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THE EFFECT OF TUMOR NECROSIS FACTOR ALPHA ANTAGONIST (ADALIMUMAB) ON GIARDIASIS IN MICE

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Abstract

Giardia lamblia is the most common worldwide intestinal protozoan infection. TNF-α has an important role in early giardiasis control. TNF-α antagonists had been used in the past few years to treat many inflammatory diseases as RA, ankylosing spondylitis, and Crohn's disease. This study aimed to investigate the effect of TNF-α antagonist (Adalimumab®) on the pathology and the outcome of giardiasis in mice. A total of 45 laboratory-bred Swiss albino mice were divided into 2 groups: tested group and control group. Tested group was *G. lamblia* infected, then treated with Adalimumab and subdivided into 3 subgroups (GIa, GIb, & GIc) according to given doses (1.5, 3, & 6mg/kg/BW respectively). Control group was subdivided into 2 subgroups, *Giardia* infected control and normal control (not infected or treated). Infection was assessed by successive stool analysis for cyst count and trophozoite count in intestinal wash, and histo-pathological changes in small intestinal sections. Serum TNF-α level was evaluated by ELISA.

The results showed that highest mean serum TNF-α in infected control on 16th dpi compared to normal control and GIb & GIc. Mean serum TNF-α didn't not show significant differences in tested group compared to control on 28 dpi. Peak of cyst count was on 15th dpi in tested group and infected control. The highest cyst count was in GIb (3mg/kg/BW). Cyst count was significantly higher in tested group compared to infected control to the experimental end. Number of trophozoites in intestinal wash in tested group was significantly higher than in infected control on 28th dpi. The intestinal sections of tested group showed moderate to severe inflammatory reaction with more inflammatory cellular infiltration in the lamina propria causing villous shortening and blunting more than in the infected control. The lymphoid follicles and sloughing of the epithelium were only in test subgroups on 16th dpi.

Keywords: *Giardia lamblia*, Adalimumab, TNF-α antagonist, ELISA, Trophozoits & cysts count, histopathology.

Introduction

Giardiasis is a diarrheal disease caused by the microscopic parasite *Giardia doudenalis or lamblia* (or *"Giardia"* for short). Once a person or animal has been infected with *Giardia*, parasite lives in intestines and is passed in stool (poop), but once outside the body, it can sometimes survive for weeks or even months (CDC, 2021). More than 300 million cases were reported annually and it was considered the third most common cause of diarrhea among children ≤5 years old after Rotavirus and *Cryptosporidium* (Lanata *et al*, 2013). In Egypt giardiasis prevalence was up to 27.3% among symptomatic children with chronic diarrhea (Taha *et al*, 2018). Human infections are mainly by the *Giardia* assemblages A and B, with rare cases of assemblages E and C (Mohamed *et al,* 2020).

Both innate and adaptive immunity play role in controlling infection and most of the people living in endemic areas are less prone to reinfection (Saghaug *et al*, 2016). This suggested that acquired immunity exists (Lujan and Svard, 2011). Interleukins (IL-6, IL-8, & IL-10) and tumor necrosis factor (TNF-α) are hormone like polypeptides, secreted by multiple cells as macrophages, lymphocytes and monocytes, they regulate the inflammatory response to parasitic infection (Ahmed *et al*, 2015).

TNF-α and IL-6 are pro-inflammatory cytokines needed for clearance and early giardiasis control (Zhou *et al*, 2007). TNF-α' s antitumor, antiviral and anti-parasitic effects

