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Molecular and Biochemical Study of some pathogenic factors of *Giardia lamblia*

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

نَرْفَعُ دَرَجَاتٍ مَنْ نَشَاءُ

وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ

صَدَقَ اللَّهُ العَلِيُّ العَظِيمِ

سورة يوسف الآية (٩٨)

Dedication

I Would like to dedicate my thesis to my beloved .....

Parents.....

Husband.....

Son Ali Redha.....

brother and sisters.....

Dhuha

1.1 Introduction

Giardiasis is a protozoan disease caused by the parasite *Giardia intestinalis* , also known as *Giardia lamblia*, this disorder is the most common parasitic gastrointestinal disease ,it is estimated that up to 2.5 million cases of giardiasis occur each year in the USA and up to 20% of the world population is chronically infected. (Giraldo-Gómez J.M. *et al*.,2005; Hesham M.S. *et al*.,2004; Savioli L *et al*.,2006). Iran is an endemic area for the infection and the prevalence is 10.9% (Sayyari *et al*., 2005).

Giardia protozoan is seen in two forms cyst and trophozoite. Trophozoites attach to upper portion of small intestine by its sucker plate and may present with severe damage affecting nutrient absorption (Oberhuber *et al*., 1997). the trophozoite cannot live long outside of the body ,therefore it cannot spread the infection to others . The infective cyst ,on the other hand ,can exist prolonged periods outside the body .when it is ingested ,stomach acid activates the cyst ,and the cyst develops into the disease –causing trophozoite. It takes ingestion of only ten cysts to cause infection Trophozoites are important not only because they cause the symptoms of giardiasis ,but because they produce the cysts that exit the body in the feces and spread the infection to others . (Adam R.D. 2001).

The severity of giardiasis may depend on multiple virulence factors. By poorly understood mechanism , the parasite is not invasive and little or no mucosal inflammation is seen during acute infection. (Roxstorm-Lindquist K.*et al* .,2006).

One of the proposal mechanisms is the attachment of *Giardia* trophozoite to enterocytes which is essential for colonization of the small intestine and is considered a prerequisite for parasite- induced enterocyte dysfunction and clinical disease. (M. Céu Sousa *et al* .,2001).

Another mechanism is secretion proteins with toxin-like activities (Kaur H.*et al*.,2001 ;Meyer E.A.*et al*.,1979). These proteins are present on the surface of *Giardia* known as cysteine rich proteins (CRPs). (Mowatt *et al*., 1991) .

The putative roles for this proteins include protease, heavy metal resistance , bile salt resistance and resistance to metronidazole , (the most common anti giardial drug) and there are two members of cyctein rich proteins family that have been founded considered as protein like-toxin in *Giardia* known as CRP136 and CRP65. (P Upcroft *et al*.,1997).

Recently several cysteine-type protease of *G. lamblia* origin were detected after incubation with rat small intestine epithelial cells (Rodriguez-Fuentes G.B. *et al*.,2006) , and other host cell .

Giardia interaction experiments and giardiasis patient data have shown that *Giardia* reduces the epithelial barrier function. (Teoh D.A. *et al*.,2000; Scott K.G. *et al.*, 2000) and induces apoptosis. (Panaro M.A. *et al*., 2007;Chin A.C. *et al*.,2002 ;Troger H. *et al*.,2007). But effecter agents have never been identified .

Some researches have been hypothesized that exposure of *G. lamblia* to human intestinal epithelial cells (IECs) might lead to release of trophozoite proteins into the medium , and they identify three major *Giardia* proteins released into the medium after only brief interaction between *G.lamblia* and (IEC) and these three enzymes (araginine deminase (ADI) , ornithine carbamoyl transferase (OCT), and enolase).

These enzymes have their function in the metabolism of the parasite (Emma R. *et al*.,2008 ; Palm J.E. *et al*.,2003) .

Another complication of giardiasis is lipid malabsorption so that steatorrhoea (foul smelling, greasy stool) is a clinical sign of giardiasis (Huang and White, 2006).

1.2Aims of the Study :

The overall goals of this study were to :

1. Detect the enzymes (Araginine deiminase (ADI) , Ornithine carbamoyl transferase (OCT)) by using polymerase chain reaction (PCR) technique.
2. Gene characterization of Cysteine rich proteins (CRP65) by using polymerase chain reaction (PCR) technique.
3. Assay of total lipids (cholesterol, triglyceride, high density lipoproteins,(HDL), low density lipoproteins (LDL) and very low density lipoproteins(VLDL)) in peripheral blood in *Giardia* cyst passers comparing the results with healthy individuals .

1.3 Review of Literature

1.3.1 Historical Review

Giardia was first described in 1681 by Antony Van Leeu Wenhoek , who found in his own diarrhea stool (Adam R.D., 2001). , it was reported in more detail in 1859 by Lambl after whom the human species was named (Lambl, 1859).

However , it has only in last 30-40 years that its real role in diarrheal syndromes has been described . (Moore *et al*.,1969).

The first major reviews of *Giardia* were published in the 1970s. (Peterson , 1972: Burke, 1975 ; Wolfe , 1975 ; Meyer and Radulescu , 1979).

Since that time there has been extensive research describing the epidemiology and biology of *Giardia* , while much is known about this parasite , there is still much to learn, particularly about how *Giardia* causes diarrhea . (David R. Hill ,2001).

1.3.2 Classification

As it is mentioned by Zibigs, (1997). *Giardia lamblia* was been classified as follows

Kingdom : Protista

Phylum : Protozoa

Sub phylum : Sarcomastigophora

Superclass : Mastigophora

Class : Zoomastigophora

Order : Diplomonadida

Family : Hexamitida

Genus *: Giardia*

Species : *lamblia*

1.3.3 Description of the Organism

Giardia is a flagellated , teardrop-shaped parasite with only two life forms , the trophozoite and the cyst (fig 1.1).

It is one of the oldest eukaryotic organisms , based on the sequence analysis of its small 16s ribosomal RNA (Sogin *et al*., 1989). It also lacks many of the organelles typical of higher eukaryotes , such as mitochondria , piroxisomes and a typical Golgi apparatus (Adam R.D 1991 ; Gillin *et al* ., 1996 ; Roger *et al* ., 1998), the trophozoite measure 9-21 um in length . 5-15 um in width contains four sets posteriorly directed flagellae , which aid in the parasites movement . The most prominent feature of trophozoite is the ventral disc . which may help *Giardia* to attach to intestinal epithelial cells . This disk is composed of a tight , clock wise spired of micro tubules , bound together by micro ribbons (Peattie , 1990 ; Marshall and Holberton , 1993). The micro tubules are critical to the functioning of the disk as well as to the movement of the flagellae. There are two apparently equal nuclei which , on stained preparations , create the characteristic face-like image (Kabnick and peattie , 1990).

traditionaly There have been only a few species designated for *Giardia* , based on host restriction and microscopic morphology *; G. lamblia* in humans and G. agilis in amphibians; *G. muris* in rodents; *G. ardeae* ; *G. psittaci* in birds; and *G. microti* in muskrats and voles (2–6). However, on the basis of host origins, *Giardia* species have been named (Campbell S.R.*et al*., 1990; van Keulen H.*et al.*, 1993).

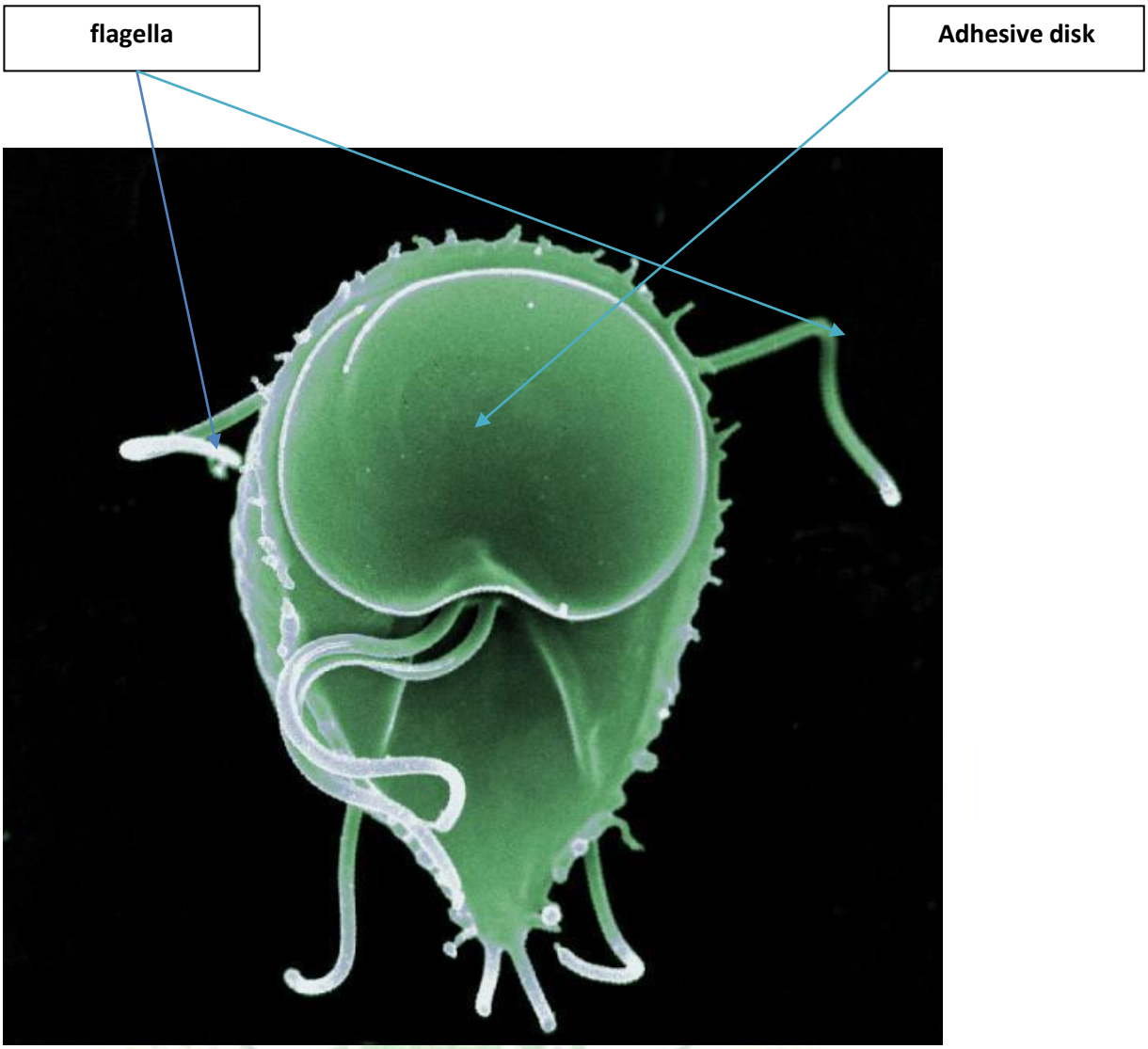


Fig 1.1 ***Giardia lamblia*** **trophozoite**

1.3.4 The Life Cycle

Giardia infection can occur through ingestion of dormant cysts in contaminated water, food, or by the fecal-oral route. The cyst of *Giardia* can survive for weeks to months in cold water, and therefore can be presented in contaminated wells and water systems, especially stagnant water sources such as naturally occurring ponds, storm water storage systems, and even clean-looking mountain streams. They may also occur

in city reservoirs and persist after water treatment, as the *Giardia* cysts are resistant to conventional water treatment methods such as chlorination and ozonolysis. (Roxstorm-Lindquist K *et al*.,2006). Zoonotic transmission is also possible, and therefore *Giardia* infection is a concern for people camping in the wilderness or swimming in contaminated streams or lakes, especially the artificial lakes formed by beaver dams (hence the popular name for giardiasis ,is "Beaver Fever").(Huanq and White ,2007).

In addition to waterborne sources, fecal-oral transmission can also occur, for example ,in day care centers, where children may have poor hygiene practices. those who work with children are also at risk of being infected, as are family members of infected individuals. not all *Giardia* infections are symptomatic, and many people can unknowingly serve as carriers of the parasite. (Exner and Gornik , 2004).

The life cycle(fig 1.2) begins with a non infective cyst being excreted with the feces of an infected individual. The cyst is hardy, providing protection from various degrees of heat and cold, desiccation, and infection from other organisms. a distinguishing characteristic of the cyst is four nuclei and a retracted cytoplasm.

Once ingested by a host, the trophozoite emerges to an active state of feeding and motility. After the feeding stage, the trophozoite undergoes asexual replication through longitudinal binary fission, the resulting trophozoites and cysts then pass through the digestive system in the feces, while the trophozoites may be found in the feces, only the cysts are capable of surviving outside the host.(Tovar J. *et al*., 2003).

Distinguishing features of the trophozoites are large karyosomes and lack of peripheral chromatin, giving the two nuclei a halo appearance.

Cysts are distinguished by a retracted cytoplasm. this protozoan lacks mitochondria, although the discovery of the presence of mitochodrial remnants organelles in one recent study "indicate that *Giardia* is not primitively amitochondrial and it has retained a functional organelle derived from the original mitochondrial endosymbiont" (Farthing M.J. *et al* ., 1996)

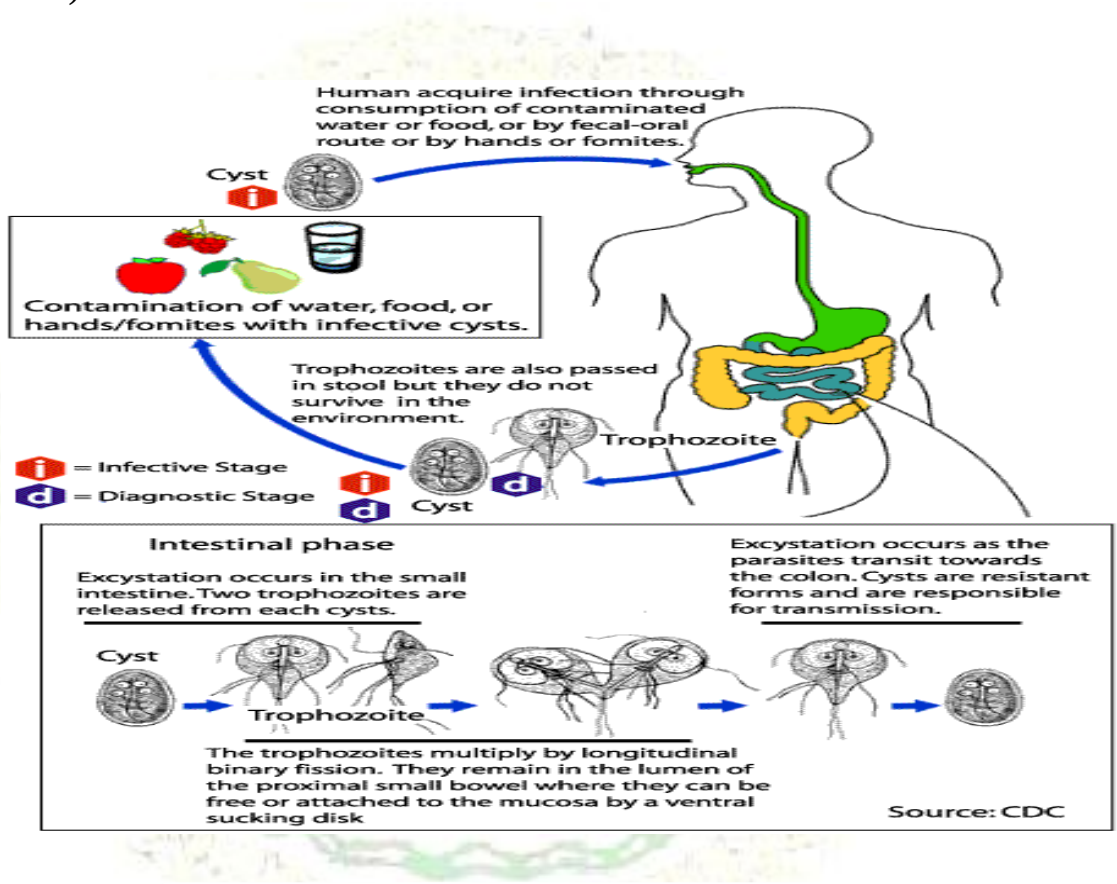


Fig 1.2 ***Giardia lamblia*** **life cycle** **(www.dpd.cdc.gov/dpdx)**

1.3.5 Transmission and epidemiology

Giardia is a parasite found in all parts of the world and in a large number of mammals , including humans , live stock, pets , wild life . and aquatic animals (Thompson , 2000; Lasek-Nessel quist *et al* , 2010). Several recent reports have also described *G*. intestinalis in various birds and even fish , although true infections remains to be confirmed in these animals (Lasek-Nesselquist *et al* .,2008;Yang *et al.*, 2010b).

