



Determining the Role of Interleukin 6 and Interleukin 10 in the Immune Response to Vaginal Candidiasis

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Abstract

The balance in the production of interleukins has an important role in regulating the immune response in infections caused by *Candida albicans*, Therefore, this study focused on determining the level of some interleukins, (interleukin-6 and interleukin-10) as an immune response against *Candida* using rats as a model. Rats were inoculated vaginally with *Candida albicans* to obtain infection and the level of interleukin was measured at different periods (2,4,6,8) days after rats immunized with *Candida albicans* using ELISA technique. four types were isolated of *Candida* genus in current study, *C. albicans* (71.43. %), *C. glabrata* (14.29), *C. parapsilosis* (8.57%) and *C. tropicalis* (5.71%) and the concentration of IL-6. The concentration of interleukin 6 increased at the second day after the immunization of rats with *C.albicans*, and its highest concentration was recorded on the sixth day (162.880 pg/ ml) after rats inoculated with *C. albicans*, while the interleukin 10 increased also on the second day after the inoculated, and its highest concentration (123.3456 pg/ml) was recorded on the eighth day. Generally, coordination between interleukin 6 and 10 is essential in the immune response to vaginal candidiasis.

Key Words: Vaginal Candidiasis, Il-6, IL-10, ELISA.

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Introduction

Candida spp. are the fourth main pathogen, and the most widespread fungal pathogen causing healthcare-associated infections (Magill *et al.*, 2018). Candidemia is a frightening complication of immunosuppression, with mortality rates of up to 20% (Pagano *et al.*, 2017). Primary infections in women with an efficient immune systems are still common diseases caused by *Candida spp.* (Sobel, 2007; Bongomin *et al.*, 2017; Denning *et al.*, 2018). *Candida spp.* are Responsible for nearly 38 million cases of recurrent vulvovaginal candidiasis (RVVC) and 3 million cases of mucosal candidiasis per year In addition to about 750 thousand cases of invasive candidiasis and candidemia per annum (Bongomin *et al.*, 2017; Denning *et al.*, 2018).

Candida albicans is One of the main pathogens of fungal diseases in humans. It is present in the oral cavity, gut and genital tract as a commensal of individuals, the perturbation in the epithelial

barriers, local microbiota and immune defences can lead to outgrowth of fungal cells and expansion of mucosal infections like vulvovaginal candidiasis or oropharyngeal, and immunocompromised patients are susceptible to life-threatening systemic infections. (d'Enfert *et al.*, 2021). VVC, a primary disease related with inadequate disinfection and Chronic fungal infections, and deficiency in host defenses against colonization leads to fungal infections (Rosati *et al.*, 2020).

The vaginal immune response involve innate immunity and acquired immune response. The vaginal mucosa composed of various lymphoid tissues and lymphocytes, which have serious immune functions and have anti-infective effects (De Bernardis *et al.*, 2018). The first line of defense against *C. albicans* infection is the innate immune system.

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In order to provide such a defense system, epithelial cells combine with innate immune cells as an immune response against *Candida* and to recognition of fungi. (Nikou *et al.* 2019; Richardson *et al.* 2019). Inflammation is usually mediated by inflammatory cytokines that include both chemokines and cytokines. (Charo and Ransohoff, 2006). Certain proinflammatory cytokines (IL-6, TNF- α , IL-12) are essential for effective infection control. IL-6 induces overexpression of acute phase proteins and thus has a pivotal role in the development of the appropriate innate immune response. (Yano *et al.*, 2012). IL-6 is secreted into the bloodstream and stimulate B and T cell, through the acute stage of infection, and also antibodies production and acute phase proteins (Fidel, 2005). When epithelial cells of Vagina are activated by *Candida albicans*, especially in its filamentous form, they produce cytokines that encourage PMN recruitment for the vaginal area. (Peters *et al.*, 2014). IL-6 and TNF- α are known to induce initiation and activation of neutrophil recruitment and activation to kill the fungi (Pietrella *et al.*, 2011). Some authors have shown that during the invasion process, *C. albicans* excretes candidalysin, which induces the immune system which activates the release of IL-6. (Richardson *et al.*, 2018; Swidergal *et al.*, 2019). IL-10 is an anti-inflammatory cytokine that inhibits the activity of Th1 cells, NK cells, and macrophages, during infection, and all are required for perfect pathogen clearance but also involved in tissue damage (Couper, 2008). Many studies indicate that dissolution of infection requires a coordinated response in which primary proinflammatory mechanisms remove the pathogen and are then modulated by IL-10 before pathology occurs. Thus, the timing and relative amounts required for the production of pro-inflammatory and anti-inflammatory cytokines are critical to the safe resolution of infection (Iyer1 and Cheng, 2012) So The current study sought to determine the concentrations of two cytokines proinflammatory IL-6 and anti-inflammatory IL-10 in the immune response to vaginal candidiasis using laboratory animals.