are mainly, through apoptosis, cell activation, induction of cytokines and induction of cell recruitment to the infection site (Johnston and Conly, 2006). TNF-α plays an important role in determination of the parasitic load of *G. lamblia* and duration of infection (Saghaug *et al*, 2016), its serum level increased significantly in acute infection i.e. less than 8 weeks (Ahmed *et al*, 2015). Its absence in common variable immune deficiency (CVID) is associated with increased risk for chronic infection (Weatherhead *et al*, 2017). Multiple TNF-α antagonists are used widely in the treatment of autoimmune diseases. Adalimumab® (Humira, trade name), is a recombinant human monoclonal antibody specific only to TNF-α (Johnston and Conly, 2006). It was approved by US/FDA in treatment of rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, ulcerative colitis and Crohn's disease (Ali *et al*, 2013). However, its use increased the risk of bacterial, viral and parasitic infections (Ali *et al*, 2013). TNF-α antagonists has been suggested to increase disease activity when given to patients with opportunistic infections as toxoplasmosis (El-Sayed *et al*, 2016) and reactivated latent tuberculosis (Sfikakis, 2010). Also, *G. lamblia* was reported to cause acute acalculous cholecystitis in a patient on regular Adalimumab therapy for rheumatoid arthritis (Araki *et al*, 2017).

The present study aimed to evaluate the effect of TNF-α antagonist, Adalimumab® (ADA) on the pathology and the infection outcome of giardiasis in mice.

Materials and Methods

The study was carried in Department of Parasitology, Faculty of Medicine, Ain Shams University, and Animal house of Theodor Bilharz Research Institute (TBRI), Giza, from November 2020 to August 2021.

Parasite: *G. lamblia* cysts were collected from patients' stool samples attended outpatient clinic of Pediatric Hospital, Ain-Shams University Hospitals.

Mice: Laboratory-bred Swiss Albino male mice, aged 5 weeks old, weighted 10-15gm each purchased from TBRI. They were housed in polycarbonate cages with paper bedding, 2 to 3 mice per cage (Visvesvara *et al*, 1988), maintained at 25°C, with a relative humidity of 40-60% and under a 12hr. light/dark. The stools of mice were examined by wet mount and Lugol's iodine-staining to exclude any infected one (Garcia, 2016).

Experimental giardiasis in mice: Viable *G. lamblia* cysts were concentrated using normal saline and centrifuged at 2,000rpm for 5 min. at 4°C several times. Mice were infected orally by gavage tube by 1.0×10^{6} cysts in 0.2ml 85% Normal saline solution aft-er an overnight starvation (Zhou *et al*, 2007).

Mice were divided into two main groups: GI (Tested): 30 mice, TNF-α antagonist (ADA) treated, subdivided into 3 subgroups of 10 mice each according to drug dose into: GIa, GIb & GIc, they received 4 equal doses of 1.5, 3 & 6mg/kg/BW/dose respectively. GII (Control): 15 normal mice and subdivided into two subgroups: GIIa (normal control): 5 mice, neither infected nor treated and GIIb (infected control): 10 mice infected, but not-treated.

Drug: Adalimumab (Humira®, AbbVie Laboratories), a monoclonal anti-TNF-α antibody in a prefilled syringe containing 40mg Adalimumab in 0.8ml. Each mouse in the test subgroups received 4 equal intraperitoneal injections of the drug in special concentrations started from 6th day post inoculation (dpi) with 3 days intervals in between and last dose was on 15th dpi (De La Cámara *et al*, 2015).

Blood was collected from mice orbital sinus (Hoff, 2000) on 16th & 28th dpi for quantitative detection of TNF-α in mice serum by quantitative Sandwich ELISA (Mouse TNF-α ELISA Kit Bioassay Technology Laboratories Cat. No E0117Mo, Shanghai/China).

Fresh fecal pellets from each mouse in all groups were collected on intermittent days from 6th dpi to 28th dpi, to count *G. lamblia* cysts. On 28th dpi small bowel was removed from all sacrificed mice, duodenum and proximal jejunum of each mouse were process-

ed to release trophozoites, mean number of viable ones in 1ml of intestinal wash by the following equation:

$\frac{A \times 10.000}{4}$ A= trophozoites no. in 4 big squares.

On 16th dpi, 2 mice from each group, and on 28th dpi remaining ones 1cm segments were dissected out aseptically, from the small intestine upper part, fixed in 10% formol saline and processed for paraffin sectioning and staining with hematoxylin and eosin (Abdel-Bary *et al,* 2012) and microscopy examined for *G. lamblia* cysts, trophozoites and histopathological changes.

