Egypt. J. Exp. Biol. (Zool.), 1: 77 – 86 (2005)

RESEARCH ARTICLE

Magdy E. Mahfouz
Nabila M. Mira
Said R. E. Amer

HISTOPATHOLOGICAL AND MOLECULAR STUDIES ON THE INTESTINE OF EXPERIMENTALLY INFECTED MICE BY *PROHEMISTOMUM VIVAX* (TREMATODA: CYATHOCOTYLIDAE)

ABSTRACT:

To study the pathological and possible molecular changes of small intestine during prohemistomumiasis, a total of 30 BALB/c mice were experimentally infected orally with 150 encysted metacerceriae / mouse of *Prohemistomum vivax*. Groups of 3 mice, each, were dissected at 6 and 12 h. and subsequently on daily basis up to 7 days post-infection (p.i.) as well as non-infected controls. Tissues of jejunum/upper intestine were immediately removed, fixed and prepared for histopathological investigation. On the other hand, tissue specimens from the above mentioned area were collected, RNAs were extracted and semi-quantitative reverse transcription-polymerase chain reaction was performed to analyze the expression of the multifunctional cytokine transforming growth factor-beta (TGF-β). Light microscopic examination showed compression and sometimes erosions of intestinal epithelial lining especially at the site of parasite attachment. The villi underwent deformation in the form of shortening, blunting and fission, which progressed with the infection time. Some villi were totally eradicated at the parasite localization. Hypertrophy of crypts was parallelly observed. In addition, inflammatory cell infiltration was recorded in the lamina propria of the parasitized intestine. These changes reached its peak value at the end of the experiment. At the same time, the molecular outcome was in accordance with the pathological findings. The expression of TGF-β was normal at baseline and increased progressively along the infection time compared with that in non-infected control. These findings strongly support a role for TGF-β during *P. vivax* infection. The recorded results were discussed in the light of the available literature.

Key words

Trematode parasite, *Prohemistomum vivax,* transforming growth factor-ß, RT-PCR.

Magdy E. Mahfouz
Nabila M. Mira
Said R. E. Amer
Dept. of Biol. Sciences, Faculty of Education, Tanta University, Kafr El-Sheikh, Egypt.

CODE ARTICLE: 12.01.05

INTRODUCTION:

Prohemistomum vivax (Sonsion, 1892) is a small trematode (Cyathocotylidae) parasite that inhabits the intestine of fish-eating birds and mammals (Shalaby, 1988; Khalil *et al*., 1998). Along the Nile River, numerous fish species such as *Mugil*, *Tilapia*, *Clarias*, *Hydrocycon*, *Alestes*, and *Mormyrus* were reported as second intermediate hosts for *P. vivax* (El-Naffar and Hassan, 1985; Khalil, 1987). The unconfined distribution of the infected fish along the Nile river and irrigation canals, in addition to the lack of specificity of the final host raise many queries about the rate and dynamics of the infection in fish-eating animals, as well as the effect of the parasite on the definitive host.

In the final host the main habitat of the parasite is the jejunum and the upper third of ileum (Amer, 1992). Intestinal infections can induce substantial pathological changes in the gut compartments. Intestinal trematodes have a great potency to induce enteritis (Lee *et al*., 1985). A number of these trematodes such as *Meatagonimus yokogawai*, *Pygidiopsis summ*, *Fibricla seoulensis*, *Centrocestus armatus* and *Gymnophalloides seoi* are known to cause villous atrophy, fusion and blunting as well as stromal changes such as crypt hyperplasia, oedema and cellular infiltration (Chai, 1979; Lee *et al*., 1981; Seo *et al.*, 1986; Hong *et al.*, 1997; Chai *et al*., 2001). In addition, *P. vivax* infection in rat resulted in efflux of mast cells as well as eosinophils in the mucosa of parasitized area (Amer, 1992). These alterations may lead to disturbance of physiological milieu of the intestine and subsequently its functional performance.

