

LOCAL AND SYSTEMIC IMMUNE RESPONSES IN RATS INFECTED WITH *GIARDIA LAMBLIA*

Entehaa Abd-Al-Zahra¹, Wafaa Sadoon Shani², Maha Khalil Al-Malak²

ABSTRACT

Objectives: This study was performed to determine the local (tissue) and systemic immune responses in rats infected with *Giardia lamblia*.

Materials and methods: *Rattus norvegicus* rats aged 2 months were inoculated with 10³ *Giardia lamblia* cyst. After 7 and 14 days of infection samples (blood and small intestine) collected from both control and infected rats. Local immune response was assessed by counting the inflammatory cells in small intestine, mast cell number and lymph node diameter. Whereas systemic immune response was carried out by measurement of total and differential WBC count, phagocytic activity of polymorphonuclear cells by NBT stain and histamine levels in blood plasma by ELISA kit.

Results: Results showed an infiltration of inflammatory cells in all parts of small intestine and an increasing in lymph nodules diameter. Also, there is an infiltration of mast cells. Results of systemic immune response revealed a high significant differences in total and differential WBC count between two studied groups. Moreover, there was an increasing in phagocytic activity of polymorphonuclear cells and histamine plasma levels.

Conclusion: Recent data indicated that there is a local and systemic immune response during acute phase of *Giardia lamblia* infection in experimental animals.

INTRODUCTION

Giardia lamblia is an intestinal protozoan parasite that causes intestinal malabsorption and diarrhea (giardiasis) in humans and other mammals worldwide.^[1] Giardiasis has been reported to mimic inflammatory bowel disease in man.^[2] It is unknown whether variation in host responses to the parasite, differences among parasite genotypes, or both, are responsible for the differences seen in clinical outcomes.^[3] *Giardia* infection in mice is usually self-limiting, which indicates the presence of an effective host defense.^[4] Immunological and non immunological factors have different levels of importance during the course of a *Giardia* infection. Immunity to *Giardia* has been shown to occur in two major phases in mice: an early B-cell independent phases in the first two weeks after infection, followed by an antibody-dependent phase.^[5,6] In addition most individuals effectively control infection within a few weeks,^[3] although the precise immune mechanisms responsible for eliminating parasites are unknown. It also remains unclear whether symptomatic disease is due to immune mediated pathology or parasite derived factors or both. So, this study was designed to analyze the local or (tissue) and systemic immune response in rats infected with giardiasis in the first and second weeks after infection especially the presence of inflammatory cells in blood and intestine mast cells and histamine levels also the

response of gut associated lymphoid tissue (GALT).

MATERIALS AND METHODS

Samples collection

Fecal samples were collected from patients with diarrhea from Al-Basrah General Hospital, Health Centers and Private Laboratories during a period between (Sept. 2008 to March 2009). All collected samples preserved in a chilled sucrose until reaching to the laboratory for examination.

Stool samples examination

The samples were examined with direct microscopical observation by using physiological normal saline to identify *G. lamblia* trophozoites and cysts.

Cysts purification and enumeration

Cystic stage of *G.lamblia* was purified with some modifications^[7-9] with some modifications.

Cysts enumerate by using neubaure chamber under oil objective lens. The sample was diluted with normal saline to (10³) cyst for each rat.

Rats inoculation

Rattus norvegicus rats were used in this study aged two months and (250–300gm) in weight. All animals were examined to be sure there is

¹MSc, Technology Institute, Basrah

² PhD, Department of Biology, College of Science, University of Basrah, Basrah, Iraq

no an infection, then divided into two groups, infected group which inoculated with (10^3) cyst according to Buret *et al.*^[10] While, control group inoculated with physiological normal saline.

Intestinal and blood samples collection

After 7 and 14 days of infection, infected and control animals were anesthetized with chloroform and blood samples were collected from heart and divided into two portions (first portion preserved with EDTA tubes and used for total and differential white blood cell count. Plasma isolated after blood centrifugation which used for histamine measurement, while (second portion) of blood preserved in heparin for study of neutrophil function or phagocytosis by using nitroblue tetrazolium test. Small intestine, (duodenum, jejunum and ileum) from control and infected animals were resected and divided into small pieces washed and fixed with formalin (10%).

Local or tissue immune response

This study include the following studies:

Rate of inflammatory cells infiltration

Inflammatory cells were counted in both infected and control animals in (7,14) day after infection. The results expressed as number of cells/mm² according to Yang *et al.*^[11] in each part of small intestine. Tissues prepared for light microscopy examination according to Luna.^[12] Specimens were fixed with formalin (10%), washed, dehydrated and then embedded with paraffin wax then sections (5 μ m) were done and stained with haematoxylin and eosin to study the local immune response.

Response of gut associated lymphoid tissue (GALT)

Diameters of lymph nodules associated with small intestine were measured as mean \pm SD in all pieces of small intestine (duodenum, jejunum and ileum) related to (15) infected rats and (10) control rats.

Counting of mast cells infiltration

Number of mast cells in each part of small intestine related to (experimental animals) was counted in (7, 14) day after infection. The mean number was counted in each (10) villi/crypt unit according to Scott *et al.*^[13], Vallance *et al.*^[14]

and McDermot *et al.*^[15] All sections were stained with specific stain (Toulidin blue).

Study of systemic immune response

This study was carried out by the measurement of total white blood cells count, differential white blood cells, the phagocytosis activity of polymorphonuclear cells (PMN) by using nitroblue tetrazolium (NBT), and measurement of histamine level in blood plasma.

Counting of total white blood cells number

The total number of white blood cells was done according to Lewis *et al.*^[15]

Differential white blood cells count

Blood smears related to infected and control animals were prepared and stained with Leishman stain according to Lewis *et al.*^[16]

Determination of phagocytosis activity of PMNs in blood

According to Murata *et al.*^[17] and Metcalf *et al.*^[18] the phagocytosis activity was determined. Blood collected from (60) infected and (30) control rats after (7,14) days post infection. 0.5 ml of blood mixed with 0.5 ml of mixture from NBT-Cl stain and Tris-Hcl solution in heparinized tubes, then transfer to incubator at (37C $^{\circ}$) for (1 hr). The specimens centrifuged and the supernatant used for absorption measurement by spectrophotometer on (515 nm) and this compare with the absorption related to control rats.

