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Estimation of the Effect of Phospholipase Extracted from Candida Albicans on Human Lymphocytes Using MTT Assay (In Vitro Study)

Saif Talib Jasim
Al-Qaim Education Dept., General Directorate of Education in Anbar, Ministry of Education, Iraq

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Annotation: Background & aim: One of the common fungal illnesses brought on by the genus Candida is candidiasis. Clinical manifestations include everything from mucocutaneous colonization to deadly infections like candidemia that spread widely. So, the current investigation was aimed to estimate of the effect of phospholipase (PL) extracted from Candida albicans on human lymphocytes using MTT assay (in vitro study).

Materials & methods: Between September 2024 and April 2025, 180 married patients with VVC symptoms had their vaginal swabs taken at Al-Yarmok Hospital in Baghdad, Iraq. The age range of the patients ranged from 20 to 60 years. After being combined with 10% KOH, the initial batch of vaginal swab samples was inspected under a microscope to check for the presence of Candida. Sabouraud Dextrose agar (SDA) was used to hold the second round of vaginal swab samples. The phospholipase production of the isolates was assayed using the egg yolk agar plate. The effect of the enzyme on lymphocytes was tested using MTT assay.

Results: Using a 10% KOH solution, 180 samples were directly inspected under a microscope; of these, 51(28.3%) were found to be positive and 129(71.7%) to be negative. The results showed that after 24 hours of incubation, lymphocyte inhibitory rate was 9.7% at 5μL of PL, while at 10μL of PL, lymphocyte inhibitory rate was 13.2%, and at 15μL of PL, lymphocyte inhibitory rate was 16.0%. After 48 hours of incubation, it was found that at 5μL of phospholipase, lymphocyte inhibitory rate was 14.60%, while at 10μL of PL, inhibitory rate inhibition was 24.70%, and at 15μL of PL, inhibitory rate inhibition was 37.40%. After 72 hours of incubation, it was found that at 5 μl of PL, the inhibitory rate of lymphocytes was 38.1%, while at 10 μl of PL, the inhibitory rate of lymphocytes was 59.2%, and at 15 μl of PL, inhibitory rate of lymphocytes was 76.34%.

Conclusion: it is concluded that the phospholipase enzyme is considered one of the most important causes that increase the risk of infection with vulvovaginal candidiasis (VVC).

Keywords: candidiasis, phospholipase, inhibitory rate, MTT assay, VVC.

Introduction

In immunocompromised patients, opportunistic yeasts called Candida species can cause a broad range of infections, from superficial to widespread [1,2]. The most aggressive of the Candida species, Candida albicans, can infect disabled humans with several types of candidiasis. A number of variables were identified as pathogenic for Candida albicans pathogenesis. The literature has focused primarily on phenotypic flipping, adhesion to host tissues, germ tube development, secreted aspartyl proteinases, and phospholipases [3,4]. Both hydrolytic enzymes have the ability to destroy cell membranes. Phospholipase A, B, C, and D are the four forms of phospholipases that have been identified in Candida albicans. The pathophysiology of infections and invasion of mucosal epithelia is significantly influenced by C. albicans extracellular phospholipases [3].

Furthermore, a number of investigations have demonstrated that clinical isolates of Candida albicans exhibit elevated extracellular phospholipase activity [4,5]. The amount of phospholipase produced by C. albicans isolated from commensals and infection cases was compared by Pinto et al. [6]. They noticed that C. albicans with infection sources had increased levels of phospholipase activity. A study by Farina et al. [7] found that 14.5% of vaginal strains of C. albicans lacked phospholipase activity, whereas 40.6% of isolates exhibited significant phospholipase production. Basu et al. [8] examined the phospholipase activity of Candida albicans isolated from vaginal and urine samples. Phospholipase activity (Pz value) was reported to be 0.82-0.86 in 66.6% of vaginal isolates and 0.84-0.89 in 60% of urine isolates. Despite the fact that Iran has a number of studies on Candida and candidiasis [5,9,10], little is known about their phospholipase activity. So, the current investigation was aimed to estimate of the effect of phospholipase extracted from Candida albicans on human lymphocytes using MTT assay (in vitro study)

Materials & methods

Sample collection

Between September 2024 and April 2025, 180 married patients with VVC symptoms had their vaginal swabs taken at Al-Yarmok Hospital in Baghdad, Iraq. The age range of the patients ranged from 20 to 60 years. Using sterilized cotton swabs, vaginal samples were taken. Two vaginal swabs were obtained from every patient. They quickly put the swab stick back in its case. After being given a number and labeled with the patient's name, it was brought to the private scientific laboratory.

