stabilizer. The cells number were adjusted equal to tube number 10 of McFarland standard ($3*10^{9}$) in a sterile 50 mM potassium phosphate buffer (pH 7.5) containing 1M NaCl. The cells were lysed by sonication in (Olympus/Japan) sonicator then centrifuged at 15000 rpm for 10 min at 4^{o}C[14]. The supernatant were collected as a whole cell fraction antigen (WCF) total protein of whole cell fraction were measured according to method of (Hudson & Hay, 1984) (1.55*absorbance at 280)-(0.77*absorbance at 260)[15].

Two groups of BalB/c mice were used and each one comprises eighteen animals were immunized subcutaneously and intradermaly with 0.2µl WCF antigen emulsified with complete Freund's adjuvant at the first week, after 7 days with incomplete Freund's adjuvant (sigma USA) and at the third week without adjuvant, then after 21 days from first injection mice were killed; serum was collected for measuring (IgG, IFN-γ and IL-4 levels by microtiterplate ELISA at OD 450 nm).

RESULTS

During the study period , a total of 75 samples of vaginal infection swaps were examined microscopically. Out of total positive cases of vaginal infections only 43 (57.3%) was caused by yeast infections, whereas *Candida albicans* was recorded more dominance (30/43) 69.77% among other yeast infections.

The prevalence of all vaginal infections and vaginal yeast infections according to age group showed that the higher infection rate was in 26-35 age group with percentage (48 and 58.1%), followed by 15-25 age group with percentage (37.4 and 34.9%) respectively (Table 1).

Table 1: **Distribution of yeast infections and *C. albicans* infections according to age groups.**

Age groups	Vaginal infections (%)	Yeasts infections (%)	*C.albicans* infections (%)
(15-25)	28 (37.3)	15 (34.9)	13 (43.3)
(26-35)	36 (48%)	25 (58.1)	15 (50)
(36-45)	8 (10.7)	2 (4.7)	2 (6.7)
(46-55)	3 (4%)	1 (2.3)	0
Total	**75**	**43**	**30**

Microscopic Examination

The results of direct examination for vaginal swaps that treated with wet preparation and stained slide smear were showed epithelial cells, blastospore(yeasts budding cells), pseudohyphae and true hyphae(Figure 1).

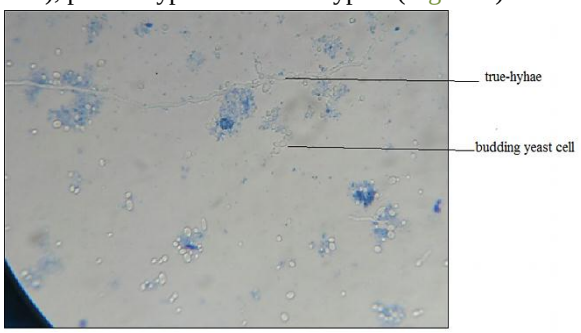

Figure 1: ***C. albicans* in vaginal swap staind by methylene blue (40x).**

Culturing data

From 75 vaginal swaps, only 43 (57.3%) were positive for yeast in culture including 40 isolates of yeast belong to *Candida* spp. were identified as follow *C. albicans* 30, (69.77%); *C. glabrata* 4, (9.3%); *C. parapsilosis* 2 (4.65%); *C. famata 2* (4.65%); *C. tropicalis* 2

(4.65%).while *Tricosporon* spp. 3 (6.98%). It was grew rapidly through 48 h.

Table 2: **Causative agents of vaginal yeasts infections**

Yeast species	Percentage (%)
C. albicans	30 (69.77)
C. glabrata	4 (9.3%)
C. famata	2 (4.65%)
C. parapsilosis	2 (4.65%)
C. tropicalis	2 (4.65%)
Tricosporon spp.	3 (6.98%)
Total	**43 (100)**

Yeast identification

All of yeast isolates were identified by culture morphology, microscopic elements, germ tube, and assimilation properties by API C kits.

Colony color on CHROMagar Candida and Brilliance ™ Candida Agar

Depending on colony color and morphology (smooth/rough) to distinguish the yeast according to enzymatic activity; variable colors on CHROMagar Candida plates appeared as follow; *C. albicans* with green color, *C. glabrata, C. famata* and *C. parapsilosis* with (off white, creamy) color, and *C. tropicalis* with brawny color. Also *Tricosporon* appeared with violet colors on this media. On Brilliance ™ Candida agar 30 isolates which appeared with green colors was identified for *C. albicans* , brown/ beige for *C. glabrata, C. famata* and C. *parapsilosis*; while *C. tropicalis* dark brown to light purple colors.

