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# Immunohistochemical evaluation of interleukin-23 and cyclooxygenase-2 in the muscles of mice infected with *Trichinella spiralis*

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**Abstract.** The course of *Trichinella* (*T.*) *spiralis* infection includes intestinal and muscle phases. The aims of this work were to evaluate IL-23 and cyclooxygenase-2 (COX-2) by immunohistochemistry in the muscles of *T. spiralis* infected mice in a time-course study and to correlate their level with the serum levels of IL-23, IFN-γ, IL-4 and IL-10 cytokines. The mice were divided into an un-infected control group (UC) (10 mice) and 5 infected mouse groups (each 10 mice/group. Each mouse was infected with 200 *T. spiralis* larvae) and sacrificed on days 7, 14, 21, 28 and 35 post-infection (dpi). IL-23 showed weak expression (+1) on the 21$^{st}$ dpi, then it became moderately expressed (+2) on the 28$^{th}$ dpi and on day 35 pi, the immunoreactivity was strong (+3). COX-2 expressed weakly on 14 dpi, while the other mouse groups (21, 28 and 35) showed strong (+3) expression. IL-23 serum concentrations increased gradually in a significant pattern, in comparison to that of UC mice, from the 21$^{st}$ dpi to the end of the experiment. IFN-γ increased gradually and was significantly higher than those of UC mice from the 7$^{th}$ dpi, reached its maximum level on the 21$^{st}$ dpi, after which it decreased non-significantly. IL-4 up-regulated significantly in all infected groups in comparison to UC mice achieving its highest level on the 21$^{st}$ dpi and decreased after that. IL-10 increased significantly on the 7$^{th}$ dpi, but dropped at the 14$^{th}$ dpi, then reached its peak on the 21$^{st}$ dpi, and decreased again on the 28$^{th}$ and 35$^{th}$ dpi. In conclusion, *T. spiralis* infection caused increased expression of IL-23 and COX-2 in the muscle of infected mice, the effect being strongest on the 35$^{th}$ day. Also, the infection induced a mixed Th1/Th2 profile with a predominance of Th2 at the early muscle phase, after which the immune repose became mainly Th2.

## INTRODUCTION

Trichinellosis is a parasitic disease caused by *T. spiralis* which affects around 11 million people worldwide (Murrell & Pozio, 2011). The disease passes into two phases: intestinal and muscle stages. It occurs after eating infected, undercooked meat, especially the meat from pigs and wild animals, containing the encapsulated muscle larvae (Cui *et al.*, 2011; Cui & Wang, 2011). In the stomach, the larvae ex-capsulate and move to the small intestine to mature into adults. After mating, *T. spiralis* females release newly born larvae that pass into the blood and invade the striated muscles. The infected muscle cells form nurse cells within 20 days from the myocytes invasion (Wu *et al.*, 2006; Wu *et al.*, 2008). The nurse cell is surrounded by cellular infiltrates composed mainly of macrophages, CD4+ T cells and some CD8+ T cells (Beiting *et al.*, 2004).

During the early intestinal phase, *Trichinella* infection induces Th1/Th2 mixed immune response with an initial

increase of Th1. At the end of this phase, the Th2 response predominates with high production of IL-4, IL-5 and IL-13. During the muscle phase of the infection, the same immune response profile persists in association with up-regulation of regulatory T (Treg) cells which produce elevated amounts of the immunoregulatory IL-10 cytokine (Ilic *et al*., 2011). Experimentally, *Trichinella* has been shown to alleviate the severity of some immune-mediated diseases, such as Crohn's disease (Khan *et al*., 2002) and ulcerative colitis (Ashour *et al*., 2013). This could be attributed to the up-regulation of Th2 immune responsiveness, a lowering IFN-γ and IL-17, and activation of natural killer cells and Treg cells by the parasite (Ashour *et al*., 2013; Gruden-Movsesijan *et al*., 2010). Many studies have found that *T. spiralis* can suppress many types of tumors, while others have shown that the infection can enhance the tumor progression (Liao *et al*., 2018).

Interleukin-23 (IL-23) is a pro-inflammatory cytokine which belongs to IL-6/IL-12 family. It consists of an IL-12p40 subunit and a novel p19 component. It is mainly produced by activated macrophages and dendritic cells (McKenzie *et al*., 2006) and its receptor is found mainly on macrophage, CD4+ T and natural killer cells (Pahan & Jana, 2009). IL-23 shows various immunological effects, such as stimulating production of IL-17 (Beadling & Slifka, 2006). This cytokine has been incriminated in various inflammatory disorders of an autoimmune background, such as Crohn's disease, psoriasis and ankylosing spondylitis (Gaffen *et al*., 2014). Cyclooxygenase (COX) is an enzyme which has three iso-forms: COX1, COX2, and COX3. COX-2 is an induced enzyme that is considered a pro-inflammatory mediator and it stimulates the generation of prostaglandins (PGs) and thromboxane which induce severe inflammation and pain (Gilroy *et al*., 1999; Cannon & Cannon, 2012).