The prevalence of *Giardia* in humans varies in and between countries , and it is higher in areas where environmental hygiene is low, about 200 million people have symptomatic giardiasis , and around 500,000 new cases occur each year (WHO ,1996).

Studies in different countries have indicated prevalence of 1-17% and up to 100% of population of infected in certain highly endemic areas ,Infective dose of *Giardia* for humans is known ,10 to 25 cysts caused infection in 8 of 25 subjects and the infection can be established by the ingestion of viable cysts (Randtorff, 1954).this allows person-to person spread which may be the most common way of transmission for humans , the infection spread by fecal oral route , venereal transmission occurs among home sexual through direct fecal-oral contamination .

Water is a major route of infection in community outbreak infection result from the ingestion of *Giardia* cysts under favorable conditions of temperature and humidity , such favorable conditions for temp between 4-10C , since the cysts may remain viable for several months (Marshall *et al* .,1997).

The cysts are relatively resistant to chlorination and disinfecting by ultra violet light. Boiling is very effective for inactivating *Giardia* cysts , but some cysts may survive in freezing for a few days , most human infection results from the contaminated water but less common transmission may occur via food contaminated by food handlers (Craun 1986; Hill , 1993).

Infected animals such as beavers have also been associated with water borne outbreaks (Faubert,1988). Humans are the main reservoir of the parasite but a variety of animals carry *Giardia spp*.

At first the genus was thought to contain numerous host-specific species ; however it is now believed that perhaps only two Morphologically distinct species infect animals one of these *duodenalis* (Synonyms G.*lamblia*) naturally infects humans , beavers , coyotes , cattle, cats and dogs can experimentally infect certain other mammals (Wolfe,1992). Where as studies analyzing isolates of *G. duodenalis* from different hosts suggest that intraspecific variation occurs within group .Host specificity is still considered unreliable as a means of classifying *G. spp* (Smith *et al* ., 1981) .

1.3.6 Pathophysiology

There is no doubt that the parasite is capable of causing disease (giardiasis) or (lambliasis) in human. During infection of *Giardia* trophozoites colonize the proximal small intestine and adhere to the apical surface of enterocytes (Farthing M.J. ,1996) .This close association between the parasite and host can be represented as the flowing :

1.3.6.1Deficeincy of Intestinal Transport

Since brush border membrane forms digestive-absorptive surface and any change that occurred in its structural and functional organization seem to provide a rational explanation of certain clinical conditions which impaired digestion and absorption (Miller ,1987). Enteric infection with *Giardia* spp.is responsible for decreased absorption of electrolytes,glucose and fluid, at least in part because of diffuse epithelialmicrovillus shortening, which may be combined or not with villous atrophy (Buret *et al*., 1991, 1992; Farthing M.J. ,1996).

Another study also explain that the disruption of functional integrity of the intestinal mucosal cells lead to disturbance in the fluxes of water and

electrolytes , together these abnormalities lead to diarrhea , Also changes in cells proliferation rate effect the numbers of absorptive epithelial cells (enterocytes) villous surface area and perhaps the rate at which cells mature as they migrate from crypts base to villous crest (potten and Loeffler,1987). In turn the number and maturity of cells in villi determine the extent and the activity of striated brush border (Pothier and Hugon , 1980) .

Furthermore microvillous-glycocalyx complex effects the potential for incorporation of membrane- associated with hydrolytic enzymes and various transporter molecules. (Ferraris and Diamond, 1986). One of the pathological changes in experimental giardiasis is inhibition of the activities of several digestive enzymes, including sucrase and maltase, during the acute phase of the infection.
(Scott K.G. *et al*.,2000).

1.3.6.2-Intestinal competition

Owen *et al*., (1979) observed that the numbers of trophozoites are very large and the lumen may be teeming with parasites which cover the epithelial surfaces especially in the patches .

Another study show that one parasite can cover three epithelial cells (Olveda *et al* ., 1982) so it quite conceivable that symptoms of malabsorption are caused by purely physical means ; the epithelial cells are blocked by the parasites which lie over them (Adam, 1991).

Tandon *et al*., (1977) noted that bacterial overgrowth is common in patients with giardiasis and malabrsorption and has been proposed as a possible cause of diarrhea . Indeed growth of bacterial is essential for virulence of pathogenic intestinal protozoa *Enatamoeba histolytica* (Wolfe,1992) , so there is precedence for the idea . However, bacterial

overgrowth is variably present and is studies of infected human volunteers bacterial overgrowth was not associated with symptomatic giardiasis (Nash *et al*., 1987). In addition treatment of bacterial overgrowth alone does not result in improvement of symptoms , this gives an expression that bacterial overgrowth is not related to the diarrhea of giardiasis (Gillon , 1985).

1.3.6.3-Malabsorption

Many authors have shown that malabsorption of glucose , folic acid , maltase , fat , vitamin B12 , lactose , sucrose and protein does occur in *G. lamblia*

Infection, Some patients have malabsorption of more than one substance but one of the feature of giardial malabsorption is that specific treatment to eradicate the parasite often lead to rapid disappearance of the malabsorption (Gillon , 1985).

Some studies have shown no effect of giardiasis on weight or nutritional status

(Schofiled *et al* ., 1991). whereas others have shown an association between giardiasis and lactose malabsorption and/or malnutrition (Mantovani and Pettoello M. ,1991).

A factor which seems to be an important at least in some cases of giardiasis with malabsorption was the deconjugation of bile salts which it's normally brought about by colonic bacteria in the colon (Tandon *et al*., 1977).

In some cases there was no bacterial overgrowth at all ,yet free bile acids were present in the duodenum aspirates this suggests that *G.lamblia* itself might be able to deconjugate bile salts (Tomkins *et al* ., 1978; Bo-Linn and Fordtran 1984).

The unconjugated bile acids might have atoxic effects on the intestinal mucosa or perhaps the reduction in the concentration of conjugated bile salts may be responsible for malabsorption (Tandon *et al*.,1977).

Malabsorption of bile acid results in increased passage of glycine and taurine conjugates of the cholic where block the net electrolyte and water transport and result in fecal water and electrolyte which lead to various diarrheal conditions

(Williams *et al*., 1991).

1.3.6.4-Lactose Intolerance

According to the New Zealand Ministry of Health, about 40 percent of people with giardiasis go on to develop lactose intolerance.(Bhatnagar S., *et al*.,1996).

Lactose intolerance is the inability to digest lactose, a sugar found in milk. The sugar is normally digested by an enzyme finds in the small intestine, when this enzyme is missing, the lactose accumulates in the intestine and ferments. This can cause symptoms similar to giardiasis: abdominal cramps, flatulence and diarrhea.

For some reason, giardiasis damages the body's ability to produce the enzyme that digests lactose. (American Academy of Pediatrics,2006).

1.3.6.5- Vitamin A Deficiency

A study had been done in India was conducted to investigate whether giardiasis is found in children with diarrhoeal syndrome, as attempt to seek an analogue with deficiency of vitamin A. The fecal samples and blood specimens from children with diarrhea of over 10 days prior to treatment were the materials used for the demonstration of pathogen,

drinking water samples were also examined for the presence of cysts from the schools of afflicted children.(Visvesvara G.S. *et al*., 1980).

The results have shown the occurrence of giardiasis among children. The frequency was one in four cases of diarrhea over long duration. The observed cysts in water samples disclose the contamination of live cysts and the quantitative estimation of serum vitamin A level in healthy children and infected children. The results showed there is a progressive loss of vitamin A with respect to the infection of the *Giardia*. The highlight of investigation is that it is difficult to diagonize the indication of vitamin A deficiency with giardiasis however, there may be a chance of impairment of dark adoption and inadequate hepatic retinol reserve when the serum level will fall below10 mg. Nevertheless, vitamin A depletion also affects the functioning of intestinal mucosa and causes loss of goblet cells. This may lead to the increasing susceptibility to the trophozoites.(K. Jayaprakash,2005).

1.3.7 Virulence Factors

In spite of significant recent advances in the knowledge on the biochemistry and molecular biology of *G. lamblia*, little is known about the pathogenesis of symptomatic infections in humans and the factors that determine the variability of the clinical outcome, a combination of parasitic factors and host responses seems to be involved ,and these factors include:

1.3.7.1 Attachment

Attachment of *Giardia lamblia* trophozoites to enterocytes is essential for colonization of the small intestine and is considered a prerequisite for

parasite -induced enterocyte dysfunction and clinical disease.(P.H. Katelaris et al.,1995).

The mechanism of attachment of trophozoites to intestinal cells has not been established definitively, evidence supports roles for the ventral disk, which is considered a unique ultra structural feature of Giardia (also called adhesive disk, sucking disk, sucker, or striated disk). The adhesive disk is a concave structure which occupies approximately two-thirds of the anterior end of the ventral surface as the names imply, this structure plays a role in the attachment of the trophozoite to the intestinal epithelium and ultra structural studies reveal close associations between the adhesive disk and the intestinal brush border. (Brugerolle, 1991; Nohýnková and Kulda , 1999).

The adhesive disk appears to be a relatively rigid structure and striations are evident by transmission electron microscopy. These striations are the result of microtubules (mT) and a unique cytoskeletal element called microribbons (mR).

Microribbons are long flattened structures and each microribbon is associated with a microtubule. The combined microtubule-microribbon structure are arranged in concentric rows that form a flatten spiral with minimal overlap ,the outer rim of the adhesive disk, called the lateral crest, contains components of the actin-myosin cytoskeleton.(Holberton D.V. ,1973).

A major component of microribbons are proteins called giardins (aka beta-giardins). These giardins play primarily a structural role in the formation of the microribbons. Interestingly, the giardins show a limited homology to a protein called 'striated fiber assemblin' from Chlamydomonas (a free-living, bi-flagellated unicellular algae), in Chlamydomonas this protein forms filamentous structures at the base of

the flagella. The giardins have evolved to play a different functional role in Giardia, but are still associated with microtubule based cytoskeletal elements. (Gillin et al., 1996; Adam, 2001).

This association of proteins involve in the generation of contractile force and other cytoskeletal elements in the adhesive disk suggests that attachment is mediated by mechanical forces generated by the parasite.(Owen R. et al., 1979).

The observation that imprints and circular dome-shaped lesions remain in the intestinal brush border (i.e., microvilli) following detachment of trophozoites is consistent with contractile forces playing a role in attachment. (Ghosh et al., 2001).

The other proposed mechanisms for the attachment of Giardia to the intestinal epithelium include hydrodynamic forces generated by the ventral flagella and receptor-mediated binding via lectins on the trophozoite surface., however, flagellar movement is poorly correlated with attachment and the surface lectins cover the entire trophozoite and are not specifically localized to the adhesive disk. (Meyer et al .,1979) .

1.3.7.2 Alteration of Host Innate Defenses

Recent studies revealed that Giardia lamblia can release special enzymes after only brief interaction between G. lamblia and intestinal epithelial cells (IECs) (Emma Ringqvista et al., 2007).these enzymes have multifunctions such as alteration of host innate defences by reduces the ability of IECs to produce nitric oxide (NO), an anti-microbial innate defense molecule.

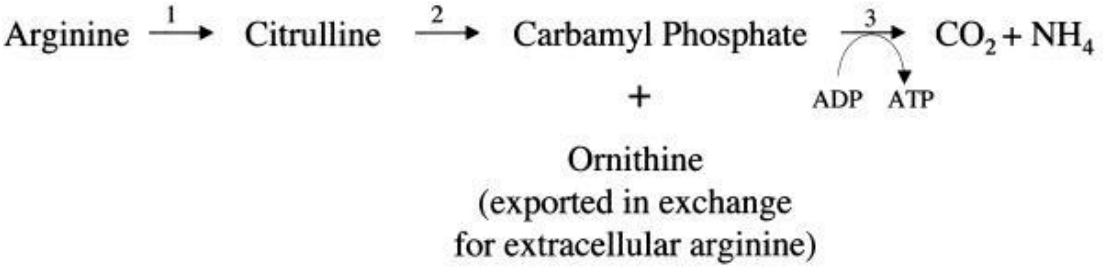
Three major Giardia proteins have been idntified (arginine deiminase (ADI), ornithine carbamoyl transferase (OCT) and enolase) function in

giardial metabolism and are immunoreactive during human and murine infections (Palm J.E., et al 2003, Tellez A. et al., 2003).

In Giardia lamblia arginine deiminase catalyzes the irreversible catabolism of arginine to citrulline in the arginine dehydrolase pathway (ADH) and serves as an important source of energy, this pathway has been regarded as being restricted to prokaryotic organisms. (Schofield *et al*., 1990).

On the other hand, higher eukaryotes utilize nitric oxide synthase (NOS) to convert free L-arginine into citrulline and nitric oxide (NO), and use peptidyl-arginine deiminases (PADs) to deiminate protein-bound arginine and convert it to citrulline (citrullination) by a Ca2+ dependent way. (Leigh A. Knodler et al.,1998).

Ornithine transcarbamoylase (OTC E.C. 2.1.3.3, also known as ornithine carbamoyltransferase) catalyzes the reversible conversion of citrulline to carbamoyl phosphate (CP) and L-ornithine in the arginine dihydrolase pathway.



1-Argninedeimnase, 2-OrnithineCarbamoyltransferase, 3-Carbamate Kinase

(Edwards M. R. *et al* ., 1992; Schofield P. J. *et al*., 1990; Schofield P. J. *et al* ., 1992).

This pathway is used by a number of microorganisms, including Giardia lamblia to generate ATP fermentatively from arginine 1,2. G. lamblia OCT was shown to be one of the 16

immunodominant proteins, underscoring the importance of the arginine catabolism pathway in the characterization of the parasite.

Moreover, recently G.lamlia OTC was identified as one of the major proteins released into the medium after brief interaction of Giardia with human intestinal epithelial cells. .(Andrey Galkin et al.,2009).

1.3.7.3 Antigenic Variation

Nash *et al* (1990) were first to report the phenomenon of antigenic variation in giardiasis. Some characteristics of this phenomenon in giardiasis are as follows:

- certain epitopes are re expressed in clones, suggesting the presence of a favored set in the repertoire of epitopes
- the repertoires of variant surface proteins (VSPs) may differ among isolates
- the same epitope detected on the surfaces of independent isolates is present in molecules with different molecular masses (Nash T. E. *et al* 1990, Nash T. E.; 1989, Nash T. E. 1992).

In contrast to other parasites in which the phenomenon has been observed, antigenic variation in giardiasis was first observed as a phenomenon occurring in vitro. most of the studies on antigenic variation were done with the WB isolate obtained from a symptomatic individual. clones of the WB isolate of G. duodenalis were exposed in vitro to a cytotoxic Monoclonal Antibody (Mab) which reacts with a 170-kDa surface antigen (Nash T. E. et al.,1987). analysis of progeny and clones of the progeny by different assays failed to detect the high-cysteine 170-kDa

antigen. In a subsequent study, it was demonstrated that the loss of this antigen was associated with the appearance of a new 64-kDa surface antigen. (Adam R. D. et al.,1988). Specific variants have been detected after 12 generations of in vitro growth of the WB isolate (Nash T. E. et al.,1990). the abundant, highly variable VSPs which cover the surface of trophozoites have been confirmed. (Zhang Y.et al.,1993). and these VSPs are capable of binding 65Zn in vitro.

The finding of a cysteine-rich protein(s) in Giardia trophozoites (Adam R. D. et al.,1988;Aggarwal A.et al.,1989) was not unexpected, since Giardia has a high nutritional requirement for cysteine (Gillin F.D.et al.,1988). the gene VSPA6 coding for the 170-kDa surface antigen has been cloned (Adam R. D.,et al.,1988)

This gene consists of three regions: a short 5′ region containing a hydrophobic leader, a repeat region comprising 4,056 nucleotides and 20.8 repeats, and a 3′ region containing a region of homology to the other VSPA6 genes. (Adam R. D,1992).

