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**Ministry of Higher Education and Scientific Research**

**University of Diyala**

**College of Veterinary Medicine**

**Department of Microbiology**



# **Immunoregulatory Effect and Molecular Detection of *Candida albicans* From Human and Pigeon in Diyala Province**

**A Thesis**

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Diyala in Partial Fulfillment of the Requirements for the Degree of Master of  
Science in Veterinary Medicine / Veterinary Microbiology**

**By**

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## بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(يَا أَيُّهَا الَّذِينَ آمَنُوا إِذَا قِيلَ لَكُمْ تَفَسَّحُوا فِي  
الْمَجَالِسِ فَافْسَحُوا يَفْسَحِ اللَّهُ لَكُمْ وَإِذَا قِيلَ انشُرُوا  
فَانشُرُوا يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا  
الْعِلْمَ دَرَجَاتٍ ۗ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ ﴿١١﴾)

صدق الله العظيم

سورة المجادلة - آية (١١)

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In view of the available recommendations , I forward this thesis to debate by the examination committee.

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## **Examination committee certification**

We, the examination committee , certify that the entitled thesis "**Immunoregulatory Effect and Molecular Detection of *Candida albicans* From Human and Pigeon in Diyala Province**" by **(Mohammed Abdul Hameed Hassan)** has been examined and read through all of its contents and related topics . The committee recommends that the student passed and awarded the degree of master of science in veterinary medicine (veterinary microbiology ).

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## **Dedication**

**To those who lavish me with unending affection... My  
dearest**

**Mother and father.**

**To my loving brother and sister.....who are always there for  
me.**

**To my friends.....who always come through for me**

**I offer my little contribution and heartfelt thanks.**

**Mohammed abdul hameed Hassan..**

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Prof. Dr Amer khazeal

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Thank you to all of the M.Sc. students and friends who helped me finish my research. Finally, I'd want to thank everyone who assisted me in my academic career, and please forgive me if I missed someone.

## **Declaration From**

I here by declare that this thesis entitled (**Immunoregulatory Effect and Molecular Detection of *Candida albicans* From Human and Pigeon in Diyala Province**) presented at the **College of Veterinary Medicine / University of Diyala in 2022**, is my original work, except for quotations and citations which have been duly acknowledged .I also declare that it has not been submitted previously or concurrently, for any other degree at the University of Diyala or other Universities.

**Mohammed Abdul Hameed Hassan**

**Date:    /    / 2020**

## ABSTRACT

Candidiasis is the most frequent fungal infection of the oral cavity in pigeons and human. As a result, they have the potential to trigger an opportunistic infection known as oral candidiasis. With or without signs or symptoms of Candidiasis, a high percentage of healthy persons have commensals *Candida* in the oral cavity.

*Candida* overgrowth is enhanced by local and systemic predisposing factors such as immunologic imbalances, heredity, and malignant illnesses. This study aimed to isolate and molecular detection of gene mutation in *Candida albicans* isolated from human and pigeon with the study of immunoregulatory effect. *Candida albicans* was isolated from oral mucosa of infected pigeon and pigeons breeders, while control from human and pigeon clinically don't have any sign of candidiasis and identification by using candida elective agar. The isolates were applied for gene sequence. Blood sample were collected from all above groups to apply for detection the level of IL-3, GM-CSF, and IL-25 by ELISA technique . The finding of isolated *Candida* spp. On Sabouraud dextrose was appeared white colored, smooth, and yeast-like appearance, *Candida* colonies on SDA are large, white to creamy, smooth and rounded while the colonies of *Candida* spp. isolated from pigeon on SDA are small , white , rough , and rounded .

The *C. albicans* colonies were growth on elective agar appeared as a small colonies with dark brown to black color. The following results were presented in this study based on the diagnosis of *Candida* spp. among study groups: control group (male) was diagnosed 59.1% (13 isolates of 22), Pigeon owners was diagnosed 52%(26 isolates of 50) , control (pigeon) was diagnosed 31.8% (7 isolates of 22), and 56%



(28 isolates of 50) for infected pigeons. *Candida albicans* in this study, diagnosed among the study groups was as follows: the control (male) was diagnosed with 45.5% (10) sample isolate from 22 samples, the pigeon owner was diagnosed with 48% (24) sample isolate from 50 samples, the control (pigeon) was diagnosed with 22.7% (5) sample isolate from 22 samples, and the infected pigeon was diagnosed with 52% (26) sample isolate from 50 samples.

