Available online at www.jpsscientificpublications.com
Volume – 6; Issue - 5; Year – 2020; Page: 2110 – 2118
DOI: 10.22192/iajmr.2020.6.5.2

Indo – Asian Journal of Multidisciplinary Research (IAJMR)
ISSN: 2454-1370

INTERACTION BETWEEN *Giardia* SPECIES AND INTESTINE: A REVIEW

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Abstract

Giardia species are intestinal parasites that infect the human and animal and cause an infestation called Giardiasis. Many species have been isolated from humans, wild animals and domestic animals. All have the same simple life cycle with two stages including: cyst stage which is usually ingested causing the infection and Trophozoite stage which transform into a cyst then spread with faeces infecting anew host. It may also cause the disease with a low ability to survive outside. Infection is asymptomatic or with many symptoms such as abdominal pain, diarrhoea and diarrhoea. Diagnosis of Giardiasis was done by stool examination, small intestine biopsy in difficult cases and also ELISA could be used for high numbers of infections. There is a high diversity for Giardiasis treatment, the common used for is metronidazole. This present study aims to review most common techniques used in Giardiasis diagnosis, and the treatment, histology and symptoms of infection.

Key words: Giardiasis, *Giardia* sp., Intestinal infection and Physiology.

1. Introduction

Giardia lamblia is a famous parasite that infects the human intestine (Petersen, 1972). Antonie van Leeuwenhoek was the first who discovered the parasite in his stool in 1681 and classified it to the order Retortomonadida. *Giardia* species are worldwide spread parasites especially where there is a poor sanitation (Barbour *et al*., 1976). All ages of people could be infected by this parasite and the more common is infants. The increased risk of infection was highly detected in areas where travelers are found such as Tropical areas in Africa, South of America, Rusia and Mexico (Wolfe, 1978).

Giardia species has a simple life cycle with two stages: STAGE 1 - Trophozoites stage *Giardia* 9.5 – 21 µm long by 5 – 15 µm wide with two nuclei, convex from the dorsal side and pear shaped with a ventral adhesive disk that gives an area for absorbing nutrients and with four pairs of flagellae (Jones, 1988). Furthermore, the Trophozoite dorsoventrally has a teardrop shape and from the side it has a spoon-like appearance. The median bodies, the nuclei and axonemes positions are similar to a" human face" with a motion as a "falling leaf ". Trophozoites multiplies by Binary fission longitudinally. According to morphological characteristic, there are three types of trophozoite "*Giardia muris*, *Giardia agilis* and *Giardia duodenalis*" (Brandborg *et al*., 1967). STAGE 2: With oval shaped, 12 µm long by 7 - 10 µm wide with a liner cyst wall and contains four nuclei. Also there are axonemes and median bodies" usually curved". The cyst can survived in wet cool place for 3 months and can also resist chlorine concentrations in purified water systems (Craun,1986).

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***Received*:** 19.08.2020; ***Revised*:** 02.09.2020;
***Accepted*:** 20.08.2020.

Giardia cysts are transmitted from one person to another by "fecal-oral contamination" by ingesting with contaminated food (Owen *et al*., 1979) and transmission by dirty hands, then cysts go to the stomach reaching the duodenum where Excystation occurs and the mature cyst gives two Trophozoite and becomes adhesive to the villi. The duodenum and the upper jejunum become inhabited by the parasite because of its alkaline pH which was optimum for *Giardia* growth. Trophozoites attach by their sucking disks firmly to the microvilli of the intestine or become free in the lumen, rare cases of Trophozoite invasion of the mucosa layer and sub-mucosa layer has been recorded. In liquid stool, Trophozoites is usually found, and even cysts could be seen in more liquid stool (Craun,1986).

2. Epidemiology

The transmission of Giardiasis from one person to another is by oral-fecal contamination or by contaminated water or food (Craun, 1986). When cysts in contaminated water "usually from wells or lakes" were ingested by Travelers, they often become infected. In America, most hiker and campers could be infected by drinking polluted water from streams (Barbour *et al*., 1976). Wide numbers of community infection were due to the contamination of water supplies with feces (Craun, 1986). Most waterborne infections may be according to the ineffective filtration process of water that causes diarrhea (Gradus, 1989) and also from water sources such as shallow wells, unfiltered surface waters and household water" whom all are contaminated with feces (Chute *et al*., 1987). Also Increased infection could be occurred in nursery children, then they may transmit the infection to their families (Pickering *et al*.,1984). To ensure human infection, it is required "100 cyst" ingestion or more, and in some experimental volunteers the infections were required a few "only 10 cysts" (Rendtorff, 1954).