Antigenic variation at the surface membrane of trophozoites occurs frequently in Giardia isolates. These antigens are made of cysteine-rich proteins (Aggarwal A. et al.,1989, Adam R. D. et al.,1988), which are controlled by 20 to 184 genes (Nash T. E.et al.,1990).

In contrast to African trypanosomiasis, where genes controlling variant surface antigens are expressed in telomere-associated sites, the VSP genes controlling the VSPs in Giardia are not telomere associated (Babb R.et al.,1971).

Another virulence factors include circumvention of the natural factors of the intestinal lumen which occur by flagellar motility which enables re-localization to new endothelial cells during colonization, and VSP potentially help to protect against luminal proteases, oxygen and free

radicals anti-inflammatory modifications this process produced by unknown trophozoite products that have anti-inflammatory roles and at last survival in stomach acid and the external environment by differentiation into cysts.(Johan Ankarklev et al ., 2010).

1.3.8 Clinical Features

The infection with *Giardia lamblia* ranges from asymptomatic passage of cysts , to acute diarrhea , to a syndrome of chronic diarrhea and malabsorption .

It can be estimated that 5-15% of infected persons will become asymptomatic cyst passers , 25-50m % will have a diarrheal syndrome , and 35-70% will have no trace of infection(Hill, 1993).

The incubation period for acute giardiasis is 1-2 weeks , symptoms may develop prior to the excretion of cysts , therefore a stool examination done at the onset of symptoms may be negative . In reality , most patients will have had diarrhea for several days before presenting to their physician , and stools should be positive. (Brodsky *et al*., 1974).

The onset of illness is generally abrupt with diarrhea abdominal cramping , bloating and flatulence the most common symptoms. In addition , patients frequently complain of malaise , nausea and experience sulfuric belching . It is unusual to have vomiting or fever . The presence of anorexia and nausea probably contribute to the important finding of weight loss, which occurs in over 50% of patients by the time they present to physician (Moore *et al*., 1969).

On average , persons suffering from *Giardia* have lost 4 kg .stools will range from profuse and watery to greasy and foul smelling , they should

not contain gross or microscopic blood , or poly morphonuclear cells since *Giardia* is not an invasive pathogen .

Another hallmark of giardiasis is the duration of symptoms by the time a patient presents . Most persons will have been sick for 7-10 days this feature , combined with weight loss ,help distinguish giardiasis from most viral and bacterial etiologies of diarrhea, which usually have shorter duration of illness .

Viral diarrhea may also be more commonly associated with vomiting and if there is an invasive bacterial etiology , inflammatory stool changes should be seen protozoal causes of diarrhea such as Cyclospora cayetanesis and cryptosporidium parvum , have clinical features that are similar to diarrhiasis.(Adel et al.,1995 ; Goodgame , 1996 ; Soave , 1996) .

Extra intestinal manifestations of Giardia have been described , These include uriticaria in about 50 of infected persons and rarely , a reactive (Shaw and Stevens , 1987 ; Cylne and eliopoules,1989; Layton *et al*.,1998).

It is possible that allergic manifestation occur because of passage across an injured gut food or other allergens. *Giardia* may infected biliary treat and the stomach however, the letter occurs only co-infection with *Helicbacter pylori* ingastric giardiasis. (Di prisco *et al* ., 1998).

Most persons with symptomatic giardiasis have an illness which is uncomfortable and requires treatment but is usually not severe of life threatening.

It is now recognized , however , that some person do have severe diarrhea with significant volume depletion , which necessitates hospitalization (Lengerich *et al*., 1994 ,Robertson , 1996).

In some of these severe cases , a hypokalemic myopathy has been described. in US study, rates of hospitalization for *Giardia* were similar to those for *Shiglla*

(approximately two cases per 100 ,000 persons and about 4600 annual admissions) and the highest for children under age of 5 and women of child bearing age(Lengerich *et al*.,1994) .

In general, the symptoms of giardiasis and diarrhea , Malaise , Flatulence , Foul-smeling greasy stools , Abdominal , Bloating , Nausea , Anorexia , weight loss (average 4 Kg) , vomiting , fever , constipation and unitecaria.

1.3.9 Laboratory Diagnosis

1.3.9.1-Microscopy

Microscopic detection of *Giardia* cysts in stool specimen , either in a wet smear or after formol-ethyl acetate concentration , is the most frequently used method for diagnosis of giardiasis worldwide ., less often , diagnosis is based on detection of trophozoites in fresh stool samples or SAF – fixed material .

Compared to identification of *Entamoeba spp* ., microscopy of *Giardia* cysts and trophzoites is more straightforward , and there is little risk of confusion with other parasites .

Moreover , only "ghost" cysts with an empty appearance are sometimes not recognized as *Giardia* parasites. (Collins *et al*.,1978).

However , the sensitivity of microscopy is quite low due to the intermittent excretion of *Giardia* cysts(Rendtorff , 1954 ; Danciger and Lopez , 1975). and thus, it is recommended that at least there are three samples have to be examined in order to rule out giardiasis .

1.3.9.2-Antigen Detection Methods

Quite a few commercial kits are available for detection of *Giardia* antigen . Two techniques that are used enzyme-linked immune-sorbent assay (ELISA) that assesses soluble antigens and a directs fluorescent antibody(DFA) test that detects intact organisms . Several studies have shown that these two methods offer greater sensitivity compared to light microscopy (Zimmerman and Needham , 1995; Garcia and Shimizu,1997) , but they are not available in all parasitology laboratories due to the high cost and substantial workload they entail , and also limited access to the required equipment .

An alternative technique involves a solid-phase immunochromstographic test card system (ImmunoCardSTAT *Cryptosporidium/Giardia* rapid assay) , which allows concurrent detection of cryptosporidium and it also fast , easy to use , and does not require extra equipment (Johnston *et al*.,2003). Unfortunately , the sensitivity of such a system is lower than microscopy , and thus this test is not recommended for follow-up patients with *Giardia* treatment failure(Stand *et al*.., 2008).

1.3.9.3-Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) is a scientific technique in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

Developed in 1983 by Kary Mullis,[1] PCR is now a common and often indispensable technique used in medical and biological research labs for a variety of applications.[2][3] These include DNA cloning for sequencing, DNA-based phylogeny, or functional analysis of genes; the diagnosis

of hereditary diseases; the identification of genetic fingerprints (used in forensic sciences and paternity testing); and the detection and diagnosis of infectious diseases. In 1993, Mullis was awarded the Nobel Prize in Chemistry along with Michael Smith for his work on PCR.[4].

Conventional single or nested PCR analysis are not often used for diagnosing giardiasis , except at specialized centers . In contrast ,there is increasing use of real-time PCR as a diagnostic tool that can detect *Giardia* , often in combination with other enteric protozoa , such as *Cryptosporidium spp*., *E. histolytica* , and *D, fragilis* . This approach has proven to provide higher sensitivity if compared to conventional methods , and it also entail a lower workload(Verweij *et al*,., 2006 ; Haque *et al*., 2007 ; Schuurman *et al*.,2007; Bruijnesteijn van Cooppenraet *et al*., 2009 ; Ten Hove *et al*.,2009 ; Calderaro *et al*., 2010). However , in the parts of the world where Giardiasis is highly endemic , microscopy will probably remain the routine procedure for detecting stool parasites , including *Giardia* , for a long time to come.

2.1 Fecal Samples Collection

Stool samples containing *Giardia lamblia* cysts were collected from 80 infected individual .The samples were from sporadic cases and food born outbreaks .

Giardia infection was diagnosed by microscopy of wet amount and concentration method, then samples stored frozen at (-20°C) until the time of experiments in DNA extraction without cyst isolation.

2.2Patient Age Groups

Giardiasis patients were subdivided into five statistically groups :

a. Group 1 between 2-10 years 15 patinets .
b. Group 2 between 11-20 years 11 patinets .
c. Group 3 between 21-30 years 10 patinets .
d. Group 4 between 31-40 years 10 patinets .
e. Group 5 between 41-50 years 34 patinets.

2.3Chemicals and Biological Materials

The chemicals and biological materials used in this work are listed in table 2.1 below :

Table 2.1 Chemicals and Biological Materials

Types of chemicals	**Manufactures name**
Ethidium bromide	BDH
Agarose	Promega (USA)

DNA marker	Promega (USA)
Stool DNA extraction kit	Bioneer (Korea)
Absolute Ethanol(96≈ 100)	BDU
Absolute isopropyl alcohol	BDU
Cholesterol kit	Linear Chemicals (Spain)
Triglycerides kit	Linear Chemicals (Spain)

2.4 Instruments and Equipments

The instruments and equipments used in this study are listed in table 2.2 below :-

Table 2.2 Instruments and Equipments

Instruments / Equipments	**Manufacture / state**
PCR sprint – thermal – cycler – IP20	MWG Biotech /Germany
Gel Electrophoresis apparatus	Shandod, Scientific Co.(UK)
Eppendoff Centrifuge	Hettich EBA 20 (Germany)
Eppendroff tubes	Sigma (England)
Micropipettes (different volumes)	Eppendroff Oxford (USA)
Water distiller	Ogawaseiki (Japan)
Light Microscope	Olympus (Japan)
Sensitive balance	Gallen Kamp (England)
Water bath	Memmert
Vortex Mixer	Memmert

Digital Camera	Sony (China)
PCR tubes	Eppendroff (USA)

2.5 Diagnostic Kits which include :

Table 2.3 The Contents of Diagnostic kits

Material	Origin
Go Tag Green master mix, 2X (PH 8.5) 1.25 µl	Bioneer Koria
Nuclease free water 1.25 µl	Bioneer Koria
1000 base pair (bp) DNA ladder 250 µl	Promega USA
Blue /orange 6X loading dye 1.25	Promega USA

2.6 Specific Primer Sequences Used for PCR Amplification

These primer were prepared according to information of company by dissolved primer in 1000 M of deionized distilled water to obtain different concentrations of picomols/µl for each primers according to providing company reccomendations.

Table2.4 Primers Used in This Study Provided by Bio CorP Co :-

Enzyme	Primer Sequence
Arginine deiminase	(forward5′-CAG TACAAC TC GCT CTC GG-3 (reverse -5′-GTT RTC CTT GCA CAT CTC C-3′)
Ornithinecarbamoyltransferase	(forward 5-CATATGCTTTCAACATGCAC-3) (reverse 5-ATCGATGGCGAGGGTCGACG-3)

cystein rich protein CRP65	(forward5-GGCACTACGAATGACCTA-3) (reverse 5-TTGTAGCACCCGCCCATG-3)

2.7 Microscopic examination

- **Wet Mount:**

A wet mount can be performed directly from fecal sample and from concentrated specimens . the wet mount was used for each of fecal sample is Iodine staining wet mount. It was used for the initial microscopic examination of stool and to demonstrated cysts .

For direct iodine mount, a drop of iodine solution was put in the centre of slide with an applicator stick picked up a small of the specimen (size of a match head) and mixed with a drop of iodine. then a drop of iodine was covered with cover slips. A Giardia cysts can be examined with high power, dry magnification. (Elizabeth, 1997).

- **Concentration Method**

This method was done when the stool was suspected G. lamblia . and the cysts were not seen in the wet mounts. (Elizabeth, 1997).

1. .Ten µl of 2% formalin was added to approximately 1g of stool and stirred using applicator stick until get a cloudy suspension.
2. A gauze filter was fitted into a funnel and the funnel was placed on top of the centrifuge tube.
3. The fecal suspension was passed through the filter into centrifuge tube until 7µl mark is reached.
4. The filter was removed and discards with lumpy residue.

5. A volume of 3µl ethyl acetate was added and mixed well for one minute.
6. Transfer back to the centrifugation tube and centrifuge for 1mintue at 1000g.
7. The fatty plug was loosed with an applicator stick, and supernatant was poured away by quickly inverting the tube.
8. The tube was replaced in its rack and the fluid was allowed on the sides of the tube to drain down to the sediment, mixed well and transfered a drop to a slide for examination, also it was made an iodine –stain preparation (WHO, 1991).

2.8 Electrophresis Solutions

1. Tris-Borate EDTA buffer (TBE buffer , 10X) :

This buffer was prepared by dissolving 3.8 gm TrisHCl , 2.7 gm Boric acid and 2 µl EDTA (0.5 M) in 50 µl of distill water , the PH was adjusted to 8 , autoclaved (121°C/1.5 Ib for 15 min) , cooled and stored at 4°C until be used (Sambrook et al., 1989).

2. TBE (1X):

This solution was prepared by mixing 10 µl (of stock) TBE-10X with 90 µl of distilled water and stored at 4C until using (Sambrook et al., 1989) .

3. Ethidium bromide solution (0.5%) :

This solution was prepared by dissolving of 0.25 gm of Ethidium bromide stain in 10 ml sterilized distilled water , stored in sterilized flask final concentration 5 milligram/milliliter . (Sambrook et al., 1989).

2.9 Agarose Gel Preparation

The agarose gel was prepared according to the method of Sambrook *et. al.*, (1989) as the following: One hundred ml of 1X TBE buffer was taken in a beaker, 1 g agarose was added to the buffer. the solution was heated to boiling by hot plate until all the gel particles were dissolved, then allowed to cool down at room temperature . It was used for electrophoresis.

2.10 Preparation of Kit Components

- Proteinase K ,lyophilized 25mg x2

One vial with 25mg lyophilized proteinase K.was dissolved in 1.25ml nuclease free water .

- Stool Lysis buffer (SL) 50ml

Stool lysis buffer was mixed thoroughly by shaking before use.

- Binding buffer (ST) 50ml

Binding buffer was mixed thoroughly by shaking before use.

- Washing buffer1 (W1) 40ml

W1 buffer was prepared by adding 30 ml of absolute ethanol .

- Washing buffer2 (W2) 20ml

W2buffer was prepared by adding 80 ml of absolute ethanol Elution buffer (E)

10mM of Tris Cl(pH8.5) was stored at room temperature

2.11 Extracting DNA from Stool: (Carter and Milton,1993).

1. 1.5 ml microcentrifuge tube was prepared and 20 µl proteinase K was added .

2. About 100-200 mg of the stool sample to the tube was added .

3. 400 µl of SL buffer to the tube was added and mixed by light vortexing for about 30 seconds .
4. The tube was incubated for 10 min at 60 °C.
5. After 10 mins , the mixture was centrifuged at 12,000 rpm for 5 minutes .
6. The supernatant was transferred to a new tube and 400 µl binding buffer was added.
7. The tube was incubated again for 10 min at 60° C.
8. 100 µl isopropanol was added , lightly vortex for about 5 seconds , then the tube was spinet down for 10 seconds to down the liquid clinging to the walls and the tube was led .
9. The binding column was fitted into the 2 µl collection tube . the liquid was transferred into the binding column .
10. Carefully the lid was closed and centrifuged for 1 min at 8,000 rpm.
11. Following centrifugation , the binding column was transferred to a new 2 µl collection tube .
12. 500 µl washing buffer 1 (W1) was added to the column , taking care so that the sides do not get wet ; the lid was closed, and centrifuged for 1 min at 8,000 rpm
13. After centrifugation , the binding column was transferred to a new 2 µl collection tube .

14. 500 µl washing buffer 2 (W2) was added , taking care so that sides do not get wet ; the lid was closed , and centrifuged for 1 min at 8,000 rpm.

15. The tube was let to spin down once more at 13,000 rpm for 1 min to completely remove ethanol ..

16. Residual washing buffer 2 left in the binding column can hinder the following steps .

17. The binding column was transferred to a 1.5 µl collection tube , and 200 µl of Elution Buffer was added , and let stand for 1 min to allow the buffer to permeate the column .