Measurement of histamine level in blood plasma

Histamin Bioassay Kit was used to measure the histamine level in blood plasma by using the method of enzyme-linked immunosorbent assay (competitive ELISA), Blood plasma which prepared from (16) infected and (10) control rats were used. The test was began by adding 50 μ l from histamine enzyme (HRP) to (50 μ l) of blood plasma in each well then incubated for 45 min at room temperature. After incubation each well washed with washing buffer, then add 150 μ l from TMB substrate and incubated at room temperature for 30 minute. The optical density (OD) was read by the microplate ELISA reader at 450 nm. Then the concentrations of histamine in studied sample were calculated by plotting a

standard curve for standards (which supplemented with a kit).

Examination and photography

All the slides were examined by light microscope (Olympus) under different magnification and photograph by microscope with digital camera.

Statistical analysis

Analysis of variance (ANOVA test) was used to

clarified the statistical differences by using minitab program version 11.^[19]

RESULTS

Immune response study

Local immune response

Rate of inflammatory cells infiltration

The study showed significant increase ($p < 0.01$) in the numbers of inflammatory cell of infected rats in comparison with control while, their number less in ileum region followed by duodenum and jejunum (Figure-1,2,3; Table-1).

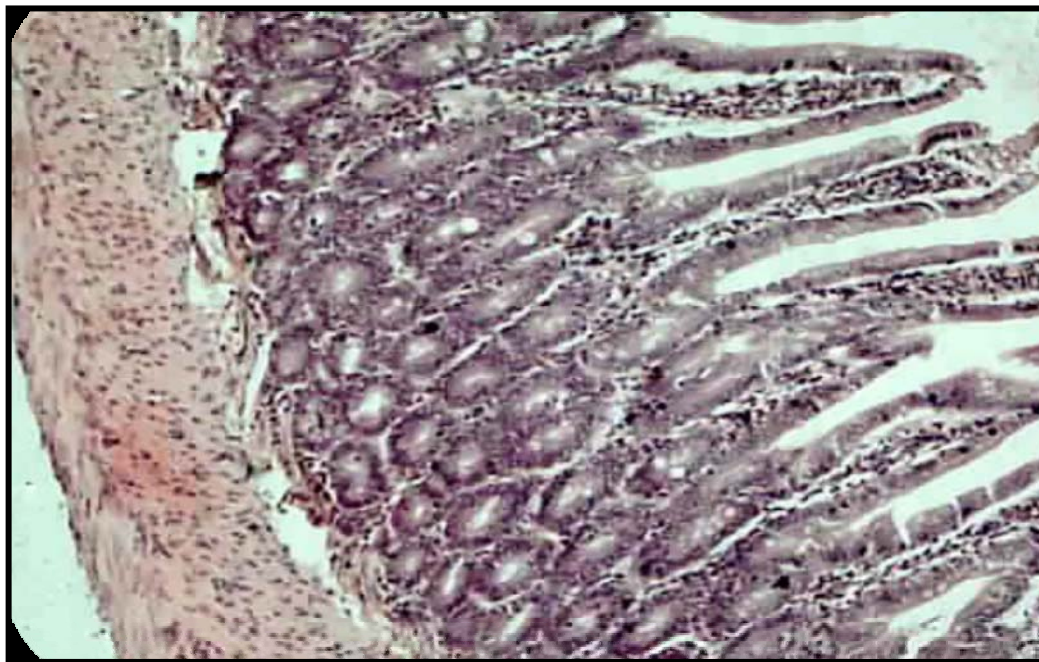


Fig 1. Section in duodenum showing dense infiltration of an inflammatory cells after (7 days) post – infection→, crypts elongation→, also showing sinternal muscular layer→ and external muscular layer. H&E stain. X (95).

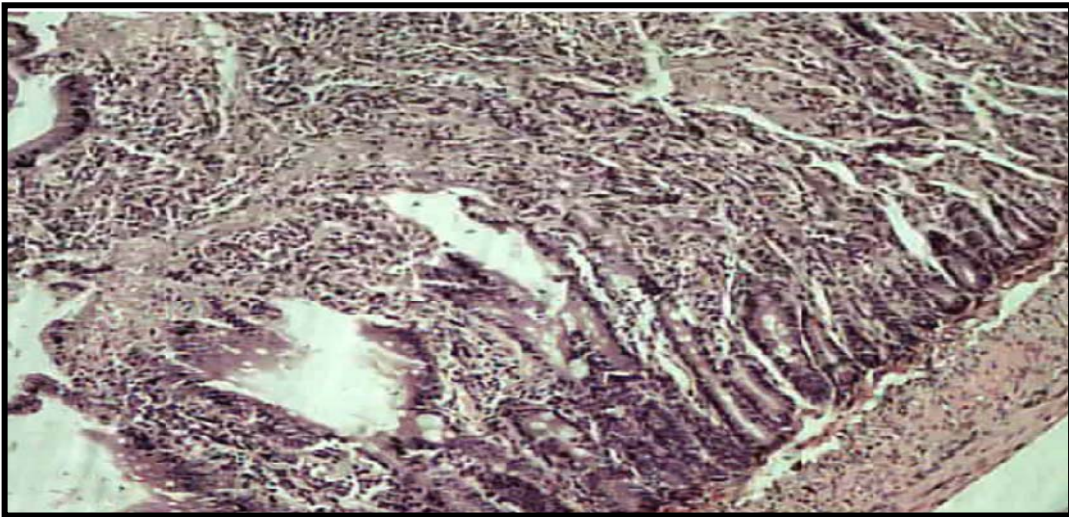


Fig2. Section in duodenum of infected rats after (14 days) clarified complete damage in villi →, dense infiltration of an inflammatory cells (→) extend between crypts, also showing internal muscular layer → and external muscular layer (→). H&E stain. X(95).