The major reservoir of *Giardia* species is Human and also another species of this parasite were carried by animals and causing the same disease. In the beginning it was thought that "The genus contain host-specific species", but now they think" it is only two different species that infect animals" (Pickering *et al*.,1984). The first species was *Giardia duodenalis* (includes *Giardia lamblia*) which infects" humans, cattle, beavers, cats, dogs, coyotes and other mammals". Other studies were detected isolated of *Giardia duodenalis* from various hosts indicating that these variables appear within the group, and these studies considering the host specificity in the classification of *Giardia* species is unreliable. The second species was *Giardia muris* which infects rats and mice (Smith *et al*.,1980). Community water supplies can be contaminated by "*Giardia duodenalis*" cysts from beavers" which have been infective for humans experimentally" and may pollute water supplies with cysts of "*Giardia duodenalis*", and subsequently transmit infection to human (Engels *et al*.,1996). Dogs infection were induced experimentally by human cysts, and there is no recorded cases for the transmission of the cyst from dogs to humans (Mason and Patterson, 1987).

3. Methodology

Giardiasis diagnostic methods give us a different sensitivity and specificity depending on many factors including: Operations type, stage wanted and the method that have been used in the diagnosis which is very important for the control and treatment (Korman *et al*.,1990). In routine laboratories it's important to use cheap, accurate and easy method especially for "large-scale population" screening. It is also very important to differentiated Giardiasis from Acute diarrhea resulted from bacteria, viruses and other protozoa (Uehlinger *et al*., 2017). Giardiasis incubation period is long compared to the enteric infections, and patient usually suffering abdominal pain, foul stool, gas, distention, and usually no mucus or blood are existing (Bertram *et al*., 1983). Giardiasis diarrhea must be differentiated from other organism infections (*Entamoeba histolytica*, *Cryptosporidium parvum*, *Isospora belli*, *Dientamoeba fragilis* and *Strongyloides stercoralis*) as well as symptoms such as Inflammatory bowel disease, Malabsorption and Irritable bowel syndromes (Bertram *et al*., 1983).

In Giardiasis, hiatal hernia, duodenal ulcer and pancreatic or gall bladder disease may be appeared. If there is a sign of Eosinophilia.

This symptom may be due to Giardiasis, and the laboratorians should suspect in other parasites, especially Helminths (Bertram *et al*., 1983). Giardiasis main diagnosis is depending on the occurrence of cyst or trophozoites stage in the stool or duodenum. Stool samples must be collected on different days to increase examination accuracy because of the huge number of organisms that may occur in the sample (Gordts *et al*., 1985).

Examination of Giardiasis differ according to the stool state either directly if the sample is fresh or by preserving sample in polyvinyl alcohol or formalism, then staining with trichrome, iodine and haematoxylin (Gordts*et al*.,1985). Stool examination can confirm the infection in most of Giardiasis cases. Three stools series are recommended in the diagnosis. The laboratorians must recognize the cysts of Giardia shedding on a peridicbasis and six stools examination or more may not reveal the parasite (Petersen,1972). Fresh sample examination allow noticing the trophozoite moving in direct wet saline field, even preserving stool help in recognition of the trophozoite morphology which is very important to avoid the organisms disintegration (Gordts *et al*., 1985).

Aspiration is also used for duodenum examination with a tube, with endoscopy or by string test for anal contents which is called the" Entero-test" (Craun, 1984). Recently antigen techniques that are commercially available is often used for detecting this parasite in the stool and unfortunately there is no serologic examination for this parasite because Giardiasis is a luminal disease, and not a systematic (Bertram *et al*.,1983). In Giardiasis diagnosis, there is no molecular methods In the routine laboratories such as PCR. It is only restricted to laboratories where researches usually made (Beal*et al*.,1970). In cases with three negative examinations of stool ELISA test will be the most helpful tool for diagnosis, and is also used in multiple samples (Beal *et al*.,1970). Histological examination is also a useful diagnostic test for patient having Giardiasis symptoms with multiple small bowel biopsies (Beal *et al*.,1970).