Biochemical test

By using Analytical profile index (API) Candida kit to identify and confirm identification of yeast, thirty isolates were identified as *C. albicans* ,four isolates were identified as *C. glabrata*, while two isolates identified as *C. parapsilosis*, two isolates of *C. tropicalis*, two isolates of *C. famata* and three isolates were identified as *Tricosporon* sp. (Figure 2).

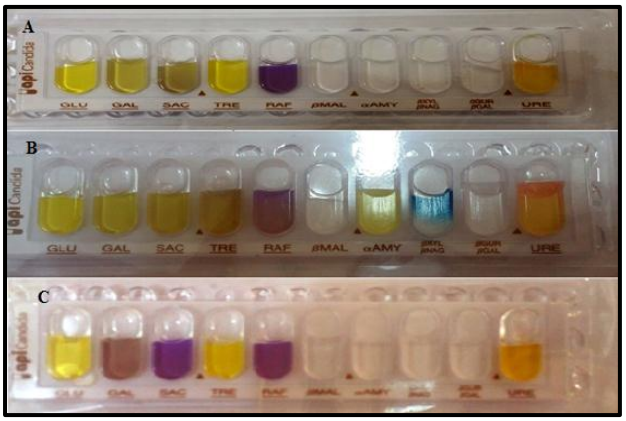

Figure 2: **Results of Analytical profile index (API C) test A=*C. famata*, B=*C. albicans*, C=*C. glabrata***

Molecular diagnosis

Three isolates of *Candida* spp. has been selected to confirm their identification by molecular analysis. According to PCR assay of ITS1 and ITS4 genes the band in Figure 3 confirmed the size of gene was 500bp.

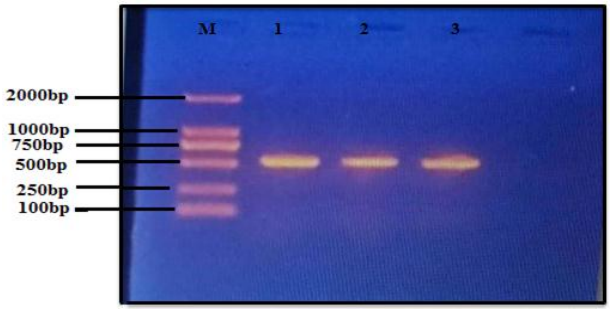

Figure 3: **2% Agarose gel electrophoresis analysis for PCR assay with two primers ITS1,ITS4 M=DNA marker Lanes:1-3 gen of *Candida* spp.**

Immunization protocol

Protein concentration of prepared antigen

The concentration of protein for (WCF) antigen was approximately 0.107 mg/ml.

Determination of humoral immune response

The level of immunoglobulin G (IgG) was measured in serum of immunized and control mice. The results as shown mean±SD revealed that the two control groups injected (SC/Id) were closely related to each other in mean 10.44±0.95 ; 10.15±1.104 respectively. IgG level in immunized group with subcutaneous injection for WCF antigen was higher than control group 34.41± 16.13 with high significant differences P<0.01. As well as IgG level in mice immunized intradermaly with WCF antigen was higher than those of control group 26.96 ±10.38 (Table 3).

Table 3: **IgG level in mice immunized subcutaneously and intradermaly with WCF antigen and control group**

Groups	Route of immunization	No.	Mean	Range	SD	SE
WCF	SC	18	34.41	17.4-55.5	16.13	3.8081
	Id.	18	26.96	11.5-44.8	10.38	2.4465
Control	SC	5	10.44	9.18-11.11	0.95	0.4248
	Id.	5	10.15	8.9-11.8	1.104	0.4937

Determination of IFN-γ concentration

IFN-γ concentration was measured in immunized and control groups after 21 day from immunization, statistical analysis confirmed that the mean concentration mean±SD of IFN-γ in subcutaneously immunized group of mice with (WCF) Ag didn't show any significant differences 332.75±73.43 comparing with control group 302.17±72.14, while mice that injected intradermal showed decreasing in IFN-γ levels 271.42±50.67 by comparison with control mice 296.22±75.46 (Table 4).

