Republic of Iraq

Ministry of Higher Education and Scientific research

University of Diyala _ College of Veterinary Medicine



IMMUNOLOGICAL ROLE OF ORAL MUCOSA AGAINST CANDIDLASIS IN HUMAN AND DOMESTICATED CATS

A Thesis

Submitted to the Council of College of Veterinary Medicine at the University of Diyala in partial of the requirements for the degree of Master of Sciences in Microbiology

By

TEEBA MUTHANA MOHAMED

B.Sc Laboratory Analaysis Technique/Al-Yarmouk University College(2014)

Supervised by

Assist. Prof. Dr. Amer Khazaal Al-azzawi

Assist. Prof. Dr. Walaa Najm Abood

1441 A.H

2019 A.D

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

{ فَتَعَالَى اللهُ الْمَلِكُ الْحَقُّ وَلَا تَعْجَلْ بِالْقُرْآنِ مِن قَبْلِ أَن يُقْضَىٰ إِلَيْكَ وَحْيُهُ وَقُل رَّبِّ زِدْنِي عِلْمًا }

صدق الله لعظيم

[طه 114]

Dedications

To my parents who are generous enough to provide me with all kinds of support and love.....

To my brother the ever best companions

To my lovely sister, Meissaa for all her gentle support ...

Finally, I dedicate this thesis to all who have given me hand throughout my studying career.....

Teeba

Acknowledgments

Thanks giving are due Allah alone.

Then I would like to express my deepest thanks to my family, my parents, my brother, my spiritual sister Meisaa for their deeply love and attention ,for helping encouraging and supporting me in critical days.

I am deeply indebted and grateful to the to the Dean Prof. Dr. (Talib Jiwad Kazim) of the college of veterinary medicine/university of Diyala.

I would like to thank my supervisors for accepting supervision and supporting me with scientific device and helping me Assist.Prof.Dr/ Amer Khazaal AL-Azzawi and Assist Prof. Dr. Walaa Najm Abood My deep thanks to Assist. prof. Dr. Zahid Ismael Al-jebory for his great support and providing all facilities required for this thesis . My deep gratitude and grateful to Assist. Prof. Dr. Mustafa Gheni Taher for his helping and supporting me in this thesis.

My deepest thanks to Prof . Dr. Kareem Saadoon Ali and Assist. prof. Dr. Ramzi Abdulghafoor Abbood and ms.c Mustafa Ahmed Jasim.

Then I would like to thank my friends Ahmed Raad Rasheed for his cooperation and helping me .Then I would like to thank DR. Mohammad Sabee Challob for his help. Also I thank all those who helped me in this study (Siaf, Mohammed, Yasir).

Finally iam indebted with great thanks to Asmaa Abass for her help in the analysis part of this thesis and ms.c Mustafa for his help.

Teeba

Supervisor Certification

We certify that this thesis entitled (Immunological Role of Oral Mucosa against Candidiasis in Human and Domesticated Cats) was prepared by (Teeba Muthana Mohamed) under our supervision at Department of Microbiology / College of Veterinary Medicine / University of Diyala in as partial fulfillment of the requirements for the Degree of Master of Science in Veterinary Microbiology.

Supervision

Assist.Prof.Dr.Amer Khazaal Saleh College of Veterinary Medicine University of Diyala / /2019 Assist.Prof.Dr.Walaa Najm Abood College of Medicine University of Diyala / /2019

In view of the available recommendation, I forward this thesis for debate by the examining committee.

Assist .Prof.Dr .Khalid Ibrahim Abd

Vice Dean of post graduate studies and science affairs College of Veterinary Medicine University of Diyala / /2019

Examination Committee Certification

We, the Examination Committee, certify that we have read the thesis prepared by the student (**Teeba Muthana Mohamed**) which was entitled (**Immunological Role of Oral Mucosa against Candidiasis in Human and Domesticated Cats**) and we examined the student in it, we found that the thesis is adequate for awarding the Degree of Master in Veterinary Microbiology

Prof. Dr. Talib Jawad Kadhim Vet .Med. College/ Diyala University (Chairman)

Assist.Prof.Dr.

Assist.Prof.Dr.