The study examined the level of IL-3, GM-CSF, and IL-25 in blood samples. The level of IL-3 in a group of pigeons breeders decreased significantly ( $P < 0.05$ ) as compared to the control (male) group ( $28.23 \pm 20.97$  pg/ml), while IL-3 levels were significantly higher in all pigeon infected groups ( $P < 0.05$ ) than in the control (pigeon) group ( $14.91 \pm 0.57$  pg/ml). The GM-CSF levels in all pigeons breeders groups were significantly higher those in the control (male) group ( $13.77 \pm 3.43$ pg/ml), while the results of GM-CSF showed a significant decreasing at ( $P < 0.05$ ) in all pigeon infected group compared with control (pigeon) group ( $53.66 \pm 40.81$  pg/ml). The results of IL-25 showed a significant decreasing in all pigeons breeders group compared with control (male) group ( $28.15 \pm 19.57$  pg/ml), also the results of IL-25 showed a significant decreasing at ( $P < 0.05$ ) in all pigeon infected group compared with control (pigeon) group ( $9.32 \pm 6.05$  pg/ml).

In this study, Molecular detection of *C.albicans* gene (chitin synthase) confirmed the isolation of *C. albicans* among the study group. The control group (male) was diagnosed with 45.5% (10 confirmed *C.albicans* from 22 samples), the pigeon owner was diagnosed with 48% (24 confirmed *C.albicans* from 50 samples), the control pigeon was diagnosed with 22.7% (5 confirmed *C.albicans* from 22 samples), and the infected was diagnosed with 52% (26 confirmed

*C.albicans* from 50 samples). The sequence of *C. albicans* isolated was applied for strains from humans and pigeons. The result shown that three strains of *C. albicans* isolated from a human source and one from a pigeon source have mutations in the gene sequence compared with the reference gene sequence. The frequency of mutant allele and genotype was detected as, an allele *T* was has highly mutant frequency (10 time), while allele *A* and *G* have 4 time and 1 time for allele *C*.

In conclusion: *Candida albicans* that caused the infection of pigeon and pigeon breeder has the same gene sequence and some isolates appeared with mutations that lead to the raised virulence of *C. albicans* resulting in the immunoregulatory effects.

## List of content

<b>Table No.</b>	<b>Titles</b>	<b>Page No.</b>
	Supervisor certification	I
	Examination committee certification	II
	Dedication	III
	Acknowledgements	IV
	Declaration From	V
	Abstract	VI-VIII
Tables	List of contents	IX-XI
	List of tables	XII
	List of figures	XIII-XIV
	List of appendix	XV
	List of abbreviation	XVI-XVII
<b>No.</b>	<b>Chapter one : Introduction</b>	
1-1	Introduction	1-2
1-2	Aim of study	2-3
<b>No.</b>	<b>Chapter two: Literature Review</b>	
2.1.1	Oral candidiasis in pigeon	4-6
2.1.2	Oral candidiasis in human	6-9
2.2	<i>Candida albicans</i>	9
2.2.1	Classification of <i>C.albicans</i>	9-12
2.2.2	Pathogenesis	12-13
2.2.3	Diagnosis of <i>candida albicans</i>	13-15
2.2.4	Clinical signs in pigeons	15
2.2.5	Clinical signs in human	15-16
2.3	Immune response of <i>C.albicans</i>	16-18
2.3.1	Immunoregulatory of <i>C.albicans</i>	18-21
<b>No</b>	<b>Chapter three : Materials and Methods</b>	
3.1	Materials	<b>22</b>
3.1.1	Equipment and instrument usrd in laboratory	22
3.1.2	Chemicals and solutions used in the laboratory	23
3.1.3	Apparatus used in the laboratory	24
3.1.4	kits used for detection the parameters	24
3.1.4.1	Human granulocyte –macrophage colony stimulating factor	24
3.1.4.2	Avian granulocyte –macrophage colony stimulating factor	25
3.1.4.3	Human interleukin- 3	25
3.1.4.4	Avian interleukin -3	25
3.1.4.5	Human interleukin-25	25