Direct examination of samples

After being combined with 10% KOH, the initial batch of vaginal swab samples was inspected under a microscope to check for the presence of Candida. The diagnosis of candidiasis was made possible by the presence of both hyphae and budding yeast cells [11].

Samples culturing and isolating Candida species

SDA containing 1% chloramphenicol (HIMEDIA, India) was used to cultivate the second set of vaginal swab samples [12]. After that, each agar plate was placed in an incubator set at 37° C, and for seven days, it was checked daily for growth [13]. To verify Candida growth, the colonies from the SDA plate were stained with Gram stain [14]. HiCrome™ Candida Differential agar (HiMedia, India) was used to inoculate all isolated colonies, and they were then cultured for 48 hours at 37°C [15].

Phospholipase production

Price et al. [16] used the egg yolk agar plate method to measure the isolates' phospholipase production. But changes were made to the process, such as using egg yolk and Sabouraud Dextrose Broth (SDB) to extract the enzyme from the fungus. The medium was removed after three to seven days, centrifuged, and the enzyme-containing filtrate was extracted. The De Haas et al. method was used to confirm the presence of the phospholipase enzyme following the extraction process [17]. The presence of the enzyme was thought to be indicated by the medium becoming acidic and the pH concentration changing.

Lymphocyte culture

After obtaining five milliliters of blood from the peripheral vein. The volunteer's blood was carefully rolled and sucked into heparinized tubes. Using the separating solution (Human lymph prep; sp. g=1.077 g/l), a general lymphocyte separation methodology [18] was carried out to gather lymphocyte pellets by centrifuging samples for 30 minutes at 2000 rpm. Following multiple washes with Roswell Park Memorial Institute (RPMI) media and centrifugation for 10 minutes at 2000 rpm, the recovered lymphocyte cells were suspended in a 10 ml complete growth medium. As part of everyday work, the haemato-cytometer chamber and trypan blue solution dye were used to count live lymphocytes.

Determining the effect of phospholipase using MTT assay

Several sterile phospholipase concentrations, including 5, 10, and 20 µl, were applied to the sample's suspended separated lymphocyte cells using a 96-well microplate. The cells were then incubated for 20 hours at 37°C in a CO2 incubator [18]. Untreated lymphocyte cells from the sample, suspended in the medium, served as the negative control. All wells were filled with 2 mg/ml of MTT dye, which was then incubated for 4 hours to allow living cells to metabolize it. After dissolving the purple crystals with a dimethyl sulfoxide (DMSO) solution, an ELISA reader was used to measure the wavelength at 620 nm. The percentage of lymphocytes viability was calculated as follows:

$$Lymphocytes\ viability\ =\ (Absorbance\ of\ the\ test/negative\ control)\ \times 100$$

Results & Discussion

Isolation of *C. albicans*

For the current study, vaginal swabs from vulvovaginitis-affected women were collected. 180 samples were examined directly under a microscope using a 10% KOH solution; 51 (28.0%) of these were determined to be positive and 129 (71.7%) to be negative. Figure 1 and Table 1.

Table (1): Distribution of positive cultured cases

Procedures	Samples	Positive samples	
		No.	%
Direct examined by 10% KOH	180	51	28.3
Culturing Procedure	180	51	28.3

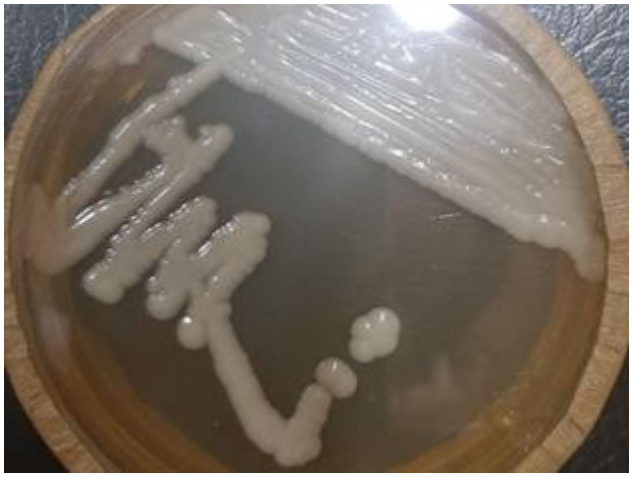

Figure (1): colonies of *C. albicans* on SDA.

