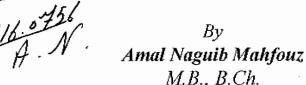
ELISA ASSAY FOR DETECTION OF GIARDIA ANTIGEN IN RECENT GIARDIASIS

Thesis

Submitted for Partial Fulfillment of Master Degree of Clinical Pathology





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LIST OF ABBREVIATIONS

DFA Direct fluorescent antibody

ELISA Enzyme linked immunosorbent assay

Exam. Examination F Female

FN False negative FP False positive

G6PD Glucose-6-phosphate HRP Horse radish peroxidase

M Male

MAO Mono amine oxidase

Mic. Microscopie

MIF Merthiolate iodine formalin

NaCl Sodium chloride Optical density OD PMN Polymorphnuclear PV Predictive value Polyvinyl alcohol PVA \mathbf{OC} Quality control Red blood cells RBCs rRNA Ribosomal RNA

SAF Sodium acetate-acetic acid-formalin

Spp Saprophytica

TMB Tetramethyl benzidine

TP True positive WBCs White blood cells

ZnSO₄ Zinc sulfate

INTRODUCTION & AIM OF THE WORK

INTRODUCTION AND AIM OF THE WORK

INTRODUCTION:

Giardiasis is an intestinal infection caused by a microscopic parasite called Giardia lamblia. Giardia has a life cycle consisting of an infective cyst and a vegetative trophozoite (Adam, 1991).

Giardiasis is implicated in 25% of cases of gastrointestinal diseases and is more prevalent in children. It is caused by ingestion of contaminated water or eating fruits and vegetables washed in cyst-infected water (Loken, 1986).

Acute giardiasis symptoms include foul watery diarrhea, nausea and dehydration. About 40% of patients demonstrate disaccharide intolerance during and up to 6 months after infection. Lactose intolerance is also frequently observed. Chronic giardiasis is associated with loss of weight, anorexia and malabsorption syndromes. Children may also display distended abdomen and growth retardation (*Bonner*, 1986).

Diagnosis of giardiasis is established by microscopic examination of stools. Examination of 3 to 6 stool specimens over 3 consecutive days for the presence of trophozoite or cyst is needed. Giardia is intermittently shed in stools, thus, approximately 10 to 50% of all Giardia infections are missed using standard ova and parasite microscopic examination (Aldeen, 1995). A less frequently applied method for diagnosis, is an invasive endoscopic method involving examination of the duodenal

aspirate for the presence of the parasite (Nazer, 1993). On the other hand, detection of IgG, IgM and IgA serum antibodies to Giardia lamblia by enzyme immunoassay (EIA) is another diagnostic approach which proved to have a low positive predictive value and cannot be relied upon as diagnostic of recent infection (Goka et al., 1989 and Chaudhuri et al., 1991).

The main line of treatment and defense against infection is chemotherapy, the main drawback of which is drug resistance. Loss of drug activation and alteration of drug targets are the mechanisms of metronidazole resistance in Giardia species (Johnson, 1993). In addition, quinacrine and metronidazole, the two most widely used drug therapies for giardiasis have been proven to cause cancer in laboratory rodents (Craft, 1984).

Hence, empirical administration of the above mentioned chemotherapeutic agents in the absence of a definitive diagnosis should be discouraged (Kanra and Ecevit, 1992).

AIM OF THE WORK:

The aim of this work is to study the clinical utility of enzyme linked immunosorbent (ELISA) assay for detection of Giardia antigen in stool specimens, aiming at diagnosis of giardiasis in gastroenteritis patients.

REVIEW OF LITERATURE

I-GIARDIA LAMBLIA

A-Historical Background:

Anton Van Leeuvenhoek discovered Giardia in (Stürchler, 1987 and Farthing, 1993). Van Leeuvenhoek discovered the trophozoites in his own diarrheal stool (Dobell, 1920 and Adam, 1991). Giardia lamblia is a flagellated protozoan that was described by Lambl in 1859 (Dobell, 1920 and Adam, 1991) with its structure being further reviewed by Feely in 1988. The protozoan initially was thought to be a normal, non pathogenic inhabitant of the upper intestinal tract of humans (Paulson and Andrews, 1930), until a series of volunteer experiments in 1950s established its infectivity (Rendtroff, 1954 and Rendtroff and Holt, 1954). The American public become aware of Giardia lamblia in the early 1970s, when outbreaks of disease were reported from the Soviet union (Walzor et al., 1971 and Stürchler, 1987). Since then, epidemiologic studies have shown Giardia lamblia to be a cause of diarrhea in children in daycare centers and in family contacts of infected children (Pickering et al., 1984 and Farthing, 1993). Fortunately, laboratory studies using endonuclease restriction analysis, evaluating surface antigens and measuring homologous and heterologous antibody responses are beginning to clarify the biology of these organisms (Pickering and Engelkirck, 1988 and Adam, 1991).

Giardia has been classified in the Phylum Sarcomastigophorea, along with the other flagellated human parasites, Trypanosoma, Leishmania, Trichomonas and Dientamoeba have been classified in the order Diplomonadida (*Levine et al.*, 1980 and Adam, 1991).