Republic of Iraq
Ministry of Higher Education
and Scientific Research
University of Diyala
College of Medicine

Immunological and Molecular Study of *Giardia lamblia* in Patients with Diarrhea

A Thesis

Submitted to College of Medicine - University of Diayla in Partial Fulfillment of the Requirements for the Degree of Master of Science in Medical Microbiology.

BY

Haider Aasad Saleh

BSc. Medical Analysis 2015\ Al-Yarmouk University

Supervised by

Assistant Professor
Dr. Rawaa Abdulkhaleq Hussein

Professor
Dr. Mehdi SH. AL-Zuheiry

2018 A.D. **1439 A.H.**

بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيم

{يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ}

صَدَقَ اللهُ العَلِيُّ العَظيم

المجادلة : 11

Approved by

College of Medicine\ Diyala University

As the thesis for the degree of

Master in Medical Microbiology

Professor

Dr. Talib Jawad Kadhim

Dean

College of Medicine \ Diyala University

Seal

Higher Studies Registration

College of Medicine\ Diyala University

Committee Certification

We, the examining committee, certify that we have read this thesis entitled (Immunological and Molecular Study of *Giardia lamblia* in Patients with Diarrhea) was prepared by (**Haider Aasad saleh**) and as the examination committee examined the student in its content and in our opinion it adequate for award of the Degree of Master of the Science in Medical Microbiology.

Professor

Dr. Abdul-lateef molan mohamad

Chairman

Date: 2018\ \

Assistant Professor

Dr. Ahmed Medab Athab

Member

Date: 2018\ \

Assistant Professor

Dr. Munim Radwan Ali

Member

Date: 2018\ \

Assistant Professor

Dr. Rawaa Abdulkhaleq Hussein

Member\ Supervisor

Date: 2018\ \

Professor

Dr. Mehdi SH. AL-Zueriry

Member\ Supervisor

Date: 2018\ \

SUPERVISOR CERTIFICATION

We certify that this thesis entitled (Immunological and Molecular Study of *Giardia lamblia* in Patients with Diarrhea) submitted by (**Haider Aasad saleh**) to the College of Medicine- University of Diyala was under our supervision as a partial fulfillment of the requirements for the Degree of Master of Science in Medical Microbiology.

Signature

Assistant Professor

Dr. Rawaa Abdulkhaleq Hussein

Department of Medical Microbiology

College of Medicine

University of Diyala

Date: 2018\ \

Signature

Professor

Dr. Mehdi SH. AL-Zueriry

Department of Pediatrics

College of Medicine

University of Diyala

Date: 2018\ \

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

Signature

Assistant Professor

Dr. Areej Atiya Hussein

Head of Medical Microbiology Department

College of Medicine

University of Diyala

Date: 2018\ \

Dedication

To our security forces (army and the popular crowd) who work for our protection …

To the light of my life my mother, to whom who gave me strength and morals my father, may God have mercy on him …

To my sisters…

To my friends…

Acknowledgements

To “ALLAH” and to his prophet “Muhammad” my praise and thanks are due for their blessings without which this research would not have been achieved. It is a pleasure to express my deep appreciation to my supervisors Assist. Prof. Dr. Rawaa Abdulkhaleq Hussein and Prof. Dr. Mehdi SH. AL-Zueriry for their invaluable advice, assistance, cooperation, and support throughout the course of preparing my thesis. Faithful thanks to are my parents who have continuously supported me to complete this study.

I would like to thank all my lecturers who taught me during the courses. My thanks are extended to the College of Medicine/ University of Diyala University for providing the chance to get the degree of Master in Microbiology.

I would also like to thank the staff of the Department of Medical Laboratories and Blood Bank at AL-Batol Hospital for their kind cooperation.

I also address my thanks to the patients and to my friends who cooperated with me during this study.

Summary

Summary

Diarrhea is one of the most common health complaints. It can range from a mild, temporary condition, to a potentially life-threatening one. Most cases of diarrhea are caused by an infection in the gastrointestinal tract. The microbes responsible for this infection include bacteria, viruses and parasites.

This study aimed to determine the infection rate of *Giardia lamblia* among other intestinal parasites in fecal samples collected from patients with diarrhea and to compare between the performance characteristics of microscopy and enzyme linked immunosorbent assay (ELISA) to identify the standard test for the diagnosis of *Giardia* in fecal samples. The study also aimed to investigate the relationship between *Giardia* infection and some of the socio-environmental factors. *G. lamblia* genotypes were identified and the role of genotypes in the establishment of different clinical signs was determined. Molecular characterization of sub genotypes and the association between these sub genotypes and the presence of sever clinical signs were studied.