The findings of this investigation are consistent with those of Saeed and Saadallah [19], who gathered samples from Duhok Province hospital patients. When these isolates were put onto CHROM agar, the most prevalent yeast was discovered to be Candida albicans. Mohsin and Ali [20] identified each isolate both macroscopically and microscopically after swab incubation on Sabour and dextrose agar (SDA). Microscopic analysis was employed by Hussain et al. [21] to identify the Candida isolates. To find yeasts, Ozcan et al. [22] employed traditional techniques including chrom agar and microscopic morphology. Of the 182 isolates, Candida albicans and Candida glabrata were detected. The chromogenic medium exhibited a 92.9% effectiveness rate against Candida albicans after 72 hours. Because members of the genus Candida are commensal, it is essential to distinguish between infection and colonization when identifying invasive candidiasis in a laboratory setting. From a serological standpoint, various kinds of the antibodies shown in each circumstance might make this separation easier [23–24].

Determining the effect of phospholipase using MTT assay

After the phospholipase enzyme was extracted from Candida albicans (Fig: 2), the test was performed. Using a 96-well microplate, the sample's suspended isolated lymphocyte cells were exposed to various sterile phospholipase concentrations, such as 5, 10, and 20 ul, and then incubated for 20 hours at 37°C in a CO2 incubator.

Figure (2): Extraction of phospholipase enzyme from Candida albicans fungi

Figure 3 showed MTT assay results for three phospholipase concentrations that affected normal human lymphocytes after 24 h exposure. The results showed that after 24 hours of incubation, lymphocyte inhibitory rate was 9.7% at 5µL of phospholipase, while at 10µL of phospholipase, lymphocyte inhibitory rate was 13.2%, and at 15µL of phospholipase, lymphocyte inhibitory rate was 16.0% (Figure 3). After 48 hours of incubation, it was found that at 5µL of phospholipase, lymphocyte inhibitory rate was 14.60%, while at 10µL of phospholipase, inhibitory rate inhibition was 24.70%, and at 15µL of phospholipase, inhibitory rate inhibition was 37.40% (Figure 4). After 72 hours of incubation, it was found that at 5 µl of phospholipase, the inhibitory rate of lymphocytes was 38.1%, while at 10 µl of phospholipase, the inhibitory rate of lymphocytes was 59.2%, and at 15 µl of phospholipase, inhibitory rate of lymphocytes was 76.34% (Figure 5).

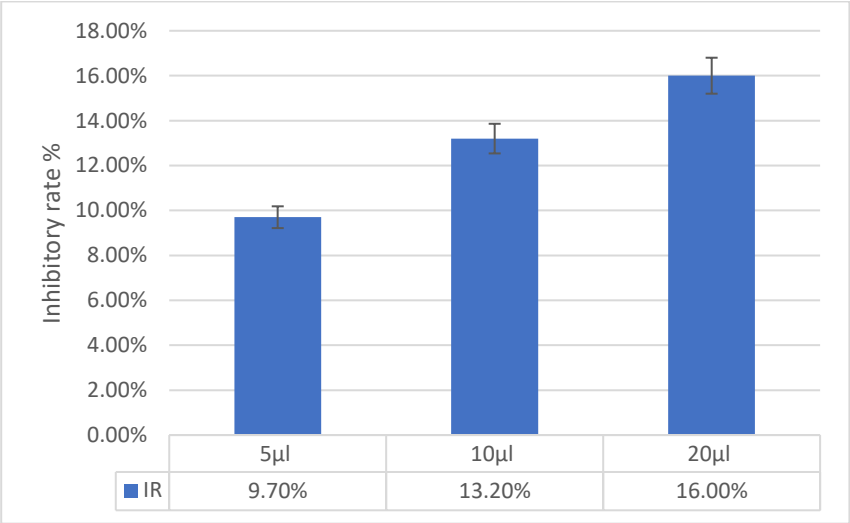

Figure (3): lymphocyte inhibitory rate after 24hr.

