

# Study the Correlation of *Cutaneous Candidiasis* and Some Immunity Parameters in Patients with Surgical Wound Infection

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**Annotation:** *Candida albicans* is a type of opportunistic fungus that poses a significant concern to individuals with compromised immune systems. *C. albicans* is the source of the skin infection known as cutaneous candidiasis. The current work is aimed to study the effect of cutaneous candidiasis on some immunity parameters in patients with wound infection. The study was conducted in Kirkuk City from August 2023 to February 2024 and carried out on 120 patients admitted in the surgical wards of 3 hospitals of Kirkuk city. The different species of *Candida* were diagnosed by using a 10% KOH solution, Chrom agar culture and polymerase chain reaction (PCR). The study reported that 19 of 120 samples were found to be positive and 101 to be negative. It included the following types: *C. albicans* 13 (68.4%), *C. tropicalis* 4 (21.1%), and *C. krusei* 2 (10.5%). The results of PCR found that 13 (92.9%) of *C. albicans* isolates out of a total of 14 isolates possessed the *ASL1* gene as a

virulence factor. The IL-6 and IL-17 levels in serum of patients demonstrated significant increases in compared to the control group ( $3.85 \pm 0.26$ ) and ( $35.17 \pm 3.94$ ) respectively. For cluster of differentiation, CD8 and CD33 levels in serum of patients demonstrated significant increases compared to control ( $295.11 \pm 25.04$ ), ( $81.64 \pm 7.74$ ), respectively. In Conclusion, cutaneous candidiasis led to changes in some immune parameters, and it was found that interleukins and CD markers play an important role in protection against candidiasis infections.

**Keywords:** Candidiasis, *Candida albicans*, interleukins, CD markers, immune marker.

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## Introduction

Wounds that do not heal at the anticipated rate for a prolonged period of time (more than six weeks) and do not restore function after three months are classified as chronic wounds (Ge and Wang 2023). When it comes to wounds, chronic wounds exhibit aspects like non-healing or delayed healing. Chronic wounds occur between 1% and 4% of the time (Rahim et al. 2017). Diabetes ulcers, arterial/venous ulcers, burn wounds, and surgical wounds that don't heal are the most prevalent forms of chronic wounds (Tomic-Canic et al. 2020). Opportunistic fungal infection is primarily seen in immunocompromised people and is primarily caused by certain fungal species, including *Aspergillus*, *Candida*, and *Zygomycetes* (Abbood and Ahmed 2019). *Candida* is one of opportunistic fungus that poses a serious concern to individuals with compromised immune systems. *C. albicans* is the source of the skin infection known as cutaneous candidiasis. Thirty of the twenty species of *Candida* are known to infect humans (Nurdin et al. 2021). The superficial infection of the skin and mucous membranes can be resulted by the causative agent of cutaneous candidiasis known as *Candida albicans*. *Candida albicans* is the most common species in humans that causes candidiasis, while other species, including *C. glabrata*, *C. tropicalis*, *C. krusei*, *C. parapsilosis*, and many more, are also responsible for skin infections (Bhattacharya, Sae-Tia, and Fries 2020). According to Sadeghi et al. 2019 (Sadeghi et al. 2019), People with nutritional inadequacies or with endocrine abnormalities and immunocompromised patients primarily can get infection by *Candida albicans* easily. According to a recent studies, cutaneous candidiasis can affect outpatient by 1% and 7% of inpatient who visit dermatological wards in a hospitals (Taudorf et al. 2019;Chen et al. 2020;Talapko et al. 2021).

Although cutaneous infections have a low mortality rate, they can lead to systemic and invasive candidiasis, which has a 25–50% mortality rate, if the infections are mysterious or untreated for an extended period of time (Tortorano et al. 2021;Barantsevich and Barantsevich 2022). The uncommon consequence of cutaneous surgery is postoperative *Candida infection*. *Candida albicans*, like many opportunistic microbes, is frequently a natural part of the skin flora, but it can

cause skin and mucosal infections. Usually exhibiting as a red eruption, satellite pustules appear 1-2 weeks following surgery in cases of postoperative candidiasis (Wallis et al. 2021).

The present study is conducted to investigate the relationship between cutaneous candidiasis and various immune parameters in patients with surgical wound infections.

## Materials and Methods

### Sample collection

The study was performed in Kirkuk city for the period of August 2023 to February 2024. The study was carried out on 120 patients admitted in the surgical wards of 3 hospitals in Kirkuk city. The age of patients was ranging from 15 to 75 years. Surgical site infection was classified according to preoperative and postoperative hospitalization. All laboratory tests were performed in private laboratories.

### Identification and Genetic Study

In order to isolate and identify the fungi, the swabs were grown in Sabouraud dextrose broth (Oxoid, England) and then plated onto Sabouraud dextrose agar (Oxoid, England). The yeasts were cultivated by streaking them onto a plate and incubating them at 30 °C. They were then identified using biochemical testing and Chrom agar culture.