Dunya Fareed Salloom

Science.College/ Baghdad University

Luma Taha Ahmed Med.College/ Diyala University

(Member)

(Member)

Appoved by the council of the College of Veterinary Medicine- University of Diyala

Assist.Prof.Dr. Amer Khazaal Al-Azawy	Prof. Dr. Talib Jawad Kadhim
Head of Department of Microbiology	The Dean College of
College of Veterinary Medicine	Veterinary Medicine
University of Diyala	University of Diyala
Date / / 2020	Date / / 2020

Declaration Form

I hereby declare that this thesis entitiled "Immunological Role of Oral Mucosa against Candidiasis in Human and Domesticated Cats" presented to the College of Veterinary Medicine-University of Diyala in 2020, is my original work, except for quotation and citations which have been duly acknowledged. I also declare that i has not been previously and is not concurrently, submitted for any other degree at University of Diyala or other Universities.

Teeba Muthana Mohammed

Date: / / 2020

ABSTRACT

Candida albicans is an opportunistic fungus that infects the mucosa of oral cavity. Oral candidiasis remains one of the most common forms of *Candida* infections. This study is carried out in the laboratory of microbiology department, College of Veterinary Medicine- Diyala University to compare density of *C. albicans* infection between cats and human. Oral samples were collected from human oral mucosa and domestic cats oral mucosa during December 2018- February 2019 to isolate *C.albicans*. The results of study based on the analysis of three groups ,six mice in each group: control (not infected), mice injected with human candida and mice group with cats candida respectively. These groups are divided depends on the time of blood serum and tissue biopsy collection (6,24 hours and 10 days) after the mice were injected with *Candida*.

The study measures levels of IL-17, HMGB1 and G-CSF level in blood sampels and tissue biopsy. The concentration of IL-17 in a group of mice injected with isolated *Candida* from mouth of human (G2) shows a significant increase at ($P \le 0.05$) and this elevation is shown in the two times of draw (24h and 10 days), while The concentration of IL-17 in the mice injected with isolated *Candida* from mouth of human (G2) shows significant increase at ($P \le 0.05$) and this elevation is significant increase at ($P \le 0.05$) and this elevation is shown in the two times periods of tissue biopsy collection (6h and 24h).While the higher increase in the levels of serum HMGB-1 recorded in mice at 6h and 24h (636.41±321.04 and 616.69±240.26) ng/mL after being injected with isolated *Candida* from mouths of cats. On the other hand the result of the concentration of G-CSF in tissue biopsy shows decrease at 10 days compared with control group. In conclusion there are different effects of candida in immunity stimulation of mice injected with *Candida albicans*.

CONTENTS

	Subject	page
	Supervisor certification	
	Dedication	
	Acknowledgments	
	Abstract	I
	Table of Contents	Ш
	List of Tables	VI
	List of Figures	VII
	List of Abbreviations	VIII
N o.	Chapter One : Introduction	Page
1.1	Introduction	1
1.2	The aim of the study	2
No.	Chapter Two : Literature Review	page
2.1	The Fungal kingdom	3
2.2	Candida albicans	3
2.2.1	Classification of Candida .albicans	3
2.2.2	Types of Candidiasis	3
2.2.3	Candida albicans overview	5
2.2.4	Morphology and clinical features	7
2.2.5	Cell wall and Morphogenesis	8
2.2.6	Virulence factors	8
2.3	Oral candidiasis	9
2.4	Candida infection of human	10
2.5	Pathogenesis	10
2.6	Immune response to <i>C.albicans</i>	13

2.7	Barrier Sites in the Human Body against <i>C.albicans</i>	14
2.7.1	Skin Immunity against C. albicans	14
2.7.2	Oral Immunity in Response to C. albicans	15
	Recognition	
2.7.3	C. albicans Recognition during Vulvovaginal	17
	Candidiasis	17
2.8	Candida infection in cat	19
2.9	Epidemiology	21
2.10	Candida. Albicans diagnosis	22
	Enzyme Linked Immune Sorbent Assay	
2.10.1	technique(ELISA) technique for detection of C .	24
	albicans	
2.10.2	Molecular detection of Candida albicans	25
2.11	Prevention and control of <i>C. albicans</i>	26
2.11 No.	Prevention and control of <i>C. albicans</i> Chapter Three : Materials & Methods	26 Page
No.	Chapter Three : Materials & Methods	Page
No. 3.1	Chapter Three : Materials & Methods Study Samples	Page 29
No. 3.1 3.2	Chapter Three : Materials & Methods Study Samples Materials	Page 29 29
No. 3.1 3.2 3.2.1	Chapter Three : Materials & MethodsStudy SamplesMaterialsEquipment and Materials of histopathology	Page 29 29 29 29
No. 3.1 3.2 3.2.1 3.2.2	Chapter Three : Materials & MethodsStudy SamplesMaterialsEquipment and Materials of histopathologyDiagnostic kits	Page 29 29 29 30
No. 3.1 3.2 3.2.1 3.2.2 3.3	Chapter Three : Materials & MethodsStudy SamplesMaterialsEquipment and Materials of histopathologyDiagnostic kitsMethods	Page 29 29 29 30 30
No. 3.1 3.2 3.2.1 3.2.2 3.3.3 3.3.1	Chapter Three : Materials & MethodsStudy SamplesMaterialsEquipment and Materials of histopathologyDiagnostic kitsMethodsSamples collection	Page 29 29 29 30 30 30
No. 3.1 3.2 3.2.1 3.2.2 3.3 3.3.1 3.3.1.1	Chapter Three : Materials & MethodsStudy SamplesMaterialsEquipment and Materials of histopathologyDiagnostic kitsMethodsSamples collectionHuman sample	Page 29 29 29 30 30 30 30

