

## Ocular Changes in Giardiasis: Human and Experimental Studies

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### ABSTRACT:

**Background/Aim:** Ocular changes associated with giardiasis were recorded in a number of studies. The aim of the present study was to evaluate the frequency of ocular manifestations in children with giardiasis in relation to ages and severity of infection. In addition, the mechanisms underlying the ocular lesions were investigated through an experimental animal study.

**Subjects & Methods:** The human study comprised 73 children (from 3 to 14 years old) attending the Outpatient Clinic of Pediatric and Tropical Medicine Departments of Tanta University Hospital. They have been diagnosed as being infected with *Giardia lamblia* through stool examination and indirect immunofluorescence assay for scoring the severity of infection. The present study also included a control group comprised 25 age-matched children. All the children were sent to the Ophthalmology Department of Tanta University Hospital to undergo an ophthalmic examination of ocular motility, slit lamp examination of the anterior chamber, direct and indirect ophthalmoscopic examination of the fundus after induction of mydriasis. In the experimental study, 90 laboratory-bred mice were used, 70 mice were infected with *Giardia lamblia* cysts while the remaining 20 mice were kept uninfected. The animals were divided into four groups: Group I (35 immunocompetent and infected), group II (35 immunosuppressed and infected), group III (10 immunocompetent control) and group IV (10 immunosuppressed control). Half the number of mice was sacrificed 2 weeks post-infection (P.I) and the remaining number was sacrificed 4 weeks P.I. At each period of examination 1- Duodenum and upper parts of jejunum of each sacrificed mouse were removed and subjected to histopathological examination 2- Both eyes of each sacrificed mouse were enucleated and subjected to both histopathological examination and indirect immunofluorescence assay as a trial to detect the parasite or its antigen in the eye tissues.

**Results:** The results of human study revealed changes in the retinal epithelium compatible with a salt and pepper appearance in 9/73 (12.3%) children. The presence of ocular changes was related to the severity of infection where the higher percentage of cases (77.8%) was from group C (severe infection). Also, it was observed that the children with retinal changes were significantly younger (mean age 3.8 ± 0.04 years) than those without lesions. In the experimental study, 2 weeks P.I, neither histopathological changes nor immunofluorescent deposits were detected in eye tissues whereas, 4 weeks P.I. mild lymphocytic infiltration was detected in 1/18 (5.6%) mice of group I and in 3/15 (20%) mice of group II with no detection of the parasite in eye tissues. On the other hand, the immunofluorescence staining of eye tissues revealed few immunofluorescent deposits in the retinal layers of 2/18 mice of group I (11.1%) and marked immunofluorescence deposits in 5/15 (33.3%) mice of group II.

**Conclusion:** the present study may throw some light on the pathology and pathogenesis of human ocular complication that may occur with giardiasis. In spite of absence of *Giardia* parasite from eye sections, the deposition of giardial antigen in the affected eyes may play a pivotal role in the pathogenesis of ocular changes in giardiasis. So, both ophthalmologists and clinicians should be aware of this link when interpreting retinal findings in children, especially those with severe giardiasis.

**Key words:** Ocular Changes, Giardiasis, Human, Experimental Studies.

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## INTRODUCTION

*Giardia lamblia* (*G. Lamblia*) is a binucleate protozoan of the subphylum *Mastigophora* that has four pairs of flagellae<sup>(1)</sup>. The prevalence of giardiasis varies depending on the population being examined, but the highest rates are found in developing countries and crowded urban areas. The infective cysts are transmitted oro-fecally or in food and water that have been contaminated with feces<sup>(2)</sup>. The protozoan colonizes the duodenum and upper third of the jejunum, where it is believed to cause direct damage to the microvilli that leads to an accelerated turnover of the mucosal epithelium and, as a result, changes in intestinal absorption<sup>(3)</sup>.

The infection is often asymptomatic in immunocompetent adults, but symptoms are much more common in children, in part due to the oro-fecal transmission and in part because of the immaturity of their immunological systems<sup>(4)</sup>. The primary gastrointestinal symptoms consist of recurrent abdominal pain, diarrhoea, and vomiting. *G. lamblia* appears as an opportunistic parasite with development of various complications as malabsorption syndrome, lactose intolerance, vitamin B12 deficiency and pancreatic insufficiency<sup>(2)</sup>. Patients with giardiasis also present extra-intestinal manifestations such as fever, cutaneous manifestations in the form of maculopapular rashes and urticaria, pulmonary infiltrates, lymphadenopathy, polyarthritis and aphthous ulcers<sup>(5)</sup>. In overwhelming experimental *Giardia* infection, parasite was detected in liver, heart and brain in about 10% of such highly infected mice<sup>(6)</sup>. Also, when highly infected mice were super-infected with *Plasmodium berghei*, *Giardia* was detected in organs outside the intestines in 80% of mice

which indicated diminished resistance to invasion<sup>(7)</sup>.

Ocular manifestations associated with giardiasis were recorded in a number of studies. The first description of ocular complications in patients with giardiasis was reported by Barraquer in 1938<sup>(8)</sup> which included cases of iridocyclitis, choroiditis, and retinal haemorrhages. Moreover, cases of anterior and posterior uveitis and retinal vasculitis have been detected in association with giardiasis in a number of studies<sup>(9-13)</sup>. The mechanisms underlying the ocular lesions associated with giardiasis are currently obscure. Although microscopic studies on ocular tissues were not performed, most authors exclude the possibility of direct invasion by the parasite<sup>(11-13)</sup>.