### DNA Extraction

The isolates of *Candida* were cultured on SDA at 37 C ° for 48 hours in accordance with the recommendations, and the DNA extraction kit (Fungi/Yeast Genomic DNA isolation kit, NorgenBiotek/Canada) was used to extract the DNA. The procedure of extraction was followed the company guideline provided.

### Detection of ALS1 gene

The multiplex PCR amplification process employed ALS1. Three microliters of genomic DNA were added into PCR master mix reaction to the total volume of 25 µl of working solution. The volume of 12.5 µl of 2X Go Taq Green Master Mix (Promega/USA) was added, along with 1 µl for each of the genes' forward and reverse primers. 5.5 µl of DNase- and RNase-free water was used to finish the volume. For the PCR, the following procedure was followed: One cycle lasting for four minutes at 94°C followed by one minute at 52°C, and ended the cycle with two minutes at 72°C in 35 cycles total. Final extension cycle was performed at 72°C for 5 minutes., The PCR products were separated on 2% agarose gel. ALS1 gene1 amplification was indicated to be effective with 419bp size in a gel (Wealtec, Dolphin-View, USA). The primers were utilized in the presented research have been listed in Table 1.

**Table 1. The primers sequence of ALS1 gene**

Primer	Primer sequence	Length (bp)	References
ALS1-F	5- GCCAATGGTACCGCTATCCC-3	580	Tariq & Abbas [10]
ALS1-R	5- CGGAGAGGTAATACCCAGG-3		

### Agarose Gel Electrophoresis of DNA

The electrophoresis was used in order to differentiate the size of PCR bundle on the agarose gel to detect the DNA fragments after the extraction procedure or to identify the PCR results.

**IL -6:** The IL-6 was measured by using a human interleukin 6 (IL-6) ELISA kit from Sun Long Company, China (catalogue number: HS-EL0277Hu).

IL -17: The IL-17 was measured by using a human interleukin 17 (IL-17) ELISA kit from Sun Long Company, China (catalogue number: SL 0978Hu).

CD8: The CD8 was measured by using a human cluster of differentiation8 (CD8) ELISA Kit from Sun Long Company, China (Catalogue number: SL0136Hu).

CD 33: The CD33 was measured by using human cluster of differentiation 33 (CD33) ELISA Kit from Sun Long Company, China (Catalogue number: SL3374Hu).

### Analytical statistics

For statistical analysis, the data was processed using GraphPad Prism Software's (San Diego, CA) statistical package for the social sciences, version 26 for Windows. ANOVA was used to compare multiple means, while the T-test was employed to compare the means between two groups. A *P*-value of less than 0.05 indicated statistical significance, and a *P*-value less than 0.001 indicated strong significance. To compare two categorical variables, the Chi-square ( $\chi^2$ ) test was applied (Saleh 2018; Abdul, Rahim, and Saleh 2023).

### Results

#### Isolation of *C. albicans*

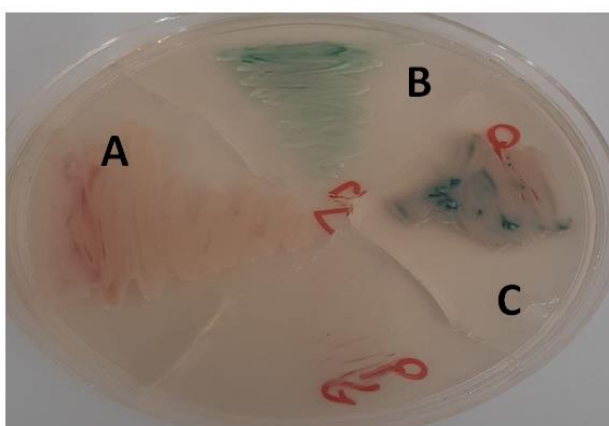
The results showed that there is 19 samples were found to be positive and 101 to be negative. Whereas, the conidia and vesicles chains were born directly, hyphae and dichotomous were seen to be branching, conidial heads, and chains basipetally from phialides in the absence of metulae Table (2).The similar results were obtained from both microscopic examination and the culturing methods.