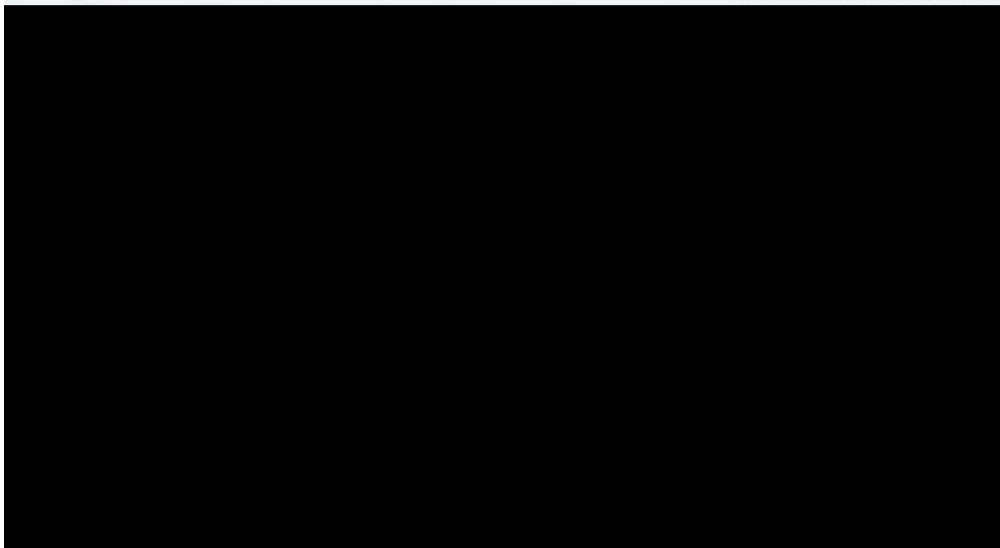


Fig 3. High power view section of jejunum villus illustrate types of inflammatory cells after (14) days post- infection, neutrophils (→), eosinophils (→), lymphocytes (→) and monocytes (→), H&E stain. X(400).

Table 1. Infiltration of neutrophils, eosinophils, lymphocytes and monocytes of infected rats with *G. lamblia* in comparison with control (values are expressed as mean±SD) on (7,14) days post infection.

Tissue type	Period /Days	Studied groups	rat numbers	Mean numbers of inflammatory cells ±SD			
				Neutrophile	Eosinophile	Lymphocyte	Monocyte
Duodenum	7	Infected	16	76.46 ± 18.90a	64.83±14.12a	199.92 ±44	4.926±7.586a
		Control	8	0.61 ±1.93b	0.61 ±1.93b	2.44±4.27b	0.00 ±0.00b
	14	Infected	16	83.93± 21.83a	74.22±15.5a	204.89±31.75a	6.445±8.730a
		Control	8	0.61 ±1.93b	0.61 ±1.93b	4.27±2.44b	0.00±0.00b
Jejunum	7	Infected	16	76.94±13.54 a	68.61±16.14a	185±4.12b	4.185±6.488a
		Control	8	0.61 ±1.93b	0.00 ±0.00b	1.83±4.12b	0.00 ±0.00a
	14	Infected	16	81.44±15.62a	73.44±20.27a	194.44±42.09a	4.24±6.50a
		Control	8	0.61 ±1.93b	0.00±0.00b	4.12±1.83b	0.00±0.00a
Ileum	7	Infected	16	71.59±25.40a	55.48 ±19.13a	161.66 ± 35.49a	2.45±4.27a
		Control	8	0.00±0.00b	0.00±0.00b	1.22±2.58b	0.00±0.00b
	14	Infected	16	79.74±26.85 a	72.56±19.32a	165.39±40.18a	2.67±5.08a
		Control	8	0.00± 0.00b	0.00±0.00b	1.22±2.58b	0.00±0.00b

- Similar letters referred no significant differences on p>0.05
- Different letters referred to significant differences on p<0.05

Mean rate of inflammatory cells reached (76.6± 18.9 cell) in duodenum for neutrophils, (199.9± 44.8) of lymphocyte cells and (64.8±14.12) of eosinophils cells after (7 day) of infection in comparison to control (Table-1). After (14 days) of infection the rate of inflammatory cells showed significant increase when compared with control rats while the monocytes number

did not show any changes in number among infected animals in comparison to control after (7,14) days post infection (Figure-3). Results of inflammatory proliferation both in jejunum and ileum related to infected rats after (7,14) days post infection revealed a significant increase in all inflammatory cells (neutrophils, eosinophils and lymphocytes) (Figure-4,a,b,c).