*Giardia lamblia* antigens were analyzed where a major surface antigen of *G. lamblia* was detected as a surface protein which was immunoprecipitated by human sera from patients with giardiasis. This surface protein was released into the culture medium during in vitro growth and so it appears to be both a major immunogen and an exoantigen<sup>(14&15)</sup>. Both humoral and cellular immune responses to giardial antigen are generated by the host as a defense mechanism. Secretory immunoglobulin A (sIgA) and IgM antibodies seem to play a role in eradicating parasites. *Giardia*-specific IgG and IgM antibodies have also been demonstrated in serum and may be helpful in differentiating patients with acute or recent infection from those with past or previously treated infection<sup>(16,17&18)</sup>. Therefore, host immune status had been found to be the major determinant of the pathogenesis of giardiasis<sup>(5)</sup>.

The aim of this study was to evaluate the frequency of ocular manifestations in children with giardiasis in relation to ages of children and severity of infection. In addition, the mechanisms underlying the ocular lesions were investigated through an experimental animal study.

## SUBJECTS AND METHODS

This study was designed to fulfill two objects; firstly, to evaluate the frequency of ocular lesions in children with giardiasis in relation to age and severity of infection through human study. Secondly, to investigate the underlying mechanism of ocular lesions through an experimental animal model.

### HUMAN STUDY

#### Patients and methods

The study population comprised 73 children attending the Outpatient Clinic of the Pediatric and Tropical Medicine Departments of Tanta University Hospital from March to August (2006). The children (44 males and 29 females) ranged in age from 3 years to 14 years (mean age  $6.3 \pm 0.4$ ). All had presented with gastrointestinal symptoms and they have been diagnosed as being infected with *Giardia lamblia*.

The diagnosis of giardiasis was made after the parasite (trophozoite or cyst) had been isolated from at least three fresh stool specimens and also through detection of serum antibodies by indirect immunofluorescence assay.

**Indirect immunofluorescence assay:** It was done according to Hu<sup>(19)</sup> as follows:

**1- Preparation of *Giardia lamblia* antigen:** *G. Lamblia* trophozoites were isolated from positive diarrhoeic stool samples of infected children and purified by the modified formol ether technique. *G. lamblia* antigen was prepared by cultivation of the isolated trophozoites on commercially obtained *Giardia* culture medium (YFS culture medium). It was obtained from Biomed Diagnostic Company (Antelope Road, white Cty, U.S.A.) through internet communication. A drop of the cultured suspended trophozoites was placed in the center

of each well of the 12-well antigen slide and allowed to dry at room temperature and then fixed in 100% acetone for 10 - 20 minutes and stored at  $-20^{\circ}\text{C}$  till use<sup>(20)</sup>.

2- Tested sera of infected and control children were collected. Serial two folds dilutions of each coded serum beginning at 1/10 were prepared in phosphate buffered saline (PBS). 50  $\mu\text{L}$  of diluted serum per well was applied to the antigen slides. The slides were incubated at room temperature.

3- Indirect immunofluorescence assay was performed by using fluorescein conjugated anti-mouse immunoglobulin, IgG product No. F-0257, Sigma. The immunoglobulin was diluted 1/50 in PBS and 50  $\mu\text{L}$  was added to each antigen well. The slides were incubated for 30 minutes at room temperature. Then, the slides were examined by the fluorescence microscope. A bright yellowish green fluorescence denoted positive results. The intensity of fluorescence was graded on a scale of 0 to 3+. Negative control sera as well as control wells of PBS were included in each run.

The severity of infection was determined by scoring the intensity of fluorescence of the tested sera which graded on a scale of 0 to +3. The infected children were divided into three groups according to the severity of infection: {Group A: mild infection (+1)}, {group B: moderate infection (+2)} and {group C severe infection (+3)}.

The present study also included a control group (group D) comprised 25 age-matched children selected between the ones who attended the Outpatient Clinic of the Pediatric and Tropical Medicine Departments of Tanta University Hospital in the same period as the infected group. They all came from the same geographic area and had socioeconomic and nutritional characteristics similar to those in the infected group. All of the control subjects were negative for giardiasis based on microscopic examination of three stool specimens and immunofluorescence assay for serum antibodies.

None of the children included in the infected and control groups had current or past histories of other infections and none had ever been

treated with any drugs which are known to cause ocular toxicity like chloroquine, chlorpromazine and thioridazine. All the infected and control children underwent an ophthalmic examination that included study of ocular motility, slit lamp examination of the anterior chamber, direct and indirect ophthalmoscopic examination of the fundus after induction of mydriasis.

#### EXPERIMENTAL STUDY

##### Materials and methods

Ninety laboratory-bred, parasite free Swiss albino mice (20 -25 gm in weight and 6-8 weeks old) were used in this study. Seventy mice were infected with *Giardia lamblia* cysts while the remaining twenty mice were kept uninfected (control group). *Giardia lamblia* cysts were isolated from the positive stool samples of the examined children attending Outpatient Clinic of the Pediatric and Tropical Medicine Departments of Tanta University Hospital. Cyst suspension was prepared according to Cheesbrough (21), in which cysts were separated from stool samples by a modified formol ether technique where normal saline was used instead of formalin. Cyst suspension was adjusted to contain  $10^6$  cysts/ml. Each mouse was infected by intra-oesophageal inoculation of 0.5 ml cyst suspension. The animals were divided into four groups:

**Group I (immunocompetent and infected):** 35 mice were kept immunocompetent and infected with *Giardia* cyst suspension.