18. The column eluted by spinning down at 8,000 rpm for 1 min the eluted DNA solution can directly be used , or stored at 4 C° or -20 C° longer storage periods

2.12 PCR Kit (Green Master Mix , Primers , Nuclease Free Water and Extracted DNA)

And these constituents put in ice container :-

- A new PCR tubes (0.5 ml) were labeled with number of sample for amplification reaction (located in ice) .
- To avoid contamination , all solutions were taken with separate clean tips under septic condition .
- 0.5 µl of DNA sample was added to PCR tube , 1.5 µl of ward primer and 1.5 µl of reverse primer , 12 µl of nuclease free water the volume was complemented to 20 µl .
- Control for primer 5 µl of extracted DNA , 15 µl of PCR matter

- Control for DNA 3 µl of primer , 17 µl of PCR matter .
- All tubes were closed , the mixture was spin for 5 second by light vertex , the PCR tubes were transferred to preheated thermocycler .

2.13 PCR Program For Enzymes

2.13.1 PCR program for Arginine deiminase Gene Amplification

Three major steps in PCR program including (35) cycles and this was done by automated thermocycler :

1. Denatuartion at 94° C for 45s : The double strand helix was melt and became single stranded DNA .
2. Annealing at 50°C for 45s primers were binded to DNA strand .
3. Extension 72° C for 10 min : Anew DNA strand complimentary to DNA template was synthesized by Taq DNA polymerase by adding dNTPs in the 5-3 direction , temperature differ according to DNA length (Leigh A. Knodler *et al*.,1998).

2.13.2 PCR program for Ornithine Carbamoyl Transferase Gene Amplification (OTC):

The PCR conditions used for the amplification OTCase gene were as follows :

1. Denaturation at 95° C for 1 min .

2. Annealing at 55° C for 1 min .
3. Extention at 72° C for 1.5 min for 30 cycles (Andrey Galkin. *et al* .,2009) .

2.13.3 PCR program For Amplification of Cysteine Rich Proteins (CRP65) :

1. Denaturation at 94° C for 2 min .
2. Annealing at 51°C for 1 min .
3. Extension at 72° C for 2 min .

All these steps are repeated for 30 cycles (P Upcroft *et al*.,1997) .

2.14 PCR Results Analysis

The results of the PCR were performed in post amplification area 10µl from amplification samples was directly loaded in a 2% agaros gel containing 0.5 mg / ml ethidium bromide with adding loading buffer in electrophoresis and the products were visualized by UV transillumination.

2.15 Agarose Gel Electrophoresis :

Gel electrophoresis was prepared in concentration (2%) to all PCR tubes as the following :

a- Casting of The Horizontal Agarose Gel:

- The gel was assembled to casting tray and the comb was positioned at one end of the tray.
- The agarose solution was poured into the gel tray and it was allowed to gel at room temperature for 30 minutes.
- The comb was carefully removed and the gel placed into electrophoresis chamber, then the chamber filled with TBE-

electrophoresis buffer until the buffer reached surface of the gel (3-5mm over the surface).

b- Loading and Running DNA in Agarose Gel:

- DNA (20µl) was mixed with bromophenol blue in the ratio at 3:1and loaded in the wells of the 2% agarose gel.
- The cathode was connected to the well side of the unit and the anode to other side.
- The gel was run at 100V until the bromophenol blue tracking dye migrated to the end of the gel.
- The DNA was observed by staining the gel with ethidium bromide and viewed under UV Tranilluminator.

2.16 Loading and Running DNA in Gel Agarose:

1. DNA (9 µl) was mixed with (3µl) bromophenol blue (loading buffer) and loaded in the wells of the 2% agarose gel.
2. The cathode was connected to the well side of the unit and the anode to the other side.
3. The gel was run at 100 Volt until the bromophenol blue tracking dye migrated to the end of the gel in about 45 mints.
4. The DNA was observed by staining the gel with ethidium bromide and viewed with UV transilluminator.

2.17 Lipid Profile

2.17.1 Blood Sample Collection

Blood samples were collected from 60 individual at different ages (2-60) years 40 of them were suffer from giardiasis , 20 were healthy and used as a control , 3 ml of venous blood was collected from each

individual transferred immediately into plain plastic tubes and the serum was obtained by centrifugation . the serum was dispensed in plastic tubes and used immediately in Lipid profile process .

Table 2.5 patients and control age groups

Age	Patients number	Control number
2-10	5	2
11-21	8	4
21-30	6	3
31-40	7	4
41-50	9	4
51-60	5	3
Total number	40	20

2.17.2 Serum Triglycerides Assay

Principle

The method is based on the enzymatic hydrolysis of serum or plasma triglyceride to glycerol and free fatty acids (FFA) by lipoprotein lipase (ATP) . The glycerol is phosphorylated by adenosin to form glycerol-3-phosphate (G-3-P) and adenosine diphosphate (ADP) . G-3-P is oxidized by glycerphosphate oxidase (GPO) to form dihydroxyacetone phosphate (DHAP) and hydrogen peroxide . red chromogen is produced by the peroxidase (POD) catalyzed coupling of 4-aminoantipyrine (4-AA) and phenol with hydrogen peroxide (H_2O_2) , proportional to the concentration of triglyceride in the sample .

$$\text{Triglycerides} + 3H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + 3\,\text{FFA}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{Glycerol-3-P} + \text{ADP}$$

$$\text{Glycerol-3-P} \xrightarrow{\text{GPO}} DHAP + H_2O_2$$

$$4 - AA + 4Phenol \xrightarrow[POD]{H_2O_2} Quinonei\,\min e + H_2O_2$$

Reagent composition

- **R1** Monoreagent .composed of 50mmol/L buffer pH6.8 LPL≥ 12KU/L,GK≥ 1KU/L,ATP 2.0mmol /L,Mg^{+2} 40mmol/L,POD≥2.5 KU/L,4-AA0.5mmol/L, phenol 3mmol/L ,non-ionic tensioactevies 2g/L(w/v) Bioacids.
- **Cal** Triglyceride standard . Glycerol 2.26mmol/L equivalent to200mg/dL of Glycerol trioleate ,Secondary standard .concentration value is traceable to standard reference material 909b.

Procedure

The reagents and samples were brought to room temperature and then. pipetted into labeled tubes .

Table 2.6 Serum Triglycerides Assay Procedure

TUBES	Blank	Sample	CAL. Standard
R1. Monoreagent	1.0 ml	1.0 ml	1.0 ml
Sample	-	10 µl	-
CAL. Standard	-	-	10 µl

1. The tubes were mixed and leaved the tubes stand 15 minutes at room temperature (16-25° C) or 5 minutes at 37° C.
2. The absorbance (A) of the samples and the standard was read at 500 nm against the reagent blank .

The color stable for at least 1 hour protected from light.(Buccole and David ,1973).

2.17.3 Serum Cholesterol Assay .

Principle

This method for the measurement of total cholesterol in serum involves the use of three enzymes : cholesterol esterase (CE) , cholesterol oxidase (CO) and peroxidase (POD) . In the presence of the former the mixture of phenol and 4-aminoantipryne (4-AA) are condensed by hydrogen peroxide to form a quinoneimine dye proportional to the concentration of cholesterol in the sample .

$$Cholestry\_lesters \xrightarrow{CE} Cholestrol + Fatty\_acids$$
$$Cholestrol + O_2 \xrightarrow{CO} Cholesenone + H_2O_2$$
$$4-AA + Phenol \xrightarrow[POD]{H_2O_2} Quinonei\min e + 4H_2O_2$$

Reagent Composition

- **R1** Monoreagent 200mmol/L 7.0pH sodium cholate ,1mmol/L cholesterol esterase >250 U/L , cholesterol oxidase >250 U/L, piroxidase >1 KU/L,4-aminoantiantipyrine 0.33mmol/L,non-ionic tensioactevies 2g/L(w/v) Bioacides.
- **Cal** cholesterol standard .Cholesterol 200mg/dL (5.18mmol/L),organic matrix based primary standard **,**

Procedure

1. reagents and samples were brought to room temperature and pipette into labeled tubes .

Table 2.7 Serum Cholesterol Assay Procedure

Tubes	Blank	Sample	Cal. Standard
R1. Monoreagent	1.0 ml	1.0 ml	1.0 ml
Sample	-	10µl	-
CAL. Standard	-	-	10 µl

2. The tubes were mixed and incubated stand 10 minutes at room temperature or 5 minutes at 37 °C.
3. The absorbance (A) of the sample and the standard was read at 500 nm against the reagent blank .

The color stable for at least 30 minutes protected from light. (Allian C.C. *et al*., 1974).

2.17.4 Serum High Density Lipoprotein Assay

Principle

The chylomicrons and lipoproteins of very low density (VLDL) and low density (LDL) contained in the sample are precipitated by the addition of phosphotugstic acid in the presence of magnesium ions .

The supernatant obtained after centrifugation contain high density lipoproteins (HDL) from which the cholesterol and phospholipids can be determined using the 'Cholesterol Enzymatique' and 'Phospholipids Enzymatique ' reagent.

Procedure

Precipitation

sera containing > 3.5 mmol / I of triglycerides was diluted in

9 g /NaCl

Serum…….500 µl

Reagent 1 (precipitant) ……..50 µl

1. Then tube was mixed and let stand for 10 min .
2. The tube was centrifuged for 15 min at 5000 rpm.
3. Determination of HDL cholesterol .(Allian C.C. *et al*., 1974).
4. The absorbance (A) of the sample and the standard was read at 500 nm against the reagent blank .

Reagent Composition

❖ **R1** phosphotungstic acid 40g/L, MgCl2 6H2O pH 6.2

Table 2.8 Serum High Density Lipoprotein Assay Procedure

	Reagent blank	Standard	sample
Distilled Reagent (HDL cholesterol	50 µl	-	-
Calibrating solution)	-	50 µl	-
Supernatant	-	-	50 µl
" Cholesterol Enzymatigue" Working solution	1 ml	1ml	1ml

2.17.5 Serum Very Low Density Lipoprotein Assay

Very low density lipoproteins were estimated by Friedewald equation as the following (Friedewald, 1972):

Very low density lipoproteins = Serum Triglycerides / 5

2.17.6 Serum Low Density Lipoprotein Assay

The low density lipoproteins were estimated according to Friedewald equation as the following(Friedewald, 1972) :

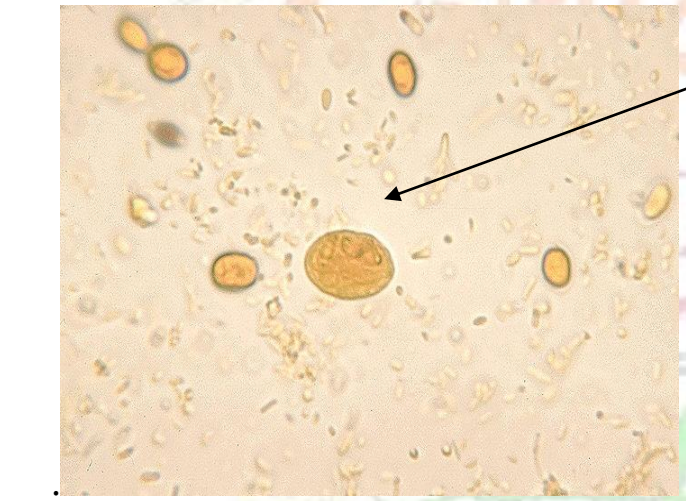
Low density lipoprotein =Serum cholesterol - (VLDL-HDL) .

2.18 Statistical Analysis:

All results of lipid profile test were performed by Chi square test at the level of significant when P-value < 0.05.The specificity, sensitivity and diagnostic accuracy of the results were also calculated by applying the following equations: (a / a + b) x 100 = sensitivity, (d / d + c) x 100 = specificity. diagnostic accuracy = (a+d / a+b+c+d)x100 . also calculated positive predictive value (PPV) =(a / a + c)x 100 and negative predictive value (NPV) =(d / b + d)100. (a = the total number of positive cases, b = false positive those bearing positive reading from negative samples, d =total number of true negatives, c=those with negative reading from positive cases (Niazi, 2000) .

3.1 Results of Microscopic Examination

The results of microscopic examination are cleared in figure (3.1), the stool samples in the field of study were taken to prepare direct smear and stained with iodine after wet mount preparation . All the stained specimens were saw by using oil immersion(100x) of microscopy showed *Giardia* in its cyst form, the figure (3.1,A) shows one mature cyst in the center of the slide and many immature cysts in the peripheral parts of the slide, the figure (3.1,B) shows one mature cyst with four nuclei in the center of the slide,the figure (3.1,C) shows one [illegible] in the center and many debris in different parts of the slide.



.

Fig 3.1A *Giardia* mature and immature cysts

Fig 3.1B*Giardia* mature cyst

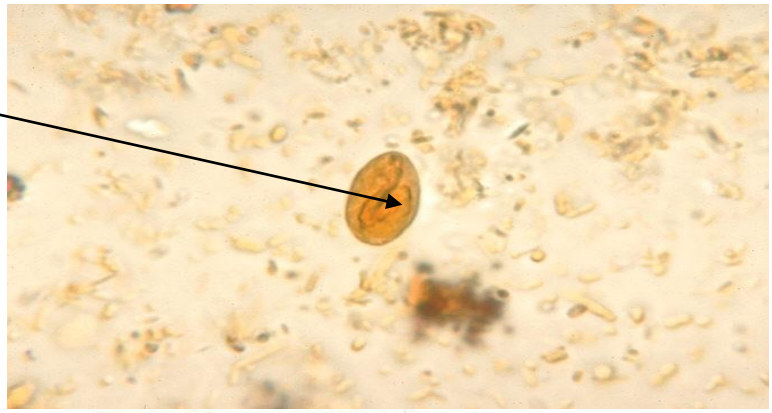


Fig 3.1C *Giardia* mature cyst with its median body

3.2 Results Obtained from Amplified Primers by Using PCR Technique

The samples used in PCR were DNA isolated from stool of human giardiasis patients at various age groups tested by (3) three primers provided by Bio CorP Co. Canada which were: Arginine deiminase gene(ADI), Ornithine carbamoyl transferase gene (OCT) and Cysteine rich protein (CRP65) .The optimum conditions in this experiment were corresponding to standardization of other previous studies (Leigh A. Knodler *et al*.,1998; Andrey Galkin. *et al* .,2009 and P Upcroft *et al*.,1997) .Results obtained from using of these primers in PCR reaction led to that those primers differ in production of amplified bands which differ in number and its molecular weights .

The araginine deminase (ADI) , ornithine carbamoyl transferase (OCT) and cysteine rich protein (CRP65) genes of *Giardia lamblia* was identified in 25 from 40 samples, the result were detected by electrophoresis on 2% agarose gel and exposed to UV light in which the DNA appears as compact bands , these bands come to form successful binding between specific primers and isolate extracted DNA .

The electrophoresis also used to estimate DNA size depending on DNA marker (1000 bp DNA ladder) . and the results of this estimation revealed that the amplified DNA has (600 bp) for ADI enzyme fig (3.2), 4 bands in sample 2; 3 bands in sample 7 ;2 in sample 12 ,3 bands in sample 13 which labeled by C_1 or Control for primer (5 µl of extracted DNA , 15 µl of PCR matter). On the other hand disappearance of amplified bands in the samples 1,3,4,5,6,8,9,10,11,12 and 14 or control 2 (C_2) which represent Control for DNA (3 µl of primer , 17 µl of PCR matter.

The amplified DNA showed that cysteine rich protein 65 has (460 bp) the figure (3.3) show this result .1band in the samples 3,4 ,7 ; 3 bands in the well 12 which represent the control C_1 also disappearance of amplified bands in case of samples 1,2,6,8,9,10,11 and 13 or (C_2).

As regarding to giardial Ornithine carbamoyl transferase gene the amplified bands were absent in all samples ,except the sample in well 5which represent the control for DNA , it was contain three bands .this result is cleared in fig (3.4).