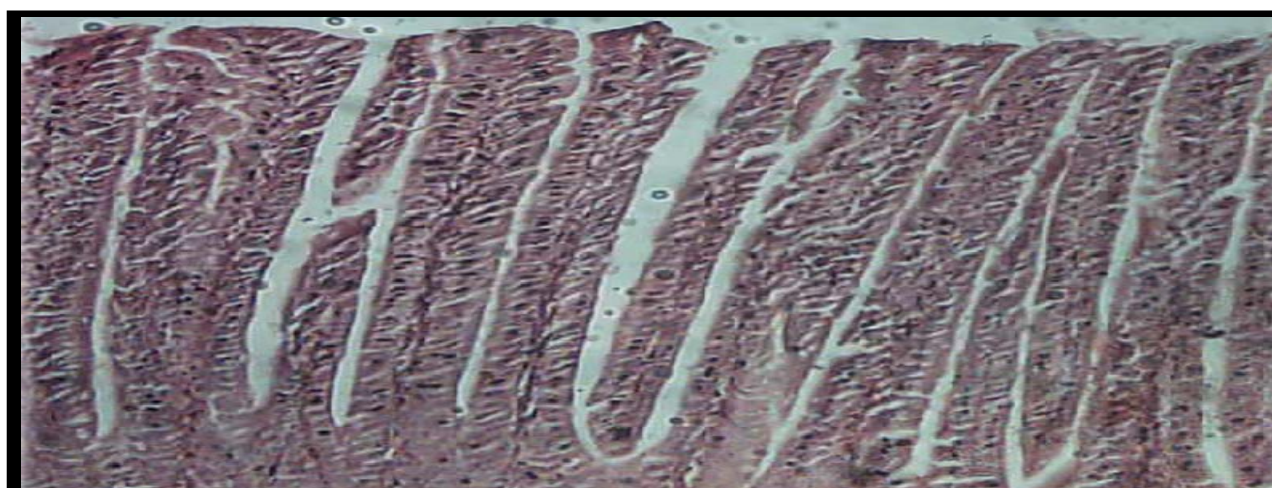
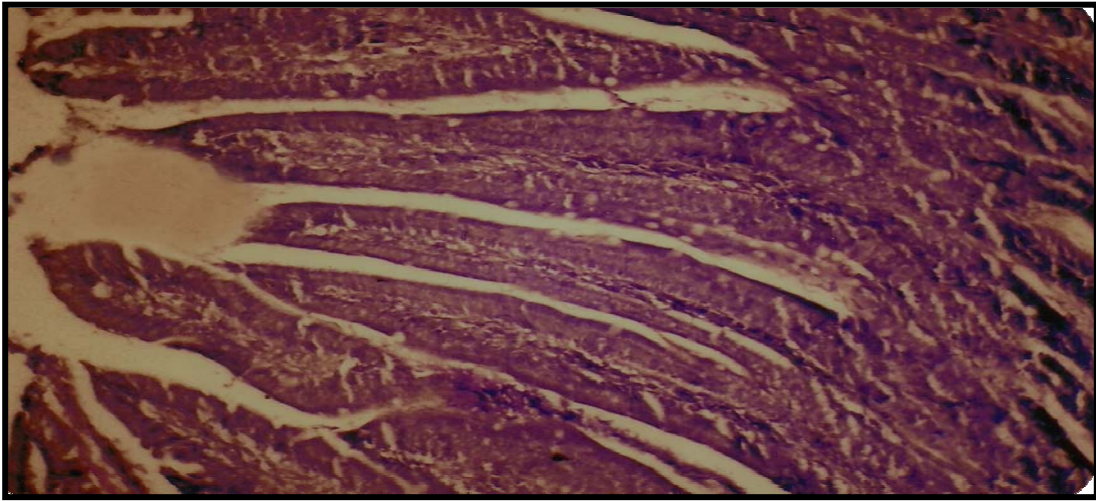
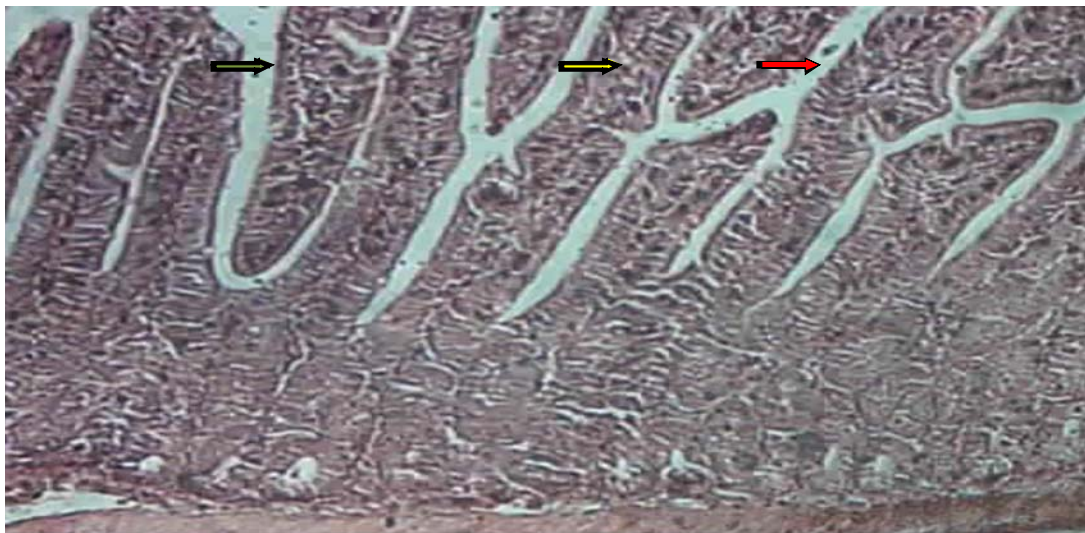


Fig 4. Small intestine of control rats showing general architecture.

A- Section in duodenum showing the mucosal layer composed of leaf shape villi (→) and crypts of leiburkuhn at the base (→). H&E stain. X(95).



B- Section in jejunum, finger like villi (→) and crypts of leiburkuhn (→). H&E stain. X(120).



C- Section in ileum illustrate short villi (→), crypts (→) and muscularis mucosa (→). H.E stain. X(95).

The study not showed any changes in various region of small intestine of control animals (Table-1).

Mean infiltration of mast cells

Results clarified that giardiasis cause an increase in rate of mast cells numbers with

significant difference ($p < 0.01$) in (duodenum, jejunum, ileum) after (7,14) day post-infection. (Figure-5, 6; Table-2) when compared with control animals which show no mast cells infiltration.