Table 4: **IFN-γ level in subcutaneously and intradermaly immunized mice with WCF antigen and control groups**

Groups	Route of immunization	No.	Mean	Range	SD	SE
WCF	SC	18	332.75	236.41-430.71	73.43	17.307
	Id.	18	271.42	184.10-355.98	50.67	11.643
Control	SC	5	302.17	184.1-348.5	72.14	32.261
	Id.	5	296.22	176.62-355.98	75.46	33746

Determination of IL-4 concentration

Concentration of IL-4 in sera of both control and immunized groups was measured after 21day from immunization. IL-4 levels in group of mice that immunized subcutaneously with (WCF) antigen was higher than control group, 183.99±20.68 ; 119.22±8.40 , respectively. Whereas IL-4 concentrations in mice which were immunized intradermal with (WCF) antigen was higher than control group 211.22±7.64 ; 108.12±15.81 with a high significant increasing (P<0.01)between immunized and control group (Table 5).

Table 5: **IL-4 concentrations subcutaneously and intradermaly immunized with WCF antigen and control groups**

Groups	Route of immunization	No.	Mean	Range	SD	SE
WCF	SC	18	183.99	153.42-213.52	20.68	4.874
	Id.	18	211.22	199.65-218-15	7.64	1.8007
Control	SC	5	119.22	111.82-130.31	8.40	2.236
	Id.	5	108.12	84.08-125.69	15.81	6.7082

Discussion

C. albicans.is the major cause for vaginal candidiasis in child bearing women[16]. It can infect approximately about 75% of women. Misdiagnosing of vaginal candidiasis is resulted by usually diagnosis without laboratory examination. Usually infection with vaginal candidiasis occurs in pregnant women because of the secretion of both progesterone and estrogen during this period[17].

The laboratory examination results for the present study revealed that *C. albicans* was the most frequent pathogenic yeast among total yeast (30/43) 69.77% because it represents a part of normal flora on epithelial surfaces in addition to the presence of virulence factors and the ability to germ tube formation. This results was comparable to other studies by Trama *et al*., (2005); Ahmed & Khan (2009), Alli (2013), Hamad *et al*., (2014) and Kalia *et al*., (2015) *C. albicans* percentage was (80.2 %), (63.6 %), (46.9%), (83.02%) and (40.3%) , respectively[18,19,20,21,22] .

API C kit was used in trash work to confirm the identification of all yeast to the species level. Different *Candida* spp. identified by observing the changing in the indicator color when cultures of the yeast utilize 1% carbohydrates for example; glucose, maltose, trehalose, sucrose and raffinose[23]. Most clinical microbiology laboratories depending on commercial identification systems for identification of germ tube negative yeasts. The API C consists of 10 tubes comprising dehydrated substrates and relies on sugar acidification or enzymatic reactions. The strips are read after 24 h of incubation at (30±2^{o}) C[24]. Results of API were read automatically and compared with the instruction of manufactured company.

The present study results were insured that the WCF Ag was efficiently stimulate IgG by both immunization routes. Subcutaneous injection appeared more efficient than intradermaly route; a comparable results insured by other researchers Vilanova *et al* ., (2004) when they improved elevation with IgG levels in mice after immunization with highly purified secretory aspartyl proteinase (Sap2)[25], and Bernardis *et al*., (2002) who documented high IgG and IgA concentration in rats which immunized with (extract of mannoproteins (MP) or secretory aspartyl proteinase (Sap)[26].

Current work indicated high concentration IL-4 for both groups, and there was no elevation in IFN-γ level against WCF antigen. Different studies recorded stimulation in Th1/2 response against systemic candidiasis represented by IFN-γ / TNF in mice injected intravenously with *C. albicans*[27], or by stimulation of Th1(IL-12 and IL-8) and Th2(IL-10) in mice immunized subcutaneously with recombinant enolase resulted in production of protection against systemic candidiasis[28]. Production of cytokine IFN-γ and activation of Th1 cells is helpful in the optimal activation of phagocytes at the site of infection, while the anti-inflammatory cytokines secreted by Th2 cells (IL-4, IL-5) and regulatory T cells(Treg) suppress the induction of Th1 response and down regulate the candidacidal activity of phagocytes[29,30].

