

## Modulation Immune Response of Thrush Disease infected Pigeons in Diyala Governorate

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### Abstract:

Candidiasis is the most frequent fungal infection in the oral cavity of pigeons. As a result, they have the potential to trigger an opportunistic infection known as oral candidiasis. This work aimed to isolate with *Candida albicans* from infected pigeon with thrush disease and the study of immunomodulatory effect. *Candida albicans* was isolated from oral mucosa and identification by using *C.albicans* elective agar. Blood sample was collected from infected pigeons and control from pigeon clinically don't have any sign of candidiasis to apply for detection the level of IL-3, GM-CSF, and IL-25 by enzyme linked immunosorbent assay test. The following results were showed that *Candida albicans* was caused infection of Thrush disease at the percentage of (22.7%) from 22 isolate for the infected pigeon. *C. albicans* induced modulation in immune response through the effect in the levels of IL-3, GM-CSF, and IL-25. The conclusion was *C. albicans* caused thrush disease in pigeon that lead to the resulting in the immunomodulatory effects.

Keywords: *Candida albicans*, thrush disease, Immunomodulatory, IL-3, GM-CSF, IL-25



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## Introduction:

*Candida albicans* (*C. albicans*) is one of the most significant pathogenic fungus in pigeon. It is present in the gastrointestinal tract as a commensal [1]. *C. albicans* mucosal infections by candida are mostly attributable to either abnormalities in host cellular immunity, particularly those induced by primary or secondary immunodeficiency, or alterations in the normal microbiota produced by antibiotic therapy [2]. More infrequently, *C. albicans* produces systemic illnesses with significant fatality rates. In addition to host factors, *C. albicans* infections may be aided by higher expression of the fungus's virulence characteristics. *C. albicans* genetic changes that result in phenotypic variability within the fungus have been shown to influence its pathogenicity at epithelial surfaces and systemically [3]. *C. albicans* was found as normal flora in the oral cavity many animals as domestic cats leads to cause their breeder in candidiasis [4]. Fungal polysaccharides are released into the bloodstream during infection, allowing for the early identification of invasive fungal infections. However, their involvement in immune response regulation, particularly that of platelets, is poorly known. The shape

and solubility of glycans influence the direction of the host's immunological response [5]. This work aimed to isolate *Candida albicans* from pigeon infected with thrush disease and the study of immunomodulatory effect.

## Methods:

The current study was included 50 pigeon with White-grey, thicker plaques or diphtheritic membrane may be evident in the tongue, mouth, crop, and esophagus. It was collected by swabbing samples from pigeon oral mucosa between October 2021 and February 2022 at the Baquba birds market. And 22 healthy pigeons did not appear any clinical sign were chosen at random for swabbing oral mucosa samples. The samples were taken from oral mucosal lesions with a sterile cotton swab, then the samples were inoculated into candida elective agar and incubation at 35°C for 24h to 48 h till the growth appears.

Blood samples was collected from all pigeons, with vein puncture collection takes place from a basilic vein in the wing at the cutaneous ulnar, was visible just underneath the skin as it passed over the medial surface of elbow. The blood sample was placed in

the vacuum gel tube , centrifugation 20 min at the speed 4000 r.p.m. the collected serum was kept in sterile tube and preserved in -20 for ELISA test to measure the level of Avian granulocyte –macrophage colony stimulating factor Elisa kit (Sun long Biotechnology , China, Cat #:SL0063BI), Avian IL-3 Elisa kit (Sun long Biotechnology , China, Cat #: SL0065BI) and Avian (IL-25) Elisa kit (Sun long Biotechnology , China, Cat #:SL0064BI) the method was done as manufacture instructions.

### Statistical Analysis

The data was analysis by using IMP Statistical Package for the Social Sciences statistics 20. T test ( A t-test is a statistical test that is used to compare the means of two groups) and ANOVA test (ANOVA tells you if there are any statistical differences between the means of three or more independent groups) were used for analyzing the data. The data was presented as mean  $\pm$  SD. Significant of variance was at P vale < 0.05.