A study of the immunological response against *T. spiralis* infection in experimental animals can clarify some finer aspects of the host-parasite relationship (Yu *et al*., 2013) which may have applicable benefits. This response has been studied extensively during the intestinal phase of *T. spiralis* infection, but that against the muscle phase has been less studied (Fabre *et al*., 2009). The aims of this work were to evaluate IL-23 and COX-2, by immunohistochemistry in muscles of *T. spiralis* infected mice in a time-course study and to correlate them with the serum levels of IL-23, IFN-γ, IL-4 and IL-10 cytokines.

## MATERIALS AND METHODS

### Mice

The mice were parasite-free Swiss albino mice (7-8 weeks old and 18-20 g in weight). They were purchased from Theodore Bilharz Research Institute (TBRI) (Giza, Egypt). The mice were given a standard food regimen and tap water *ad libitum*. Animal experimentation was performed following the regulations of TBRI ethical committee.

### Parasites and infection

*T. spiralis* larvae were originally obtained from infected pork meat from a Cairo abattoir, and maintained in the laboratories of TBRI by consecutive passages in rats and mice. The infective larvae were collected from the previously infected mice or rats around the $35^{th}$ dpi using the artificial digestion method. The infected muscles were cut into small pieces, then digested overnight in a 6% pepsin-HCl mixture at 37°C with stirring. After that, the collected larvae from the digested fluid were washed several times in sterile phosphate-buffered saline (PBS) (Li *et al*., 2010). Each mouse was orally infected with 200 larvae (Dunn & Wright, 1985).

### Mouse groups

Fifty mice were distributed equally into five infected groups, and in addition 10 mice were used as an un-infected control group (UC). The infected mouse groups were sacrificed on day 7 (end of the intestinal phase of *T. spiralis* infection), day 14 (initiation of muscle cell invasion), day 21 (early stage of nurse cell formation), day 28 (formation of most nurse cell was completed) and day 35 (convalescent period), while UC mice were sacrificed on day 0 (Yu *et al*., 2013).

**Specimen collections**

At the time they were killed, the mice were anaesthetized with ether and killed by heart puncture. The collected blood was allowed to coagulate, centrifuged and the sera so obtained were stored at -20°C until use. Muscle specimens were excised from skeletal muscles of the scarified mice and they were fixed with 10% neutral buffered formaldehyde until examination.

**Histopathological examination**

The fixed specimens were embedded in paraffin blocks and the prepared paraffin sections were stained by haematoxylin and eosin (H&E). The sections were examined microscopically following standard procedures.

**Immunohistochemical detection of IL-23 and COX-2**

IL-23 and COX-2 expressions were analyzed in the muscle tissues by immuno-histochemical staining using the avidin biotin immune-peroxidase complex technique following the manufacturer's instructions. Briefly, sections from the prepared paraffin blocks were mounted on glass slides and kept overnight. Endogenous peroxidase activity was blocked by heating at 100°C. Antigen retrieval was done by putting the slides in citrate buffer (pH 6.0). Then, the slides were incubated overnight at 4°C with either the primary polyclonal rabbit anti-mouse antibody for COX-2 (Thermo-Scientific, USA, diluted 1:200) or primary polyclonal rabbit anti-mouse antibody for IL-23 (BioLegend, U.K, diluted 1:400). The sections were incubated with biotinylated secondary anti-mouse antibody and avidin-biotin complexed horseradish peroxidase solution was added. After that, 3,3'-diaminobenzidinetetrahydrochloride (DAB) chromogen was added and the slides were counterstained with Meyer's haematoxylin. Negative control slides were prepared following the same protocol but without the primary antibodies and all the slides were examined under a light microscope (Olympus, Japan).

**Immunohistochemical evaluation of IL-23 and COX-2**

The immunohistochemical score (IHS) was used for immunohistochemical evaluation of IL-23 and COX-2 by examination of 10 high-power fields (magnification × 400) for each slide. The average IHS was calculated by combining % of positive stained cells (the quantity score) with the staining intensity score. The quantity score ranged from 0 to 4 (0 = no immunostaining; 1 = 1-10% of cells positive; 2 = 11-50% positive; 3 = 51-80% positive and 4 = ≥81% positive). The staining intensity was scored from 0 to 3 (0 = negative; 1 = weak; 2 = moderate and 3 = strong). The quantity score was multiplied by the staining intensity score and the resulting IHS ranged from 0 to 12. The IHS of 9-12 = strong immunoreactivity (+3); 5-8 = moderate (+2); 1-4 = weak (+1) and 0 = negative (Gou *et al*., 2011).

**Measurement of the serum cytokines**

The serum concentrations of IL-23 (BioLegend, UK), IFN-γ, IL-4 and IL-10 (Phar Mingen, San Diego, USA) cytokines were measured by quantitative sandwich ELISAs using the specific monoclonal anti-cytokine antibodies (Li *et al*., 2018) following the manufacturer's guidelines. The supplied recombinant cytokines were used as standards. The flat-bottom plates were coated with the diluted capture antibodies (100 µl/well) overnight at 4°C. Between each step, the plates were washed with PBS/Tween 20. The blocking buffer (PBS/T20/1% low-fat dry milk) was added for 2 hours (hrs) after which the serum samples or the recombinant cytokine standards (100 µl/well) were added to the wells. The standards were serially diluted and the plates left for 3 hrs at room temperature. The specific biotinylated anti-cytokine antibody was added (100 µl/ well) and left for 1 hr. Then, streptavidin-alkaline phosphatase conjugate was added for 30 min. Finally, para-nitrophenyl phosphate (Sigma, U.K.) was added to develop the reaction and the absorbance (at 405 nm) was read using a microplate ELISA reader. The cytokine

concentrations were calculated from standard curves.