Fecal Concentration methods is a good procedure used in routine laboratories for detecting *Giardia* cyst stage in a small numbers, which may be difficult to detect by "wet mount direct smear", and it is also separate the parasite cysts and helminthes eggs, from fecal debris (Graham and Diamond, 2002). Two concentration types have been used in the laboratories of parasitology: Flotation and Sedimentation methods, Flotation methods for separation of the protozoa cysts and eggs of helminthes by using a high specific gravity liquid (1.20) such as"$NaNO_3$, NaCl, $ZnSO_4$" for the diagnosis of *Giardia* cyst Zinc sulfate is usually used as a saturated solution (Beal *et al*.,1970). *Giardia* cysts will float on the surface and the debris will be at the bottom of container, a centrifugation step was added to the procedure to increase the efficiency of the test. This procedure is cleaner than sedimentation methods but cyst walls will be collapsed and the sedimentation procedure is the easiest one with less technical errors (Beal *et al*.,1970). In this method, centrifugation is usually used to detect the cyst stage of *Giardia* in fecal sediment. It is an easy method but containing more debris (Beal *et al*.,1970). For *Giardia* species detection many Sedimentation methods have been used, one of them was the (formalin-ethyl acetate) Sedimentation technique which is the easiest method in which (10 % formalin) is used for preserving and fixing cyst stage. The mechanism of this method is by Fat drop removing, it is better than Zinc sulfate flotation because of the less distortion of *Giardia* cysts (Beal *et al*.,1970). In a Comparison between Formalin - ether concentration techniques and Wet mounts smear in intestinal parasite diagnosis "Formalin-ether concentration technique" showed (65.26 %) detection rate of positive specimens for one or more intestinal parasites while the "direct wet mount smear" was only (34.74 %) (Craun, 1984).

A large number of the infected cases were mistaken by the use of Wet mount smear method (Beal *et al*.,1970). The best identification of the parasite is by using "permanent stained smear" for the distinguishing of the cyst or trophozoite morphological features which is sometimes cannot be seen without

staining (Beal *et al*.,1970). Temporary stains such as "Iodine or Methylene blue" have been used for that purpose after the Wet mount or Concentration techniques (Palmer, 1991). Giemsa stain is also used in "routine clinical laboratory" for staining the flagella and nuclei where they appear "reddish pink", and the cytoplasm looks "grey-blue". Additionally, Iron hematoxylin is a beneficial stain for detecting cyst and Trophozoite (Craun,1984).

Recently the use of Culture methods for detecting the protozoa that infect the human intestine were very effective for diagnostic purpose (Gordts*et al*.,1985). *Giardia* species cultivation were used in the research laboratories were they used xenic and monoxenic cultures for the growth of a huge number of Trophozoite (Gordts*et al*.,1985). Sometimes, it is difficult to detect *Giardia* trophozoite in some cases of the routine laboratories, so they gain fluid by either string test (entro-test) or by Endoscopy from "duodeno-jejunal". Bouyou-Akotet *et al*. (2016) studies have recorded that "the use of the string test has led to an increase of the sufficient axenic cultivation of *Giardia* species than other diagnosis techniques (Gilman *et al*.,1985). Furthermore permanent staining could be used for a staining a mucus drop that is fixed directly on a slide (Mason and Patterson,1987).

4. Physiology

Histological studies have been detected no abnormality In the mucosa of the jejunal and the duodenal in infected person with no symptoms, and infection could be detected by finding crypt hyperplasia, atrophy in the villi, damage in the epithelial cell and infiltration of lymphocytes, leukocytes, plasma cells, and polymorphonuclear in lamina propria. Furthermore, parasites distributions on the villi and intervillous space have been detected in Biopsies (Bouyou-Akotet *et al*., 2016). The histological appearance of jejunal with no inflammatory exudate could be seen in Giardiasis infection (Korman *et al*.,1990). *Giardia lamblia* trophozoite interferes with the brush border of the small intestine (Owen *et al*.,1979). The mechanism of why some infected persons are asymptomatic and others becomes ill is still unknown (Owen *et al*., 1979). The trophozoites cover the mucosa of the intestine making a Mechanical obstruction, yielding in fat soluble and fat absorption blockage, then attachment by the sucking disk directly to the mucosa could lead to irritation (Imre *et al*., 2017). Host factors such as the motility of bowel, nutritional status and parasite characteristics are responsible for the parasite pathogenesis. The effects of the parasites includes toxigenic, immunologic and mechanical factors even interactions with other intestinal flora (Owen *et al*., 1979).