REFERENCES

1. Rex J.H., Walsh T.J., Sobel J.D., Filler S.G., Pappas P.G., Dismukes W.E. and Edwards J.E. "Practice guidelines for the treatment of candidiasis. Infectious Diseases Society of America". J. Clin. Infect. Dis. 2000; 30(4): 662-678. DOI:10.1086/313749.
2. Akinbami N.A., Babalola O.G., Shittu O.M., Tijani M.A. and Adekola A.S. "Detection and Epidemiology of Vulvovaginal Candidiasis among Asymptomatic Pregnant Women Attending a Tertiary Hospital in Ogbomoso, Nigeria". Int. J. Biomed. Res. 2015; 6(07): 18-23. doi.org/10.7439/ijbr.v6i7.2242.
3. Ferreira C., Silva S., Faria-Oliveira F., Pinho E., Henriques M. and Lucas C. "*Candida albicans* virulence and drug-resistance requires the O-acyltransferase Gup1p". BMC Microbiology. 2010; 10(1): 238-251. DOI:10.1186/1471-2180-10-238.
4. Faraji R., Rahimi M.A., Rezanmadani F., Hashemi M. "Prevalence of vaginal candidiasis infection in diabetic women". Afr. J. Microbiol. Res. 2012; 6(11):2773-2778. DOI: 10.5897/AJMR11.1443.
5. Gonzalez M., Elizondo M. and Ayala J. "Trends in Species Distribution and Susceptibility of Blood stream Isolates of Candida collected in Monterrey Mexico to Seven Antifungal Agents". J. Clin. Microbiology. 2008; 46(9): 2902-2905.
6. Wang X., Sui X., Yan L., Wang Y., Cao Y. and Jiang Y. "Vaccines in the treatment of invasive candidiasis". J. Virulence. 2015; 6(4): 309-315. doi.org/10.4161/21505594.2014.983015.
7. Vilanova, M., Teixeira L., Caramalho I., Torrado E., Marques A., Madureira P., Ribeiro A., Ferreira P., Gama M. and Demengeot