**Group II (immunosuppressed and infected):** 35 mice were immunosuppressed and infected with *Giardia* cyst suspension. Immunosuppression was done by subcutaneous injection of methyl prednisolone acetate (Depo-medrol, 40 mg/ml, Upjohn) adjusted so that 1 mg was suspended in 0.1 ml sterile 0.9% NaCl solution. Each mouse was injected with a dose of 2 mg/day for five days before infection. A weekly dose was given to the mice through the period of experiment (22).

**Group III (control immunocompetent):** Ten mice were kept immunocompetent and not infected.

**Group IV (control immunosuppressed):** Ten mice were immunosuppressed and not infected.

The stools of the infected mice were examined for the presence of *Giardia lamblia* trophozoites or cysts from the second day post-infection daily up to one week. Only mice with positive stools for *G. lamblia* were included in the study where 15 mice from group I, 15 mice from group II, 5 mice from group III and 5 mice from group IV were sacrificed two weeks post-infection while all the remaining mice were sacrificed four weeks post-infection. At each period of examination all sacrificed mice were subjected to the following assessments:

##### Histopathological examination

- Formalin-fixed, paraffin-embedded sections (5  $\mu$ m) from the small intestines (duodenum and upper jejunum) were prepared and stained with haematoxylin and eosin (H&E). The sections were examined for histopathological changes and assessment of parasitic colonization by counting *G. Lamblia* trophozoites/oil immersion field. To assess the intensity of *G. Lamblia* infection, ten oil immersion fields were examined and the mean number of trophozoites was calculated to score the severity of infection(6).

- Both eyes of each sacrificed mouse were enucleated and formalin-fixed, paraffin-embedded eye sections (5  $\mu$ m) were prepared and stained with H&E for histopathological examination (23).

##### Indirect immunofluorescence (IIF) assay of eye sections (24 & 25):

1-Formalin-fixed, paraffin-embedded eye smears were left to dry at room temperature, then fixed in acetone for 10 - 20 minutes and stored at -20 °C till used.

2- Preparation of hyper immune anti-*Giardia* serum in a white rabbit by initial intradermal injection, at multiple sites, of *Giardia* cysts ( $3 \times 10^6$  cysts) mixed with 1:1 complete Freund adjuvant followed by intramuscular booster injection of  $2 \times 10^6$  cysts in incomplete Freund adjuvant at 3, 6 and 8 weeks after the initial injection. The rabbit was bled by cardiac puncture under anesthesia 5 days after the last

booster dose. The sera were stored at  $-20^{\circ}\text{C}$  till the time of use.

3- The smears were flooded with the hyper immune anti-*Giardia* serum and incubated for 30 minutes at room temperature in a humid chamber.

4- Fluorescein labeled anti-mouse IgG product (No. F-0257, Sigma) was diluted 1/50 in PBS and applied to the slides which incubated for 30 minutes at room temperature. The slides were rinsed three times in PBS, air dried and examined with the fluorescence microscope.

N.B: Number of mice died during the course of the experiment was 8 mice; 2 mice from group I, 5 mice from group II and one mouse from group IV

**Statistical analysis:** Results were expressed as means  $\pm$  SD. Differences were statistically analysed and compared for significance using Student *t* test and analysis of variance (ANOVA) test by using SPSS program version 13.

## RESULTS

**RESULTS OF HUMAN STUDY:** As regards the results of ophthalmic examination, the following data were recorded:

- Ocular motility and anterior chamber examination appeared normal in all children of both infected (73 children) and control groups (25 children).
- Changes in the retinal epithelium compatible with a salt and pepper appearance were confirmed by the ophthalmologist in 9/73 (12.3%) children {6 male, 3 female} (Table 1). The lesions were more visible at the posterior pole, where there were distributed along the paths of the major blood vessels. These lesions presented as punctate areas of hyperpigmentation against a lighter coloured fundus (Fig 1). Involvement was bilateral in 7/9 cases.
- Concerning the severity of infection as scored from the intensity of fluorescence of the tested

sera, the infected children was classified into 3 groups:

- 1- Group A: 23 cases with mild infection (+1, Fig 2)
- 2- Group B: 31 cases with moderate infection (+2)
- 3- Group C: 19 cases with severe infection (+3, Fig 3).

• As regards the relation between ocular lesions (9 cases) and the severity of *Giardia* infection, table (1) showed that no cases (0 %) were detected in group A, 2 cases (22.2%) were detected in group B and 7 cases (77.8%) were detected in group C. Thus, it was observed that the presence of ocular changes was related to the severity of infection where the higher percentage of cases (77.8%) was detected in group C (severe infection).