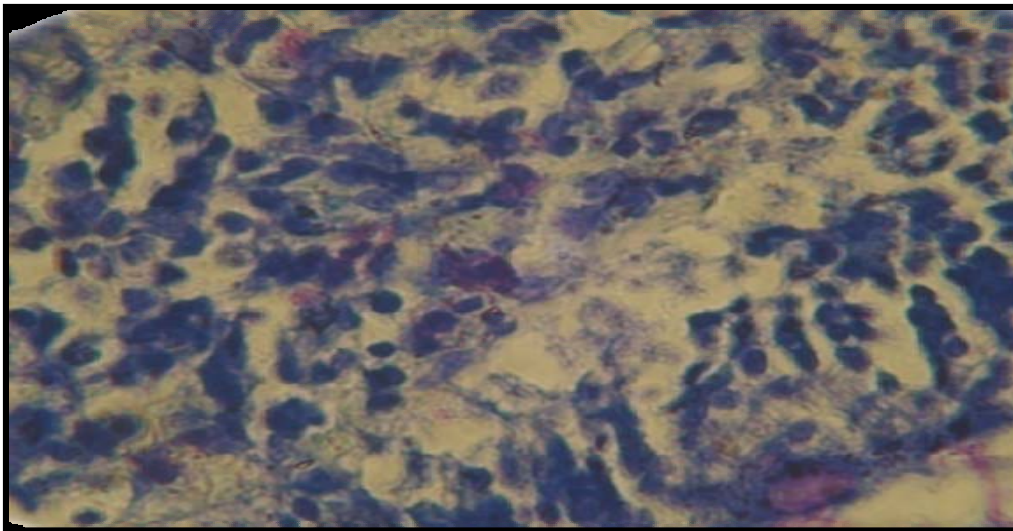


Fig 5. Longitudinal section in duodenum villus showing mast cell infiltration (→) after (14) days post infection. Toulidin blue stain. X(480).

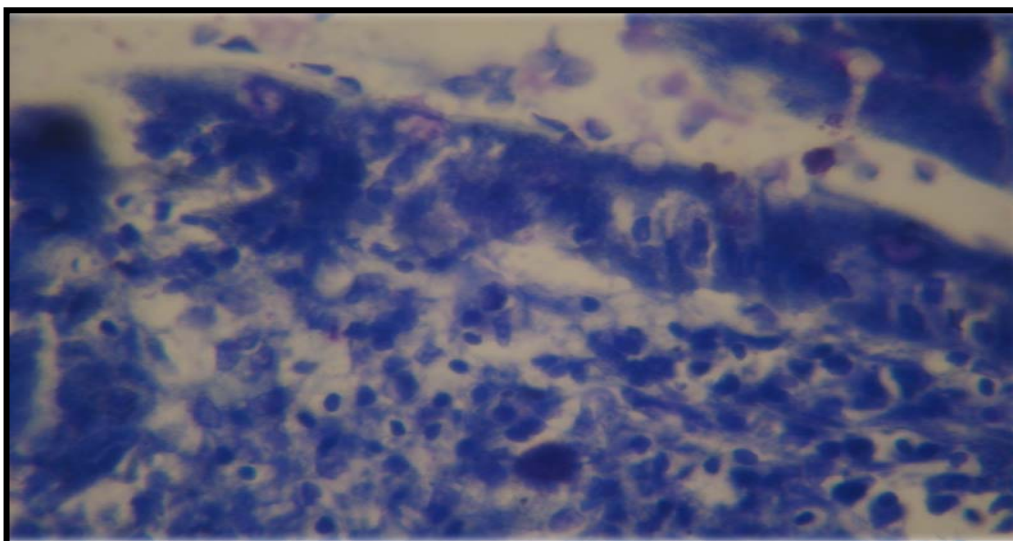


Fig 6. Tissue section in jejunum illustrate mast cells in villus connective tissue (→), the parasite well visible (→) after (14) days post- infection. Toulidin blue stain. X(400).

Table 2. Clarified mean infiltration of mast cells in an infected rats with *G. lamblia* in comparison with control after (7 and 14) days post infection.

Days	Mean number of mast cell in small intestine ± SD					
	Duodenum		Jejunum		Ileum	
	Infected	Control	Infected	control	Infected	control
Animals number	15	10	15	10	15	10
7	38.7 ±10.242 a	0.1 ±0.316 B	31.70 ±7.319 a	0 ±0 b	18.3 ±6.832 a	0 ±0 a
14	39.6 ±12.825 a	0.1 ±0.316 b	32.8 ±8.456 a	0 ±0 b	19.1 ±8.517 b	0 ±0 b

(Values expressed as mean ± SD).

- Similar letters referred no significant differences on $p > 0.05$
- Different letters referred high significant differences on $p < 0.01$

Number of these cells were more in duodenum and jejunum in comparison with their number in ileum, the mean rate reached to (38.7±10.4, 31.70±7.31 cells) in duodenum, jejunum and (18.3±6.3) cells in ileum in 7 day after infection. Also the mean rates of mast cells infiltration after 14 day post infection show the same pattern with high significant differences. The results didn't show any significant differences (p>0.05) in mean number of mast cells when

compared in infected animals after 7,14 day post-infection.

Response of gut associated lymphoid tissue (GALT)

Results of present study related to (GALT) response in intestine infected with giardiasis concluded an increasing in lymph nodules diameter (Table-3).

Table 3. Illustrate mean of lymph nodules diameter in infected animals and control after (7 and 14) day post infection (values expressed as mean ± SD).

Days	Mmean number of lymph nodules diameter (µ) in different parts of small intestine ± SD					
	Duodenum		Jejunum		Ileum	
	Infected	Control	Infected	Control	Infected	Control
Animals number	15	10	15	10	15	10
7	39.793 ±8.095 a	6.91 ±7.396 b	35.667 ±6.174 a	0 ±0 b	59.267 ±10.107 a	24.02 ±7.933 b
14	46.313 ±5.819 c	8.36 ±7.633 b	38.553 ±4.441 a	0 ±0 b	74.45 ±24.02 c	28.6 ±7.85 b

- Similar litters referred no significant differences on p>0.05
- Different litters referred to significant differences on p<0.01

The mean diameter of lymph nodule in infected duodenum is (39.74±8.095) µm and (46.31±5.819) µm in compare to control

(7.39±6.91), (8.36±7.633)µm with high significant difference (p<0.01) in 7,14 day after infection respectively (Figure-7).