Materials and Methods

Collection of Specimens

110 samples were collected from women infected with vaginal candidiasis from Bint Al-Huda Hospital for the period from 1-3-- 2021 to 10-15 2021. The samples were cultured using Petri dishes containing SDA medium and incubated in the

incubator under temperature 37^o for 48 hour. *Candida* species have also been diagnosed according to biochemical tests or traditional methods (sugar fermentation, formation of germ tube and by using CHROM agar *Candida*). (Barnett *et al.*, 2000; Gupta *et al.*, 2019).

Ethical Approval

It was obtained from College of Education for pure science, thi Qar University. Written agreement had been obtained by participants.

Yeast Suspension

The fungal suspension was prepared from two-day-old *C. albicans* yeast colonies by taking a part of the colony using a loop and adding it to a tube containing 5 ml of normal saline solution. The tube was shaken to obtain a homogeneous fungal suspension and its turbidity was modified using McFarland to obtain a concentration of 5x 10⁵cell/ml. (Collee *et al.*, 1996).

Laboratory Animals

Fifty laboratory female Albino rats (*Rattus norvegicus*) were used in this study, with an age 8 weeks, and a weight (150 to 200 gm). These animals were divided into four groups in addition to the control group, each group containing ten animals, all animals were grew under hygienic conditions.

Experimental Designs

The rats were injected with the immunosuppressant (cyclosporine 10 mg) for one time, then Rats were intravaginally inoculated with 20 μ l of 5 \times 10⁵ *C. albicans* suspension, 50 rats were divided into four equal groups each group contain 10 rats, were prepared to detect level of IL-6 and IL-10 in serum post-inoculation as well as control group. Rats in the control group inoculated with normal saline, Blood was drawn from rats on the second, fourth, sixth and eight days after infection and the serum stored at -20^oC to determent level of IL-6 and IL- 10 by using (Enzyme Linked Immunosorbent Assay) ELISA kit. IL-6 and IL-10 concentrations were quantitatively determined in serum of infected animal and healthy control subjects by means of ELISA using ready kits manufactured by USBiological company (USA).

Statistical Analysis

Data were expressed as the means of three independent experiments. Statistical comparisons of the results were performed by one-way ANOVA



using SPSSver.19. Significant differences (P<0.05) among the time were analyzed by Duncan triplicates range test.

Results and Discussion

Diagnosis of Candida spp

The results of the current study showed that 70 out of 110 samples with a rate of 63.63% were positive for culture on culture media, and all of these isolates belong to the genus *Candida*, and four species belonging to this genus were recorded. *C. albicans* also recorded the highest incidence among other species (Table 1).

Table 1. Total numbers and percentages of incidence for *Candida* isolates

Type of yeast	Number of isolates	Percentage %
<i>C.albicans</i>	50	71.43
<i>C.glabrata</i>	10	14.29
<i>C.parapsilosis</i>	6	8.571
<i>C.tropicalis</i>	4	5.714
Total	70	100

X²= 114.160 df= 3 P-value = 0.000

Estimation Level of IL-6 and IL-10 in Serum

The results showed that the concentration of IL-6 began to rise from the second day of infection until it reached the highest concentration on the sixth day of infection and then began to gradually decrease on the eighth day (Table 2).

Table 2. Concentration (pg/ml) of IL- 6 in serum of infected animal with VVC

Time (day)	IL- 6 concentration (pg/ml)
control	7.45 ± 3.56 e
2	18.07 ± 1.02 d
4	29.07 ± 11.29 c
6	162.88 ± 7.09 b
8	34.74 ± 4.39 a

a-e Different letters within each colum indicate significant difference P< 0.05.