**Table 2. Distribution of positive cultured cases**

Procedures	Samples	Positive samples		<i>P. value</i>
		No.	%	
Direct examined by 10% KOH	120	19	15.83	0.194

#### *Candida spp.* identification

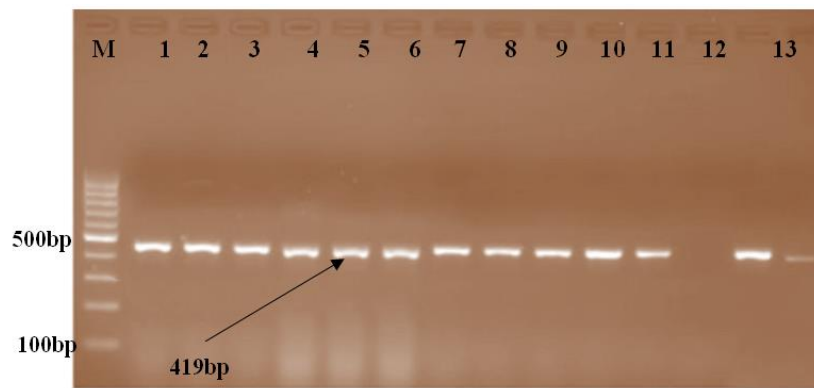
The highest record detected were represented in the *Candida spp.* made up of 19 (15.83%). It included the following types: *C. albicans* 13 (68.4%), *C. tropicalis* 4 (21.1%), and *C. krusi* 2 (10.5%). The different species of *Candida* were diagnosed by using Chrom agar culture as shown in Figure 1.



**Figure 1. Chrom agar culture A: *C. krusi*, B: *C. albicans*, C: *C. tropicalis***

#### *ASL1* gene

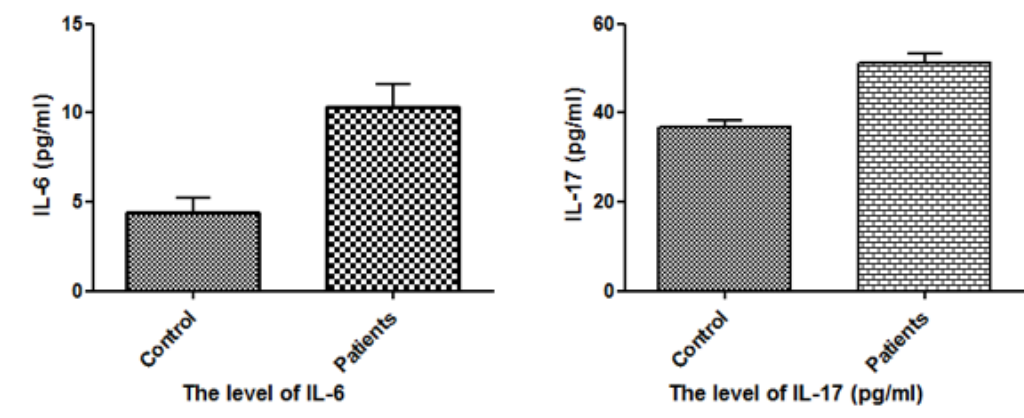
*C. albicans* isolates possessed *ASL1* gene by 13 (92.9%) out of 14 sample diagnostic as virulence factor which shown in Figure (2).



**Figure 2. Agarose gel electrophoresis of *C.albicans*** (2% agarose, 7 v/cm<sup>2</sup> for 60 min) for *ASL1* gene (419bp amplicon): M: represent M 100bp DNA Ladder, lanes 1-14 represent *C.albicans* isolates.

### IL-6 and IL-17

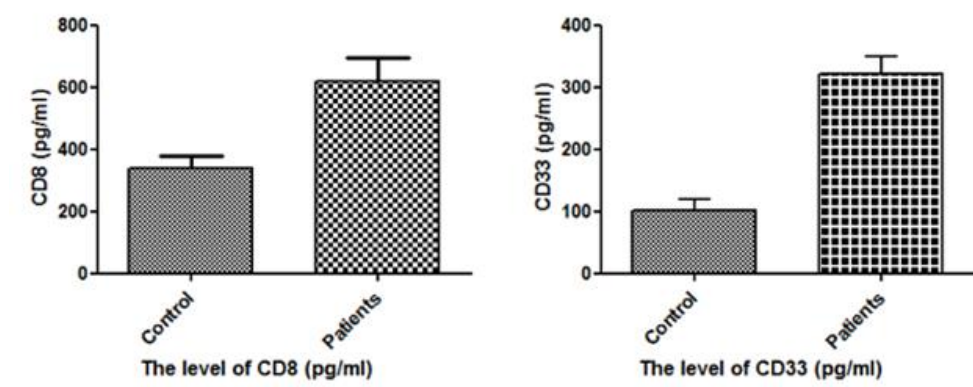
Figure (3) shows the levels of IL-6 and IL-17 in patients and control group, were IL-6 levels in the serum of patients ( $9.43 \pm 1.57$ ) demonstrated a significant ( $P < 0.05$ ) increasing compared to control ( $3.85 \pm 0.26$ ), and the levels of IL-17 demonstrated a significant ( $P < 0.05$ ) increasing in patients ( $49.33 \pm 4.61$ ) compared to control ( $35.17 \pm 3.94$ ).