**Statistical analysis**

The statistical package for social sciences (SPSS), version 23 was used in this study. The cytokine concentrations in serum were presented as means ± standard deviation (SD). After homogeneity testing, the one-way ANOVA test followed by post-hoc tests were used for analysis of the statistical difference between the various mouse groups. Chi-square test or Fisher's exact test determined the probability of significant differences between the mouse groups for the immunohistochemical expression. $p < 0.5$ was considered statistically significant.

## RESULTS

**Histopathological findings**

There was normal muscle histology in UC and the 7$^{th}$ dpi mouse groups. The larvae were barely seen in muscle sections on the 14$^{th}$ dpi which also showed few inflammatory cells, but with normal muscle architecture. The infected mice on day 21 pi revealed severe areas of muscle damage around many *T. spiralis* larvae. Each larva was surrounded by a collagenous capsule forming a nurse cell. The nurse cells were surrounded by inflammatory cellular infiltrations consisting mainly of macrophages and lymphocytes. Additionally, the infected muscle cells showed cytoplasm mainly of a basophilic type. At the 28$^{th}$ dpi, the nurse cells increased in size and most of them had completed their development. There was a linear alignment of satellite cell nuclei around the periphery of the infected cells along their longitudinal axis. The nurse cell cytoplasm was mainly eosinophilic with less basophilic area. Most of the muscle bundles were normal and muscle regeneration predominated. On the 35$^{th}$ dpi, cytoplasm of the nurse cells was mainly of eosinophilic kind and the inflammatory cells were concentrated at the poles of the capsule. There was less muscle fiber degeneration, which was limited around the capsule. Also, at the periphery of infected cells and along their longitudinal axis the nuclei of satellite cells were aligned linearly (Figure 1).

**Immunohistochemistry results of IL-23 and COX-2**

In UC and the 7$^{th}$ dpi mouse groups, there was no immunoreactive evidence of either protein in the muscle sections. Regarding the expression of IL-23, it was increased in correlation with the duration of infection. The expression was weak (+1) on the 21$^{st}$ dpi mouse group, then moderate (+2) on the 28$^{th}$ dpi and strong (+3) on day 35 pi (Figure 2 & Table 1). With regard to COX-2 immunoreactivity, there was weak expression in the 14 dpi mouse group, while the other groups (21, 28 and 35 dpi) showed strong (+3) expression. The enzyme was found in the cytoplasm of the surrounding infiltrating inflammatory cells and in the cytoplasm of nurse cells (Figure 3 & Table 2).

**Serum cytokines**

IL-23 serum concentration did not differ significantly ($p > 0.05$) at the 7$^{th}$ dpi (19.11 ± 5.21 pg/ml) or 14$^{th}$ dpi (23.42 ± 3.98 pg/ml) from that of UC mice (20.22 ± 2.80 pg/ml). Thereafter, it increased gradually and significantly to 42.31 ± 7.13 pg/ml at the 21$^{st}$ dpi, 67.90 ± 10.94 pg/ml at the 28$^{th}$ dpi and reached a peak at 109.42 ± 12.31 pg/ml at the 35$^{th}$ dpi ($p < 0.001$) (Figure 4).

IFN-γ increased significantly ($p < 0.001$) at the 7$^{th}$ dpi (98.8 ± 12.7 pg/ml) in comparison to UC mice (52.41 ± 6.82 pg/ml). At the 14$^{th}$ dpi it had again increased significantly to 147.82 ± 10.55 pg/ml, and it reached its maximum level at the 21$^{st}$ dpi (245.9 ± 15.3 pg/ml) ($p < 0.001$). After that, its concentration decreased and was not significantly different ($p > 0.05$) from levels in UC mice (62.45 ± 14.60 and 58.15 ± 6.40 pg/ml on the 28$^{th}$ and 35$^{th}$ dpi, respectively (Figure 4).

IL-4 production in serum was up-regulated in all infected mice groups significantly ($p < 0.001$) in comparison with UC mice (89.76 ± 11.82 pg/ml). Its concentration was 241.55 ± 14.92 pg/ml on the 7$^{th}$ dpi and it decreased to be 183.91 ± 14.97 pg/ml at day 14 pi. Then, it increased afterwards to achieve its highest level on