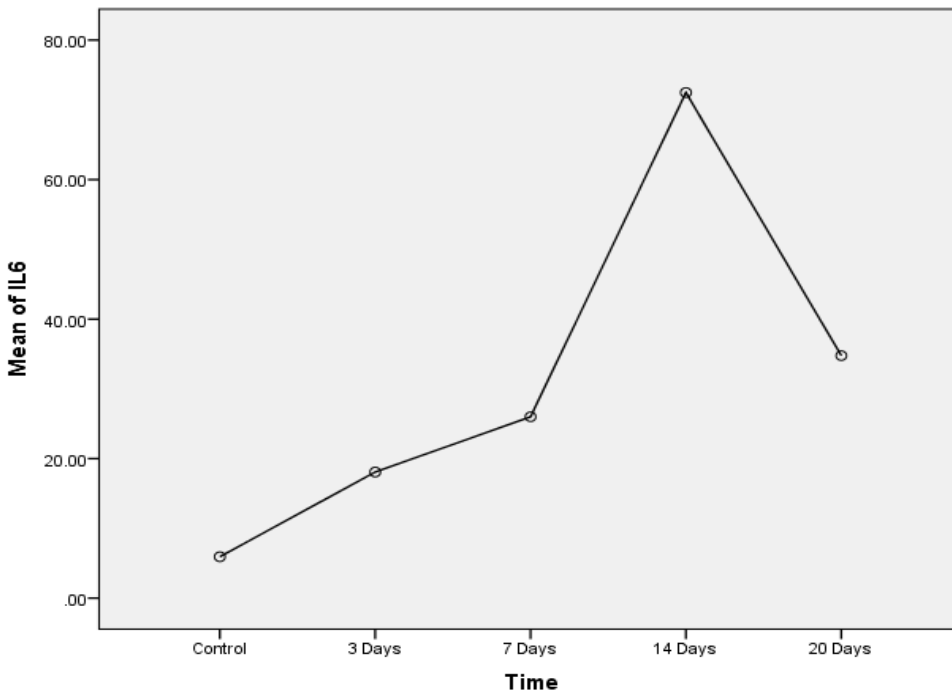


Figure 1: Level of interleukin 6 in serum of infected rats with VVC

As for interleukin 10, an increase in concentration was also observed when compared with the control group, as we notice an increase in the concentration of interleukin on the eighth day of infection (Table 3).

Table 3. Concentration (pg/ml) of IL- 10 in serum of infected animal with VVC

Time (day)	IL- 10 concentration (pg/ml)
control	9.43 ± 0.80 d
2	15.07 ± 2.79 cd
4	21.09 ±1.63 b
6	99.11 ± 10.14 a
8	1.23 a99 .16 ±