Statistical analysis: Data were collected data, tabulated and analyzed by using Statistical package for Social Science (SPSS) version 20.0. Quantitative data were presented as mean ±SD, while Qualitative ones were expressed as frequency and percentage. Statistical significance was determined by Student "t" test for differences between two groups, Chi-Square test examined the relationship between two qualitative variables and Fisher's exact test was used when expected count between two qualitative variables was <5 in more than 20% of cells. A P-value below 0.05 & 0.01 indicated significant and highly significant differences, respectively.

Ethical considerations: The study was done according to regulations of the Egyptian Ministry of Higher Education, and the ethical committee of the Faculty of Medicine Ain Shams University, which went with the Helsinki Guidelines (2008), when dealing with experimental animals.

Results

Serum TNF-α level: On 16th dpi infected control (GIIb) showed higher serum TNF-α levels than in non-infected ones (GIIa) with highly significant difference (P= 0.001). Infected mice received TNF-α antagonist in different doses (GIa, GIb & GIc); 1.5, 3 & 6 mg/kg BW, mean serum TNF-α levels were higher than in GIIa with significant difference than in GIa & GIb, and lower than in GIIb with significant difference in GIb & GIc. On 28th dpi serum TNF-α level in infected control (GIIb) decreased, but still higher than in GIIa without significant difference. Infected mice received ADA in different doses (GI), mean level of serum TNF-α in (GIa), was non-significant and slightly higher, but without differences in GIb & GIc, when compared to GIIa. But, serum TNF-α level in tested groups (Ia, Ib & Ic) were low without significant difference than in GIIb, or in mean values of serum TNF-α in them, either on 16th or 28th dpi.

G. lamblia cysts were first detected in stools on 5th dpi and counting started on 6th dpi in all infected mice. In normal control (GIIa), no cysts were in stools till the experimental end. In control infected (GIIb), cyst count increased to reach a peak on 15th dpi, then started to decline to reach least value on 28th dpi. All tested mice had peak cyst count in stool on 15th dpi. The count in GIa was slightly higher, but without significant difference than in GIIb on 6th (P= 0.976) and 28th (P= 0.203) dpi, otherwise mean counts were higher with significant (9th & 21st dpi) and highly significant (12th, 15th & 18th dpi) differences versus GIIb**.** The highest cyst counts were recorded in GIb with either significant (9th & 12th dpi) or high significant differences on other days**.**

Trophozoite in intestinal wash: **T**rophozoites numbers in tested group were greater than in infected control (GIIb) with highly significant differences (<0.001). Trophozoite numbers in intestinal wash of all tested mice showed non-significant slight increase in numbers in GIb.

Histopathological examination of duodenal and proximal jejunal sections of normal control on 16th & day 28th dpi showed normal architecture and mucosa with well-formed columnar epithelial lining, arranged finger-like villi giving the brush border of the intestine normal appearance, preserved and normally distributed goblet cells and typically normal intestinal crypts. On 16th dpi, intestinal sections of infected control (IIb) showed moderate inflammation with inflammatory cells infiltrating lamina propria causing expansion and widening of villous core compar-

ed to moderate to severe inflammatory reaction with more inflammatory cellular infiltration in lamina propria causing villous shortening and blunting with expansion of villus core in all infected treated mice. Inflammatory changes were moderate in mice of GIa & GIc, but severe in GIb. Also, sloughing of mucosa was recorded in all infected treated mice, but lymphoid aggregation and follicles were only in GIb. Neither mucosal sloughing nor lymphoid aggregation or follicles were in control infected GIIb.

Pathological changes were more common and severe in tested mice treated with TNF-α antagonist than in infected control, changes were more severe in GIb received 3mg/ Kg/BW. In control infected mice, trophozoites were in lumen of small intestine in few numbers (+), in 50% and moderate intensity (++), in 50% of infected mice. No intense presence of trophozoite (+++) was recorded in these mice compared to tested ones that trophozoites were moderately (++) in intestinal sections of GIa and excessively present (+++) in GIb & GIc.