3.1.4.6	Avian interleukin-25	26
3.1.5	DNA extraction kit	26
3.1.6	Master mix kit	27
3.1.7	Primers	27
3.1.8	Agarose gel electrophoresis materials	28
3.2	Methods	28
3.2.1	Subjects and sample collection	28
3.2.1.1	Study samples	28
3.2.2.1	Control samples	29
3.2.2.2	Pigeon sample	30
3.2.3	Identification of <i>Candida albicans</i>	31
3.2.3.1	Sabouraud dextrose agar	31
3.2.3.2	Candida elective agar to Nickerson	31
3.2.4	Glycerine preparation for storag	31
3.2.5	Preparation of blood serum	31
3.2.6	Elisa method for identification the level of cytokines	32
3.2.6.1	Principle for all Elisa kits used in this study	32-39
3.2.7	Molecular detection of <i>Candida albicans</i> based on PCR technique	40
3.2.7.1	DNA extraction kit for fungi	40-41
3.2.7.2	PCR Reaction	42-43
3.2.7.3	Gel red	43
3.2.7.4	DNA Sequencing for <i>C.albicans</i> isolates	44
3.3	Statistical Analysis	44
<b>No.</b>	<b>Chapter four result</b>	<b>45</b>
4.1	Isolation of candida spp.	<b>45</b>
4.1.1	Isolation of Candida spp. from human sample	45
4.1.2	Isolation of Candida spp. from pigeon	45-46
4.2	identification of <i>Candidia albicans</i>	46
4.3	Distribution of C.albicans among pigeons breeders and control (male)	47
4.4	Distribution of <i>Candidia albicans</i> among infected pigeon and control (pigeons)	48
4.5	Distribution of <i>Candidia albicans</i> among Human study groups related to smoking habit	49
4.6	Molecular detection of <i>C. albicans</i>	50
4.7	Immunoregulatory effect by <i>C. albicans</i>	51
4.7.1	The relation of IL-3 level and c. albicans infection	51-52
4.7.2	The relation of GM-CSF level and c. albicans	52-53

	infection	
4.7.3	The relation of IL-25 level and <i>c. albicans</i> infection	54
4.8	Whole genomic and Sequencing gene of <i>C. albicans</i> isolate	55-58
<b>No.</b>	<b>Chapter five Discussion</b>	
5.1	<i>Candida</i> spp. and <i>C. albicans</i> identification	59-60
5.2	Distribution of <i>Candida</i> spp and <i>C. albicans</i> among study groups	60-62
5.3	<i>Candidia albicans</i> related to smoking habit	62-63
5.4	Molecular detection of <i>C. albicans</i>	63-64
5.5	Immunoregulatory effect by <i>C. albicans</i>	64-69
<b>No.</b>	<b>Chapter six Conclusions and Recommendations</b>	
6.1	Conclusions	<b>70</b>
6.2	Recommendations	71
	Reference	72-95
	Appendix	96-101
	Summary in Arabic	

## List of Tables

Table No.	Titles	Page No.
3.1	Equipment and instrument used in laboratory	<b>22</b>
3.2	Chemicals and solutions used in the laboratory	<b>23</b>
3.3	Apparatus used in the laboratory	<b>23-24</b>
3-4	candida DNA-extraction kit	26
3-5	Primers and markers used in the study	27
3-6	The dilution factor of human GM-CSF	33
3-7	the dilution factor of avian GM-CSF	33
3-8	the dilution factor of human interleukin -3	34
3-9	the dilution factor of avian interleukin -3	34
3-10	the dilution factor of human interleukin -25	35
3-11	the dilution factor of avian interleukin -25	35
3-12	the reaction of PCR	42
3.13	Thermocycler condition	42
4-1	the distribution <i>Candida</i> spp among pigeons breeders and control (male)	47
4-2	the distribution <i>C. albicans</i> among infected pigeon and control (pigeons)	48
4-3	Showed the distribution <i>C.albicans</i> among study groups related to the smoking	49
4-4	the molecular detection of <i>C.albicans</i> among study group	50
4-5	Mutant frequency to Allel type in gene strain sequence for <i>C. albicans</i>	58
4-6	Mutant frequency to genotypes in gene strain sequence for <i>C. albicans</i>	58