Experimental infection of mice with various intestinal parasites has facilitated dissection of the immunological events associated with both parasite clearance and the pathology of parasitic infection. Cytokines are small, low molecular weight, secreted proteins which mediate and regulate host responses to infection, immune responses, inflammation, and trauma (Finkelman *et al*., 1997). They are known as one of the major effectors in the expulsion of helminthes (Else and Finkelman,

1998; Onah and Nawa, 2000). Cytokines are actively secreted by the immune cells as well as other cell types in response to an antigen and function as chemical messengers for regulating the innate and adaptive immune systems. Some cytokines act to make disease worse (proinflammatory), whereas others serve to reduce inflammation and promote healing (anti-inflammatory) (Dinarello, 2000). Among many cytokines involved in cell growth and differentiation, the anti-inflammatory transforming growth factor-beta (TGF-β) is noteworthy because of its multiple functions in a variety of cells (Letterio, and Roberts, 1998; Taipale *et al*., 1998). TGF-β is believed to be essential in wound healing as well as in regulation of cell growth and differentiation and is known to be involved in tissue repair and remodelling (Tominaga *et al*., 1997; Beck *et al*., 2003). *In vivo* and *in vitro* studies have indicated that TGF-β plays important roles in several physiological and pathological functions (Blobe *et al*., 2000*;* Beck *et al*., 2003), in addition to its increase in association with intestinal inflammation (Shull *et al*., 1992).

Since each species of trematode parasites has its characteristic mode of damaging host tissue (Lee *et al*., 1985), the pathogenesis of individual trematode infection might be associated with particular species. Although *P. vivax* has a significant medical importance due to its ability to infect human beings (Nasr, 1941), the associated pathological alteration has received little attention. The present study was undertaken to demonstrate, on a daily basis, the pathological alteration in mice intestine after experimental infection with *P. vivax* and to determine the changes in expression, if any, in the multifunctional cytokine, TGF-β in response to this trematode parasitic infection.

MATERIALS AND METHODS:

Experimental animals:

Thirty (8 week-old) male BALB/c mice, weighting about 21 gm in average were obtained from the animal house of Theodore Bilharz research Institute, Giza, Egypt. Animals were housed at room temperature (28 ± 2ºC) in plastic cages (6/cage) with wire covers. Saw dust was used as bedding and changed daily along the course of the experiment. Animals were acclimatized for one week before the start of the experiment. Food, powdered casein-based diet formulated to meet National Research Council (1978) requirements for the laboratory mouse, and water were available *ad libitium*.

Parasite and infection:

Prohemistomum vivax encysted metacercariae were obtained from the skeletal muscles of naturally infected *Claris lazera* (cat-fish), collected from the local markets at Kafr El-Sheikh city. Each mouse was infected orally with 150 freshly isolated metacercariae, counted by the aid of stereo-microscope. Three animals were kept non-infected and served as controls and were killed at the end of the experiment. Before the infection, mice were deprived of food for 12 h., while it was provided 4 h. p.i. A group of infected mice (n=3) as well as age-matched non-infected control were autopsied by cervical dislocation at 6, 12, and 24 h. post infection and on daily basis onward up to 7 days p.i.

Gross examination:

The whole intestine of each mouse, starting from the jejunum up to the colon, was removed, rinsed in cold saline solution and divided into three equal portions, referred as jejunum/upper intestine, mid intestine and hind intestine. Each intestinal segment was opened separately in Petri dish containing saline, along the mesenteric border, and inspected for the presence of parasites under dissecting microscope (Bausch & Lomb, USA).

Histopathological examination:

For light microscopy, tissue samples, from both infected and non-infected control, of about 1 cm were excised from the jejunum/upper intestine, fixed in 10% buffered formalin, dehydrated in alcohol, embedded in paraffin and sectioned at 5 µm thicknesses. Finally, sections were stained by Haematoxylin and Eosin (HE) stain according to Drury *et al.* (1976).

For scanning electron microscopy (SEM), tissue samples were fixed in 2% glutaraldehyde in 0.1M sodium cacodylate buffer, pH 7.2 for 1 h, rinsed in buffer three times before post-fixed in 1% Osmium tetroxide for one hour. Following washing in double-distilled water, the samples were dehydrated in an ascending series of acetone concentrations. After critical-point drying, the specimens were mounted on aluminium stubs, coated with gold and viewed in a JEOL JSM-5300 scanning electron microscope operating at an accelerating voltage of 10 keV in the Electron Microscopy Unit, Faculty of Science, Alexandria University.