3.3.2.2	Germ tube test	31
3.3.3	Induction the inflammation by Candida albicans	31
3.3.4	Glecryne preparation for storage	32
3.3.5	phosphate buffer saline by manufacturing	32
3.3.5.1	Reagents	32
3.3.5.2	Methods	32
3.3.6	Tissue samples, Homogenization	33
3.3.7	Preparation of blood serum	33
3.3.8	Mouse (IL-17) ELISA Kit	34
3.3.8.1	Materials supplied in the test kit	34
3.3.8.2	Principle of the Test	34
3.3.8.3	Assay procedure	35
3.3.9	Mouse (HMGB-1) ELISA Kit	36
3.3.9.1	Materials supplied in the kit	36
3.3.9.2	Principle of the test	37
3.3.9.3	Assay procedure	37
3.3.10	Mouse (G-CSF) ELISA kit	38
3.3.10.1	Materials supplied in the kit	38
3.3.10.2	Principle of the test	39
3.3.10.3	Assay procedure	39
3.3.11	Histotechnique	40
3.3.11.1	Tissue preparation	40
3.3.11.2	Staining	41
3.3.12	Statistical analysis	41
NO.	Chapter Four: Results	Page
4.1	Morphological and Physiological Characteristics	42

	Of Candida spp. Isolated From Human	
	Morphological and Physiological Characteristics	
4.2	Of Candida spp. Isolated From cat	42
	Estimating the concentration of interleukin-17 in	42
4.3	studied groups relative to the times of blood	43
	collection	
	Estimating the concentration of interleukin-17 in	
4.4	studied groups relative to the times of tissue biopsy	44
	collection	
	Estimating the concentration of HMGB-1 in studied	
4.5	groups relative to the times of blood collection	45
	Estimating the concentration of HMGB-1 in	
4.6	studied groups relative to the times of tissue biopsy	46
	collection	
47	Estimating the concentration of G-CSF in studied	47
4.7	groups relative to the times of blood collection	47
	Estimating the concentration of G-CSF in studied	
4.8	groups relative to the times of tissue biopsy	48
	collection	
4.9	Histopathological results	50
4.9.1	The source of Candida albicans from human smear	50
4.9.2	The source of Candida albicans from cat smear	53
NO.	Chapter Five: Discussion	Page
	Estimating the concentration of interleukin-17 in	
5.1	studied groups relative to the times of blood	56
5.1	collection and the concentration of interleukin-17 in	50
	studied groups relative to the times of tissue biopsy	

	collection	
	Estimating the concentration of HMGB-1 in studied	
5.2	groups relative to the times of blood collection and	57
5.2	the concentration of HMGB-1 in studied groups	57
	relative to the times of tissue biopsy collection	
	Estimating the concentration of G-CSF in studied	
	groups relative to the times of blood collection and	
5.3	Estimating the concentration of G-CSF in studied	58
	groups relative to the times of tissue biopsy	
	collection	
5.4	Discussion of histopathology results	60
.NO.	Chapter six: Conclusions and Recommendations	Page
6.1	conclusions	63
6.2	Recommendations	63
	References	64
	Appendices	83

List of Tables

No.	Title	Page
3.1	Laboratory equipment and instruments of	29
	histopathology	
3.2	Laboratory equipment and instruments	83
3.3	Laboratory diagnostic kits	30
3.3.8.1	Materials supplied in the test kit IL-17	34
3.3.9.1	Materials supplied in the test kit HMGB1	36
3.3.10.1	Materials supplied in the test kit G-CSF	38