The present study included 100 patients who attended the parasitology laboratories in AL-Batol Hospital, AL-Khalis Hospital, Health Centers in Dali Abbas and Khan Bani Saad in Diyala, suffering from gastrointestinal complaints with acute diarrhea, together with additional 100 persons who had no diarrhea or other gastrointestinal complaints and considered as a control group. Age ranged from >2 year to ≤19 years, 61 males and 39 females. All patients were infected with different intestinal parasites. The information regarding socio-demographic, health factors were collected from healthy subjects and patients. All samples of fresh feces were examined by light microscopy; the remaining samples were kept at -20 °C for ELISA test and DNA extraction were analyzed with nested polymerase chain reaction (PCR).

The rates of intestinal parasite infection detected by microscopy from patients with diarrhea were *Entamoeba histolytica\ dispar* (51%), *Giardia*

lamblia (34%), *Entamoeba coli* (6%), *Cryptosporidium spp.* (4%), and *Hymenolepis nana* (3%).

The infections in males were more than in females in all types of intestinal parasites. The highest positivity rate was observed in children aged 2-5 years (46 cases). Whereas the age group 12-18 years revealed the lowest infection rate (6 cases).

Giardia lamblia antigen was detected in 39 out of 100 samples (39%) by ELISA. The light microscopy was compared with ELISA and the sensitivity was 87.17%, while the specificity was 100%. *G. lamblia* infection was associated with socio-demographic risk factors that include residence in urban area, family size, other family members infected with *G. lamblia*, presence of patients, water sources, type of feeding (1&2 years), presence of animals, washing of hands and vegetables\ fruit before consumption. The abdominal pain was the most frequent clinical symptoms of giardiasis infections which appeared in 20 cases (51.28%) cases, while vomiting only in 4 (10.25%) cases. The highest incidence was in April with 13 cases, while no infection with giardiasis were detected during December. Most common co-infections was between giardiasis cases and *E. histolytica*/ *dispar* with 3 cases (7.69%), while 2 (5.12%) cases of co-infection among giardiasis with *Cryptosporidium spp.*

Molecular characterization of 39 giardiasis patients was done by nested PCR was performed for detecting *G. lamblia* genotype by amplification triose phosphate isomerase gene (tpi). Twenty one samples amplified out of 39 samples (53.84%). However, the amplification of these samples showed that 5 (23.80%) contained genotype A and 15 (71.42%) samples contained genotype B, while 1(4.76%) sample contained mixed A and B genotypes. Regarding to the gender, *Giardia* genotypes A and B were more significant in males than in females. The highest distribution of genotypes were in patients aged 2-5 years. Regarding to the clinical presentations of giardiasis in this study, there were

differences in the genotypes weather it was A, B or mixed genotype. In type of diarrhea, assemblage B was associated with fatty diarrhea (80 %), while assemblage A was associated with watery diarrhea (60 %).

Sequence of the assemblage A isolates yielded genetic variation, one over one position was noticed for all the isolates: C to T at position 926 with silent mutation D Aspartic acid 309 to D Aspartic acid (according to the GenBank isolate accession no. L02120). One subtype of *G. lamblia* based on these sequences was evident. The isolates representing this subtype were as follows: ST1 (A1, A2, A3, A4, A5). These isolates were identical with isolate sequences of *G. lamblia* an assemblage A available in the GenBank database (accession no. KF963573). However, assemblage B isolates yielded a degree of sequence polymorphisms. One heterogeneous mixed base substitution over one position was noticed: A to G at position 752 with silent mutation E glutamic acid 251 E glutamic acid (according to the GenBank isolate accession no. L02116). Two distinct subtypes of *G. lamblia* based on these sequences were evident. The isolates representing these subtypes were as follows: ST1 (B3) in the present study was identical with isolates sequences of *G. lamblia* an assemblage B available in the GenBank database references (accession no KY320582). While the ST2 (B4, B5) had not been reported before and showed high level of similarity (99%) with isolates from GeneBank database. Therefore, these isolates were considered new types of sequences and recorded in National Center Biotechnology Information (NCBI).

List of Contents

List of Tables

List of Figures

List of Abbreviations

Abbreviation	Meaning
ASH	Allelic sequence heterozygosity
AF	Anterior flagella
BLAST	Basic local alignment search tool
Bp	Base pair
Bg	Beta giardin
CF	Caudal flagella
CDC	Centers for Disease control and prevention
CWP	Cyst wall proteins
COX-2	Cyclooxygenase-2
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytosine triphosphate
dNTP	Deoxynucleotide triphosphate
dTTP	Deoxythymidine triphosphate
DNA	Deoxyribonucleic acid
D. fragilis	*Dientamoeba fragilis*
DFA	Direct fluorescent antibody
DCs	Dendritic cells
ELISA	Enzyme- link immunosorbient assay
Esvs	Encystation-specific vesicles
E. coli	*Entaoemeba coli*
E. histolytica\dispar	*Entaoemeba histolytica\dispar*
E. vermicularis	*Enterobuis vermicularis*
E-64	L-trans-epoxysuccinyl-Lleucylamido-(4-guanidino)-butane