RNA isolation and purification:

Freshly isolated tissue samples of jejunum/upper intestine (0.5 cm) were collected, snap-frozen in liquid nitrogen and processed directly for RNA isolation or stored at -70 ºC until use. Total RNA was extracted from tissue samples using TRIzol reagent. Disruption and homogenisation was accomplished in 1.5 ml TRIzol, two times each for 2 min, and total RNA subsequently isolated using chloroform and isopropanol under standard procedures according to the manufacturer's recommendations (Gibco BRL). Contaminating DNA was removed using 2 units of deoxyribonuclease (DNase I, RNase free) for 30 min at 37ºC. RNA concentrations were measured spectrophotometerically at 260 and 280 nm (average ratio 1.89 ± 0.1).

Reverse transcription-polymerase chain reaction (RT-PCR):

Of each tissue sample, 1 µg RNA was reverse transcribed into complementary DNA (cDNA) in a volume of 25 µl of reaction mixture containing random hexanucleotides primer (0.2 µg/reaction), deoxyribonucleotide triphosphate (dNTP-0.5 mM of each), avian myeloblastosis virus reverse transcriptase (RT, AMV) (20 units/reaction), RNasin (20 units/reaction) and 2.5 µl of 10x PCR buffer. After an initial 5 min at 70 °C in order to dissociate secondary RNA structures, samples were incubated at 42 °C for 60 min. Reactions were terminated after 5 min at 95 °C.

PCR was performed initially using primers for mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) housekeeping gene (sense, 5'-GAAGGGCTCATGACCACAGTCCATG - 3'; antisense, 5'-TGTTGCTGTAGCCGTATTCATTGTC-3') (Knight *et al*., 2000), which served as a positive control for RT-PCR due to its constitutive expression. Products obtained using 25 cycles of amplification were within the linear range of signal amplification and allowed titration of the amount of template to be subsequently used in order to obtain consistent amounts of product between samples. The adjusted cDNA volumes were then used in the succeeding PCR reactions employing gene-specific primers for TGF-β (sense, 5'-GCCCTGGATACCAACTATTGC-3'; antisense, 5'-AGCTGCACTTGCAGGAGCG-3') (Sharmin *et al*., 2004). Negative control reactions without cDNA template were performed in order to ensure that no products arose due to contamination or primer-dimer effects.

The PCR reaction mixture was set up in a total volume of 50 µl, containing 3 µl of reverse transcriptase product (50–100 µg of cDNA), 5 µl of a 10x PCR standard buffer, 1.5 µl 50 mM $MgCl_2$, 1 µl 10 mM dNTP mix, 1.5 units Taq DNA polymerase (Promega) and the selected primer pair (30 pmol/primer/reaction). Amplifications were performed using 35 cycles of denaturation (40 seconds at 95°C), annealing (40 seconds at 55°C), and extension (60 seconds at 72°C) followed by a final extension at 72 °C for 10 min.

Semi-quantitative analysis of gene expression:

Following PCR, a 10-µL aliquot of each PCR product was analyzed by electrophoresis on 1.5% agarose gel containing ethidium bromide and the bands were examined under ultraviolet light for the presence of amplified DNA. Semi-quantitative assessments of mRNA levels were determined by quantifying the intensity of each band of PCR product through using Gel Analyzing Imager (Sharp-100). This relies on the quantification of the investigated genes expression on the basis of optical density of detected bands. The relative intensity of TGF-β to that of GAPDH was then determined. Corrected values were obtained by dividing the measured value for TGF-β by that of GAPDH. Mean values of three measurements of TGF-β expression relative to the corresponding GAPDH expression are presented. Differences of gene specific expression (relative to GAPDH) in infected mice were tested against corresponding values in non-infected controls at respective days p.i. using an unpaired *t*-test.