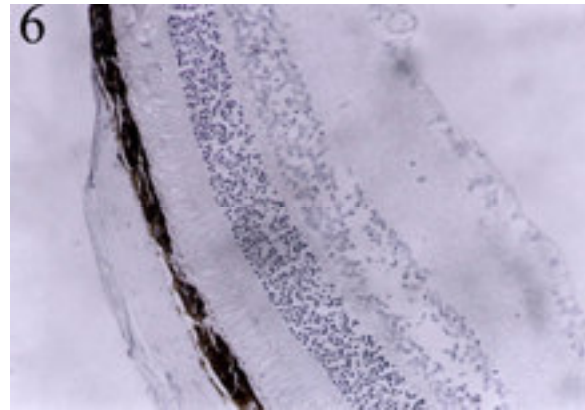
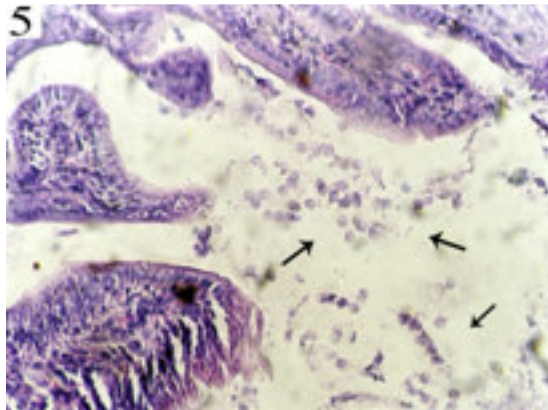
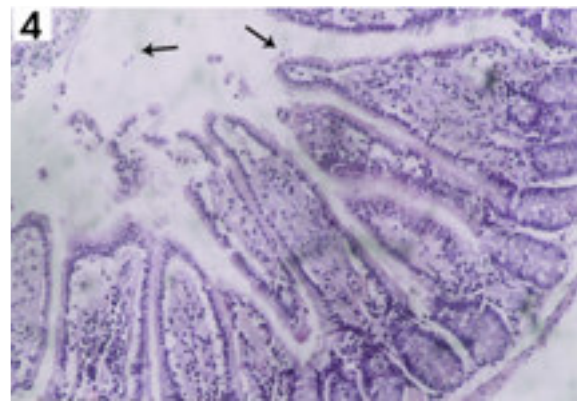
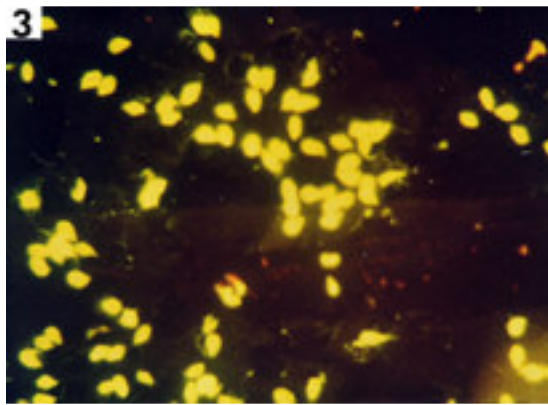
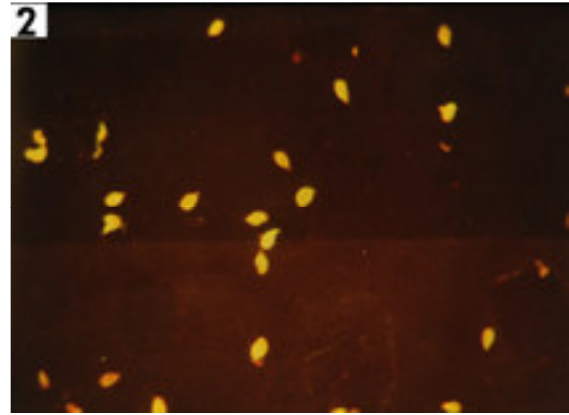
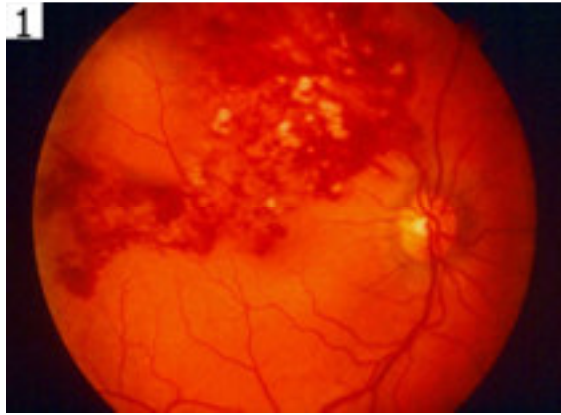
• Regarding ages of the 9 children with positive findings, it was observed that the children with retinal changes were significantly younger than those without lesion (mean age  $3.8 \pm 0.4$  years) (Table I).