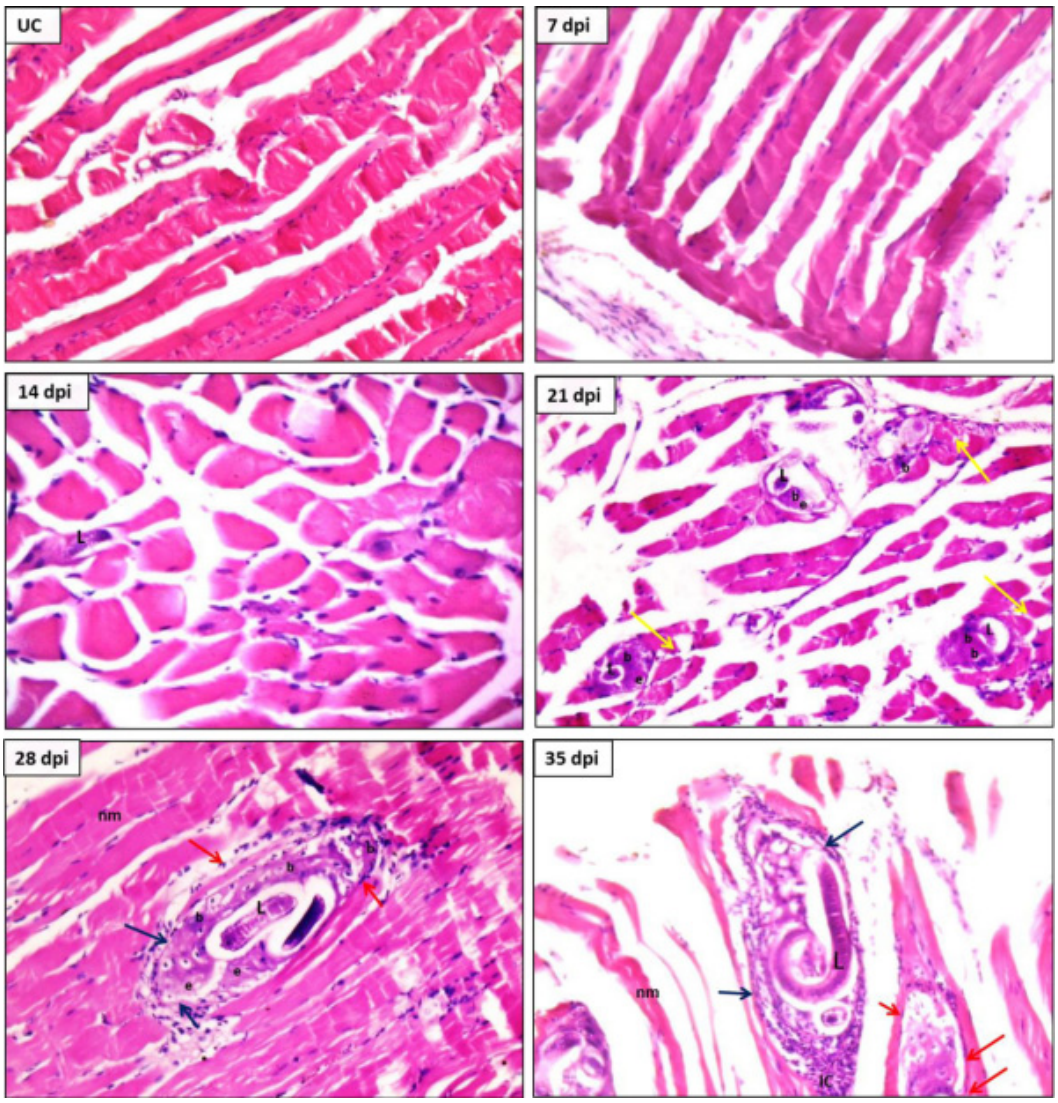


Figure 1. H & E staining of *T. spiralis*-infected muscles on days 0, 7, 21, 28 and 35 pi (×200). Infected groups showing numerous larvae within the muscles surrounded by intense inflammatory reaction. Degenerated muscle bundles (yellow arrows). The infected muscle cell is separated from undamaged muscle cells by a capsule wall (blue arrows). Linear alignment of nuclei of satellite cells in the periphery of infected cells along their long axis (red arrows). L=larva; b=basophilic cytoplasm; e=eosinophilic cytoplasm; nm=normal muscle cells.

the 21st dpi (601.44 ± 33.98 pg/ml) which did not differ significantly from that on the 28th (522.8 ± 42.6 pg/ml) or the 35th (495.8 ± 49.1 pg/ml) dpi (Figure 4).

IL-10 increased significantly on the 7th dpi (48.81 ± 3.21 pg/ml, $p < 0.001$), but on the 14th dpi it dropped to 29.30 ± 3.00 pg/ml ($p < 0.001$), though still significantly different compared with UC mice (20.13 ± 2.21 pg/ml). It then increased to reach its peak on the 21st dpi (113.7 ± 5.3 pg/ml), but dropped again on the 28th dpi (83.4 ± 7.2 pg/ml) and continued to drop till the 35th dpi (53.9 ± 3.5 pg/ml) (Figure 4).

Collectively, *T. spiralis* infection induced mixed Th1/Th2 with a predominance of Th2 at the early muscle phase, then it became mainly Th2 after that, with gradual increase of IL-23 from the early muscle phase till the end of the experiment.