RESULTS:

Gross examination:

It was observed that six h. p.i., most of the metacercariae were passed to the duodenum/jejunum, but still encysted; few were observed excysted producing immature flukes moving in the intestinal lumen. At 12 h. p.i., all the metacercariae were excysted and the immature flukes were found doweling between the villi (Fig. 1). One day p. i. and onward the worms proved to be entrapped between the intervillous spaces. However, the attachment seemed not to be so firmly, that the parasites detach from the site within 30 min to 2 h. after opening the intestine. The infection was localized at the jejunum/upper intestine for 5 days p.i. On the other hand, six and seven days p.i., few parasites were observed in the mid intestine. The parasite was never seen in the hind intestine.

Histopathological examination:

Histological examination of the intestine of the non-infected controls through the entire period of the experiment revealed normal architecture, characterized by long and slender villi with intact columnar epithelial lining; however, lamina propria of the villi occasionally had few inflammatory cells. Crypts were in normal figure with long columnar and remarkable paneth cells. Sub mucosal layer was thin and delicate without cellular infiltration (Fig. 2).

In the infected mice, the intestinal architecture showed disfigurement with progressive pathological alterations up to 6 days p.i., which coincides with the maturity of the parasite. Examination of intestinal sections on the first day p.i. revealed that the parasite was deeply inserted in the intervillous spaces and attached with the intestinal mucosa.

On the second and third days p.i., the lining epithelial cells at the parasite attachment were thickened and/or compressed. Some cells showed pyknotic changes. The villi became blunt with mild oedema. The lamina propria was infiltrated with a considerable number of inflammatory cells. Furthermore, fusion and atrophy of the villi were remarkable. In addition, increased inflammatory cell infiltration as well as occasional stromal oedema were recorded (Fig. 3-6).

On the fourth and fifth days p.i., the parasite was seen frequently entrapping the

villi, and sometimes engulfing mucosal fragments, and some necrotic debris was observed in the sucker (Fig. 7 - 8). The villous changes become more sever than that recorded before. Tremendous cell infiltration was found in the lamina propria. Thickening of the layer with obvious oedema were seen in the parasitized intestine. On the sixth day p.i., facultative epithelial changes along with sever villous atrophy, as well as complete loss of some villi at the parasite attachment were observed.

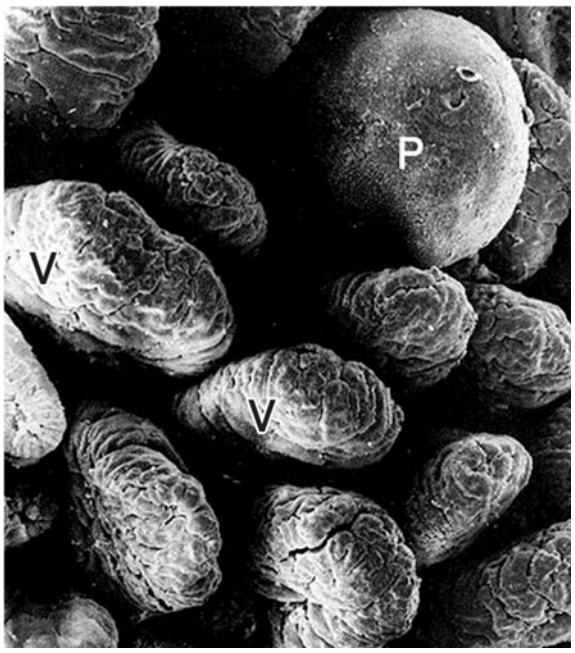

Fig. 1: Scanning Electron Micrograph (SEM) showing the parasite (P) inserted between the villi (V) (X200).