On 28th dpi (experimental end), mice were euthanized. Infected control showed various inflammation degrees. One of seven mice had neither inflammation, nor villous changes or sloughing and no trophozoite in lumen. Five of seven showed mild inflammation with mild villous changes and sloughing and one mouse showed moderate inflammation with more infiltration cells causing more villous shortening with blunting and more expansion in intestinal villi core lacking finger-like appearance. Superficial ulceration and sloughing were mild in 5 of 7, but moderate in one. The *G. lamblia* trophozoites were common in the intestinal lumen of all mice, except one was without lymphoid follicle in GIIb.

In infected treated, intestinal sections showed severe villous changes in 62.5% in Ib, but mild and moderate changes were in others. Epithelial sloughing was in Ia (71.25%), followed by Ic (25%) then (14.28%) in IIb. Pathological lesions marked decreased on the 28th dpi in all mice, which didn't receive the TNF-α than in mice that received drug.

Details were given in tables (1, 2 &3) and figures (1, 2, 3, 4, 5, 6 & 7).

Table 1: Mean serum TNF-α level ±SD in ng/L in tested and control groups on 16th & 28th dpi by Sandwich ELISA.

Date	Tested group			Control group		Student t-test *P*-values		
dpi.	GIa	GIb	GIc	GIIa	GIIb			
16th dpi	800 ± 226.27	480 ± 113.14	230 ± 70.71	43 ± 38.18	1260 ± 28.28	IIa vs. IIb= 0.001** IIa vs. Ia= 0.043 * IIa vs. Ib= 0.035 * IIa vs. Ic= 0.081	IIb vs. Ia= 0.104 IIb vs. Ib= 0.011* IIb vs. Ic= 0.003**	Ia vs. Ib = 0.216 Ia vs. 1c = 0.077 Ib vs. Ic = 0.118
28th dpi	64.29 ± 17.18	69.75 ± 98.23	74.25 ± 53.07	66.67 ± 15.28	164.86 ± 183.84	IIa vs. IIb= 0.398 IIa vs. Ia= 0.842 IIa vs. Ib= 0.959 IIa vs. Ic= 0.818	IIb vs. Ia= 0.175 IIb vs. Ib= 0.255 IIb vs. Ic= 0.204	Ia vs. Ib = 0.881 Ia vs. Ic = 0.644 Ib vs. Ic = 0.911

Dpi= Day post- infection, vs.: versus, *significant difference, P <0.05, **highly significant difference, P<0.001.

Table 2: Fecal cysts count / HPF on intermittent days pi. (dpi) in stools of infected control and tested groups.

Doses of TNF-α given	Fecal cysts count dpi	Normal control	Infected control	Tested group			Student t-test *P value*					
		GIIa	GIIb	GIa	GIb	GIc	IIb vs. Ia	IIb vs. Ib	IIb vs. Ic	Ia vs. Ib	Ia vs. Ic	Ib vs. Ic
		Mean ± SD										
1st dose	6th dpi	0	13.8 ± 1.32	13.78 ± 1.86	20.2 ± 1.69	17.1 ± 2.47	0.976	<0.001 **	0.002 *	<0.001 **	0.004 *	0.004 *
2nd dose	9th dpi	0	16 ± 1.83	19.11 ± 3.22	22.3 ± 1.64	19.3 ± 1.64	0.018 *	<0.001 **	<0.001 **	0.013 *	0.872	0.001 **
3rd dose	12th dpi	0	16.33 ±2.0	20.67 ± 3.28	24 ± 1.15	20.2 ± 1.48	0.004 **	<0.001 **	<0.001 **	0.008 *	0.689	<0.001 **
4th dose	15th dpi	0	26 ± 2.12	31.78 ± 2.33	39.7 ± 2.58	34.6 ± 2.63	<0.001 **	<0.001 **	<0.001 **	<0.001 **	0.025 *	<0.001 **
	18th dpi	0	19.89 ±1.9	30.33 ± 2.18	34.9 ± 2.18	31.1 ± 1.79	<0.001 **	<0.001 **	<0.001 **	<0.001 **	0.412	<0.001 **
	2st dpi	0	10 ± 2.16	12.86 ± 2.34	20.13 ± 2.03	15.25 ± 1.67	0.035 *	<0.001 **	<0.001 **	<0.001 **	0.038 *	<0.001 **
	28th dpi	0	5.71 ± 2.29	7.71 ± 3.2	15± 1.69	10.5 ± 2.07	0.203	<0.001 **	0.001 *	<0.001 **	0.063	<0.001 **