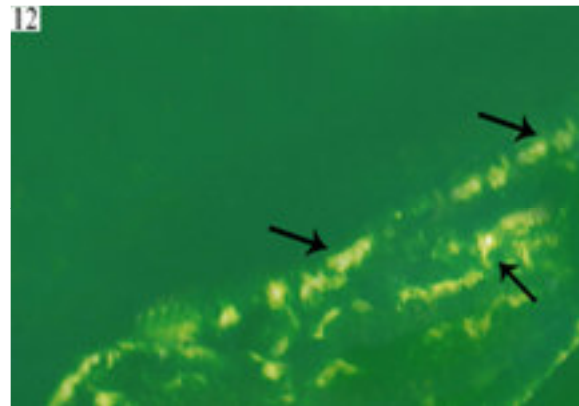
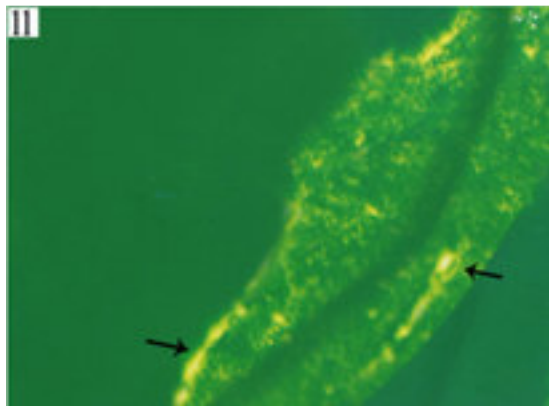
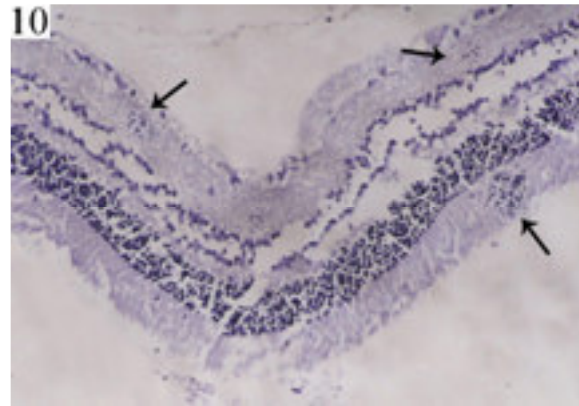
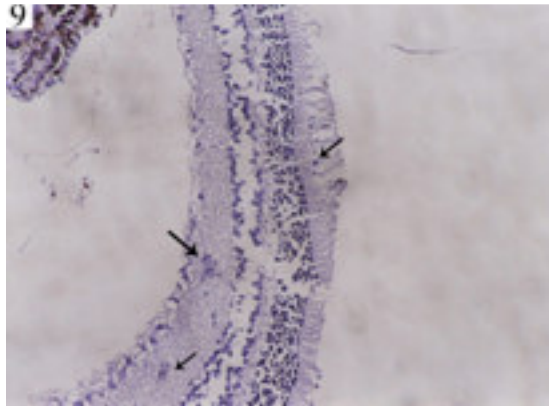
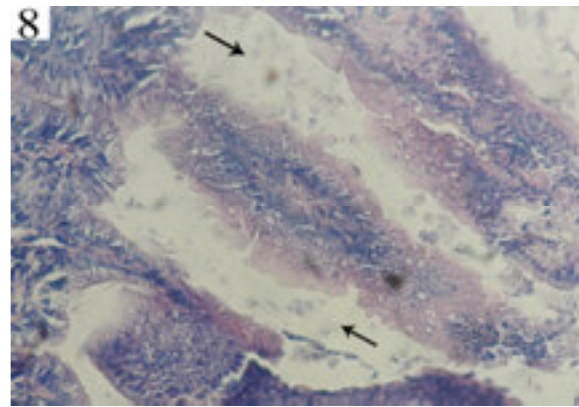
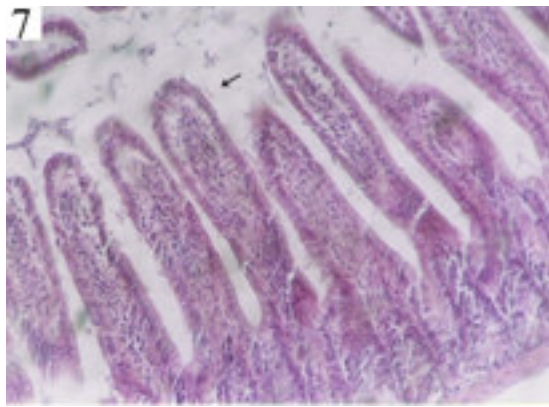
• Retinal changes could not be detected in any of the 25 child of the control normal group (group D).

### RESULTS OF EXPERIMENTAL STUDY:

#### At 2 weeks post-infection (acute phase):

- The histopathological examination of the small intestine of group I (immunocompetent and infected) revealed moderate number of *Giardia* trophozoites ( $4.25 \pm 1.37$ ) attached to the villous surface (Table 2), most of the villi are finger-shaped with mild increase of crypt depth and also, there were lymphocytic in the lamina propria (Fig. 4). Group II (immunosuppressed and infected) showed large number of trophozoites ( $7.21 \pm 2.34$ ) attached to the villous





surface and in the lumen with broadening and shortening of the villi with marked increase in depth of crypts and lymphocytic infiltration of lamina propria (Fig 5). There was significant difference between the number of trophozoites in group I and group II ( $P < 0.05$ ). The histopathological examination of the small intestine of control groups (III & IV) showed normal intestinal architecture.

- The histopathological examination of eyes of all groups of mice revealed no changes with preservation of the normal retinal architecture. No parasite or pathological changes could be detected in the histopathological sections (Fig 6).
- Regarding the immunofluorescence staining of eye tissues, no immunofluorescent deposits could be detected in any eye sections of all groups.