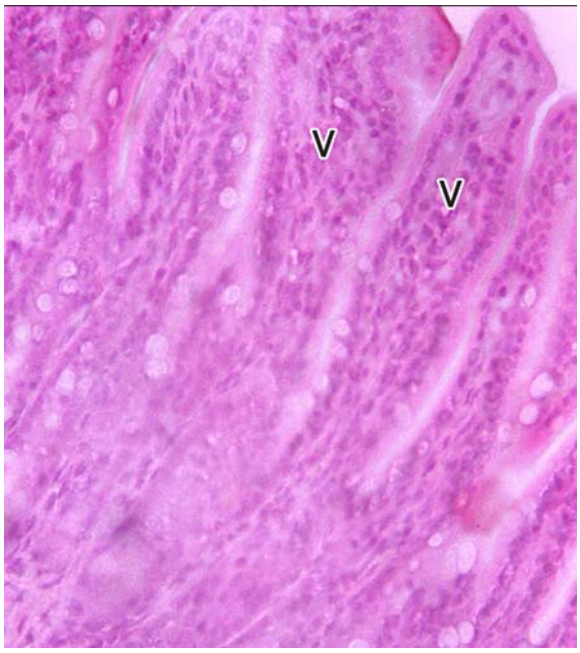

Fig. 2: Photomicrograph of jejenual/upper intestinal transverse sections (Ph.J./U. T.S.) of control mice showing normal, slender, finger-like villi, HE stain (X100).

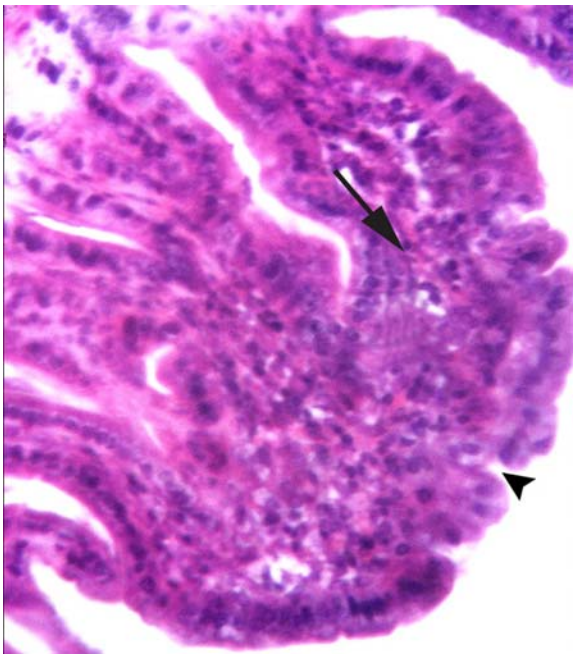

Fig. 3: Ph.J./U. T.S. of two days post infection (p.i.) showing fusion of villi (arrow head) with inflammatory cells into lamina propria (arrow), HE stain (X400).

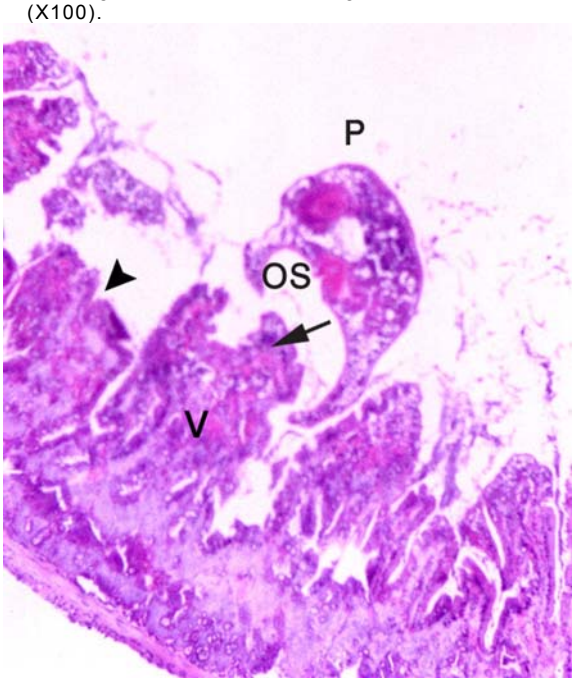

Fig. 4: Ph.J./U. T.S. of three days p.i. showing the parasite (P) pinching the villi (arrow) with the oral sucker (OS). Fusion of villi is remarkable (arrow heads), HE stain (X40).