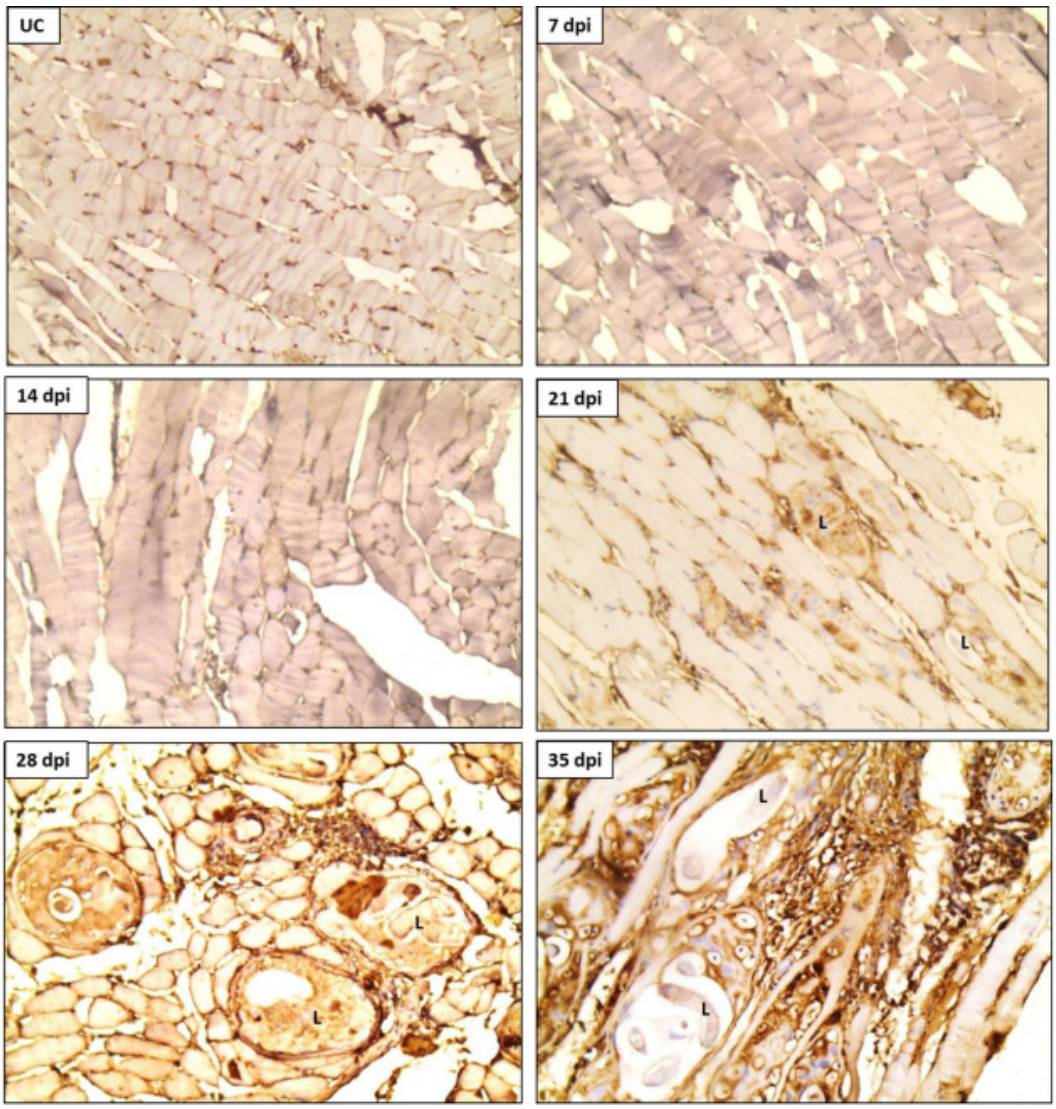


Figure 2. Immunohistochemical expression of IL-23 in the skeletal muscles of the infected mice. UC, 7th dpi and 14th dpi mouse groups were negative for the cytokine (×200). The 21st dpi mouse group shows weak (+1) expression. The 28th dpi mouse group shows moderate (+2) expression, while the infected mouse group at the 35th dpi shows strong (+3) immunoreactivity. The cytokine was found in the cytoplasm of the inflammatory cells. dpi=day post-infection, L=larva.

Table 1. Immunohistochemical expression of IL-23 in the muscles of the mouse groups

| Mouse groups (n=10 mice/group) | 0 (negative) | +1 (weak) | +2 (moderate) | +3 (strong) | p-value |
|---|---|---|---|---|---|
| UC | 10 | 0 | 0 | 0 | – |
| 7 dpi | 10 | 0 | 0 | 0 | – |
| 14 dpi | 10 | 0 | 0 | 0 | – |
| 21 dpi | 3 | 7 | 0 | 0 | 0.001 |
| 28 dpi | 0 | 2 | 8 | 0 | <0.001 |
| 35 dpi | 0 | 0 | 1 | 9 | <0.001 |

UC = un-infected control and dpi = days post-infection.

