Evaluation of Conventional, Immunological and Molecular Methods for the Diagnosis of Human Giardiasis

Thesis
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Abstract

The present study was carried out on a total of 85 cases presenting with symptoms that might be suggestive of intestinal giardiasis. All stool samples were subjected to: a) microscopical examination using direct wet smear, concentration technique and Iron Hematoxylin (Fe-Hx) staining. b) Direct immnnofuorescent assay (DFA). c) DNA extraction followed by a PCR, using nested primers specific for *Giardia*.

Considering the nested PCR (nPCR) as the gold standard test, sensitivity of stool microscopy using the direct examination and concentration technique and that of stool microscopy using permanent Iron Hematoxylin stain was found to be 66.7% and 72.9% respectively with a statistically significant difference. While the sensitivity of DFA was 85.4% in detection of *Giardia* infection with a statistically significant difference compared with the nested PCR. No false positive results were recorded; therefore, specificity for all tests used was 100%.

Key Words:

Giardia, microscopy, stain, DFA, nested PCR.

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LIST OF ABBREVIATIONS

Ab	antibody
Ag	antigen
bg	β- giardin
bp	Base pair
cDNAs	Complementary Deoxyribonucleic acid
CTC	5-cyano-2, 3-ditolyl tetrazolium chloride
CVID	common variable immunodeficiency
DFA	direct immunofluorescent assay
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
dNTP	deoxynucleotide triphosphates
EB dye	Ethidium bromide
EDTA	ethylenediamminetetraacetate
Ef-1α	elongation factor-1alpha
EIA	Enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
EPCR	extended PCR
ER	endoplasmic reticulum
ES	Ecostain
ESV	encystation-specific secretory vesicles
Fe-Hx	Iron haematoxylin stain
FITC	Fluorescein isothiocyanate
G	*Giardia lamblia*
G+, P-	Positive *Giardia*, Negative Parasites

G-, P+	Negative *Giardia*, Positive Parasites
G-, P-	Positive *Giardia,* Negative Parasites
gdh	glutamate dehydrogenase
glorfc4	G. lamblia open reading frame c4
gp60	glycoprotein 60
GSA	*Giardia* stool antigen
HIV/AIDS	Human immunodeficiency virus/ cquired immunodeficiency syndrome
IFA	Immunofluorescence assays
IFN-γ	interferon-γ
IC	Immunochromatography
Ig	Immunoglobulin
IIF	Indirect immunofluroscence assay
IL	Interleukin
KCB	Chlorazol black E stain
LAMP	Loop-Mediated Isothermal Amplification
mAbs	Monoclonal antibodies
MIF	Merthiolate (thimerosal)-iodine-formalin
N	Nuclei
n	number
NO	Nitric oxide
n(PCR)	Nested PCR
O&P	Ova and Parasite
P	Parasite
pAbs	Polyclonal antibobodies
PBS	phosphate buffered saline

PCI	phenol/chloroform/isoamyl alcohol
PCR	Polymerase chain reaction
PCR-RFLP	PCR-restriction fragment length polymorphism
PVA	Polyvinyl alcohol
PVs	peripheral vacuoles
qPCR	Quantitative Polymerase Chain Reaction
RFLP	Restriction fragment length polymorphism
RNase	Ribonuclease
ROS	Reactive oxygen species
RT-PCR	Real-Time Polymerase Chain Reaction
SAF	Sodium acetate-acetic acid-formalin
ssu-rRNA	small subunit ribosomal RNA
TAE buffer	Tris-Acetate EDTA buffer
TNFα	Tumor necrosis factor α
tpi	Triose phosphate isomerase
Tris-HCl	Tris Hydrochloride Buffer
TRITC	Tetra methyl rhodamine isothiocyanate
UV	Ultra-violet
VSPs	Variant specific surface proteins
WHO	World health Organization
WT	Wheatley trichrome stain
XLA	X-linked agammaglobulinelia

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Introduction

Giardia intestinalis (syn. *G. duodenalis*, *G. lamblia*) is flagellated, protozoan parasite and a leading cause of a human diarrheal disease worldwide **(Asher *et al.,* 2012).** The prevalence of giardiasis is 2 to 5% in developed countries and 20 to 30% in developing countries **(Júlio *et al.,* 2012).**

Infection is transmitted by the ingestion of cysts, which are the parasitic stages that resist chlorination and can remain viable for several weeks **(Nguyen *et al.,* 2012).** Most infected people remain asymptomatic, which contributes to the spread of the disease. In some cases, especially in children, infection can lead to a wide range of symptoms, including abdominal discomfort and watery diarrhea; in severe cases, malnutrition, malabsorption and disruption of the weight curve may be observed **(Escobedo and Cimerman, 2007)**.

Traditional diagnostic methods are fecal smears and must include concentration procedures along with specific staining techniques for proper microscopic detection and identification of the parasite. However, these methods are laborious, time consuming and require specialized trained personnel. In addition, microscopic examination of three consecutive samples on different days is required to achieve sensitivity up to 85% **(Schuurman *et al.*, 2007; Chakarova, 2010 and Goñi *et al.,* 2012).**

In recent years, antigen detection immunoassays, such as enzyme immunoassays (EIAs) and immunochromatography (IC) and immunofluorescence assays (IFA) to detect *Giardia* have been developed. IFA have the advantage of improving the sensitivity for detection of

giardiasis however, they are expensive and are not routinely applied in all laboratories **(Chavez, 2008).**

Molecular techniques to detect *Giardia* are now available for research purposes and several studies recommended their use to diagnose giardiasis **(Goñi *et al.*, 2012).**

Aim of Work

The aim of the present work was to evaluate different laboratory investigative methods for diagnosis of giardiasis in human cases.

Objectives:

1- Evaluate the usefulness of different diagnostic techniques; Conventional microscopy, Immunodiagnosis by Direct immnnofuorescent assay (DFA) and Molecular diagnosis using the nested PCR for identification of giardiasis.

2- Compare the sensitivity, specificity, cost and feasibility of each method.