#### At 4 weeks post-infection (elimination phase):

- The histopathological examination of the small intestine of group I revealed few number of *Giardia* trophozoites ( $2.13 \pm 1.46$ ); most of the villi and crypt retained their normal shape with little lymphocytic infiltration in the lamina propria (Fig. 7). Group II still showed marked broadening and shortening of the villi with marked increase in depth of crypts, lymphocytic infiltration of lamina propria and a large number of trophozoites ( $9.14 \pm 2.3$ ) were observed (Fig. 8). There was a significant difference between the number of trophozoites in group I and group II ( $P < 0.05$ ). The histopathological examination of the small intestine of control groups (III & IV) showed normal intestinal architecture.
- The histopathological examination of the eyes of group I revealed mild lymphocytic infiltration of the retinal layers in one mouse only 1/18 (5.6%) (Fig 9). On the other hand, 3/15 (20%) mice of group II revealed lymphocytic infiltration in the retinal layers with edematous changes and looseness of the innermost layers of the retina (Fig 10). *Giardia* parasite could not be detected in any of the examined eye sections.

Regarding the immunofluorescence staining of eye tissues, immunofluorescent deposits were observed in the retinal layers of 2 mice (2/18) of group I (11.1%) (Fig. 11) (one mouse of them showed histopathological changes). While, 5/15 (33.3%) mice of group II, showed marked immunofluorescent deposits in the retinal layers with variation in the intensity (Fig. 12) (3 mice of them showed histopathological changes). No immunofluorescent deposits could be detected in any sections of the control groups

#### Legends of figures:

**Fig. (1):** A photograph showing typical salt and pepper appearance of the retina in a child infected with *Giardia lamblia*.

**Fig. (2):** An indirect immunofluorescence reaction of a positive serum of a child with giardiasis (mild infection +1).

**Fig. (3):** An indirect immunofluorescence reaction of a positive serum of a child with giardiasis (severe infection +3). Most of trophozoites are uniformly stained.

**Fig. (4):** A section of the small intestine of a mouse of group I (immunocompetent & infected), 2 weeks P.I., showing mild number of trophozoites attached to the villous surface with moderate lymphocytic infiltration (H&E stain, X 250).

**Fig. (5):** A section of the small intestine of a mouse of group II (immunosuppressed & infected), 2 weeks P.I., showing large number of trophozoites attached to the villous surface and in the lumen with loss of the normal villous architecture (H&E stain, X 400)

**Fig. (6):** Eye section of a mouse from group I (immunocompetent & infected), 2 weeks P.I. showing normal retinal architecture (H&E, X250).

**Fig. (7):** A section of the small intestine of a mouse of group I, 4 weeks P.I., revealed few number of *Giardia* trophozoites; most of the villi and crypt retained their normal shape with little lymphocytic infiltration in the lamina propria (H&E, X 250).

**Fig (8):** A section of the small intestine of a mouse of group II, 4 weeks P.I., showed large number of trophozoites attached to the villous surface and in the lumen with lymphocytic infiltration of lamina propria (H&E, X 400).

**Fig (9):** Histopathological section of the eye of a mouse of group I, 4 weeks P.I. revealed mild lymphocytic infiltration of retinal layers (H&E, X 250).

**Fig (10):** Histopathological section of the eye of a mouse of group II, 4 weeks P.I. showing lymphocytic infiltration in the retinal layers

with edematous changes and looseness of the innermost layers of the retina. *Giardia* parasite could not be detected in any of the examined eye sections (H&E, X 250).

**Fig. (11):** Immunofluorescence stained section of the eye of a mouse from group I showing moderate immunofluorescent deposits in the retinal layers (X 250).

**Fig. (12):** Immunofluorescence stained section of the eye of a mouse from group II showing marked immunofluorescent deposits in the retinal layers (X 250).

**Table (1): Frequency of "salt and pepper" (SP) retinal changes among different groups of children in relation to age and severity of infection.**

Children with giardiasis	Number	No. of children with SP	Mean ages of total groups ( $\pm$ SD)	Mean ages of children with SP
Total no.	73	9 (12.3%)	6.3 $\pm$ 0.4	3.8 $\pm$ 0.4 *
Group A	23	0 (0.0%)	7.2 $\pm$ 0.2	
Group B	31	2 (22.2%)	5.5 $\pm$ 0.7	4.0 $\pm$ 0.3 *
Group C	19	7 (77.8%)	5.0 $\pm$ 0.4	3.2 $\pm$ 0.3 *
Group D (control)	25	0	4.1 $\pm$ 0.5	

\*:  $P < 0.05$  (significant difference) between mean ages of total group and mean ages of children with SP.

**Table (2): Mean Numbers of *Giardia Lamblia* trophozoites/oil immersion field in H&E stained small intestinal sections of different groups of mice.**

Period of exam.	Group I	Group II	Group III	Group IV
2 weeks P.I.	4.25 $\pm$ 1.37	7.21 $\pm$ 2.34*	0	0
4 weeks P.I.	2.13 $\pm$ 1.46	9.14 $\pm$ 2.3 *	0	0

\*  $P < 0.05$  :Significant difference between group I & II (2 and 4 weeks P.I.).