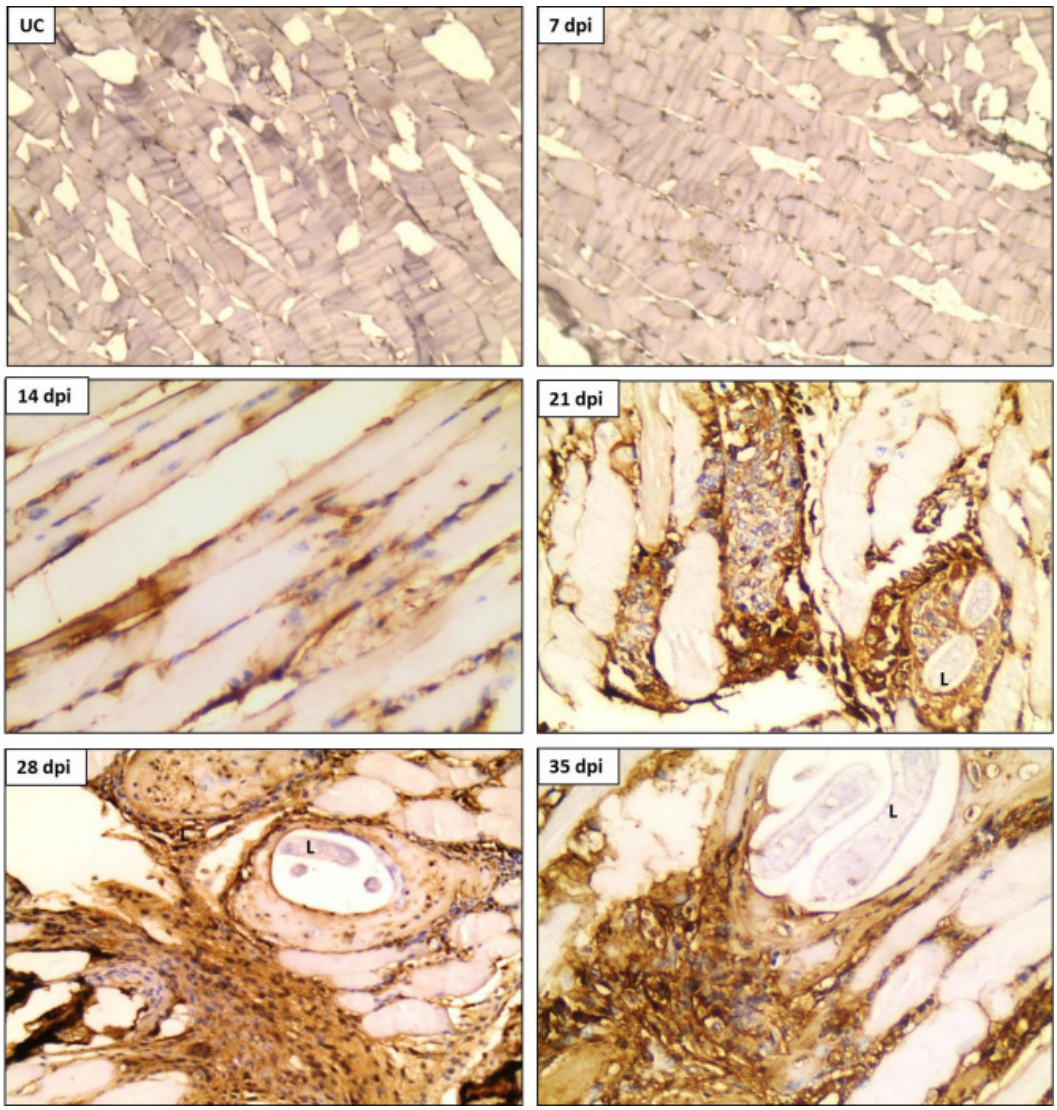


Figure 3. Immunohistochemical expression of COX-2 in the skeletal muscles of the infected mice. UC and 7$^{th}$ dpi mouse groups were negative for the enzyme (×200). The 14$^{th}$ dpi mouse group shows weak (+1) expression (×400). The other mice groups, 21$^{st}$ dpi (×400), 28$^{th}$ dpi (×200) and 35$^{th}$ dpi (×400) reveal strong (+3) enzyme expression. COX-2 was detected in the cytoplasm of the inflammatory cells. dpi=day post-infection, L=larvae.

Table 2. Immunohistochemical expression of COX-2 in the muscles of the mouse groups

| Mouse groups (n=10 mice/group) | 0 (negative) | +1 (weak) | +2 (moderate) | +3 (strong) | p-value |
|---|---|---|---|---|---|
| UC | 10 | 0 | 0 | 0 | – |
| 7 dpi | 10 | 0 | 0 | 0 | – |
| 14 dpi | 2 | 8 | 0 | 0 | <0.001 |
| 21 dpi | 0 | 0 | 2 | 8 | <0.001 |
| 28 dpi | 0 | 0 | 1 | 9 | <0.001 |
| 35 dpi | 0 | 0 | 0 | 10 | <0.001 |

UC = un-infected control and dpi = days post-infection.