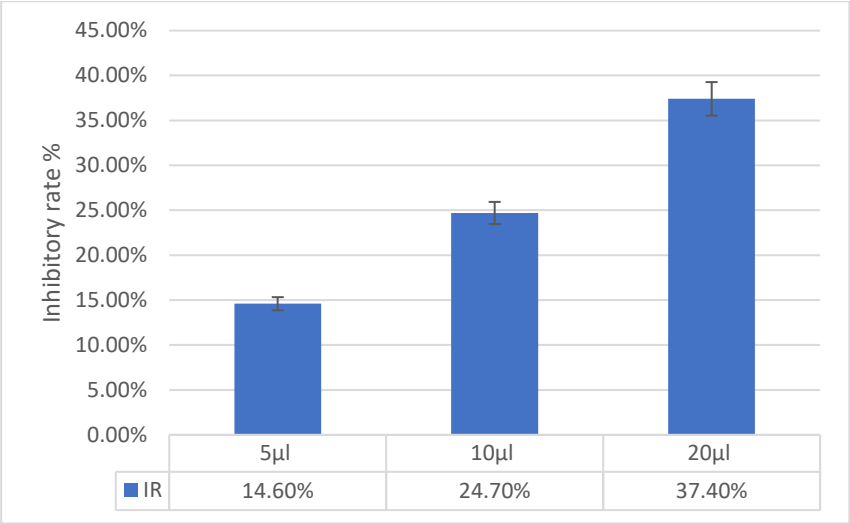

Figure (4): lymphocyte inhibitory rate after 48hr.

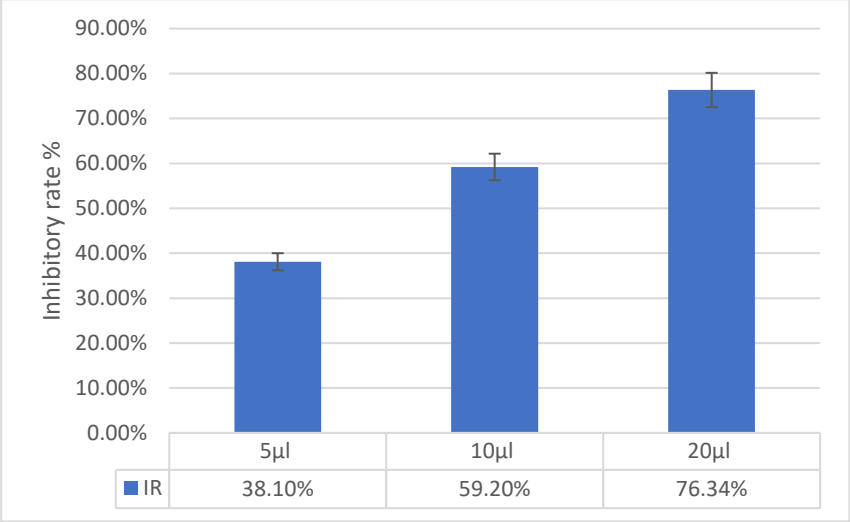

Figure (5): lymphocyte inhibitory rate after 72hr.

Infections with candidiasis have been much more common over the past 20 years [25, 26]. Due to this rise, there is now a great deal of interest in studying the pathophysiology of Candida as well as methods for preventing and controlling this clinically significant fungus. Additionally, there has been interest in using candidate virulence factors as a potential tool for creating innovative treatments to combat candidiasis [27, 28]. These virulence factors include phenotypic switching, extracellular proteinases and phospholipases, adhesion, and germination [29,30,31]. The phospholipase enzyme was discovered to be very effective in preventing the proliferation of isolated human lymphocytes, which raises the risk of Candida albicans infection. According to a study, C. albicans blood isolates often release significantly more PL than isolates from healthy people's oral cavities [32], wounds, or urine [33]. Depending on the host milieu, the three phospholipase activities (PLB, LPL, and LPTA) may have varying proportional roles in fungal pathogenesis. It has been demonstrated that phospholipase's LPL and LPTA activities can reacylate lysophospholipids and undo the harm that other released cryptococcal components have done to neutrophils [34]. To enter host cells, microbes must break through and destroy the outer cell envelope. This transmigration process is most likely mediated by physical, enzymatic, or a combination of the two. Phospholipids and proteins are the primary chemical constituents of the host cell membrane. Therefore, enzymes like phospholipases and proteinases that can hydrolyze these chemical kinds are most likely responsible for the membrane disruption processes that occur during host cell invasion. By cleaving phospholipids, phospholipases result in membrane instability and cell lysis [35]. Phospholipases have been implicated in the lysis, damage, and

penetration of host cells by Candida albicans [36]. Phospholipases are therefore among the virulence agents that damage host cells [35,37]. This explains the high inhibition rate of lymphocytes by phospholipase isolated from *Candida albicans* in the present study.

Conclusions

Based on the study results, it is concluded that the phospholipase enzyme is considered one of the most important causes that increase the risk of infection with vulvovaginal candidiasis (VVC), as it has the ability to inhibit and kill lymphocytes, which are a major part of the immune cells against infection with various microorganisms.

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