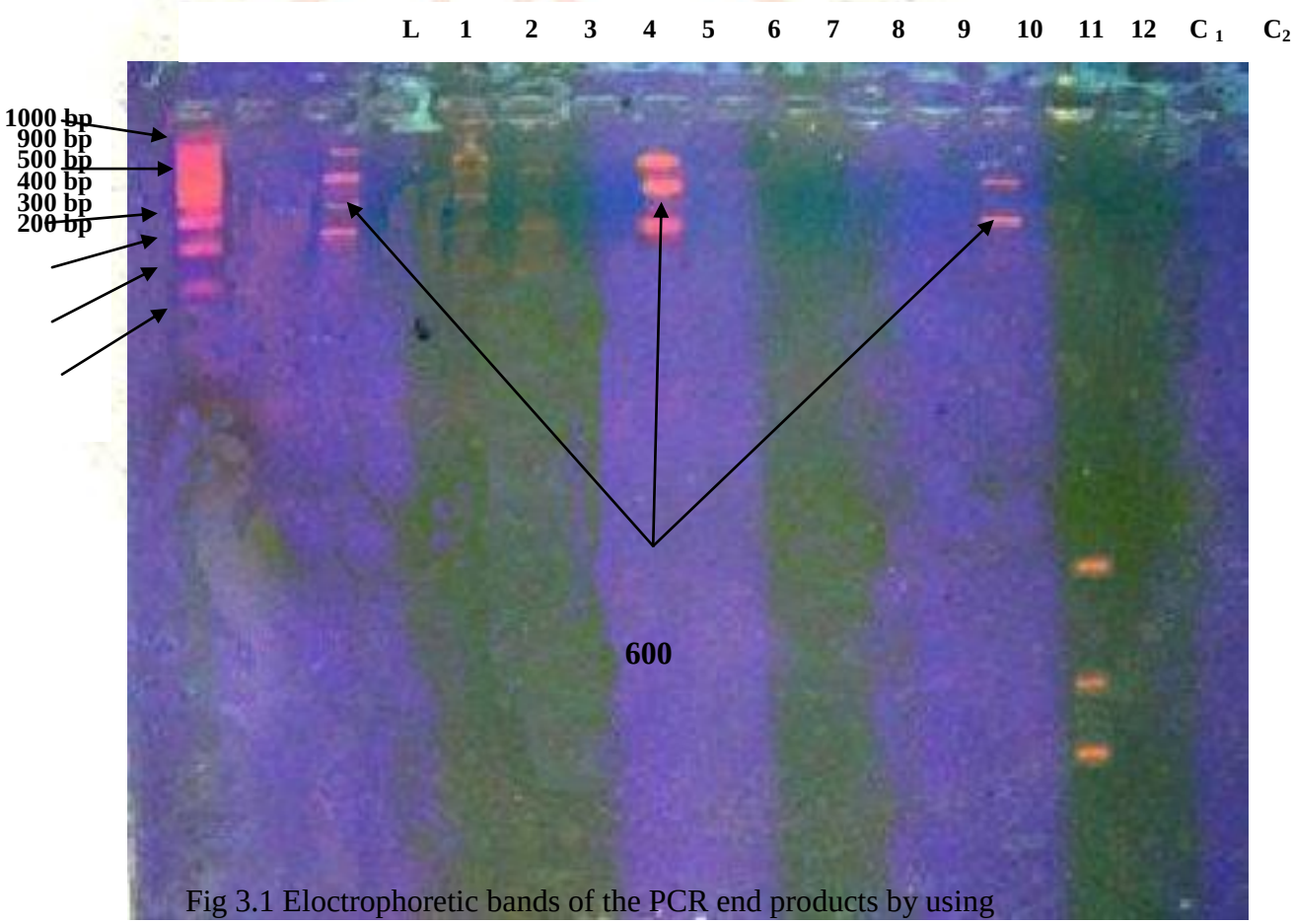


Fig 3.1 Eloctrophoretic bands of the PCR end products by using

Fig 3.2 Eloctrophoretic Bands of the PCR End Products by Using

Giardial Araginine Deiminase Primers

1-9	Stool samples from patients with chronic giardiasis
10-12	Stool samples from patients with accidental giardiasis
L	Ladder
C_1	Control for primer (5 µl of extracted DNA , 15 µl of PCR Matter).
C_2	Control for DNA (3 µl of primer , 17 µl of PCR matter).

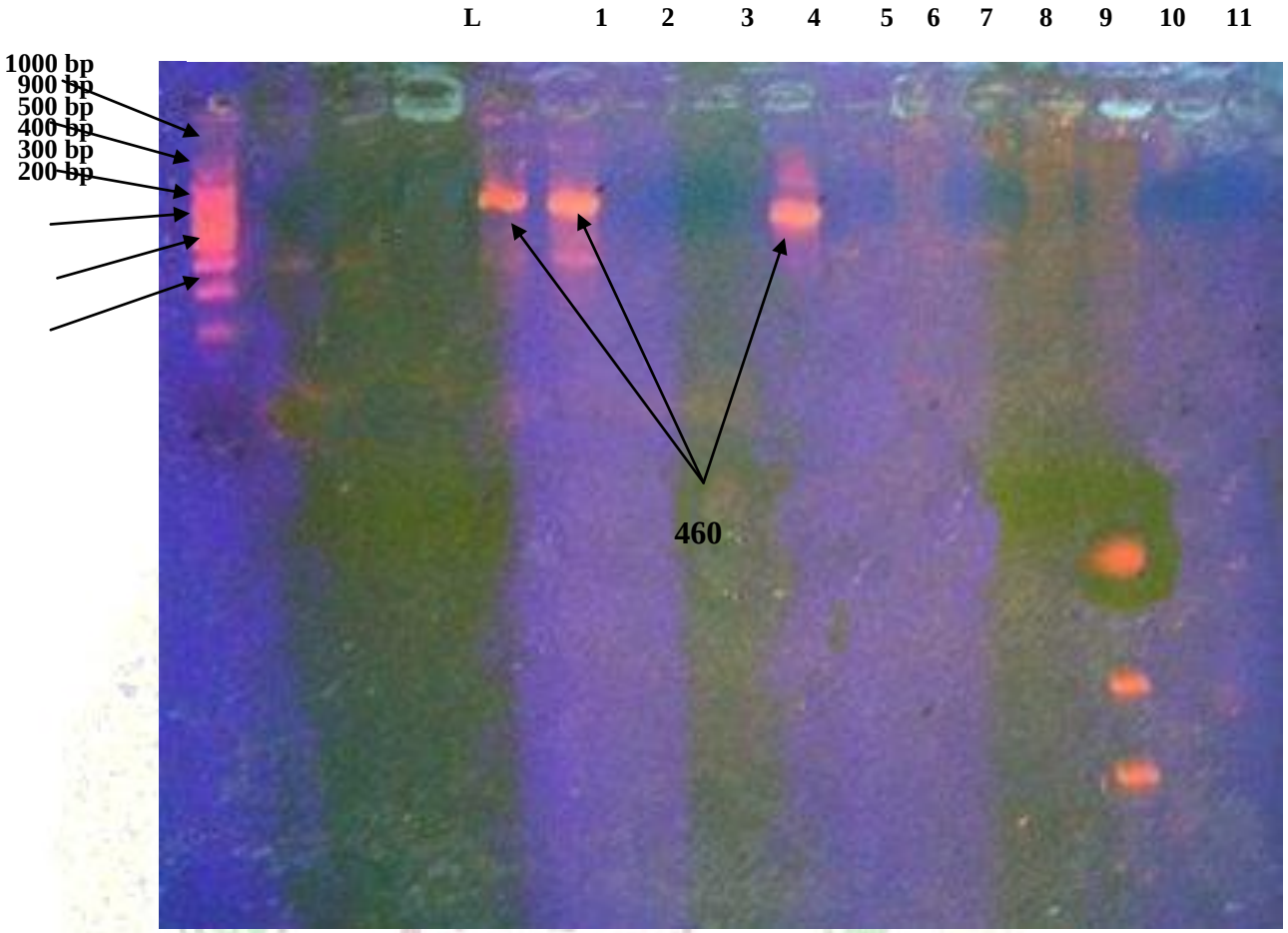


Fig 3.3 Eloctrophoretic Bands of the PCR End Products by Using Giardial Cysteine Rich Proteins 65 Primers

1-9 Stool samples from patients with chronic giardiasis

10,11 Stool samples from patients with accidental giardiasis

L Ladder

C_1 Control for primer (5 µl of extracted DNA , 15 µl of PCR Matter).

C_2 Control for DNA (3 µl of primer , 17 µl of PCR matter).

L 1 2 3 4 C_1 C_2

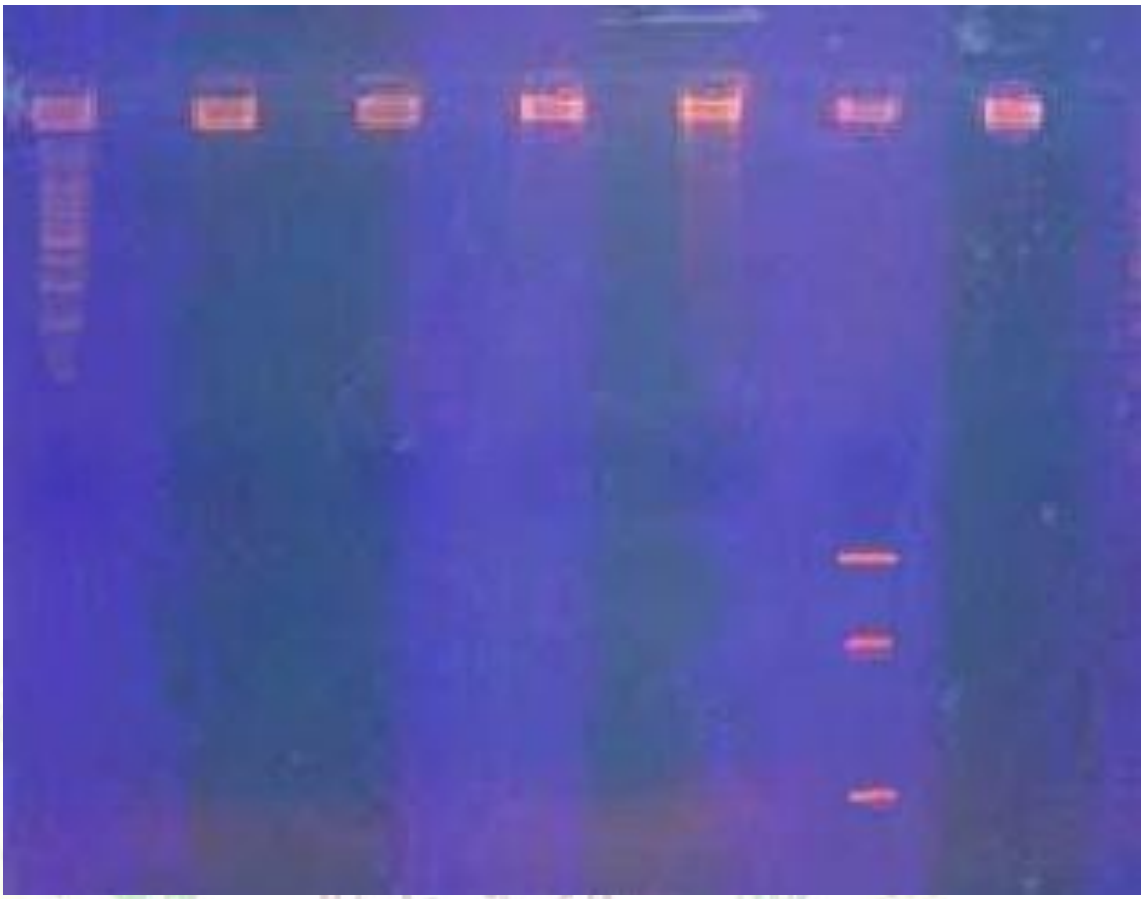


Fig 3.4 Eloctrophoretic Bands of the PCR End Products by Using Giardial Ornithine Carbamoyl Transferase Primers.

1-3 Stool samples from patients with chronic giardiasis

4 Stool samples from patients with accidental giardiasis

L Ladder

C1 Control for primer(5 µl of extracted DNA , 15 µl of PCR matter).

C2 Control for DNA (3 µl of primer , 17 µl of PCR matter).

3.3 The Results of Lipid Profile

Blood samples which were used in lipid profile test in the present study were taken from giardiasis patients. Serum was obtained by centrifugation of these blood samples for10 minutes at 3000 rpm, then total Cholesterol ,Triglyceride and High Density Lipoproteins were estimated by using their special kits provided from Linear Chemicals Company /Spain) , while Low and Very Low Density Lipoproteins were calculated depending on Friedewald, 1972 equation .(Very low density lipoproteins = Serum Triglycerides / 5 , Low density lipoprotein =Serum cholesterol - (VLDL-HDL))

The absorbance of the above samples were measured at wave length 500 nm with spectrophotometer , serum cholesterol levels for giardiasis patients were clearly decreased under the normal ranges when compared with serum cholesterol levels of healthy individuals who used as control. table (3.1),figure(3.4).

Table 3.1 The Results of Serum Cholesterol

	number	Mean ± S.E.	
control	20	5.0145	0.31 ^{A}
Patients	40	2.6431	0.19 ^{B}
A,B the different letters refers to the significant differences under (P≤0.05)			

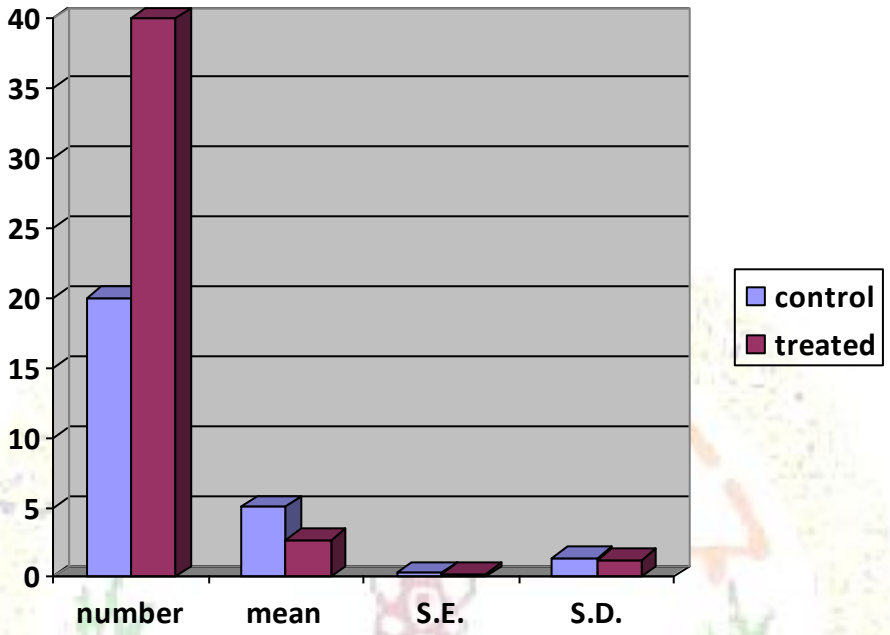


Fig 3.5 Serum Cholesterol Results

Number the number of individuals in the field of study

Mean the mean of individuals in the field of study

S.E. Standard Error

S.D. Standard Deviation

The results also showed that serum triglyceride and high density lipoproteins levels in the same patients were normal and sometimes were higher than the normal value this result agrees with Hugo D. *et al*., (1996).the results of serum T.G. and serum HDL are cleared in tables (3.2;3.3) and figures (3.6;3.7)

Table 3.2 The Results of Triglyceride .

	number	mean	S.E.
control	20	1.571	0.12^{A}
Patients	40	1.736	0.2^{A}

A,A the similar letters refers to the non-significant differences under($P \leq 0.05$).