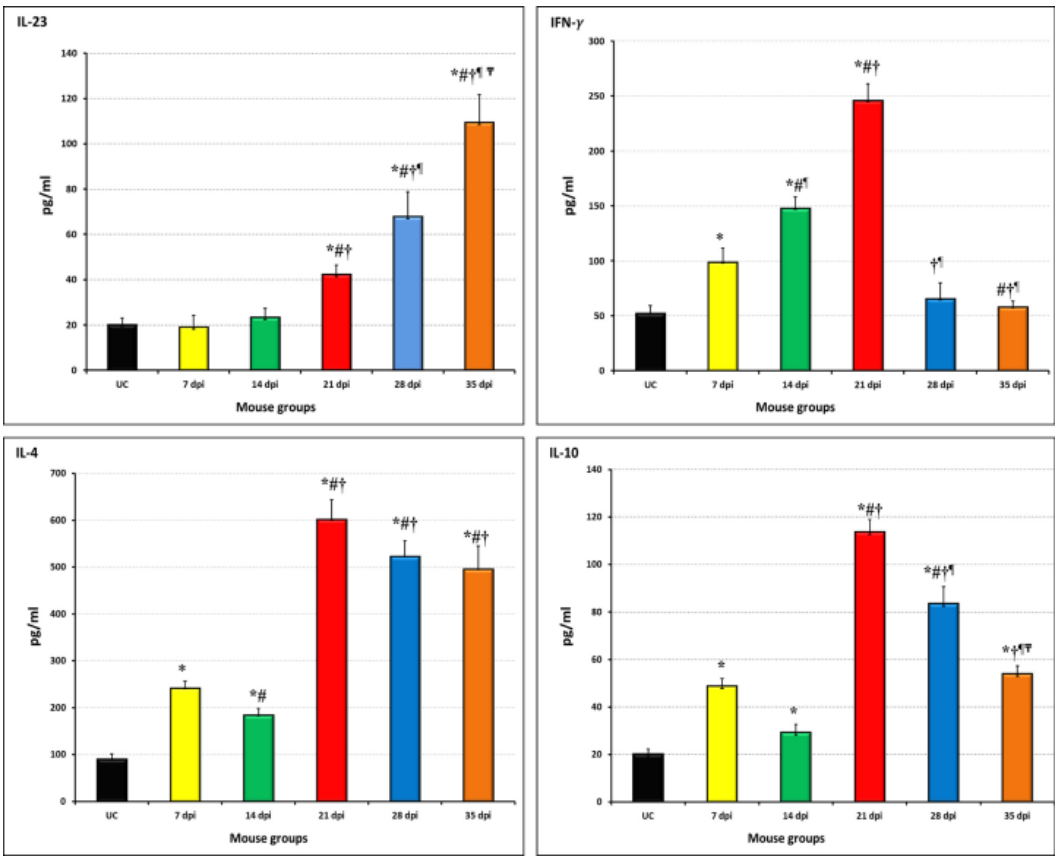


Figure 4. Serum concentrations of IL-23, IFN-γ, IL-4 and IL-10 cytokines. Results are presented as mean ± SD for each mouse group. *p < 0.05 indicates statistically significant difference from UC mouse group; #p < 0.05 indicates statistically significant difference from the 7th dpi mouse group; †p < 0.05 indicates statistically significant difference from the 14th dpi mouse group; ¶p<0.05 indicates statistically significant difference from the 21st dpi mouse group and p<0.05 indicates statistically significant difference from the 28th dpi mouse group using one-way ANOVA followed by a post-hoc test.

## DISCUSSION

*T. spiralis* can establish long-lasting infection in skeletal muscles of its hosts, where it may stay whole life of some hosts like rodents or remains for several years in human cases (Ilic *et al.*, 2012). The immunological response during the muscle phase of *T. spiralis* infection has been studied less than the intestinal phase. This work is the first to study the immune reactivity of IL-23 and COX-2 in muscles of mice infected with *T. spiralis* infection in a time-course study. Measurements of serum levels of IL-23 and IFN-γ, IL-4 and IL-10 were also performed.

The histopathological findings in the infected *T. spiralis* muscles in this study were similar to those other studies (Matsuo *et al.*, 2000; Beiting *et al.*, 2004; Boonmars *et al.*, 2005). At the early stage of the muscle invasion by *Trichinilla* larvae (14 dpi), the muscle produced basophilic cytoplasm which reduced in amount and was replaced gradually, and eventually completely replaced by the eosinophilic material (35 dpi) which is generated by satellite cells, a scenario similar to that described by Blotna-Filipiak *et al.* (1998) and Wranicz *et al.* (1998).

*Trichinella* infection causes apoptosis of the infected muscle cells, rather than necrosis (Wu *et al.*, 2008) and the underlying

injury of the muscles is attributed to the invading larvae and to the accumulated inflammatory cells which produce increasing amounts of reactive oxygen species and other toxic free radicals (Chiumiento *et al.*, 2009). At the same time, the infection causes activation and proliferation, but miss-differentiation of satellite cells which form nurse cells (Matsuo *et al.*, 2000; Wu *et al.*, 2001). The recruited inflammatory cells may have a beneficial function by taking part in the process of muscle regeneration by helping in activation of the satellite cells (Tidball, 2005) and by secreting growth factors and prostaglandins (PGs) which have well-documented roles in the muscle regeneration process (Robertson *et al.*, 1993; Merly *et al.*, 1999).

Regarding IL-23 immunoreactivity, its expression increased gradually during the infection time from the early stage of nurse cell formation (21$^{st}$ dpi) to become moderately elevated after complete formation of the nurse cells (28$^{th}$ dpi) and becoming highest at the convalescent period (35$^{th}$ dpi). This pattern of expression *in situ* correlated with its serum concentration. There is only one reported work on the study of IL-23 in *T. spiralis*-infected animals, performed by Fu *et al.* (2009). They found that the expression of IL-23, detected by Western blot in the mouse jejunum on 14, 56, and 84 dpi, showed non-significant changes when compared to controls. Fu and his colleagues suggested that IL-23 in *T. spiralis* infection is not important in mediating the associated intestinal inflammation.