## Result

### Isolation and identification of *Candida albicans*

Candida elective agar to Nickerson was utilized to isolate and identification *Candida. albicans* ( *C. albicans*) from candida colonies on sabouraud dextrose agar that isolated from all groups in this study. The colonies were growth on Nickerson agar appeared as a small colonies with dark brown - to black color (Figure 1).



Figure1: *Candida. albicans* colony appearance on candida elective agar to Nickerson

**disease effect by *C. albicans***

**Serum concentration of IL-3 with *C. albicans* infection**

The results of IL-3 showed a significant elevation at ( $P < 0.05$ ) in all pigeon infected group compared with control (pigeon) group at (mean  $\pm$  SD): (14.92  $\pm$  0.58) pg/mL. The concentration of IL-3 in a group of pigeon infected showed a significant increasing at ( $P < 0.05$ ) and this degradation compared with the control (pigeon) group at (Mean  $\pm$  SD), (29.52  $\pm$  22.11) pg/mL as shown in (Figure 2).

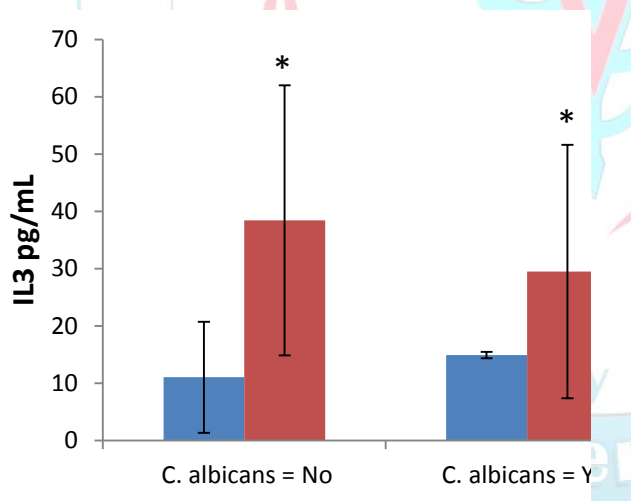


Figure 2: mean serum concentration of IL3 pg/mL in the control (pigeon) and pigeon infected groups with *C. albicans*.  
\*Significant at P value  $< 0.05$ .

**Serum concentration of GM-CSF with *C. albicans* infection**

The results of GM-CSF showed a significant degradation at ( $P < 0.05$ ) in all pigeon infected group compared with control (pigeon) group at (mean  $\pm$  SD): (53.67  $\pm$  40.81) pg/mL. The concentration of GM-CSF in a group of pigeon infected showed a significant decrease at ( $P < 0.05$ ) and this degradation compared with the control (pigeon) group at (mean  $\pm$  SD), (39.99  $\pm$  19.31) pg/mL as shown in Figure (3).

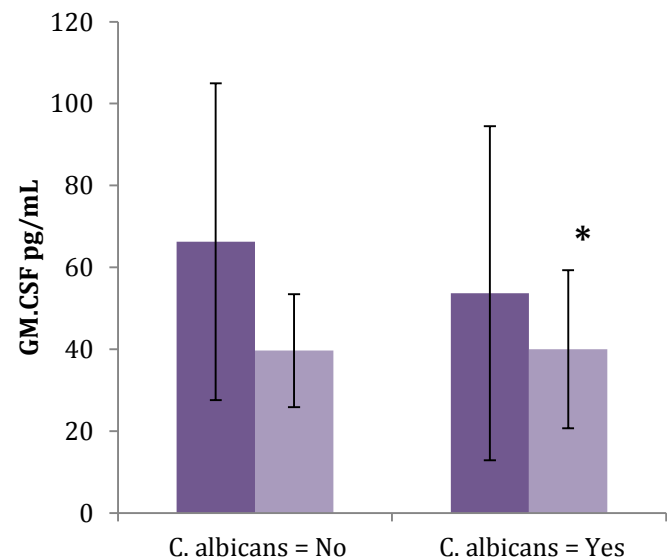


Figure 3 : mean serum concentration of GM-CSF pg/mL in the control pigeon and pigeon infected groups with *C. albicans*.  
\*Significant at P value  $< 0.05$ .