4.3	The level of IL-17 in serum of mice injected with	44
	Candida isolated from human and cats	
4.4	The level of IL-17 in tissue of mice injected with	45
	Candida isolated from human and cats	45
4.5	The level of HMGB1in serum of mice injected	46
	with Candida isolated from human and cats	
4.6	The level of HMGB1in tissue of mice injected	47
	with Candida isolated from human and cats	
4.7	The level of G-CSF in serum of mice injected	48
	with Candida isolated from human and cats	
4.8	The level of G-CSF in tissue of mice injected	49
	with Candida isolated from human and ca	

List of figures

No.	Title	Page
2.2.3	Agar plate culture of <i>C. albicans</i>	6
2.2.4	Microscopic appearance of <i>C. albicans</i> shape	7
4.1	Candida spp. From Cats	43
4.2	Candida spp. from human	43
4.9.1.1	Photomicrographs shows the histopathological observations of oral mucosa induced infection with <i>Candida</i> <i>albicans</i> after 6 hours from human smear	51
	Photomicrographs shows the histopathological observations of oral	

4.9.1.2	mucosa induced infection with Candida	52
	albicans after 10 days from human smear	
	Photomicrographs shows the	
4.9.2.1	histopathological observations of oral	54
	mucosa induced infection with Candida	
	albicans after 6 hours from cat smear	
	Photomicrographs shows the	
4.9.2.2	histopathological observations of oral	55
	mucosa induced infection with Candida	
	albicans after 10 days	

List of Abbreviations

Abbreviation	Meaning
GIT	Gastrointestinal tract candidiasis
OPC	Or opharyngeal candidiasis
DCs	Dendritic cells
VVC	Vulvovaginal candidiasis
PAMPs	associated molecular patterns -Pathogen
PMNLs	polymorphonuclear leukocytes
SAD	Sabouraud agar media
PBS	Phosphate buffer saline
H&E	Hematoxylin and Eosin stain
HMGB-1	High mobility group protein Box-1
IL-17	Interleukin 17
G-CSF	Granulocyte colony stimulating factor
D. w.	Distal water

OD	Optical density
ICUs	intensive care units
PRRs	pattern-recognition receptors
PCR	polymerase chain reaction
RT-PCR	real-time polymerase chain reaction
REA	restriction endonuclease enzyme analysis
RFLP	restriction fragment length polymorphism
MLST	multilocus sequence typing
SAPS	secretory aspartate proteases
PFGE	pulsed field gel electrophoresis
TLR2	Toll-like receptor-2
TLR4	Toll-like receptor-4
NADPH	Nicotinamide adenine dinucleotide phosphate
ELISA	Enzyme Linked Immune Sorbent Assay technique

CHAPTER ONE

INTRODUCTION

Chapter one	• • • • • • • • • • • • • • • • • • • •	Introduction
-------------	---	--------------

Chapter one: Introduction

1.1.Introduction

The oral cavity is a place where different type of microorganisms be found as viruses, bacteria, fungus, and protozoa. This area provide a good environment for growing of different microorganisms. These microorganisms could cause disease, when they have opportunities (Olsen *et al*, 2010).

One of the microorganisms is *Candida albicans* which found normally in mouth, upper respiratory tract, female genital tract and gastrointestinal tract, d (Coronado-Castellote *et al*, 2013). Oral candidiasis is the disease that caused by opportunity of *C.albicans*. The Oral candidiasis lesion differ in size, colour and shape in oral cavity. It can cause superficial and serious systemic disease in immune compromised patients (Alka Nerurkar *et al*, 2012).

From 1995, Candida species was the fourth most common to cause nosocomial blood stream infection related with highest mortality (Anurage *et al*, 2005). Candida has effective virulence factored (biofilms) made it to colonize and cause an infection by damaging membrane and extracellular proteins and enhancing entrance of *Candida* to the host and coating with platelet in this impaired immune system (Ramage *et al*, 2005), it is found in about 70% in human leading to cause disease for immune compromised patients as diabetic patients, HIA patients and chemotherapy patients (Sudbery *et al.*, 2004). Chapter one Introduction

1.2. Aim of the study :

The aim of the study was to compare density of infection and virulence, histopathological changes and level of IL-17,HMGB-1 and G-CSF of *C.albicans* isolates from human and cats oral cavity and injected in mice.