5. Immunology

Both cellular and humoral immune responses are generated by the host in Giardiasis infected host. An important role for the Immunoglobulin M (IgM) and Immunoglobulin A (IgA) in invading *Giardia* (Elsafi *et al*.,2013). The trophozoite motility will be reduced in the intestinal lumen of mice and rats, when they coated by IgG and IgA antibodies. Then, they will be prevented from adhesion to the epithelium layer (Elsafi *et al*., 2013). Detecting specific antibodies such as IgG and IgM in the serum of infected person is important for differentiating the acute from the previously treated person (Jones,1988). Studies indicated that waterborne infections with diarrhea, the antibody which present in the serum is useful for diagnosing Giardiasis. Experiments have found that in mouse model. The function of T-cell is very important in the infection resistance (Mekaru *et al.*, 2007).

6. Histology

Many reports for the histological studies of Giardiasis have been recorded. Oberhuber and Stolte recorded the biopsy specimens from duodenal from 1986 - 1988 (Uehlinger *et al*., 2017). Also they chose 80 cases and used the histological examination of `normal' on their biopsies of the duodenal, then they stained section with eosin and haematoxylin, then by Giemsa stain, showing that there is no significant difference between cases with control cases and infection that have been studied (Petersen, 1972). The patients percentage with villous abnormality was about 41 % compared to 34 % in normal, with no patient having villous atrophy (Craun,1984). In Compared to 23 % of

control patients, 25 % of infected cases were expressing intraepithelial lymphocytes, and in 39 % of cases showed Lymphoid follicles comparing with the 22 % of control cases. Furthermore, granulocytes and lymphocytes dose not appear in any infected cases in the Lamina propria. In summary, *Giardia* presence in the lumen of the small intestine does not alter the enterocyte histology. It was recorded that "Giardiasis diagnosis is available by taking two biopsies of the duodenal by endoscopy" and "the trophozoite may be found in every biopsy of 76 % Giardiasis infected patients" (Elsafi *et al*., 2013).

7. Clinical features

Giardiasis symptoms differ from one to other due to several factors such as individual host, duration of infection, inoculum size and parasite factors (Rendtorff,1954). The period of incubation is about 9 – 15 days, the acute stage begins first with intestinal pain, then anorexia and nausea. Also chills and fever may appear at the beginning of symptoms followed by "foul-smelling" watery diarrhea, gas, belching and epigastric cramps. This stage last for 3 – 4 days. This infection may clear spontaneously followed by the development of chronic infection which lasts for 2 years or more of intermittent diarrhea (Elsafi *et al*.,2013). The symptoms of this period includes headache, myalgia, malabsorption, weight loss and cholecystitis and arthritis (Goudal *et al*., 2019). Urticaria, pancreatitis (Pestechian *et al*., 2014), also thrive failure may occur in children (Hiatt *et al*.,1995; Soares and Tasca, 2016). In Giardiasis case the Eosinophilia does not occur and the hemogram is normal. Lactose, Glucose, Vitamin A, Vitamin B12 and Malabsorption of fat may occur in some cases of Giardiasis (Imre *et al*., 2017). In infected group of people with ethnic groups who are have lactose intolerance. Clinical symptoms such as intolerance of Lactose is found in the infection period and for this type of people further treatment should be provided (Hiatt *et al*., 1995).

8. Prevention

Giardia species (from 1986 – 1988) were detected as the result of disease in more than 500 person. According to the "United States centers for Disease Control surveillance" reports Infection is usually related with the poor water treatment capabilities and infiltration of surface water system (Pestechian *et al*.,2014). Using chlorine in water treatment system is only effective on enteric organisms because the cyst stage of *Giardia* needs a high concentrations and more period of contact to be killed especially In cold water (Faubert, 2000), boiling of water for 1 min could destroy Giardia cyst stage and that's important for human protection in areas where there were no qualified water treatment systems. If boiling is not available, 2 – 4 chlorine drops or 0.5 ml of iodine /1 liter of water could be effective in killing and the water must be used after 60 min of adding. An overnight treatment is required if the water is cold (Faubert, 2000). There is no available drugs for preventing Giardiasis only Cooking food well can prevent ingestion of viable cyst which was brought from contaminated food (Faubert, 2000).