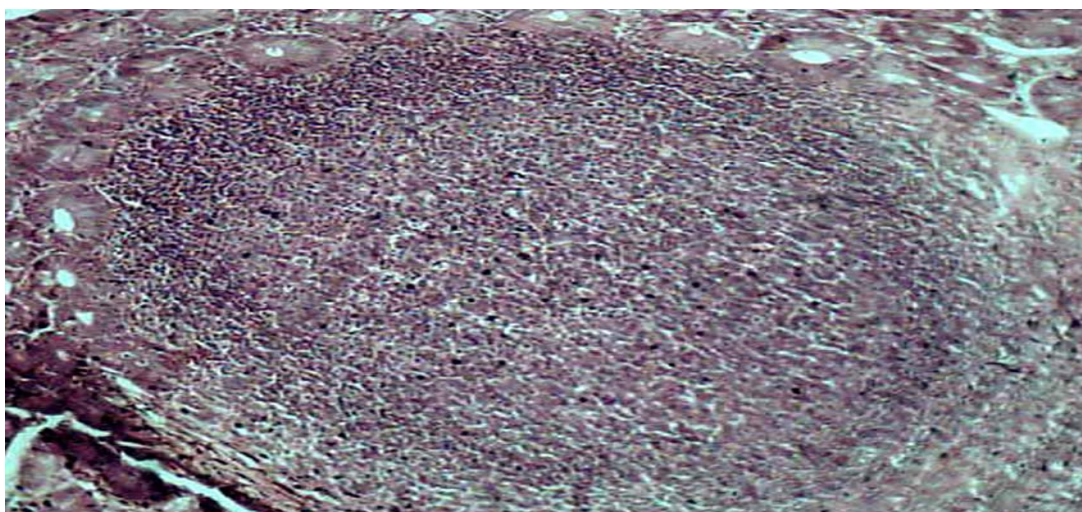


Fig 7. Photomicrograph of duodenum showing single lymph nodule composed of cortex (→) and germinal centre (→) penetrated by lymphocytes in (14 day) after infection. H&E. stain. X(95)

The infected ileum showed an increase with lymph nodules diameter reached $(74.45 \pm 24.02 \mu\text{m})$, $(59.26 \pm 10.107 \mu\text{m})$ in relation to control animals (24.02 ± 7.933) , $(28.6 \pm 7.85 \mu\text{m})$ for the same period (Table-3, Figure-8).

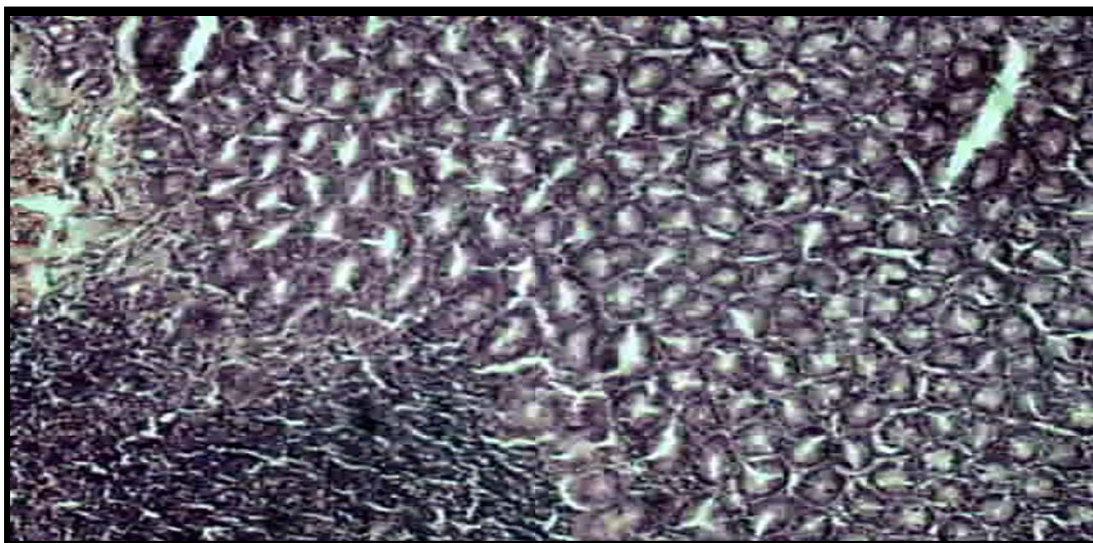


Fig 8. Section of infected jejunum showing undifferentiated lymphoid tissue as lymph node (→) diffusing through crypts (→) on (14 day) post infection. H.&E stain. X(95).

The data recorded a significant increased $(p < 0.01)$ with lymph nodules diameter at the jejunum region among infected animals when compared with control, while there were no significant differences in lymph nodule diameters between 7 and 14 days post infection (Figure-9).