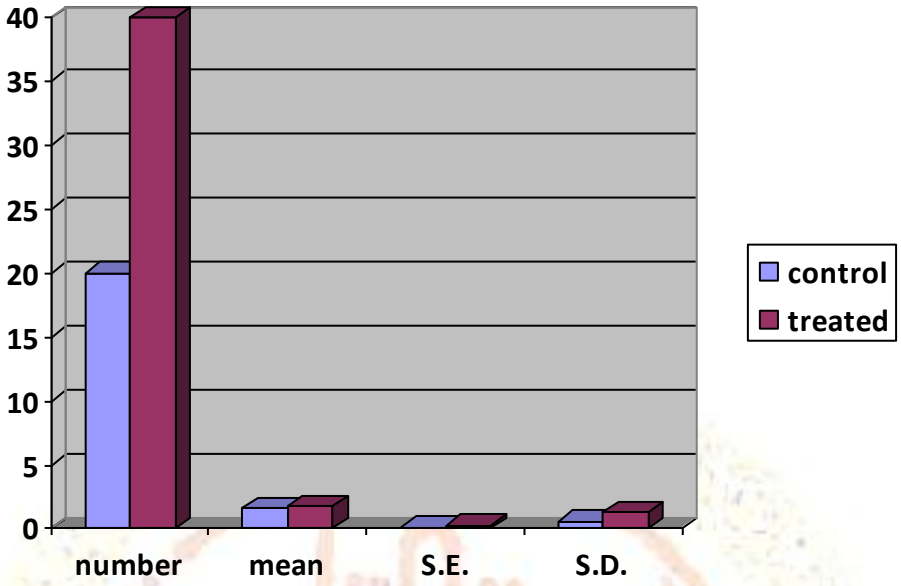


Fig 3.6 Serum Triglyceride Results

Number	the number of individuals in the field of study
Mean	the mean of individuals in the field of study
S.E.	Standard Error
S.D.	Standard Deviation

Table 3.3 Serum High Density Lipoproteins Results

	number	Mean ± S.E.	
control	20	1.362^{A}	0.02^{A}
Patients	40	0.836^{B}	0.02^{B}
A,B the different letters refers to the significant differences under (P≤0.05)			

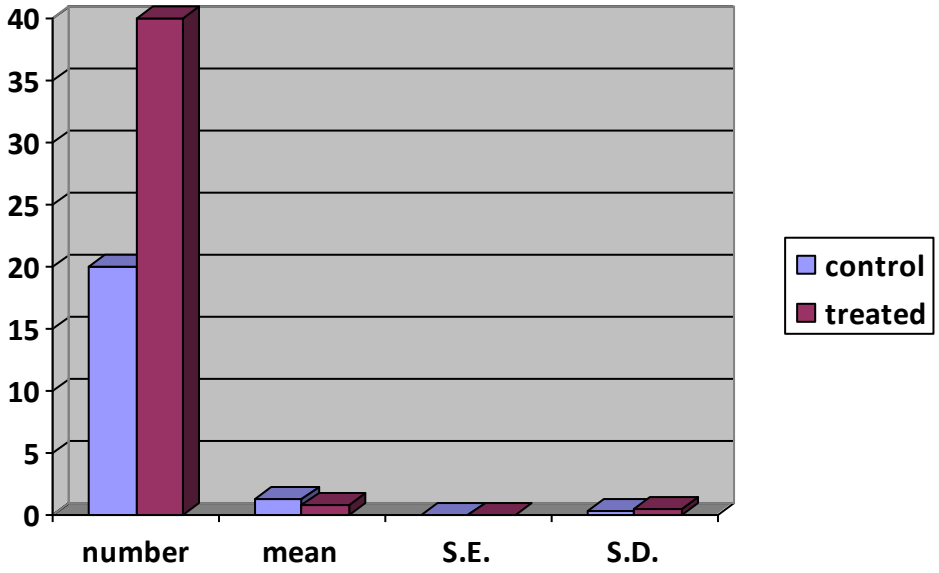


Fig 3.7 Serum High Density Lipoproteins Results

Number the number of individuals in the field of study

Mean the mean of individuals in the field of study

S.E. Standard Error

S.D. Standard Deviation

As regarding with serum LDL and serum VLDL levels the results were depended on serum cholesterol levels therefore they were also decreased in giardiasis patients while they were normal in healthy individuals (control).the tables (3.4;3.5) and figures (3.8;3.9) show these results

Table 3.4 Serum Low Density Lipoproteins Results

	number	Mean ± S.E.	
control	19	2.5989	0.23^{A}
Patients	40	1.2790	0.14^{B}
A,B the different letters refers to the significant differences under (P≤0.05)			

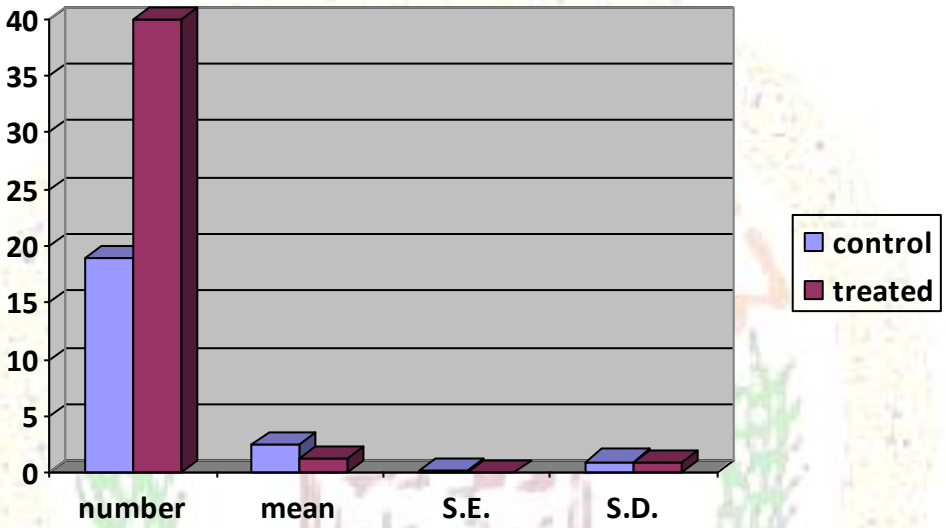


Fig 3.8 Serum Low Density Lipoproteins Results

Number the number of individuals in the field of study

Mean the mean of individuals in the field of study

S.E. Standard Error

S.D. Standard Deviation

Table 3.5 Serum Very Low Density Lipoproteins Results

	number	Mean ± S.E.	
control	20	1.0025	0.06^{A}
Patients	40	0.5285	0.03^{B}
A,B the different letters refers to the significant differences under (P≤0.05)			

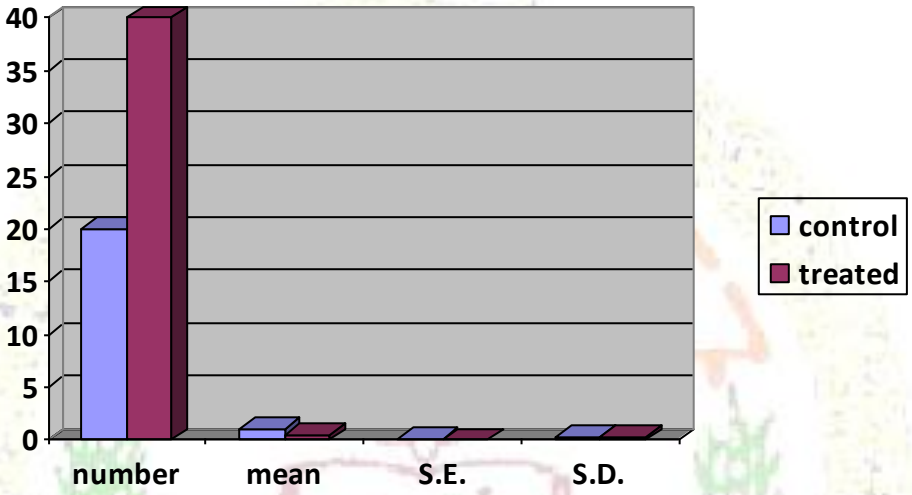


Fig 3.9 Serum Very Low Density Lipoproteins Results

Number the number of individuals in the field of study

Mean the mean of individuals in the field of study

S.E. Standard Error

S.D. Standard Deviation

4.1 PCR Discussion

This study was used to detect some of virulent proteins in *Giardia lamblia* parasite by using PCR technique , some of these proteins has been identified successfully (Argnine deimnase (ADI) and Cysteine Rich Protein (CRP65)) and the other had not (Ornithine Carbamoyl Transferase OCT) , these proteins have many characteristics gave them the ability to make complications can be consider as a mechansim of giardiasis and these complications are:

4.1.1 Argnine Deimnase (ADI)

Argnine deimnase (ADI E.C 3.5.3.6) is the enzyme that catalyze the irreversible catabolism of argnine to citrulline in the argnine dehydrolase pathway and serve as important source of energy (Schofiled *et al* ., 1990).

In the present study ADI was identified at 600 bp molecular weight this result agrees with Leigh A. Knodler *et al*.,(1998). but does not agree with Kareem H.N.*et al*.,(2011). who identified the same enzyme at 432 bp. This may belong to the conditions of PCR amplification process.

The functions of ADI goes beyond energy production, it plays an essential role in the control of antigenic variation via variant surface protein citrulination (the post-translational modification of the amino acid arginine in a protein into the amino acid citrulline) and influences of

the process of encystation . this result agree with Maria Carolin ., *et al* (2008).

Another complication of ADI is that human giardiasis patients show an increased rate of apoptosis of intestinal epithelial cells because the trophozoite of *Giardia* use ADI and OCT to actively metabolize argnine for energy production . argnine depletion is known to induce apoptosis in human cell lines, this can be considered as a major disease mechanism, also this depletion reduces the ability of IECs to produce Nitric Oxide (NO) an anti-microbial innate defense molecule previous studies implicate NO toxicity to *Giardia* because *in vitro* NO donors inhibit giardial growth but not viability. This result agree with Buret A.G. (2007) and Troger H *et al*.,(2007).

Eckman L. *et al*.,(2000) suggested that , NO also inhibits both encystation and excystation of *Giardia* and could thus interfere with parasite transmission. Interestingly NO levels in the intestinal epithelial cells have also been shown to be important in the regulation of adsorption / secretion of water suggesting that it could be associated with symptoms of giardiasis .

4.1.2 Ornithine Carbamoyl Transferase (OCT)

Ornithine Carbamoyl transferase (OCT EC 2.1.3.3) catalysis the reversible conversion of Citrulline to Carbamyl Phosphate (CP) and L-ornithine in the argnine dihydrolase pathway. This pathway is used by number of microorganisms, including *Giardia lamblia* to generate ATP fermentatively from argnine (Biagini G.A. *et al*., 2003) . recently (OCT) was identified as one of the major proteins released into the medium after brief interaction of *Giardia* with human intestinal epithelial cells. (Andrey Galkin *et al* .,2009).

The mechanisms of this enzyme which associate in causing giardiasis are :

- The secreted OCT might reduce the levels of intestinal argnine further and lower NO production by intestinal epithelial cells IECs and this agree with Emma Rigqvist *et al*., (2008).
- OCT enzyme participate in Argnine dihydrolase pathway

The results of this pathway are ATP , CO2 and NH4 the increasing amounts of CO2 make the medium of the intestine is alkaline which help in the survival of the trophozoite stage thus colonization of the parasite in the small intestine this conclusion is agree with Atkinson (1980) who proved that during its life cycle, Giardia encounters a wide range of environmental conditions. trophozoites in the intestine are maintained at

high concentrations of dissolved CO2 and at levels of O2 that fluctuate between 0-60 µm .but does not agree with Gillin et al.,(1988). who demonstrated that the slightly alkaline pH, which is characteristic of much of the small intestine greatly increases the ability of bile salts and fatty acids to induce differentiation of G. lamblia trophozoites into water-resistant cysts.

In the present, study the disappearance of amplified bands in the experiment of identification of Ornithine Carbamoyl transferase gene in Giardia may be due to absence of the enzyme in the cyst stage of the parasite.

4.1.3 Cysteine Rich Protein 65(CRP65)

Giardial Cysteine Rich Protein is a protein with high content of the amino acid cysteine ,this protein has multiple properties like heavy metal

resistances, metronidazole resistance, bile salt resistance and toxin like protein activity.(P Upcroft *et al*.,1997).

The first three characteristics may depend on the chemical structure of cysteine which has a thiol group (SH) which is an active or unstable group ,the hydrogen can bind to hydroxyl group to form water (Alessio, H.Mand E.R. Blasi,1997), leaving the free sulfur (S) in the medium which may bind to the heavy metals like Mg^{+2} ,Ca^{+2}, Cd^{+2} and Zn ,the binding of sulfur to the heavy metals prevent the small intestine from take these metals and lead to metal malabsorbtion, this result agree with Flachuk *et al*.,(1991).

On the other hand, the binding of free (S) with zinc may play an important role in trophozoite survival and colonization in the small intestine because zinc binding by CRP can lead to inhibition of a number of intestinal enzymes ., and this result agrees with Nash, (1992).

The resistant to metronidazole may come from binding of giardial CRP by its free sulfur to this common anti giardial drug , or *Giardia* appear to have defective pyruvate:ferredoxin oxidoreductase (PFO) activity transfers electrons to *Giardia* ferredoxin (Fd) with simultaneous reduction of metronidazole, this result agrees with Townson S.M. *et al*.,(1996) and Upcroft J *et al*.,(1993).

The free sulfur also may binds with bile salts preventing lipid metabolism in the small intestine resulting in steatorrhea .

The fourth characteristic of CRP is toxin like protein activity. This property depends on the whole protein ,by this activity *Giardia* may form a tube helps it to enter anther organ like bile duct in cases of heavy infection with giardiasis and when the nutrients of host small intestine

become not enough for the parasite .Many evidenced recorded that *Giardia* has been seen in the bile duct ,this result agree with Aronson N.E. *et al*.,(2001).

4.2 Lipid Profile Discussion

In the present study, the lipid profile used to understand the relationship between the infection with *Giardia lamblia* and steatorrhea (the most diagnostic symptom of giardiasis). and to determine whether that steatorrhea affect the levels of lipids in the blood of giardiasis patients and whether this impact is considered significant or not.

The association of steatorrhea with the infection of Giardia may be observed on the basis of damaging the intestinal mucosa , causing functional derangements , reducing brush border enzymes along with other factor such as synergism with agents like Salmonella and rotavirus this result agrees with K. Jayaprakash,(2005).

Recent studies showed that CRPs which is produced by Giardia lamblia can bind to heavy metals like zinc in the small intestine and as this binding inhibit the enzymes of small intestine Nash (1992) thus prevent lipids metabolism.

Giardia may consume only cholesterol and neglect the other lipids and this agree with Hugo D. *et al*., (1996) who showed that cholesterol starvation consider a trigger for trophozoite differentiation into cyst he was also proved that the affect of T.G. or HDL or another lipid on the growth was neglected . This result agrees with the present study and it may explain why only serum cholesterol levels were decreased in patients with giardiasis while the values of triglyceride or high density lipoproteins were normal in the same patients considering that low density

lipoproteins (LDL) and very density lipoproteins(VLDL) levels depend primarily on cholesterol and triglyceride levels.

Or *Giardia lamblia* trophozoites may inhibit lipolysis (the process of lipids degredation) and the degree of inhibition increased with longer duration of lipase exposure to trophozoites as P. katelaris *et al*., (1991) has suggested but this result does not agree with lipid profile results in the present study which showed only the levels of serum cholesterol in giardiasis patients was clearly decreased and the levels of triglyceride and high density lipoproteins were normal or sometimes rising more than normal rate

Giardia obtains cholesterol which consider necessary for membrane biogenesis from the serum of the host because giardiasis is unable to synthesize cholesterol it must therefore obtain this compound from the milieu of the upper small intestine , which particularly rich in biliary and dietary cholesterol this agrees with Jarroll, E. L *et al*.,(1981); Field, F. J *et al*., (1990) ;and Thomson.,*et al.*, (1993).

5.1 Conclusions

1. The precise pathogenic mechanisms of *Giardia lamblia* to cause giardiasis are not known but appear to be multifactorial .
2. Argnine diemnase plays multiple regulatory roles in the biology of *G. lamblia*. like control of antigenic variation, influences of the process of encystation and reduces the ability of intestinal epithelial cells to produce nitric oxide.
3. Cystiene rich protein is one of the most important virulence factors in *Giardia* .such as toxin like activity .
4. Ornthine carbamoyle transferase has an important role in the pathogenesis of *Giardia lamblia*. as helping in colonization and survival of trophozoite as long as possible in the small intestine .
5. *Giardia* consumed cholesterol from the host during colonization therefore it cause decreasing in the rate of serum cholesterol in chronic giardiasis patients.

5.2 Recommendations

1. Study the other virulence factors produced by *Giardia lamblia* like adhesive disk, variant surface proteins and circumvention of the natural factors.
2. Understand the association between giardiasis and lipid soluble vitamins (K, A, D, E) deficiency and the relationship between colonization of *Giardia lamblia* in the jejnum and lack of secretary IgA in the patient.

3. compare the active sites of giardial OTC and ADI with human OTC and ADI to identify potential structural features, which may be exploited to develop selective inhibitors of the parasitic enzyme.

4. Study the activity of albendazole which seems most promising, but additional studies are needed. It has the advantage of having broad antiparasitic effect, which may be beneficial in developing-world settings.