Table 3: Number of trophozoites x10^4 /ml in intestinal wash on 28th dpi

Variants	Control group		Tested group			Student t-test. *P*-value					
	GIIa	GIIb	GIa	GIb	GIc	IIb vs. Ia	IIb vs. Ib	IIb vs. Ic	Ia vs. Ib	Ia vs. Ic	Ib vs. Ic
Trophozoites No.	0.0	1.71 ± 1.5	7 ± 2.16	8.75± 2.71	7.5 ± 3.42	<0.001**	<0.001**	0.001**	0.195	0.745	0.432

*= Significant, **= highly significant, D= day, p.i. = post infection, SD= standard deviation, *vs= versus.*

Discussion

In the present study, 1×10^6 cysts were inoculated orally using the gavage tube. This agreed with Abd Al-Khaliq (2019); Li *et al*. (2020), but lower doses of 1×10^4 & 1×10^5 cysts were used by Dyab *et al*. (2016) and Al-Megrin *et al*. (2021). Roberts-Thomson *et al*. (1976) reported that cyst inoculum size affected the maximal trophozoite and cyst counts and that large inoculum increased the antigenic stimulation earlier in infection causing earlier parasite elimination.

In the present study, *G. lamblia* infection was demonstrated on the 5th dpi and ADA administration started on the next day. Zhou *et al.* (2007) started anti TNF-α administration on first infection day. Also, the present study showed that the time of drug administration coincided with the peak of trophozoites in the small intestine. This agreed with Mahmoud *et al*. (2014), Li *et al*. (2017) and Al-Ghandour *et al*. (2020).

In the present study, in normal control serum TNF-α was 43±38.18 & 66.67±15.28 ng/L on 16th & 28th dpi, respectively, ranged between 5 to 81ng/L on 16thdpi, & 51 to 81ng/L on 28th dpi. This might be due to an acquired slight infection or by long experimental time. This more or less agreed with 40pg/ml by Ore *et al*. (2020), equivalent to the present study. Li *et al*. (2021) reported significant change in TNF-α level on 12th dpi in *G. lamblia* infected mice.

In the present study, on 16th dpi, serum TNF-α level in infected mice was significantly higher than control. This agreed associated elevation of IL-6 & IFN-γ in giardiasis infected mice (Abd-Elhamid *et al*, 2021; Holthaus *et al*, 2022). Muñoz-Cruz *et al*. (2018) reported the TNF-α role in the early control of giardiasis, by significantly released by mast cells on infection time. Mahmoud *et al*. (2018) found that peak up-regulation of gene expression of TNF-α in intestinal tissue was 7th dpi. Zhao *et al*. (2022) showed that mRNA levels of TNF-α, IL-1, & IL-6 transcription and protein expression were significantly higher in giardiasis than controls. In humans TNF-α was significantly elevated in *G. lamblia* patients compared to control (Hussein and Shakir, 2014; Ahmed *et al*, 2015; MatowickaKarna *et al*, 2009). In *G. lamblia*-infected patients there was more TNF-α expressing cells than healthy ones, and derived antigens boosted TNF-α production (Saghaug *et al*, 2016).