IL-23 stimulates production of IL-17 (Beadling & Slifka, 2006), but many studies have reported that *T. spiralis* infection reduces IL-17 levels (Kang *et al.*, 2012; Yu *et al.*, 2013). It seems that IL-23 does not have the ability to increase IL-17 production during *Trichinella* infection.

IL-23 has been associated with the development of many types of tumors, such as colorectal cancer (Hu *et al.*, 2018) and hepatocellular carcinoma (Zang *et al.*, 2018). Its anti-tumor immune effects may be mediated by inhibiting the Th1 and natural killer cell functions (Teng *et al.*, 2010; Smyth & Teng, 2014) and it may induce these cells to induce pro-tumor effects (Zang *et al.*, 2018). Also, a high level of IL-23 has been found to be associated with many auto-immune diseases like Crohn's disease (Elson *et al.*, 2007) and lupus nephritis (Dedong *et al.*, 2019).

From the above results, it can be proposed that *T. spiralis* infection down-regulates the severity of many autoimmune diseases by inducing Th2 immune responsiveness and this appears to counteract the autoimmune-promoting impacts of IL-23. Also, in cases of tumor suppression, the mechanisms induced by the parasite may combat the tumor-initiation effect of IL-23. However, in cases of *Trichinella*-inducing tumorogenesis, this cytokine might play an important role in tumor progression, a point that needs more consideration.

Herein, the immunoreactivity of COX-2 was weak at the initiation of muscle invasion by the larvae (14$^{th}$ dpi), but it increased afterwards. This result is similar to that of Othman *et al.* (2016) who reported a strong expression of COX-2 in the muscles of *T. spiralis*-infected mice on day 35 pi. During the enteric phase of *T. spiralis* in animals, COX-2 shows strong expression, detected by immunohistochemistry in the intestinal epithelial cells (Othman *et al.*, 2016) or in the intestinal muscularis externa, accompanied by a significant increase of the serum PG E2 (Muñoz-Carrillo *et al.*, 2017). This suggests that COX-2 contributes to the muscle hyper-contractility which helps in the expulsion of the adult *T. spiralis* from the infected intestine (Barbara *et al.*, 2001; Muñoz-Carrillo *et al.*, 2017). Also, COX-2 production is up-regulated during many parasitic infection like *Cryptosporidium* (Sadek & El-Aswad, 2014).

The expression of COX-2 is affected by many cytokines, as inflammatory cytokines such as IL-17 (Ye *et al.*, 2001) and TNF-α (Feng *et al.*, 1995) induce synthesis and high expression of COX-2. On the other hand, IL-10 decreases this enzyme expression and consequently PG production (Berg *et al.*, 2001). The production of IL-23 is increased

by COX-2 and vice versa. The released PG-2 from healthy human fibroblasts mediates IL-23 secretion (Schirmer *et al*., 2010), and IL-23 initiates a significant increase in COX-2 mRNA expression in peritoneal cells in an experimental mouse model of rheumatoid arthritis (Lemos *et al*., 2009).

Bondesen *et al*. (2004) showed that COX-2-dependent PG has an important role in the early stages of muscle regeneration. Also, PG can activate muscle satellite cells (Pilbeam *et al*., 1993) and it has the ability to induce myoblast proliferation and differentiation (Zalin, 1987). We suggest that COX-2 may have a role in muscle regeneration during *Trichinella* infection and its importance in muscle generation could be investigated in COX-2 knockout mice infected by *Trichinella*.

We found that early *T. spiralis* infection induced a mixed Th1/Th2 immune response with a predominance of Th2. Then, with complete formation of most nurse cells (28$^{th}$ dpi), the systemic immune response shifted to Th2. In addition, IL-10 reached its peak in the early stages of nurse cell formation and decreased after that. These results are in accordance with other studies (Ishikawa *et al*., 1998; Helmby & Grencis, 2003; Yu *et al*., 2013). Also, serum IL-23 increased gradually from the early muscle phase till the end of the experiment. This implies that a complicated immune response is induced by the *T. spiralis* infection (Ding *et al*., 2017).

IFN-γ has a role in the early phase of the parasite infection until the early stages of nurse cell formation (Yu *et al*., 2013). IL-4 has the ability to induce hyperplasia of mast cells and goblet cells in the intestine of the infected mice (Kang *et al*., 2012), and these cells take part effectively in expelling adult *T. spiralis* from the intestinal wall (Finkelman *et al*., 2004). Added to that, IL-4 can stimulate production of the IgG1 isotype which has anti-*T. spiralis* effects (Kang *et al*., 2012). IL-10 is an anti-inflammatory cytokine which shifts the immune response toward a Th2 response (Manickasingham *et al*., 2003). During *T. spiralis* infection, IL-10 decreases the inflammatory response during the early stages of muscle infection by down-regulation of a Th1 response. With progression of the infection, its role in controlling the chronic infection becomes limited and is replaced by the emergence of a potent Th2 response (Beiting *et al*., 2004; Beiting *et al*., 2007) and this correlated with our findings.

In conclusion, *T. spiralis* infection caused expression of IL-23, with an increase in its serum concentration, and COX-2 in the muscle of infected mice. Also, the infection evoked mixed Th1/Th2 with a predominance of Th2 at the early muscle phase, after which the immune response became mainly Th2 with increasing serum IL-23.

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**Conflict of Interest**
None.

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