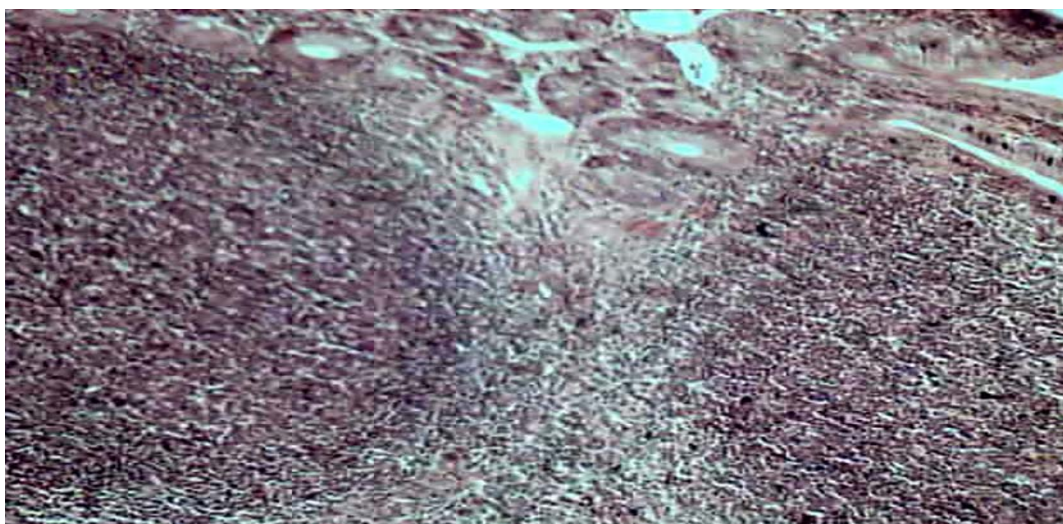


Fig 9. Section in infected ileum illustrate two lymph nodules (→) invaginated epithelial layer (→). of villus → and crypts (→). H.&E stain. X(95).

Systemic immune response

Total white blood cells count

The results showed highly significant difference ($p < 0.01$) in mean number of white blood cells in the infected animals (13265 ± 1673) and controls (6186 ± 1842) after 7 days of infection.

Also the same difference was noticed after 14 days post-infection while there was no significant difference showed when infected animals compared with each other after 7 and 14 days post-infection (Table-4).

Table 4. Illustrate mean number of total white blood cells count in infected rats with *G. lamblia* and control in (7 and 14) day post infection. (values expressed as mean \pm SD).

Days	Studied groups	No. of animals	Mean	Range	\pm SD	SE
7	Infected	15	13265 a	11150-17655	± 1673	432.076
	Control	11	6186 b	9450-4000	± 1842	555.488
14	Infected	15	14084 c	11350-19100	± 2255	582.386
	control	11	6777 d	9600-4650	± 1939	584.740

- Similar letters referred no significant differences on $p > 0.05$
- Different letters referred high significant differences on $p < 0.01$

Differential white blood cells count

Data on (Table-6) clarify an increasing percentage rate of counting cells (lymphocytes and eosinophils) which reached (59.75 ± 9.98), (6.65 ± 2.20) in infected rats after 7 days post infection in comparison with control animals. The same increase in percentage rate of these cells recorded after 14 day post infection with significant difference ($p < 0.01$). The results also

clarified the decrease in percentage rate of neutrophils and monocytes with significant difference ($p < 0.01$) in relation to control after (7, 14) days, the rate reached to (29.35 ± 9.32) for neutrophils, (1.450 ± 1.48) for monocytes after 7 days of infection in comparison to control (58.65 ± 2.39) for neutrophils and (10 ± 1.44) for monocytes (Table-5).

Table 5. Mean percentage of differential white blood cells in infected rats and control after (7,14 days) post infection. (values expressed as mean \pm SD).

Days	Studied groups	Animals number	Mean of cells percentage \pm SD				
			Neutrophils	Lymphocyte	Eosinophils	Monocytes	Basophils
7	Infected	20	29.350 ± 9.320 a	59.750 ± 9.989 A	6.650 ± 2.207 a	2.750 ± 1.482 a	0.600 ± 0.502 a
	Control	20	58.650 ± 2.390 b	31.300 ± 2.736 B	4.450 ± 1.468 b	5.100 ± 1.447 b	0.500 ± 0.513 a
14	Infected	20	17.450 ± 10.318 c	79.150 ± 11.246 C	7.550 ± 2.762 a	1.450 ± 1.572 c	0.300 ± 0.470 a
	control	20	58.650 ± 2.390 b	31.300 ± 2.736 B	4.450 ± 1.468 b	5.100 ± 1.447 b	0.500 ± 0.513 a

- Similar litters referred no significant differences on $p > 0.05$
- Different litters referred high significant differences on $p < 0.01$

There were no change in basophils number in infected animals (0.60±0.50) compare with control (0.50±0.51) after 7 days of infection. Highly significant differences in lymphocytes number showed in infected animals after (7,14) days post infection. The basophils didn't show any significant difference (p>0.5) when infected groups compared with each other on (14 days) post infection and the eosinophils didn't show any significant differences among infected animals (Table-5).

Determination of phagocytic activity of PMN in blood

Results of the study dealing with phagocytic activity of PMN cells showed high significant difference (p<0.01) in optical density rate which reflect the NBT stain reduction among infected rats (0.64±0.37), (0.735±0.28) and control (0.07±0.05), (0.076±0.07) after 7, 14 day post infection respectively. In spite there was no significant difference in absorption when compared between infected animals after 7 and 14 day of infection (Table-6).

Table 6. Optical density mean of (NBT) stain by neutrophils cells in both infected rats and control. After (7, 14 day) post infection. (values expressed as mean ±SD).

Days	Studied groups	No. of animals	Mean of optical density	Range	± SD	SE
7	Infected	60	0.640 a	0.02-1.62	±0.3773	0.048
	Control	30	0.070 b	0.002-0.2	±0.0515	9.311
14	Infected	60	0.759 a	0.07-1.54	±0.2888	0.037
	control	30	0.076 b	0.01-0.3	±0.0744	0.013

- Similar letters referred no significant differences on P> 0.05
- Different leitters referred high significant differences on P<0.01.

Measurement of histamine level in blood plasma

Systemic immune response was indicated an increase of plasma histamine level with high significant difference (p<0.01) among infected animals (1.78±0.50), (3.067±0.61) and control

animals (0.826±0.), (1.33±0.16) after 7 and 14 day of infection respectively (Table-7). The plasma histamine level related to infected animals after 14 days of infection showed high significant difference (p<0.01) comparative with first period (7days) of infection.

Table 7. Illustrate histamin level in blood plasma of infected rats with G. lamblia and control measured by ELISA technique after (7, 14) day post infection. (values expressed as mean±SD).