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Dhuha Mahdi

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List of Abbreviations

CRPs	Cysteine rich proteins
IECs	Intestinal Epithelial Cells
ADI	Araginine deiminase
OCT	Ornithine Carbamoyl Transferase
mT	microtubules
mR	microribbons
NO	Nitric Oxide
ADH	Arginine dehydrolase pathway
NOS	Nitric Oxide Synthase
PADs	Peptidyl-arginine deiminases
CP	Carbamoyl Phosphate
VSPs	Variant surface proteins
ELISA	enzyme-linked immune-sorbent assay
DFA	Directs fluorescent antibody
PCR	Polymerase Chain Reaction
TBE	Tris-Borate EDTA buffer
CE	Cholesterol esterase

POD	Peroxidase
4-AA	4-aminoantipryne
A	Absorbance
VLDL	very low density lipoproteins
LDL	Low density lipoproteins
HDL	High density lipoproteins
T.G	Triglycerides
FFA	Free fatty acids
G-3-P	Glycerol-3-phosphate
ADP	Adenosine diphosphate
GPO	Glycerphosphate oxidase
DHAP	Dihydroxyacetone phosphate
S.E.	Standard Error
S.D.	Standard Deviation
bp	base pair
SAF	Sodium acetate-acetic acid-formalin solution
MAb	Monoclonal Antibody
PFO	Pyruvate:ferredoxin oxidoreductase
Fd	Ferredoxin

Summary

This present study was undertaken to detect some of virulence proteins in the intestinal parasite *Giardia lamblia* and characterize them by polymerase chain reaction (PCR) technique .Stool samples were collected from 80 patients with chronic or accidental giardiasis in the period between (20/ December /2010 and 15 /June/ 2011) 50 of them were adult (18-50) years old and the other 30 were children (2-17) years old.

Giardia were diagnosed in stool samples by wet mount and concentration method and stained with iodine and the cysts had been seen by microscopy by using oil immersion (100x).

The *Giardia* cysts positive samples were stored frozen at (-20C) ,then these samples were used in DNA extraction by stool DNA extraction kit without cysts isolation and only 40 of the 80 samples were gave a clear DNA ,those 40 samples were at different ages (15-45) . after then the detection for the enzymes Argnine deiminase (ADI) , Ornthine carbamoyl transferase(OCT) and Cystiene rich protein 65(CRP65) was examined by polymerase chain reaction for the extracted DNA .

The results show that ADI has 600 bp and CRP has 460 bp while the amplified bands for OCT by using DNA marker (1000bp).

This present study also was undertaken to estimate the lipid profile in giardiasis patients therefore blood samples were collected from 60 individual 20 of them were healthy and used as control years old and the other 40 were patients with giardiasis the control and patients were both with range of ages between (2-60) .

The serum were obtained and a total cholesterol, triglyceride ,high density lipoproteins ,low density lipoproteins and very low density lipoproteins were estimated in the patients and control.

The results showed only the patients with giardiasis have clear decreased in their serum cholesterol levels while triglyceride (TG) and

high density lipoproteins(HDL) levels were different for the same patients. because the parasite consumed cholesterol from the host duri

الخلاصة

هذه الدراسة الحالية اجريت للتحري عن بعض البروتينات الممرضة التي يفرزها طفيلي *Giardia lamblia* وتشخيص تلك البروتينات باستخدام تقنية التفاعل التضاعفي لسلسلة الدنا حيث تم جمع ٨٠ عينة براز خلال المدة من (٢٠/كانون الأول ٢٠١٠ إلى ١٥/حزيران ٢٠١١) مكونة من ٣٠ عينة براز لأطفال (٢-١٧) سنة و ٥٠ للبالغين(١٨-٥٠ مصابين بداء الإسهال ألدهني (الجيارديات) وتم الكشف عن وجود الطفيلي باستخدام طريقة المسحةالرطبة وطريقة التركيز ثم التصبيغ بالايودين iodine stain وقد شوهد الطفيلي بطوره المكيس تحت الجهر الضوئي باستخدام العدسة الزيتية (١٠٠x).

وقد حفظت عينات البراز التي تحتوي مكيسات الطفيلي بدرجة حرارة -٢٠°م ،ثم تم اجراء عملية اسخلاص الدنا لعينات البراز بدون عزل المكيسات باستخدام Stool DNA Extraction Kit .وتم الحصول على دنا نقي ل٤٠ عينة براز من اصل ٨٠ عينة لاشخاص مصابين بداء الجيارديات تراوحت اعمارهم بين (١٥-٤٥) سنة .

وتم الكشف عن وجود إنزيمات Argnine deiminase ، Ornithine Carbamoyl transferase و Cystine rich protein 65 باستخدام تقنية التفاعل التضاعفي لسلسلة الدنا أظهرت النتائج وجود ADI بحجم ٦٠٠ زوج قاعدي CRP65 بحجم ٤٦٠ زوج قاعدي في حين لم تظهر نتائج ايجابية لإنزيم OCT باستخدام DNA marker 1000bp .

وتضمنت الدراسة الحالية أيضا اجراء اختبار تقدير الدهون للاشخاص المصابين بداء الجيارديات لذلك جمعت عينات الدم من ٦٠ شخص ٢٠ منهم كانوا اصحاء وتم استخدامهم كمعامل سيطرة و٤٠عينة دم لأشخاص مصابين بداء الجيارديات ، كلا المجموعتين (المصابين والاصحاء)تراوحت اعمارهم من ٢الى ٦٠ سنة .

ويعد استحصال المصل من عينات الدم قيد الدراسة اجري تقدير الكوليستيرول ، والدهون الثلاثية ،البروتينات الدهنية عالية الكثافة ،البروتينات الدهنية واطئة الكثافة والبروتينات الدهنية الواطئة جدا و قد أظهرت النتائج إن الأشخاص المصابين بداء الجيارديات يعانون في نقص ملحوظ في مستوى الكولسترول والبروتينات الدهنية الواطئة جدا في الدم ،واختلاف بنسبة الدهون الأخرى . وذلك لان الجيارديا تستهلك الكولسترول من المضيف خلال فترة نموها داخل الأمعاء الدقيقة لأنها غير قادرة على تصنيعه بنفسها.

Adam R .D. (1991). The biology of *Giardia spp*. Microbiol Rev,55: 706–32.

Adam R. D. (1992). Chromosome-size variation in *Giardia lamblia*: the role of rDNA repeats. Nucleic Acids Res. 20:3057-3061.

Adam R.D.(2001). Biology of *Giardia lamblia*. Clin. Microbiol. Rev. 14,447–75.

Adam R. D. ,Nash T. E. and Wellems T. E. (1988). The *Giardia lamblia* trophozoite contains sets of closely related chromosomes. Nucleic Acids Res. 16:4555-4567.

.

Aggarwal A. Adam R. D., and Nash. T. E.(1989). Characterization of a 29.4-kilodalton structural protein of *Giardia lamblia* and localization to the ventral disk. Infect. Immun. 57:1305-1310.

Alessio, H.M., and E.R. Blasi.(1997). Physical activity as a natural antioxidant booster and its effect on a healthy lifestyle. Res. Q. Exerc. Sport. 68 (4): 292-302, 1997.

Allian C.C. ,Poon L.S., Calu C.S.G. and Rhichomnd W. F.u. (1974).P.D. Clin. Chem. 20:407 .

American Academy of Pediatrics .(2006). Committee on Nutrition. Optimizing bone health and calcium intake of infants, children, and adolescents. Pediatrics117:578 –58.

Andrey Galkin, **Liudmila Kulakova, Rui Wu, Maozhen Gong, Debra Dunaway-Mariano and Osnat Herzber.(2009).** X-ray Structure and Kinetic Properties of Ornithine Transcarbamoylase from the Human Parasite *Giardia lamblia***.**

Aronson N.E. , **Cheney C., Rholl V., Burris D.and Hadro N. (2001).** Biliary giardiasis in a patient with human immunodeficiency virus.

Andrew J. Roger, Staffan G. Svard, Jorge Tovar, C.Graham Clark Michael W. Smith, Frances D. Gillin, and Mitchell L. Sogin (1998). A mitochondrial-like chaperonin 60 gene in *Giardia lamblia*:Evidence that diplomonads once harbored an endosymbion related to the progenitor of mitochondria.

Atkinson, H. J. (1980). Respiration. In Nematodes as Biological Models , pp. 101-138. Edited by B. H. Zuckerman. London:Academic Press.

Barbour A.G., Nichols C.R. and Fukushima T. (1976). An outbreak of giarediasis in a group of campers. Am J Trop Med Hyg 25:38-9.

Babb R.R., **Peck O.C., Vescia F.G.(1971).** Giardiasis. a cause of traveler's diarrhea. J.A.M.A. Sep 6;217(10):1359–1361.

Bhatnagar S., Bhan M.K., Singh K.D., Saxena S.K. and Shariff M .(1996). Efficacy of milk-based diets in persistent diarrhea: a randomized, controlled trial. Pediatrics ;98:1122 –1126.

Biagini G.A., **Yarlett N., Ball G.E., Billetz A.C., Lindmark D.G., Martinez M.P., Lloyd D. and Edwards M.R.(2003).** Bacterial-like energy metabolism in the amitochondriate protozoon Hexamita inflata. Mol Biochem Parasitol. ;128(1):11–19.

Bo-Linn G.W.and Fordtran J.S.(1984). Fecal fat concentration in patients with steatorrhea. Mol Biochem Parasitol ; 98:1122 – 1126.

Brodsky R.E., Spencer H.C. and Schultz M.G. (1974). Giardiasis in American travelers to the Soviet Union. .J Infect Dis I3):3l-B.

Brugerolle G., **.(1991).** Flagellar and cytoskeleton systems in amitochondrial flagellates: Archamoeba, Metamonada and Parabasala. Protoplasma164, 70–90.

Bruijnesteijn van Coppenraet, **L.E., Wallinga, J.A., Ruijs, G.J.Bruins, M.J.and Verweij J.J.(2009).** Parasitological diagnosis combining an internally controlled real-time PCR assay for the detection of four protozoa in stool samples with a testing algorithm for microscopy. Clin Microbiol Infect 15, 869-874.

Buccole G. and David H. (1973). Clin.Chem.19:476.

Buret A., Gall D.G.and Olson M.E. (1991). Growth, activities of enzymes in the small intestine, and ultrastructure of microvillousborder in gerbils infected with *Giardia duodenalis* Parasitol Res 77: 109-114.

Buret A., Hardin J.A., Olson M.E. and Gall D.G.(1992). Pathophysiology of small intestinal malabsorption in gerbils infectedwith *Giardia lamblia*. Gastroenterology 103: 506-513.

Buret A.G .(2007). Mechanisms of epithelial dysfunction in giardiasis. Gut 56:316–7.

Burke JA .(1975). Giardiasis in childhood Am J Dis Child 129: 1304-10.

Calderaro A., Gorrini, C., Montecchini, S., Peruzzi, S., Piccolo, G., Rossi, S., Gargiulo, F., Manca, N., Dettori, G. and Chezzi, C. .(2010). Evaluation of a real-time polymerase chain reaction assay for the laboratory diagnosis of giardiasis. Diagn Microbiol Infect Dis 66, 261-267.

Campbell S.R., van Keulen H, Erlandsen SL, Senturia J.B., and Jarrol E.L.(1990). *Giardia sp*: Comparison of electrophoretic karyotypes. Exp Parasitol 1990;71:470–82.

CarterM.J. and Milton I.D.(1993). Nucleic Acids Res.,21,1044

Chin A.C., Teoh D.A. and Scott K.G.(2002). Strain-dependent induction of enterocyte apoptosis by *Giardia lamblia* disrupts epithelial barrier function in a caspase-3-dependent manner. Infect Immun; 70:3673–3680.

Collins, J.P., Keller, K.F.and Brown L .(1978). "Ghost" forms of *Giardia lamblia* cysts initially misdiagnosed as Isospora. Am J Trop Med Hyg 27, 835-836.

Craun G. (1986). Waterborne giardiasis in the United States 1965-1984. Lancet:513-514.

Cylne C.A.and Eliopoulos G.M. (1989). Fever and uritcaria in acute giardiasis .Arch Intern Med 149:939-40.

Danciger, M. and Lopez M. (1975). Numbers of *Giardia* in the feces of infected children. Am J Trop Med Hyg 24, 237-242.

David R. Hill (2001). *Giardia lamblia* Principles and Practice of Clinical Parasitology © 2001 John Wiley and Sons Ltd print ISBN 0-470-97729-2

Di Prisco M.S.,Hagel I.,Lynch N.R. (1998). Association between giardiasis and allergy . Ann Allergy Asthma Immunul 81:557-8.

Eckmann L., Laurent F.and Langford T.D. (2000). Nitric oxide production by human intestinal epithelial cells and competition for arginine as potential determinants of host defense against the lumen-dwelling pathogen *Giardia lamblia*. Immunol;164:1478–87.

Edwards M R, Schofield P J, O'Sullivan W. J. and Costello M.(1992). Arginine metabolism during culture of *Giardia intestinalis*. Mol Biochem Parasitol. ;53:97–103.

Elizabeth A. And Zeibig (1997). clinical Parasitology by Elizabeth A. Zeibig (1997, Paperback) .

Emma Ringqvista, **J.E. Daniel Palma, b, Hanna Skarina, Adrian B. Hehlc, Malin Weilandb, Barbara J. Davidsd, David S. Reinerb, d., William J. Griffithse, Lars Eckmannf, Frances D. Gillind and Staffan G. Svärda, ©.(2008).** Release of metabolic enzymes by *Giardia* in response to interaction with intestinal epithelial cells.

Exner M.and Gornik, V (2004). Parasitic zoonoses transmitted by drinking water.Giardiasis and cryptosporidiosis" . Bundesgesundheitsblatt Gesundheitsforschung, Gesundheitsschutz 47 (7): 698–704. doi:10.1007/s00103-004-0863-y. PMID 15254826.

Falchuk K. H. (1991). Disturbances in trace element metabolism Infect Immun 443-445

Farthing M.J.(1996). Giardiasis. Gastroenterol Clin North Am;25:493- 515.

Faubert G. (1988). *Giardia* and giardiasis. Bull Can Soc Zool 19.

Friedewald W.T., **Levy R.I. (1972).** Estimation of the plasma, without use of the preparative ultracentrifuge. Clin Chem 18:499-502.

Garcia L.S. and Shimizu R.Y.(1997). Evaluation of nine immunoassay kits enzyme immunoassay and direct fluorescence) for detection of *Giardia lamblia* and *Cryptosporidium parvum* in human specimens. J Clin Microbiol; 35:1526–1529.

Ghosh, S., **Frisardi, M., Rogers, R. and Samuelson, J. (2001).** How *Giardia* swim and divide. Infect. Immun 69, 7866–7872.

Gillin F. D., D. S. Reiner, and S. E. Boucher. (1988). Small-intestinal factors promote encystation of *Giardia lamblia in vitro*. Infect Immun. 1988 March; 56(3): 705–707.

Gillin F.D., Reiner D.S.and McCaffery J.M. (1996). Cell biology of the primitive eukaryote *Giardia lamblia*. Annu Rev Microbiol;50:679-705.

Gillon J.(1985). Clinical studies in adults presenting with giardiasis to a gastro-intestinal unit. Scott Med. J. 30:89-95.

Giraldo-Gómez J.M., **Lora F., Henao L.H., Mejía S., Gómez-Marín J.E.(2005).** Prevalenc of giardiasis and intestinal parasites in pre-school children from homes being attended as part of a state programme in Armenia, Colombia. Rev. Salud. Publica (Bogota)., 7(3):327-338.

Haque, R., Roy S., Siddique A., Mondal U., Rahman, S.M., Mondal D., Houpt E. and Petri W.A., J.r. (2007). Multiplex real-time PCR assay for detection of *Entamoeba histolytica, Giardia intestinalis*, and *Cryptosporidium spp*. Am J Trop Med Hyg 76, 713-717.

Hesham M.S., **Edariah A.B., and Norhayati M. (2004).** Intestinal parasitic infections and micronutrient deficiency: a review. Med. J. Malaysia., 59: 284-293.