GC	Guanine-cytosine
gdh	Glutamate dehydrogenase
gm	gram
HIV	Humain Immunodeficiency Virus
ICAM-1	Intracellular adhesion molecule-1
IFN-γ	Interferon gamma
IL-1	Interluckin-1
iNOS	Inducible NOS
MCH	Major Histocompatibility
NF-κB	Nuclear Factor-kB
NO	Nitric oxide
NCBI	National Center Biotechnology Information
PCR	polymerase chain reaction
PLF	Posterior/lateral flagella
rRNA	Ribosomal ribonucleic acid
SSU	Small subunit
SPP.	Species
Tm	Melting Temperature
Th	T helper cell
TNF-α	Tissue necrosis factor–alpha
TFT	Triple feces tester
TBE	Tris Borate EDTA electrophoresis buffer
µm	Micrometer
VCAM-1	Vascular cells adhesion molecule-1
VSPs	variant-specific surface proteins
WHO	World Health Organization

Chapter one
Introduction

1.1 Introduction

Diarrhea is the reversal of the normal net absorptive status of water and electrolyte absorption to secretion. Acute diarrhea is defined as the abrupt onset of 3 or more loose stools per day and lasts no longer than 14 days; chronic or persistent diarrhea is defined as an episode that lasts longer than 14 days (WHO, 2017). Diarrhea is the main cause of morbidity and mortality among infants and young children, particularly in low-resource settings (Basmaci *et al*., 2018). Diarrhea is a symptom of infections caused by several bacterial, viral and parasitic organisms, over 350 million with intestinal protozoan parasitic infection (Scanes and Toukhsati, 2018). The parasitic diseases are responsible for causing a significant amount of morbidity and mortality, most of which are located in the tropical and subtropical regions (Sah *et al*., 2013). However, with regard to developed countries, the prevalence of intestinal protozoan parasites is higher than that of intestinal helminthes (Rai *et al*., 2017).

Infection with *G. lamblia* is one of the most important non-viral infections causing diarrheal illness in humans. It has been recognized as the most common intestinal protozoan parasite infecting humans in Iraq (Abd-Al-Zahra *et al*., 2012).

Giardiasis is traditionally considered an epidemic and zoonosis disease between human and animals (farm animals, dogs, cats, birds and rodents) (Thompson *et al.,* 2008). The infection in humans is usually asymptomatic or mild enough to escape diagnosis, most cases are self limited, yet significant acute and chronic infection can occur (Wicki *et al*., 2009). Acute infection can produce bloating, cramp abdominal pain and explosive diarrhea, with pale, frothy, steatorrheic stool (foul smelling, greasy stool often mixed with mucus but not blood) (Nyamngee *et al*., 2009). Transmission of *Giardia* occurs from person to person, hand to mouth transmit of cysts from the feces of infected person. Outbreaks of *Giardia* infections in institutions and families, such as

nursing homes and day care centers, especially those with diapered children, have been linked with fecal-oral route (Efstratiou *et al*., 2017).

Traditionally, the diagnosis of *Giardia* infection is performed through the identification of trophozoites and cysts by microscopy in faecal samples. However, this parasite presents variable patterns of excretion which can cause a false-negative outcome (Cama and Mathison, 2015). Beside the microscopy, a variety of different diagnostic tests have been reported: immunoassays such as enzyme-linked immunosorbent assay (ELISA), rapid tests (immunochromatographic tests), and the detection of *Giardia* specific genes by conventional polymerase chain reaction (PCR) (Soares and Tasca, 2016). *Giardia* isolates are morphologically identical; they vary significantly in their biology, virulence and genetics **(**Lalle *et al*., 2005).

However, molecular methods like PCR are used to classify *G. lamblia* into genotypes and subgenotypes. Most studies use methods which depend on single or multiple genetic loci. However, the use of a different gene, or even a different set of PCR primers, can occasionally present the same isolate to a various genotype (Haque, 2007). It was shown that *G. lamblia* consists of eight assemblages (or genotypes). Only assemblages A and B infect humans. Human infections of assemblage B (~58% of the cases) are more common worldwide compared to assemblage A (~37%). (Ryan and Cacciò, 2013).

Recent investigation indicates the importance of studying *G. lamblia* assemblages and sub-assemblages. These findings increase our knowledge of transmission dynamics, dispersion of drug-resistant alleles, and evolutionary patterns of giardiasis in different geographical regions of the world (Spotin *et al*., 2018).

1.2 Aims of the study:

1- Study the infection rate of *G. lamblia* among other intestinal parasites in fecal samples collected from patients with diarrhea.

2- Compare the test performances characteristics of Microscopy and ELISA in order to identify the best diagnostic method of *Giardia* in fecal samples.

3- To investigate the relationship between *Giardia* infection and some socio-environmental factors.

4- Identification of *G. lamblia* genotypes and then determine the role of genotypes in the establishment of different clinical symptoms.

5- Molecular characterization of subgenotypes and to study the association between these subgenotypes and the presence of sever clinical symptoms.