Days	Studied groups	No. of animals	Mean ng/ml	Range	± SD	SE
7	Infected	16	1.78 a	0.98-2.84	±0.505	0.122
	Control	10	0.826 b	0.82-0.83	±0.005	2.041
14	Infected	16	3.067 c	1.72-4.03	±0.615	0.149
	control	10	1.333 b	0.82-0.83	±0.160	0.065

- Similar litters referred no significant differences on P> 0.05
- Different litters referred high significant differences on P<0.01.

DISCUSSION

The immune response against *G. lamblia* parasite plays an important and active role during infection.^[20] The results of related studies interested in local immune response represent a significant infiltration within small intestine lamina propria and this may be due to the importance of these cells in innate and adaptive immune response against parasites (were these cells either engulf the parasite or secrete reactive substances to kill it in addition to different mechanisms participating in parasite elimination.^[21] Inflammatory cells (neutrophils, eosinophils, monocytes) infiltrated in all parts of small intestine (duodenum, jejunum, ileum) after (7, 14) days post infection and this may be related to fact that these cells considered as a first line of defense against pathogen, because they are an important source for cytokines (IL-1 β , TNF- α) which responsible for the switching on the early inflammation.^[22] Neutrophils and eosinophiles infiltration showed the same model in all parts of small intestine and in two studied period whereas monocyte didn't show the same model because their increasing don't revealed any significant differences in comparison with control group and the most acceptable explanation that the infection still acute, and this is similar to Kaplan *et al.*^[23] whom indicated the phagocytic activity of these cells with the aid of antibodies. Activation of inflammatory cells (Neutrophils, eosinophils) in acute phase represent a most important event during a defense process whereas the IL.8 play a significant role in this activation and attraction of neutrophiles to damaged area of small intestine.^[24] Highly significant infiltration of lymphocytes was also illustrated in all studied parts of small intestine infected with giardiasis after (7,14) days post infection. Some result was also indicated by Vinayak *et al.*^[25] when they recorded heavy lymphocyte infiltration in lamina propria. Moreover Biez^[26] confirmed the T. cells and IL-6 action (during first two weeks of infection) in *Giardia* expulsion and elimination of infection. Recent work showed mast cells accumulation in infected small intestine and this clearly demonstrate an importance of these cells in elimination and control of infection. The presence of parasite act as an activator of mast cells which then lead to degranulation of their granules and with a mast

cell dependent process the cholycytokinin was secreted resulting in smooth muscle contractility, then coupled contractions with nitric oxide-mediated muscle relaxation, promote intestinal transit and parasite elimination.^[3] D'anchino *et al.*^[27] and Zhou *et al.*^[28] were also recorded the importance of mast cells during giardiasis because these cells are a source of histamine, leukotriens and serotonin which facilitate the *Giardia* and helminthes expulsion.^[29] Our data reffered to an increasing in lymph nodules diameter (which is a compartment of GALT) in an infected rat in comparison with control group and this may be suggested that there is an induction of cellular inflammatory response because these nodules are the source of T-cells.^[30] In addition, to stimulation of local immune response which was proved in present study, systemic immune response takes place and was documented as a significant increase in leukocytes total number in all experimental animals compared to control group. This give an indicator to cellular immune response against circulating antigens which released from parasite during acute infection and this result is more parallel with the proved thought which indicate the importance of cellular immune response during the first two weeks of infection or (acute infection) in comparison with the humoral immune response which present during the chronic phase of giardiasis.^[31] The same results also recorded by Al-Kubassi^[32] during *Entamoeba histolytica* infection. Data of differential WBC count was clarified a significant decrease ($P < 0.01$) in neutrophils and monocytes number in all infected groups in comparison with control group and this results may be occurred as a result of cell filtration and migration from blood to an infected area (lamina propria and mucosal layer). These results also agreed with Chadee and Meerrvitcho^[33] Boirivant *et al.*^[34] clarified the role of these cells in immunity against *E.histolytica* and their ability to kill the vegetative phase of the parasite, in addition to its toxic activity and lymphokines production which share in events of immune response. An increase with significant difference ($P < 0.01$) in eosinophils number in all infected rats in comparison with control group was recorded. This result resembling with study done by Al-

Kubassi^[31] on amoebiasis patients because these cells effective against parasite. Basophilic cells did not show any changes in their number in all infected rats (after 7 and 14) day post-infection and this related to their role through chronic infection. Phagocytic activity is other studied an important factor which support the systemic immune response and was done by polymorphonuclear cells (PMN), that ingested the vegetative phase *in vitro*, present study revealed the significant phagocytic activity in all infected rats, at (7,14) day post infection. These results are acceptable with other studies suggested that his activity act as defense mechanism against the parasite.^[35] Measurement of phagocytic activity of polymorphonuclear cells in blood was determined by NBT stain reduction and this results agreed with Hill and Phol^[36] who used the NBT stain reduction and formazan granules formation as an indicator for oxidative burst. The study also indicated an increasing of histamine level in blood plasma as a marker to systemic immune response by using ELISA test, which showed high significant difference in histamine level in all rats infected with giardiasis in comparison to control group, this due to mast cell increasing which is proved in recent work because the histamine which secreted from mast cell represent as an important factor in intestinal canal^[37] and increased the blood vessels permeability^[38] which in turn increase cells migration to infected region especially neutrophils and monocytes which proved in recent results through an elevation in number of inflammatory cells in lamina propria and at the same time decreasing of its number in blood stream (specially neutrophils and monocytes). Serna *et al.*^[39] was illustrated that intestinal movement increased after treatment with histamine and this lead to parasite expulsion and controlling the infection. Elevated level of histamine which represent as an indicator to mast cells response may be shared in giardiasis pathophysiology in addition to its participating in parasite expulsion.^[40] Our data referred to an increasing in histamin level after (14) days post infection more than 7 days post infection and this is the time of mast cells activity increasing which recorded in this study which clarified the

important of mast cells and histamine in parasite expulsion in order to control the infection.

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