a-dDifferent letters within each colum indicate significant difference P< 0.05



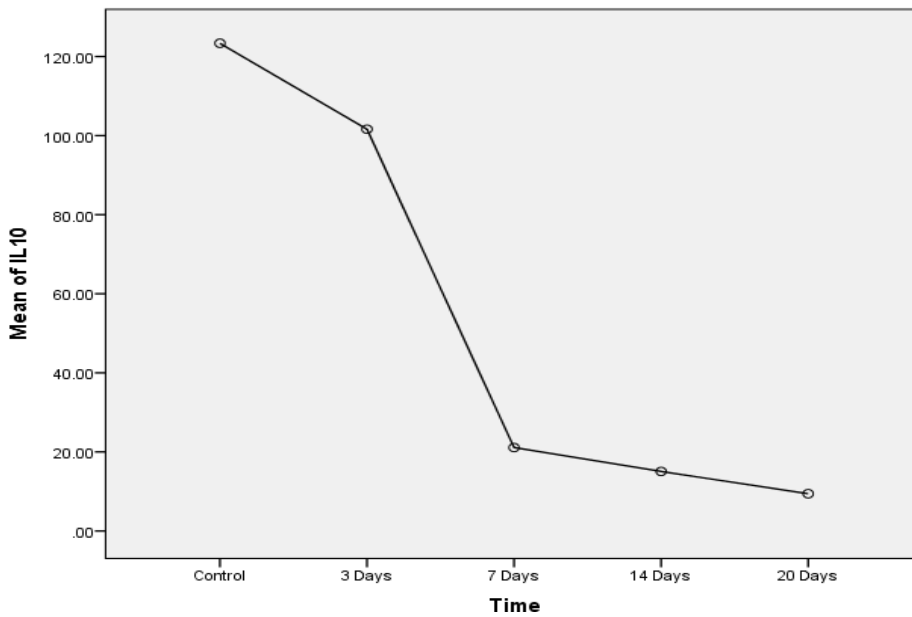


Figure 2. Level of interleukin 6 in serum of infected rats with VVC

Discussion

The dominance of VVC varies from one study to another, as it is the second most popular infection in women and represents about 17%- 42%. (Olowe et al., 2014). According to our study the prevalence rate of infection was 63.63% which was higher than rates reported by Shekhany *et al.*, (2021) and Seyoum *et al.*, (2020) with rates (33.53%) and (49.8 %) respectively. During this study, four species belonging to the genus *Candida* were diagnosed, the most prevalent of which was *C. albicans*, with an appearance rate of 71. 43%. Indeed, this species is more pathogenic between the sexes, is associated with most cases of VVC, and generally represents more than half of the isolates identified in other studies. (Gunther *et al.*, 2014). Shekhany, 2021 identification of this species in Sulaimani in 56.14% and in 78.6% of infected women with VVC in Iran Arastehfar et al., 2021. Similarly, Krishnasamy et al., 2018 identified *C. albicans* in more than 44% of the isolates from patients with VVC in india.

In order to understand the immune response to vaginal candidiasis, the levels of some interleukins (IL-6 and IL-10) have been measured by inoculating some rats with *Candida albicans* at different time periods after infection (2,4 , 6, and 6 days) Rats vaccinated with *Candida albicans* produced higher of IL-6 at 14 days, while IL-10 at 20 day. There is an interaction between the types of *Candida* and the epithelial tissue in the vagina, *Candida albicans* secrete candidalysin during the invasion process that induce immune system to produce interleukin-

6. (Richardson et al., 2018; Swidergal et al., 2019). According to Kovacs et al (2014) serum IL-6 levels had important relationship with systemic infection of *C. albicans* whereas, the immune response to interleukin-6 is limited and prolonged in local infection with *Candida* in the vagina. Vargas et al., 2020 indicate that higher level of IL6 was produced at 5 days after Mice vaccinated with fungal EVs (Vargas et al., 2020). According to Ciurea (2021) IL-6 can effect on growth rate of many species belonging to the genus *Candida*, and also effects on their many virulence factors (Ciurea, 2021). many of proinflammatory cytokines such as IL-6 facilitate the transmigration of PMNs to the infection site which are dominant phagocytes in immune response against *Candida* pathogen in the innate cellular immune system (Gabay , 2006 , Rudkin et al., 2013), The results also showed an increase in the levels of interleukin 10 compared to the control group, Ahmedi et al., 2018 indicated that the levels of IL-10 increased in the Candidiasis, IL-10 play as a key immunoregulator during infection with bacteria, viruses, fungi and protozoa (Wilson et al., 2005). The results of the statistical analysis showed that the relationship between interleukin-6 and interleukin-10 is inverse, which indicates that interleukin-6 is considered proinflammatory, while interleukin-10 is anti-inflammatory in vulvovaginal candidiasis. IL-10 can suppress proinflammatory responses in tissues this leads to poor control of pathogens and decrease immunopathology. As well as IL-10 regulates the innate and acquired immune response by decreasing the activity and

differentiation of T cells in the lymph nodes. (Couper, 2008). Several studies indicate that the disintegration of infection requires a coordinated response that, the primary proinflammatory mechanisms remove the pathogen and then modulated by IL-10 before pathology occurs. Thus, the timing and relative amounts required to produce pro inflammatory and anti-inflammatory cytokines are critical for the safe healing of the infection. (Iyer and Cheng, 2012).

Conclusion

The results demonstrated that Serum IL-6 level had strong connection with *C. albicans* infection (VVC) and the regulatory mechanism between proinflammatory (IL-6) and anti-inflammatory (IL-10) interleukins had an essential role in the immune response in vaginal candidiasis.

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