**Figure 3. The levels of IL-6 and IL-17 in group**

### CD8 and CD33

Figure (4) shows the levels of CD8 and CD33 in patients and control groups, were CD8 levels in the serum of patients ( $542.71 \pm 32.41$ ) significantly increasing in compared to the control ( $295.11 \pm 25.04$ ) at ( $P < 0.05$ ), and the levels of CD33 demonstrated a significant ( $P < 0.05$ ) increasing in patients ( $291.52 \pm 14.35$ ) compared to control ( $81.64 \pm 7.74$ ).



**Figure (4): The levels of CD8 and CD33 in groups.**

## Discussion

The fungal samples would not be impacted by using the aforementioned potassium hydroxide alkaline solutions (KOH) and would still be distinguishable from other combined compounds (Tariq and Abbas 2022). According to this study, 92.9% of surgical wound swabs from all *Candida albicans* isolates show the presence of the ALS1 gene. Using the RT-PCR technique, Nas et al. (Nas et al. 2008) examined the expression of the ALS1 gene in urine, oral, and vaginal swabs. They discovered that, in 70%, 75%, and 67% of the isolated *Candida albicans* from these samples, the ALS1 gene was expressed. According to Ali et al. 2015 (Ali, Al-Attraqhchi, and Al-Ahmar 2015), *C. albicans* isolated from the vaginal region had higher levels of the ALS1 gene, suggesting that these isolates were the most virulent, and according to Mohammed et al. 2017 (Mohammed, Ajah, and Abdulbaqi 2017), all isolates of *Candida albicans* from oral and vaginal infections were positive for ALS1.

Identification of pro-inflammatory IL-6 cytokine was tested as response to the instance of cutaneous candidiasis during our investigations. Certain pro-inflammatory cytokines are necessary for effective infection control during a *Candida albicans* infection. Since IL-6 increases the release of acute-phase proteins, it is essential for the normal development of the innate immune response (Conti et al. 2009; Mohammed, Jafar, and Mahammad 2019) IL-17 levels should also be anticipated to rise in fungal infections. The study's findings demonstrated that cutaneous candidiasis considerably raised IL-17 plasma concentrations. Based on the data shown here, it seems that IL-17 is important for defense against cutaneous candidiasis. Through the production of a characteristic gene profile that includes pro-inflammatory cytokines, antimicrobial peptides, and chemokines, IL-17 is involved as a crucial regulator of antifungal immunity (Yano, Noverr, and Fidel Jr 2012). The current study's findings corroborated those of a study by Conti et al. 2009 (Conti et al. 2009b), which found that patients with candidiasis had elevated levels of IL-17. They mentioned a *Candida* infection induces IL-17, which then affects pro-inflammatory cytokines, chemokines, and antimicrobial proteins that, through neutrophil influx and candidacidal actions, protect against fungal infection. It has been observed that IL-6 and IL-17, when they effectively boost neutrophils and macrophages in the killing of fungus during the host's innate reactions, play a significant role in defense against fungal infections (Mohammed, Al-azzawi, and Abood 2020). Finally, the primary IL-17 family of cytokines for protection against *Candida albicans* appears to be IL-17A and IL-17F, according to human data and outcomes from animal models of otitis media and cutaneous candidiasis (Puel et al. 2010; Gladiator et al. 2013; Ling et al. 2015).

The human genome has the highest number and diversity of immune response genes, showing the evolutionary benefits of a complex immune response to a broad spectrum of infectious pathogens. Infectious disorders represent a strong selective pressure (Ad'hiah, Al-Rikaby, and Jasim 2012). According to the study's findings, patients with cutaneous candidiasis had significantly more CD8T cells than the control group. The existence of IL-2 from Th1 and macrophages, which promotes CD8+ division, may help to explain this. Our findings demonstrated a considerable increase in the CD values of the different types examined. The results of additional studies showing a link between different CDs and atopic dermatitis (Alsaimary, Bakr, and Alhamdi 2013) were consistent with and supported these findings. The significance of researching CD functions and their impact on the immunopathology of allergy and/or atopic illnesses is undermined by their relationship to atopic dermatitis (Abdullah et al. 2019; Mohammed et al. 2024)

## Conclusions

Based on the current results, it was found that cutaneous candidiasis, especially in patients with wound infections, led to changes in some immune parameters, and it was found that interleukins (IL-6 and IL-17) and CD markers (CD8 and CD33) play an important role in protection against candidiasis infections.

## Author Contributions

This manuscript was done by participating of all authors above, so the Conceptualization, methodology and writing the first draft were done by Anmar Sael Hussein and Sanaa H. Mohammed. Writing—review and editing and prepare the final version for submitting were done by Thaer Abdullah Hasan while, Bushra Amir Alarim completed the statistical analysis. All authors have read and agreed to the published version of the manuscript

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## Conflict of Interest

The authors declare there is no conflict of interest

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