## DISCUSSION

*G. lamblia* is a common human intestinal parasite, it is responsible for numerous waterborne outbreaks and travellers' diarrhea, but its incrimination to be a cause of ocular complication is a matter of interest. The increased prevalence of giardiasis and its ocular complications in children has brought into light a major problem facing both ophthalmologists and pediatricians. The association between

*Giardia lamblia* infection and ocular changes has been described by a number of authors. Among those authors, Knox and King<sup>(11)</sup> who reported the presence of retinal arteritis and iridocyclitis in three patients infected with *Giardia lamblia*. Moreover, Anderson and Griffith<sup>(26)</sup> suggested that there was a relationship between intestinal giardiasis and ocular inflammatory conditions which may result in significant vision loss.

In the present study, 9/73 (12.3%) children with giardiasis revealed retinal changes in the form of salt and pepper appearance. It was observed that the children with retinal changes were significantly younger (mean age  $3.8 \pm 0.4$ ) than those without lesion. Nearly, similar studies were conducted by Pettoello-Mantovani *et al.*<sup>(12)</sup> who described eight cases of salt and pepper retinal degeneration in a group of 90 children with active giardiasis (8.8%). Moreover, in a study of Corsi *et al.*<sup>(13)</sup>, which conducted on a larger population of paediatric giardiasis patients (141 children), the percentage of children with salt and pepper retinal findings was even higher (19.9%), which may reflect the younger age of the children included in their study (mean age 4.7 years *v* 6.9 years in the study of Pettoello-Mantovani *et al.*<sup>(12)</sup>). Thus, the results of the present study were in accordance to the results Corsi *et al.*<sup>(13)</sup> as regards the increased frequency of retinal lesions in younger children with giardiasis. Corsi *et al.*<sup>(13)</sup> suggested that small children are more susceptible to this type of damage because of the immaturity of the retinal epithelial cells.

In the present study, the presence of ocular changes with giardiasis were related to the severity of infection where the higher percentage of cases (77.8%) was detected in group C (severe infection) while children of group A (mild infection) couldn't reveal any retinal changes. On the contrary, Corsi *et al.*<sup>(13)</sup> denied this relation and added that the risk of ocular complications has not related to the duration of infection, or the use of anti-parasitic therapy but may reflect a genetic predisposition.

The results of previous studies have demonstrated that structural alterations of the retinal pigment epithelium are the most common ocular findings in pediatric patients with giardiasis<sup>(9,-13)</sup>. The typical salt and pepper lesions of giardiasis differ from those of the more severe disease known as retinitis pigmentosa, in which the pigment granules are generally distributed in an osteoblast pattern around blood vessels<sup>(26)</sup>. The pathogenesis of the salt and pepper lesions in giardiasis was explained by Corsi *et al.*<sup>(13)</sup> who suggested that it was caused by damage to or necrosis of the cells of the retinal pigment epithelium (represented

clinically by the paler areas of the retina) with the release of pigment granules that migrate to the deeper retinal layers, where they can be seen as blackish dots.

The mechanisms underlying the ocular lesions associated with giardiasis are currently obscure. Although microscopic studies on ocular tissues were not performed, most authors exclude the possibility of direct invasion by the parasite<sup>(11, 13&26)</sup>. Indeed, the protozoan has never been isolated from any of the lesions caused by giardiasis, including urticaria, which is the manifestation that most strongly resembles the retinal arteritis and ocular inflammation seen in patients with giardiasis<sup>(27)</sup>. The histopathological examination of urticarial lesions caused by giardiasis has demonstrated the presence of infiltrates composed of polymorphonuclear cells, lymphocytes, and eosinophils<sup>(28)</sup>.

In the experimental part of this study and as a trial to detect the underlying mechanisms of ocular lesions of giardiasis, histopathological sections and immunofluorescence staining of eye tissues were made searching for the parasite or its antigen in eye tissues. Moreover, to detect the link between the presence of any ocular changes and the immune status, both immunocompetent and immunosuppressed animal models were used. Also, to detect the correlation between ocular changes and stage of the disease, the mice were examined 2 weeks PI. (acute phase) and 4 weeks PI. (elimination phase). The present study showed histopathological changes in the eye sections in the form of cellular infiltration in only 1/18 mice of group I (immunocompetent) and in 3/15 mice of group II (immunosuppressed) (4 weeks PI.). Additionally, few immunofluorescent deposits were observed in the retinal layers of 2/18 mice of group I and marked immunofluorescent deposits were detected in 5/15 mice of group II (4 weeks PI.). These results have raised the concept of giardial antigen deposition in the retinal layers which may be responsible for the pathogenesis of ocular manifestations of giardiasis.

In the current study, the association of ocular changes to giardiasis has increased in

immunosuppressed mice, 4 weeks PI., indicating that the immune status and stage of the disease could play an important role in the pathogenesis of ocular lesion. The coexistence of histopathological changes and immunofluorescence deposits in retinal layers gave strong evidence to the deposition of giardial antigen in the retinal layers. In addition, the detection of giardial antigen in the eye tissue, in the present study, gave a specification for the probability of subsequent immune complex formation which may be responsible for the presence of ocular changes in giardiasis. Wania<sup>(29)</sup> suggested that the retinal changes associated with giardiasis are more likely caused by immune mechanisms and reported that circulating immune complexes were found in all of the patients with ocular complications. The role of immune complexes deposition in the pathogenesis of different organ lesions of parasitic infections had been proved by many studies<sup>(30-34)</sup>.