Hill D.R. **(1993).** Giardiasis. Issues in diagnosis and management. Infect Dis Clin North Am. Sep;7(3):503-25.

Holberton D.V. (1973). Fine structure of the ventral disk apparatus and the mechanism of attachment in the flagellate *Giardia muris*. J. Cell Sci. 13, 11-41.

Huanq D.B., **White A.C. (2006).** An updated review on *Cryptosporidium* and *Giardia*. Gastroenterol. Clin. North Am., 35 (2): 291–314.

Jayaprakash.K. (2005) . Cause of night blindness in children – Alarming effect of water pollution Indian Journal of Occupational and Environmental Medicine - December 2005.

Johan Ankarklev, Jon Jerlström -Hultqvist, Emma Ringqvist, Karin Troell, Staffan G.and Svärd .(2010). Behind the smile: cell biology and disease mechanisms of *Giardia* species Microbiology ,Vol.8, No.6.pp.413422 ., doi:10.1038/nrmicro2317.

Kabnick, K. S., and D. A. Peattie .(1990). In situ analyses reveal that the two nuclei of *Giardia lamblia* are equivalent. J. Cell Sci. 95:353-360.

Kareem Hatam Nahavandi, **Esmaeel Fallah and Mohammad. (2011).** Asgharzadeh3, Nasrin Mirsamadi2, Behr Mahdavipour Glutamate dehydrogenase and triose-phosphate-isomerase codin genes for detection and genetic characterization of *Giardia lamblia* in human feces by PCR and PCR-RFLP.

Kaur H., Ghosh S.and Samra H.(2001). Identification and characterization of an excretory–secretory product from *Giardia lamblia*. Parasitology;123:347–56.

Lasek-Nessel quist,**E. Welch. and Sogin M.L. (2010).**The identification of anew *Giardia duodenalis* assemblage in marine vertebrates and preliminary analysis of *G duodenalis* population biology in marine systems . .Int J.Parasitol.in press .

Lasek-Nesselquist ,E.Bogomolni,A.L.,Gast, R.J., Welch ,D.M .,Ellis ,J.C. , Sogin M.L., Moo and M.J.(2008). Molecular

characterization of *Giardia intestinalis* ._19 Aug 2008 – DAO 81:39-51

Layton M.A.,**Dziedzic K .and Dawes P.T.(1998).** Sacroiliits in an HLA B27-negative patient following giardiasis (letter). BrJ Rheumatol 37:2611-16.

Leigh A. Knodler, **Eric O. Sekyere, Thomas S. Stewart, Philip J. Schofield and Michael R. Edwards. (1998).** Cloning and Expression of a Prokaryotic Enzyme, Arginine Deiminase, from a Primitive Eukaryote *Giardia intestinalis*. Biol Chem, Vol. 273, Issue 8, 4470-4477, February 20 1998.

Lengerich E.J., **Addis D.G. and Jurnaek D.D. (1994)** . Severe giardiasis in the United states Clin Infect Dis 18:760- 3.

Sousa M. Céu , **Gonçalves C. A., Bairos V. A. and Poiares J.-da-Silva.(2000).** Adherence of *Giardia lamblia* Trophozoites to Int-407 Human Intestinal Cells

Maria Carolina Touz1, **Andrea Silvana Rpolo1, Maria Romina Rivero1, Cecilia Veronica Vranych1,John Thomas Conrad2, Staffan Gunnar Svard and Theodore Elliott Nash.(2008).** Arginine deiminase has multiple regulatory roles in the biology of *Giardia lamblia*. Accepted 16 June 2008 Journal of Cell Science 121, 2930-2938 Published by The Company of Biologists 2008

Marshall J., Holberton D. V .(1993) . Sequence and structure of anew coiled protien from amicrotubule bundle in *Giardia* . J Mol .Biol 231:521-30.

Marshall, M.M., Naumovitz, D., Ortega, Y., and Sterling C.R. .(1997). Waterborne protozoan pathogens. Clin. Microbiol. Rev. 10, 67–85. mechanisms of cell migration and the proliferation hierarch.

Mantovani M. Pettoello (1991). Mebendazole in giardial infections.Transactions of the Royal Society of Tropical Medicine and Hygiene. (Letter). 85:557.

Meyer E.A., **Radulescu S. (1979).** *Giardia* and giardiasis. Adv Parasitol;17:1–47.

Meyer E.A. (1990). Taxonomy and nomenclature In Meyer (ed.),Giardiasis .Elsvier Sciene:The Netherlands;51-60.

Miller R. L., D. J. Nelson, S. W. LaFon, W. H. Miller, and T. A. Krenitsky.(1987). Antigiardial activity of guanine arabinoside. Mechanism studies. Biochem. Pharmacol. 36:2519-2525.

Mintz E.D., Hudson-Wragg M., Mshar P. (1993). Foodborne giardiasis in a corporate offiice setting. J. Infect Dis l67: 250—3.

Moore G.T., **Cross W.M.,McGurine D.(1969).**Epidemic giardiasis at ski resort .N Engl J Med 281:402-7

Mowatt, M.R., Aggarwal, and T.E.Nash.(1991). C-terminal sequence conservation among variant –specific surface proteins of *Giardia lamblia.*Mol Biochem,Parasitol 49:215-228.

NashT. E. (1992). Surface antigen variability and variation in *Giardia lamblia*. Parasitol. Today 8:229-234.

Nash T.E., Conrad J.T., Merritt J.W. J.r.(1990). Variant specific epitopes of *Giardia lamblia*. Mol Biochem Parasitol. 1990 Aug;42(1):125–132.

Nash. T. E., D. A. Herrington, and M. M. Levine. **(1987)**.Usefulness of an enzyme-linked immunosorbent assay for detection of *Giardia* antigen in feces. J. Clin. Microbiol. 25:1169-1171.

Nash T.E., Herrington, D.A., Losonsky, G.A. and Levine M.M. (1987). Experimental human infections with *Giardia lamblia*. J Infect Dis 156, 974-984.

Niazi, A.D. (2000). Statistical Analysis in medical research. Republic of Iraq. Al- Nehrien University. P.148.

Nohýnková, E. and Kulda, J., (1999). *Giardia intestinalis*: effect of albendazole on cytoskeletal systems. J. Euk. Microbiol 46 (Suppl. 74)

Oberhuber G. Kastner N. and Stolte M.A.(1997). Giardiasis Histologic analysis of 567 cases Scand. J. Gastroenterol., 32: 51–54.

Olveda R.R., Andrews J.S.and Hewlett E.L.(1982). Murine giardiasis:localization of trophozoites and small bowel histopathology during course of infection .Am J Trop Med Hyg 31:60-6.

Owen R. L., P.Nemanic, and Stevens. **D. (1979).** Ultrastructural observations in giardiasis in a murine model. Intestinal distribution, attachment and relationship to the immune systemof *Giardia muris*. Gastroenterology 76:757-769.

Upcroft P. , Chen N. and Upcroft. J.A (1997). Telomeric organization of variable and inducible toxin gene family in the ancient eukaryote *Giardia lamblia* Genome Res 7:37-46.

Katelarisa P. , **F. Seowa1 and M. Ngua1.(1991).** The effect of *Giardia lamblia* trophozoites on lipolysis *in vitro*. Parasitology. 1991 Aug;103 Pt 1:35-9.

Katelaris P. H. , A Naeem, Farthing M. J.(1995). Attachment of *Giardia lamblia* trophozoites to a cultured human intestinal cell line

Palm, J.E.(2003). Identification of immunoreactive proteinsduring acute human giardiasis. J. Infect. Dis. 187, 1849–1859

Panaro M.A., Cianciulli A., Mitolo V. (2007). Caspase-dependent apoptosis of the HCT-8 epithelial cell line induced by the parasite *Giardia intestinalis.* FEMS Immunol Med Microbiol;51:302–9.

Peattie D.A .(1990). The giardins of *Giardia lamblia :*genes and protiens with promisen.parasitolToday 6:52-6.

Petersen H .(1972). Giardiasis (lamliasis) .Scand J. Gastroenterol 7(Suppl.14):1-44.

Pothier P. and Hugon, J. S. (1980). Characterization of isolated villous and crypt cells from the small intestine of the adult mouse. Cell and Tissue Research 211, 405-418.

Potten C.S., Loeffler M .(1987). A comprehensive model of the promote encystation of *Giardia lamblia in vitro*. Infect. Immun. 56:705–707.

Rendtorff R.C(.**1954).** The experimental transmission of human intestinal protozoan parasites II. *Giardia lamblia* cysts given incapsules. Am J Hyg, 59:209–20.

Robertson L.J.(1996). Severe giardiasis and cryptosporidiosis in Scotland, UK. Epidemiol. Infect., 117:551-561.

Roxstrom-Lindquist K, Palm D., Reiner D. (2006).*Giardia* immunity updateTrends Parasitol;22:26–31

.

Sambrook, J. Fritsh, E.F., and Maniatis .(1989). Molecular cloning, laboratory manual, 2nd ed. Cold Spring Harbor Laboratory.

Savioli L., Smith H., Thompson A. (2006). .*Giardia* and *Cryptosporidium* join the "Neglected Diseases Initiative". Trends. Parasitol., 22: 203-208.

Sayyari A.A., Imanzadeh F., Bagheri Yazdi S.A., Karami H., Yaghoobi M. (2005). Prevalence of intestinal parasitic infections in the Islamic Republic of Iran. East. Mediterr. Health J., 11(3): 377.

Schofield P. J., Edwards M. R. Kranz P. (1991). Glucose metabolism in *Giardia intestinalis.* Mol Biochem Parasitol.;45:39–47.

Schofield P. J., Edwards M R, Matthews J, Wilson J R.(1992). The pathway of arginine catabolism in *Giardia intestinalis*. Mol Biochem Parasitol. ;51:29–36**.**

Schofield P. J., M. Costello, M. R. Edwards, and W. J. O'Sullivan. (1990). The arginine dihydrolase path way is present in *Giardia intestinalis*. Int. J. Parasit e arginine dihydrolase pathway ol. 20:697-699.

Schuurman, T., Lankamp, P., van Belkum, A., Kooistra-Smid, M. and van Zwet, A. (2007). Comparison of microscopy, real-time PCR and a rapid immunoassay for the detection of *Giardia lamblia* in human stool specimens. Clin Microbiol Infect 13, 1186-1191.

Scott KG, Logan MR, Klammer GM, Teoh DA, and Buret AG.(2000). Jejunal brush border microvillous alterations in *Giardia muris*-infected mice: role of T lymphocytes and interleukin-6 Infect Immun 68: 3412–3418, 2000.

Shaw R.A., Stevens M.B., (1987). The reactive arthritis of giardiasis. A case report. JAMA 258, 2734-2735.

Shaw P.K., Brodsky R.E. and Lyman D.O. (1977). A community wide outbreak of giardiasis with evidence of transmission by a municipal water supply. Ann intern Med 87: 42&32.

Saki J., Khademvatan S.1, Maraghi S. and Soltani S.1(2011). Serum lipid profiles and eosinophilia among *Giardia* cyst passers. African Journal of Microbiology Research Vol. 5(27), pp. 4881-4884, 23 November.

Smith T.F. and M.S. Waterman .(1981). Comparsion of Biosequnces. Adv.Appl.Math 2:482-489.

Soave R.(1996) .*Cyclospora* :an overview . Clin Infect Dis 23:429-35

Sogin, M. L.., Gunderson, J. H., Elwood, H. J., Alonson, R. A., and Peattie D. A. (1989). Phylogenetic meaning of the kingdom concept: an unusual ribosomal RNA from *Giardia lamblia.* Science 243, 75-77.

Tandon B. N., R .Tandon, B. Satpathy and S. Shriniwas. **(1977).** Mechanism of malabsorption in giardiasis: a study of bacterial flora and bile salt deconjugation in upperjejunum. Gut 18:176-181.

Ten Hove R.J., van Esbroeck, M., Vervoort, T., van den Ende, J., van Lieshout, L., Verweij J.J. (2009). Molecular diagnostics of intestinal parasites in returning travellers. Eur J Clin Microbiol Infect Dis 28, 1045-1053.

Tellez, A., Winiecka-Krusnell, J., Paniagua, M. & Linder, E. (2003). Antibodies in mother's milk protect children against giardiasis. Scand J Infect Dis 35, 322–325.

Teoh D.A., Kamieniecki D., Pang G., Buret A.G.(2000). *Giardia lamblia* rearranges F-actin and alpha-actinin in human colonic and duodenal monolayers and reduces transepithelial electrical resistance. J Parasitol;86:800–6.

Thomson A. B. R., Schoeller C., Keelan M., Smith L. & Clandinin M. T. (1993). *Giardia* and Giardiasis Can. J. Physiol. Pharmacol. 71, 531-555.

Thompson R.C.**(2000)**. Giardiasis as a re -emerging ifectious disease and its zonotic potential .Int J.Parasitol.30,1259-1267.

Tomkins A.M., Wright S.G., Drasar B.S., James W.P.T. (1978). Bacterial colonization of jejunal mucosa in giardiasis **.**Trans R Soc Trop Med Hyg 72:33-6.

Townson S. M., **Upcroft J. A.and Upcroft P.(1996).** Characterization and purification of pyruvate:ferredoxin oxidoreductase from *Giardia duodenalis*. Mol Biochem Parasitol. 79:183–193.

Troeger H., Epple H.J.and Schneider T.(2007). Effect of chronic *Giardia lamblia* infection on epithelial transport and barrier function in human duodenum. Gut 56:328–35.

Upcroft J. A.and Upcroft P.(1993). Drug resistance and *Giardia*. Parasitol Today;9:187–190

Visvesvara G.S., Smith P., Healy G., Brown W.(1980). An immuno fluorescence test to detect serum antibodies *to Giardia lamblia*. Ann Intern Med 93:802-5.

Verweij J.J, VisserL.G.,Van Esbroeck, M., Edeling, W.M., Clerinx, J.and Polderman, A.M., 2006. Diagnostic methods for differentiation of *Entamoeba histolytica* and *Entamoeba dispar* in carriers: performance and clinical implications in a non-endemic setting. Int J Med Microbiol 296, 397-403.

WHO.(1991). The World Health Report

WHO. (1996). The World Health Report **.**

Wolfe M.S. (1975). Giardiasis Jam Med Assoc 233:1362-5.

Williams A.J.K., Merrick M.V. and Eastwood M.A. (1991). Idiopathic bile acid malabsorption—a review of clinical presentation, diagnosis, and response to treatmen? Gut 32: 1004–1006.

Wolfe M.S. (1975). Giardiasis.J Am Med Assoc 233:1362-5

Wolfe M.S. (1992). Giardiasis. Clin Microbiol Rev**.** Jan;5(1):93-100.

Yang R., Reid A.,LymberyA., Ryan U. (2010). Identification Of zonotic *Giardia* genotypes in fish .Int.Parasitol 40,779-785.

Zhang Yu-ying, Stephen b. Aley, Samuel, Stanley J.r., and Frances d. gillin.(1993). cysteine-dependent zinc binding by membrane of *giardia lamblia* (Infection and Immunity, p. 520-524.

1. **Zimmerman S.K., Needham C.A.(1995).** Comparison of conventional stool concentration and preserved-smear methods with Merifluor *Cryptosporidium/Giardia* Direct Immunofluorescence Assay and ProSpecT *Giardia* E.Z. Microplate Assay for the detection of *Giardia lamblia*. J Clin Microbiol; 33:1942–1943. ^ Bartlett, J. M. S.; Stirling, D. (2003). "A Short History of the Polymerase Chain Reaction". PCR Protocols. 226. pp. 3–6.doi:10.1385/1-59259-384-4:3. ISBN 1-59259-384-4. edit
2. ^ a b Saiki, R.; Scharf, S.; Faloona, F.; Mullis, K.; Horn, G.; Erlich, H.; Arnheim, N. (1985). "Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia". Science 230 (4732): 1350–1354. doi:10.1126/science.2999980.PMID 2999980. edit
3. ^ a b Saiki, R.; Gelfand, D.; Stoffel, S.; Scharf, S.; Higuchi, R.; Horn, G.; Mullis, K.; Erlich, H. (1988). "Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase". Science 239 (4839): 487–491.doi:10.1126/science.2448875. PMID 2448875. edit
4. ^ a b Kary Mullis Nobel Lecture, December 8, 1993