J. "Protection against systemic candidiasis in mice immunized with secreted aspartic proteinase 2". J. Immuno. 2004; 111(3):334–342.
8. Viudes A., Lazzell A., Perea S., William R.K., Peman J., Thomas F.P., Jose P.M. and Jose. L. "The C-terminal antibody binding domain of Candida albicans mp58 represents a protective epitope during candidiasis". J. FEMS Microbio. Letters. 2004; 232(2): 133-138. DOI:10.1016/S0378-1097(04)00042-4.
9. Raska M., Belakova J., Horynova M., Krupka M., Novotny J., Sebestova M. and Weigl E. "Systemic and mucosal immunization with Candida albicans hsp90 elicits hsp90-specific humoral response in vaginal mucosa which is further enhanced during experimental vaginal candidiasis". J. Med. Mycol. 2015; 46(1): 411-420. DOI:10.1080/13693780701883508.
10. Cho T., Toyoda M., Sudoh M., Nakashima Y., Calderone R.A. and Kaminishi H. "Isolation and sequencing of the Candida albicans MSI3, a putative novel member of the Hsp70 family". J. Yeast. 2003; 20: 149–156. DOI:10.1002/yea.952.
11. Lain A., Moragues M.D., Ruiz J.C., Mendoza J., Camacho A. and Del Palacio A. "Evaluation of a novel enzyme-linked immunosorbent assay to detect immunoglobulin G antibody to enolase for serodiagnosis of invasive candidiasis". J. Clin. Vaccine Immun. 2007; 14(3): 318-9. doi:10.1128/CVI.00396-06.
12. Yadav K. and Prakash S. "Prevalence of Vulvovaginal Candidiasis in Pregnancy". Glob. J. Med. Med. Sci. 2016; 4(1):108-116. DOI:10.1016/j.mycmed.2017.08.006.
13. Mirhendi H., Makimura K., Khoramizadeh M. and Yamaghushi H. "A one –enzyme PCR-RFLP assay for identification of six medically important Candida Species". J. Med. Microl. 2006; 47(3):225-229.
14. Mizutani S., Endo M., Ino-Ue T., Kurasawa M., Uno Y., Saito H., Onogi K., Kato I. and Takesako K. "CD4+-T-Cell-Mediated resistance to systemic murine candidiasis by a membrane fraction of Candida albicans". J. Antimicrobial agents and Chemotherapy. 2000; 44 (10): 2653-2658.
15. Hudson L. and Hay F.C. "Practical immunology" 3rd ed., Blackwell scientific publication. 1989.
16. Roudbary M., Roudbarmohammadi S., Bakhshi B., Farhadi Z. and Nikoomanesh F. "Identification of Candida species isolated from Iranian women with vaginal candidiasis by PCR-RFLP method". European J. Experimental Biology. 2013; 3(6):365-369.
17. Adad S.J., De Lima R.V., Sawan Z.T., Silva M.L., De Souza M.A., Saldanha J.C., Falco V.A., Da Cunha A.H. and Murta E.F. "Frequency of Trichomonas vaginalis, Candida sp and Gardnerella vaginalis in cervical-vaginal smears in four different decades". Sao Paulo Med. 2001; 119(6): 200-205.
18. Trama J.P., Mordechai E. and Adelson M.E. "Detection and identification of Candida species associated with Candida vaginitis by real-time PCR and pyrosequencing". Mol. Cell Probes. "2005; 19(12): 145-152. DOI:10.1016/j.mcp.2004.10.004.
19. Ahmad A. and Khan A.U. "Prevalence of Candida species and potential risk factors for vulvovaginal candidiasis in Aligarh, India". Eur. J. Obstet .Gynecol. Reprod. Biol. 2009; 144(1):68-71. DOI:10.1016/j.ejogrb.2008.12.020.
20. Alli J.A.O., Okonko I.O., Odu N.N., Kolade A.F. and Nwanze J.C. "Detection and prevalence of Candida isolates among patients in Ibadan, Southwestern". J. Microbiol. Biotech. Res. 2011; 1(3): 176-184.
21. Hamad M., Kazandji N., Awadallah S. and Allam H. "Prevalence and epidemiological characteristics of vaginal candidiasis in the UAE". Mycoses. 2014; 57(3): 184-190. DOI:10.1111/myc.12141.
22. Kalia N., Singh J., Sharma S., Singh Kamboj S.K., Arora H. and Kaur M. "Prevalence of Vulvovaginal Infections and Species Specific Distribution of Vulvovaginal Candidiasis in Married Women of North India". Int. J. Curr. Microbiol. 2015; 4(8): 253-26.
23. Madhavan P., Jamal F. and Chong P. "Laboratory Isolation and Identification of Candida Species". J. Appli. Sci. 2011; 11(16): 2870-2877. DOI: 10.3923/jas.2011.2870.2877.
24. Verweij P.E., Breuker I.M., Rijs A.J.M.M., Meis J.F.G.M. "Comparative study of seven commercial yeast identification systems". J. Clin. Pathol. 1999; 52(4):271–273.
25. Vilanova M.,Teixeira L., Caramalho I., Torrado E., Marques A., Madureira P., Ribeiro A., Ferreira P., Gama M. and Demengeot J. "Protection against systemic candidiasis in mice immunized with secreted aspartic proteinase 2". Immunology. 2004; 111(10):334–342.
26. De Bernardis F., Boccanera M., Adriana D., Girolamo A. and Cassone A. "Intravaginal and intranasal immunization are equally effectives in inducing vaginal antibodies and conferring protection against vaginal candidiasis". J. Infec. and Immu. 2002; 70: (5) 2725-2729.
27. Sampaio P., Santos M., Correia A., Amaral F.E., Julio Chave´z-Galarza J., Costa-de-Oliveira S., Castro A.G., Pedrosa J. and Pais C. "Virulence Attenuation of Candida albicans Genetic Variants Isolated from a Patient with a Recurrent Bloodstream Infection". plos one. 2010; 5(4): 1-10. DOI:10.1371/journal.pone.0010155.
28. Adad S.J., De Lima R.V., Sawan Z.T., Silva M.L., De Souza M.A., Saldanha J.C., Falco V.A., Da Cunha A.H., Murta E.F. "Frequency of Trichomonas vaginalis, Candida sp and Gardnerella vaginalis in cervical-vaginal smears in four different decades". Sao Paulo Med. J. 2001; 119(6): 200-205.
29. Vazquez-Torres A., Jones-Carson J., Wagner R.D., Warner T. and Balish E. "Early resistance of interleukin-10 knockout mice to acute systemic candidiasis". Infect. Immun. 1999; 67(35): 670–674.
30. Mencacci A., Del Sero G., Cenci E., d'Ostiani C.F., Bacci A., Montagnoli C., Kopf M. and Romani L. "Endogenous interleukin 4 is required for development of protective CD4+ T helper type 1 cell responses to Candida albicans". J. Exp. Med. 1998; 187(3) 307-317.