9. Treatment

The most famous drugs for Giardiasis treatment are furazolidone, quinacrine, and Metronidazole. Furthermore nitroimidazole compounds such as nimorazole, tinidazole and ornidazole have been detected as effective drugs for treating Giardiasis (Owen *et al*.,1979). Metronidazole is also used in doses (about 250 mg 3 times a day for 7 days). For infected adult and 5 mg/kg 3 times a day for 7 days. For infected children, and this give a approximately (85 – 95 %) cure rate and should be used in low dose first then increased dose (Chute*et al*.,1987). Metronidazole drug is better in activity than quinacrine (Chute *et al*.,1987). Side effects includes dark urine, metallic taste, bowel overgrowth and gastrointestinal symptoms (Craun,1986).

The Metronidazole mechanism is inhibiting microtubules function within the parasite cytoskeleton, paralysedit, leading to its excretion (Beyhan and Tas Cengiz, 2017). Another Giardiasis drug is Furazolidone (Furoxone) which is liquid, being the best for infants and children with a cure rate less than Metronidazole between 75 – 90 % range, the drug side reactions include rash, urticaria, gastrointestinal symptoms, fever, and brownish urine in some cases. Furthermore, Hemolysis

may occur when patient with "glucose-6-phosphate dehydrogenase deficiency" take this medication (Beyhan and Tas Cengiz, 2017). Even Furazolidone medication has led to tumors in the mammary glands of rats, so it is not safe and remained not allowed as a Giardiasis drug. Usually (100 mg 4 times a day) is used for adult for 7 days, and (1.25 mg/kg four times a day) for children for 7 days (Beyhan and Tas Cengiz, 2017). The drug tinidazole (Fasigyn) with a single dose was also used outside America, and was effective as those found in America (35) when available. It is the best drug for Giardiasis, and a single dose of (2 g) is usually taken by adult and 30 – 35 mg/kg by children, its side effect could be vary from vertigo and headache in approximately (10 %) of the treated patients (Fink and Singer,2017).

Young children and food handlers may be cyst carrier with no obvious symptoms, and may infect others, and may spontaneously develop infection to themselves. For fetus none of these described medications were used because they may be not safe. However, treatment within pregnancy period is very important, and the medication quinacrine is the preferred one because it is effective on Giardiasis (Owen *et al*.,1979). The antibiotic paromomycin (Humatin) is also used in treating of Giardiasis during pregnancy, but it is poorly absorbed by the intestine (Petersen,1972). To check the medication activity the stool samples should be checked for 4 weeks after treatment, and if there is a parasite, different medications should be used (John*et al*.,2006). Albendazole medication may be the other choice if the case felt with no cure and the parasite still present on samples (John *et al*.,2006). However, it is impossible to eradicate the disease, as Giardiasis is worldwide spread in human and animal population and even in environment (Saha *et al*.,1977).

10. Conclusion

Giardia species is the most prevalence water borne parasite that infect human in the world. Cyst stage of the parasite has been found in lakes, ponds and rivers. The most important way of parasite transmission to human is by ingesting the cyst stage with contaminated food and drinking waters. Human Giardiasis major symptoms are Epigastric cramps, vomiting, diarrhea, weight loss and flatulence. All *Giardia* infection cases could not be detected by new or traditional methods. In the last three decades, Immunodiagnostic tests for antigen detection. Fecal samples were used for Giardiasis rapid detection, inspite of that the stool examination by microscope using concentration methods, still the best procedure in the world for diagnosing this parasite .In the large laboratories where a huge number of stool specimen must be examined daily. A diagnostic Non-morphological technique such as immunoassay is necessary for detecting coproantigen which is a complementary to the traditional methods. In developing countries, the diagnostic medical laboratories usually using technique such as Formalin-ether method "which depends on stool concentration" routinely because it is economical cheaper.

Conflict of interests

The authors declare that they have no conflict of interest.

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DOI Number	**DOI: 10.22192/iajmr.2020.6.5.2**
Thomson Reuters Researcher ID	**K-4194-2016**
ISI Impact Factor	**3.652**

How to Cite this Article:

Shaimaa A. Alsamir and Sundus W Alabdullah. 2020. Interaction between *Giardia* species and Intestine: A Review. *Indo - Asian Journal of Multidisciplinary Research*, 6(5): 2110 – 2118.

DOI: 10.22192/iajmr.2020.6.5.2