## List of Figures

Figure No.	Titles	Page No.
3.1	oral candidiasis in human	29
3.2	oral candidiasis in pigeon	<b>30</b>
3.3	human interleukin -3 standard curve	<b>37</b>
3.4	human GM-CSF standard curve	<b>37</b>
3.5	human interleukin-25 standard curve	<b>38</b>
3.6	avian interlukein-3 standard curve	<b>38</b>
3.7	avian GM-CSF standard curve	<b>39</b>
3.8	avian interleukin-25 standard curve	<b>39</b>
4.1	Candida spp. from human sample on SDA medium	<b>45</b>
4.2	Candida spp. from pigeon on SDA medium	<b>46</b>
4.3	<i>Candida albicans</i> colony appearance on candida elective agar to Nickerson	<b>46</b>
4.4	Presented the level of IL3 pg/ml in the control and pigeons breeders groups with <i>C. albicans</i>	<b>51</b>
4.5	Presented the level of IL3 pg/ml in the control(pigeons) and pigeon infected groups with <i>C. albicans</i> Data was presented as (mean $\pm$ SD). *Significant at P value < 0.05.	<b>52</b>
4.6	Presented the level of GM-CSF pg/ml in the control and pigeon owner groups with <i>C. albicans</i>	<b>53</b>
4.7	Presented the level of GM-CSF pg/ml in the control pigeon and pigeon infected groups with <i>C. albicans</i>	<b>53</b>
4.8	Presented the level of IL-25 pg/ml in the control and pigeon breeders groups with <i>C. albicans</i> .	<b>54</b>
4.9	Presented the level of IL-25 pg/ml in the control	<b>54</b>

	pigeons and pigeons infected groups with <i>C. albicans</i>	
4.10	Genomic DNA extraction	<b>55</b>
4.11	PCR DNA products migrated on on 1% Agarose supplemented	<b>56</b>
4.12	Multiple sequence alignment of amplified PCR product comparing with the reference sequence obtained from gene data bank of NCBI	<b>57</b>



## **List of appendix**

Appendix no.	Titles	Page no.
Appendix I	Material provided with the ELISA kit of human GM-CSF.	96
Appendix II	Material provided with the ELISA kit of avian GM-CSF.	97
Appendix III	Material provided with the ELISA kit of Human interleukin- 3	98
Appendix IV	Material provided with the ELISA kit of avian interleukin- 3	99
Appendix V	Material provided with the ELISA kit of Human interleukin- 25	100
Appendix VI	Material provided with the ELISA kit of avian interleukin- 25	101

## List of abbreviations

Abbreviate	definition
A	Adenine
AIDS	Acquired immunodeficiency syndrome
°C	Celsius
CD4	Cluster of differentiation 4
D.W.	Distal water
DNA	Deoxyribonucleic acid
EB	Ethidium bromide
EDTA	Ethylenediamine tetraacetic acid
ELISA	Enzyme linked immune sorbent assay
G	Guanine
GM-CSF	Granulocyte macrophage colony stimulating factor
GSH	Glutathione s- transferase
GSSG	Glutathione disulfide
HAART	highly active antiretroviral treatment
HIV	Human immunodeficiency virus
Ho1	Heme oxygenase
HRP	Horseradish peroxidase
IFN	Interferon
IL-25	Interleukin 25
IL-3	Interleukin 3
MDA	Malondialdehyde
MHC1	Major histocompatibility complex 1
mRNA	Messenger ribonucleic acid
NAC	Non-albicans Candida
NCBI	National center for biotechnology information
NK	Natural killer
Nm	Nanometer
NQO-1	Nad quinine oxidoreductase
NRF2	Nuclear factor erythroid 2-related factor 2
Nrf2	nuclear factor- erythroid factor 2
OC	Oral candidiasis
OD	Optical density
OPC	Outpatient commitment
PAMP	Pathogen associated molecular pattern
PCR	Polymerase chain reaction