Moreover, some reports have linked giardiasis to certain HLA isotypes since retinal pigmented epithelium degenerations are often genetically transmitted, it is therefore possible that the appearance of retinal complications depends, in part, on a genetic predisposition or it may be allergic in nature<sup>(35,36)</sup>. Diagnosing ocular giardiasis is a challenge and is generally made by exclusion. Retrospective diagnosis may be made by demonstration of improvement in ocular findings following a course of treatment with anti-giardial agents<sup>(27)</sup>.

In conclusion, the present study may throw some light on the pathology and pathogenesis of ocular complication that may occur with giardiasis. In spite of absence of *Giardia* parasite from eye sections, the deposition of giardial antigen in the affected eyes may play a pivotal role in the pathogenesis of ocular changes in giardiasis. So, both ophthalmologists and pediatricians should be aware of this link when interpreting retinal findings in children, especially those with severe giardiasis.

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## تغيرات العين المصاحبة للإصابة بطفيلي الجيارديا

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سجلت بعض الدراسات تغيرات في العين مصاحبة للإصابة بطفيلي الجيارديا لامبليا ولكن ميكانيكية حدوث هذه التغيرات مازالت غير واضحة. والهدف من هذه الدراسة هو مدى حدوث التغيرات العينية في الأطفال المصابين بطفيلي الجيارديا لامبليا في ضوء التباين في شدة المرض وكذلك أعمار الأطفال المصابين بالإضافة إلى بحث سبب حدوث هذه التغيرات من خلال دراسة تجريبية على الفئران.

وقد اشتملت الدراسة الآدمية على ٧٣ طفلا مصابا بطفيلي الجيارديا لامبليا ممن يترددون على العيادة الخارجية لقسمي طب الأطفال و المتوطنة بمستشفيات جامعة طنطا، ويتراوح أعمارهم ما بين ٣ - ١٤ سنة وقد تم تشخيص الإصابة بالجيارديا من خلال الفحص الميكروسكوبي لعينات البراز وكذلك اكتشاف الأجسام المضادة لطفيلي الجيارديا في مصل الأطفال المصابين باستخدام الاختبار المناعي الوميض غير المباشر. كما تضمنت الدراسة مجموعة أطفال ضابطة قياسية مكونة من ٢٥ طفلا سليما تعرضت لنفس طرق الفحص والتشخيص. وقد تم فحص كل الأطفال في قسم طب و جراحة العيون بكلية طب طنطا حيث تم فحص العين بالميكروسكوب المباشر الغير مباشر للبحث عن وجود تغيرات في قاع العين.

واشتملت الدراسة التجريبية على ٩٠ فأرا صغيرا، تم عدوى ٧٠ منهم بجرعات طفيلي الجيارديا بينما تركت باقي الفئران بدون عدوى كمجموعة قياسية ضابطة وقسمت الفئران إلى المجموعات الآتية:

- المجموعة الأولى: ٣٥ فأرا ذات جهاز مناعي سليم ومعدية بطفيلي الجيارديا
- المجموعة الثانية: ٣٥ فأرا ذات جهاز مناعي مبط ومعدية بطفيلي الجيارديا
- المجموعة الثالثة: ١٠ فئران ذات جهاز مناعي سليم وبدون عدوى (مجموعة قياسية ضابطة)
- المجموعة الرابعة: ١٠ فئران ذات جهاز مناعي مبط وبدون عدوى (مجموعة قياسية ضابطة)

وقد تم ذبح نصف عدد الفئران بعد أسبوعين من العدوى والنصف الآخر بعد ٤ أسابيع وأخذت عينات من الأمعاء الدقيقة بالإضافة إلى العينين وتم وعمل مقاطع منهم وصيغها بصيغة اهيما توكسلين والإيوسين لإجراء الفحص الهستوباثولوجي. كما تم صيغ بعض مقاطع العين بالصيغة المناعية الوميضية غير المباشرة وذلك كمحاولة للكشف عن وجود الأجسام المستضدة لطفيلي الجيارديا.

وقد أسفرت النتائج على وجود تغيرات في الشبكية في ٩ أطفال (٣. ١٢%) من ٧٣ طفلا مصابا بالجيارديا وكانت النسبة الكبرى في مجموعة الأطفال شلبيدي العدوى وكذلك الأقل سنا. أما بالنسبة للدراسة التجريبية على الفئران فقد ظهرت بعض التغيرات الهستوباثولوجية في طبقات الشبكية بعد ٤ أسابيع من العدوى وذلك في فأر واحد من المجموعة الأولى (السليمة مناعيا) و ٣ فئران من المجموعة الثانية (المبططة مناعيا) ولكن لم يظهر الطفيلي في الأنسجة وبالنسبة لاختبار الوميض المناعي غير المباشر فقد ظهرت ترسبات وميضية في الشبكية في فأرين من المجموعة الأولى و ٥ فئران من المجموعة الثانية بعد ٤ أسابيع من العدوى مما يدل على وصول الأجسام المستضدة لطفيلي الجيارديا إلى أنسجة العين والذي قد يفسر حدوث مضاعفات العين مع الإصابة بالجيارديا وفي غياب الطفيلي في العين. ولذلك لا بد أن يوضع طفيلي الجيارديا في الاعتبار عند التشخيص التفريقي لأية حالة تعاني من تغيرات في العين وخصوصا في الأطفال الصغار ذوي العدوى الشديدة.