In the present study, on 6th dpi, serum TNF-α level in tested mice were significantly higher than in normal control. Meanwhile, it was lower in tested mice by TNF-α antagonist ADA action compared to infection control. This may be due to the ADA blocking effect, as TNF-α neutralized cytokine bioactivity and apoptosis in TNF-α expressing mononuclear cells decreased serum level (Vena and Cassano, 2007). Also, De La Cámara *et al*. (2015) reported that the ADA acted on the molecular level and decreased up regulation of TNF-α genes.

In the present study, while the serum TNF-α level was lower on 16th dpi in tested mice than in infected control by more cysts in stools. This agreed with Zhou *et al.* (2007) who reported that *Giardia*-infected mice by anti-TNF-α, trophozoites in small intestines were more than 10-fold greater on 5th dpi than in control that was eliminated by 12th dpi, treated mice had parasite load up to 28th dpi.

In the present study, TNF-α serum levels decreased in test group on 28th dpi. as in infected control group. This agreed with Zhou *et al.* (2007), they found that TNF-α was important mainly during the early phase of infection, and that immunological effector mechanisms led onwards to clear infection. Also, Mahmoud *et al.* (2018) found that in giardiasis TNF-α gene express- ion was up-

regulated till 14th dpi and down-regulated at subsequent time periods starting from 21st to 28th dpi.

In the present study, stool examination of mice showed first appearance of cysts on 5th dpi and count increased with peak on day 15th, then, declined gradually till 28th dpi in infected and control mice. This agreed with Jiménez *et al*. (2014); Mahmoud *et al*. (2018); Abo-Zaid and Hamdi (2022). Shukla and Sidhu (2011) reported an earlier peak of cyst excretion on 7th dpi, after gradual decline and disappear on 29th dpi, but mal-nourished mice had a higher parasitic load with peak cyst excretion on 11th dpi, but became parasite free on 48th dpi. Chen *et al*. (2013) reported that peak and clearance of infection were on the 6th & 21st dpi, respectively. This may be due to differences in genotype, infective dosage, and animal model type.

In the present study, significant higher cyst counts were detected in *G. lamblia* infected mice that received ADA in most stool samples examined on alternate days compared to infected untreated mice. Increased cyst counts coincided with decrease in serum TNF-α level. This indicated lowering in TNF-α serum level used its antagonist caused flourishing of parasitic infection. Also, El-Sayed *et al.* (2016) reported that reactivation of latent toxoplasmosis occurred in a mice model where Etanercept caused increase in frequency and size of cysts in brain. De Almeida *et al.* (2022) reported that a 62 year old RA patient developed cerebral toxoplasmosis after ADA initiation, which was chronically treated with other non-biologics. Serradell *et al*. (2018) reported that trophozoites peak was on 7th dpi. followed by gradual decrease till 28th dpi, but Chen *et al.* (2013) found that trophozoite diminished on 14th to 2st dpi.

In the present study, intestinal wash for trophozoites was done on 28th dpi to assess TNF-α antagonist on giardiasis. Yordanova *et al.* (2021) in Germany reported that for parasitic load, contrasting other reports on overt small intestinal Th17 activity in eosinophil-deficient mice, IL-17A production was checked in the absence of eosinophil during giardiasis. Abo-Ziad and Hamdi (2022) reported that trophozoites and cysts significantly increased in infected mice before diminished after 28th dpi, and Th1 & Th2 immune responses protected against giardiasis.

In the present study, a high intestinal trophozoite count was in infected mice, with significant difference as compared to infected control that still existed in the 28th dpi. This may be explained by lowering serum TNF-α by ADA in the first two weeks of infection led to its flourishing and persistence in animals till later time than in non-treated ones. The tested (GIb) showed highest cyst count among others. Shortening of intestinal microvilli in a lymphocyte-mediated manner in giardiasis deplete activity of disaccharidases and other digestive enzymes caused indigestion and mal-absorption of nutrients (Halliez and Buret, 2013). The depletion of bile salts consumed by trophozoites caused delay in micellar solubilization of fats in small intestine and inhibited pancreatic lipase, as bile salts has hydrolytic effects on ingested lipid (Kelly, 2014) caused mal-absorption and weight loss in patients (Buret *et al*, 2015).