R.P.M.	Round per minute
R.T.	Room temperature
SD	Standard deviation
SDA	Sabouraud dextrose agar
SNP	Single nucleotide polymorphisms
SOD	Superoxide dismutase
T	Thymine
T.cell	T helper 1 cell
TH1	Thymus helper 1
TLRs	Toll like receptors
TMB	tetramethylbenzdine

## 1. Introduction

Human is sensitive to fungal diseases, which can range from mild to severe, frequent from minor surface infections to potentially fatal invasive diseases. Most fungal pathogens are opportunistic and infect immunocompromised persons, such as those suffering from HIV positive infection, tumors, radiation, transplants, or who use immuno suppression medicines. To prevent the majority of deaths, the most important priority remains effective diagnosis and treatment of high-risk populations. *Candida*, *Aspergillus*, and *Cryptococcus* species cause the most majority of invasive infections. The absence of effective vaccinations, standard diagnostic techniques, effective antifungal drugs, and also the development of antibiotic resistant species variants pose a worldwide threat that must be handled invasion of fungal diseases (Pathakumari *et al.*, 2020).

The immunoglobulin structural genes and the products of at least two main gene clusters, and the major histocompatibility complex genes are expressed as active and passive recognition structures on immune system cells and at least some of their released products. Macrophages are essential in the regulation of immunological effector cells, regulatory subsets of thymus-derived lymphocytes interact with macrophages and with each other in the start of immunological responses (Yanping Wu *et al.*, 2022). Cell interactions need that these regulatory cells identify gene products of the major histocompatibility complex at every stage of the immune response (McDevitt, 2000).

*Candida albicans* (*C. albicans*) is one of the most significant pathogenic fungus in humans and pigeon. It is present in the gastrointestinal tract as a commensal. *Candida albicans* mucosal

infections are generally caused by either defects in host cell - mediated immunity, especially that caused by both primary and secondary immunodeficiency, or by antibiotic-induced changes in the intestinal flora. *C. albicans* causes systemic infections with high mortality rates less frequently. *Candida albicans* diseases may be enhanced through overexpression of the fungus's pathogenicity characteristics, in addition to host factors. *C. albicans* genetic alterations which result in phenotype diversity inside the fungal have also been found to impact its virulence at mucosal surface and throughout the body. The mannans, glucans and chitin found in the cell wall of *Candida albicans*, an opportunistic pathogenic yeast of humans, play a significant role in regulating the host immune response during candidaemia (Mora-Montes *et al.*, 2011). During infection, the fungal polysaccharides are released into the bloodstream, enabling for the early recognition of infection by invasive fungi. The glucans molecular structure, which includes the polymer length and degree of branching, solubility, and impact on the activation or inhibition of leukocytes, is crucial for determining their immunological characteristics. receptors. It has been demonstrated that yeast-derived soluble -glucans can inhibit receptors such Dectin-1 and M2 inhibit multivalent binding, which is required for robust leukocyte triggering. (Jawhara *et al.*, 2020).

## 1.2 Aim of the study

This study was aimed to isolate and molecular identify of *C. albicans* isolated from Pigeon and Pigeons breeder with study of immunoregulatory effect .

The objectives to achieve the aim of the study are:

1. Isolation *C. albicans* from oral cavity for infected pigeon and breeder pigeon with control non infected pigeon and not pigeon breeder.
2. Preliminary differentiating of *C .albicans* according to NICKERSON agar.
3. Evaluation the status of IL-3,GM-CSF,IL-25 in the serum of pigeon breeder , pigeon and control groups .
4. Determination the mutation of *C. albicans* gene (chitin synthase) in the isolate from all groups in this study.