### serum concentration of IL-25 with *C. albicans* infection

The results of IL-25 showed a significant degradation at ( $P < 0.05$ ) in all pigeon infected group compared with control (pigeon) group at (mean  $\pm$  SD): ( $9.32 \pm 6.1$ ) pg/mL. The concentration of IL-25 in a group of pigeon infected showed a significant decrease at ( $P < 0.05$ ) and this degradation compared with the control (pigeon) group at (mean  $\pm$  SD), ( $7.93 \pm 5.49$ ) pg/mL as shown in (Figure 4).

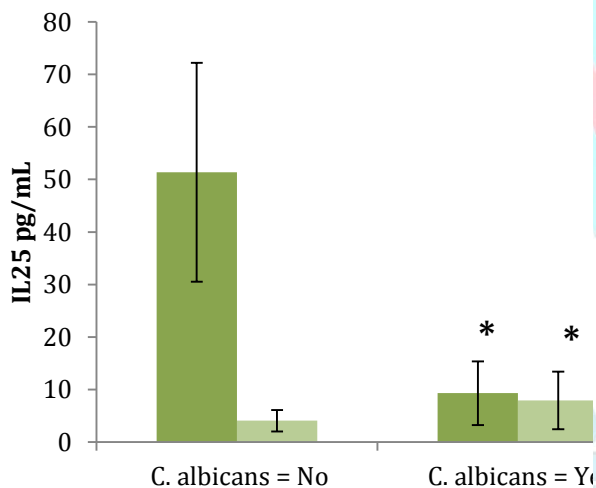


Figure 4: mean serum concentration of IL-25 pg/mL in the control pigeon and pigeon infected groups with *C. albicans*.  
\*Significant at P value  $< 0.05$ .

### Discussion:

The innate immune response is one of the first lines of defense against pathogens. Some receptors and techniques of microbial infection resistance are shared by all species, whereas others are exclusive to a specific class of organisms.

Fungi are eukaryotic organisms found all over the earth. There are around one million distinct species of microfungi, according to conservative estimates [6]. The outcomes of this study demonstrated that infection with *Candida albicans* might result in immune response modification via changes in the levels of interleukin-3 (IL-3), Granulocyte-macrophage colony-stimulating factor (GM-CSF), and interleukin-25 (IL-25). Furthermore, the composition of the cell wall may change as the organism matures. As a result, multiple receptors for innate antifungal immunity are predicted [7]. Many fungi communicate using C-type lectin receptors (CLRs) and toll-like receptors (TLRs). PAMPs from fungi are recognized by C-type lectin receptors and TLRs. Although the exact mechanism of these connections is unknown, activation of both pathways frequently leads to increased production of protective cyto-

kines. All of these interactions do not take place at the plasma membrane. Dectin-1 phagosomes, for example, include TLR-9, which may facilitate endosomal detection of fungal CpG DNA after phagocytosis and fungus eradication [8]. GM-CSF and IL-3 have long been recognized as regulators of emergency myelopoiesis, but current research indicates that they also have a role in regulating innate immune effector functions in mice and humans. This new understanding has uncovered novel aspects of the genesis of a wide range of disorders, including viral, neoplastic, autoimmune, allergy, and cardiovascular issues. GM-CSF and, to a lesser extent, IL-3 play critical roles in experimental models of trained immunity, operating not just on bone marrow precursors but also directly on mature myeloid cells. Identifying GM-CSF and IL-3 as significant mediators of innate immune activation has the potential to open up novel therapy pathways for a wide range of immunological-mediated disorders and define their potential in the context of immunotherapies [9]. GM-CSF and IL-3 are produced by a variety of hematological (e.g., lymphocytes) and non-hematopoietic (e.g., epithelial cells and fibroblasts) cell subsets [10]. The activation of chitotriosidase, a crucial and tightly regu-

lated enzyme active against chitin-containing infections, by GM-CSF may boost the efficacy of fungicidal activity. Steroid-induced alveolar and tissue macrophage dysfunction is also reversed by GM-CSF. GM-CSF therapy may promote anti-fungal immune resistance in those on systemic corticosteroids [11].

**Conclusion:** *C. albicans* caused thrush disease in pigeon that lead to the resulting in the immunomodulatory effects. *Candida albicans* might result in immune response modification via changes in the levels of interleukin-3, Granulocyte-macrophage colony-stimulating factor and interleukin-25.

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