In the present work, sections of proximal part of mice small intestine on days 16 & 28 PI showed that giardiasis induced pathological effect on receiving TNF-α antagonist (ADA). On 16th dpi more destructive changes occurred than on day 28th dpi. This high degree of inflammation and intestinal pathological changes in ADA treated mice implied the crucial role of TNF-α in protection against pathological changes (Paranjpe *et al*, 2016). Decreased goblet cells number and lamina propria lymphonuclear cell infiltration were reported (Shukla *et al*, 2012; 2016). Also, Abd-Elhamid *et al*. (2021) found that intestinal pathological changes in *G. lamblia* infected hamsters caused villi shortening, shedding, and desquamation .

In the present study, on 16th dpi trophozoites were moderately to excessively present in intestinal sections of infected mice. But, in infected control, trophozoites were in lu-

men of small intestine in few to moderate numbers without intense presence in mice. This might be explained as a non-qualitative response to gastrointestinal mucosal irritation brought on by trophozoites adhering or cytotoxicity effects. Besides, abundance of bacteria coupled with fungi and cytokines involved in the inflammation of small intestine in *G. lamblia* infection may also play a role. One of the factors that affect intestinal pathology during giardiasis was intestinal microbiota as causing low-dose IFN-γ or TNF-α stimulation that internalized nonpathogenic bacteria and endocytosed by epithelial cells (Chen *et al*, 2013). Abd-Elhamid *et al*. (2021) reported that decreased TNF-α level in *G. lamblia* infected mice was associated with better outcome in intestine with significantly reduced cytes and normal villi. El-Kady *et al.* (2021) on giardiasis infected rats found that high TNF-α levels caused intestinal villi's destruction and inflammation.

Conclusion

TNF-α antagonist (ADA) administration to *Giardia lamblia* infected mice led to exacerbation of infection as proved by increased cyst shedding, increased trophozoite count and more inflammatory changes. Effect was more pronounced in mice received 3mg/Kg/BW of drug. Thus patients on regular ADA therapy for inflammatory diseases are at a high risk of giardiasis infection flourishing.

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Explanation of figures

Fig. 1: Bar chart showed mean serum TNF-α level in mice on 16th & 28th dpi in each group.

Fig. 2: Mean *Giardia*-cyst count on intermittent days in groups.

Fig. 3: Mean numbers of trophozoites in intestinal wash of infected mice on 28th dpi.

Fig. 4: Section of small intestine of *Giardia* normal control showed finger-like villi with intact epithelial lining and preserved goblets cells, crypts normal. BB: brush border, C: crypt, IG: intestinal gland, GC: goblet cell, LP: lamina propria, V: villi, (H & E, x 200).

Fig. 5: Section of small intestine of *Giardia*-infected non-treated control on 16th dpi showed crypt hyperplasia, moderate degree of villous atrophy with moderate inflammatory cells infiltrate causing widening of core of villi, and few trophozoites (IC: inflammatory cells, T: trophozoites (H & E, x200).

Fig. 6: Section of small intestine of *Giardia*-infected mice in GIa day 16th dpi. showed moderate inflammation with inflammatory cellular infiltrate causing expansion of villus core, shortening, and blunting of villi, sloughing on top of villi (SV: shortened villi, H & E, x200).

Fig. 7: Sections in small intestine of *Giardia*-infected control on 28th dpi showed variable inflammation degrees; A- Mild inflammatory reaction and maintenance of finger-like villi, B- Moderate inflammation with cellular infiltrate, fusion, blunting and shortening of villi, widening of villus cores, sloughing of epithelium, crypt hyperplasia and trophozoites in lumen (BV: blunting of villi, IC=inflammatory cells, S: sloughing, H & E., 200), C- *Giardia lamblia* trophozoites in lumen (in red circle) (H & E, x1000).