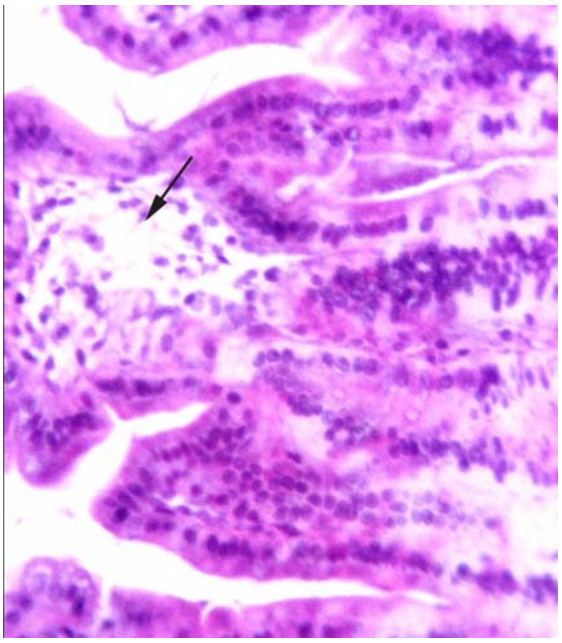

Fig. 5: Ph.J./U. T.S. of three days p.i. showing infiltration of inflammatory cells in the lamina propria and stromal oedema (arrow) of villi, HE stain (X400).

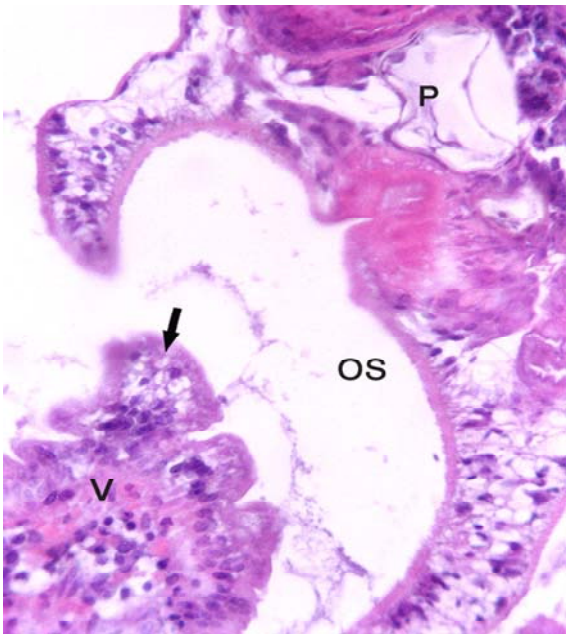

Fig. 6: Ph.J./U. T.S. of three days p.i. showing facultative necrosis (arrow). The parasite (P) is pinching the villi with its oral sucker (OS), HE stain (X400).

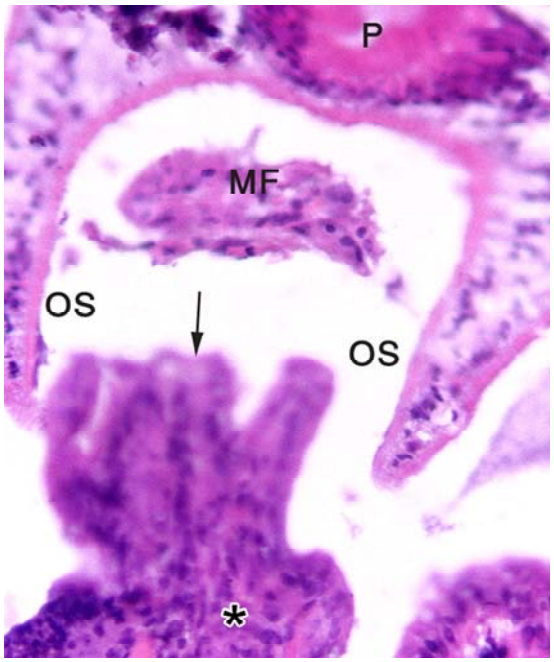

Fig. 7: Ph.J./U. T.S. of four days p.i. showing the parasite (P) entrapping fused villi (arrow). Mucosal fragments (MF) are being engulfed in the oral sucker (OS). An asterisk indicates cell infiltration in the lamina propria, HE stain (X400).

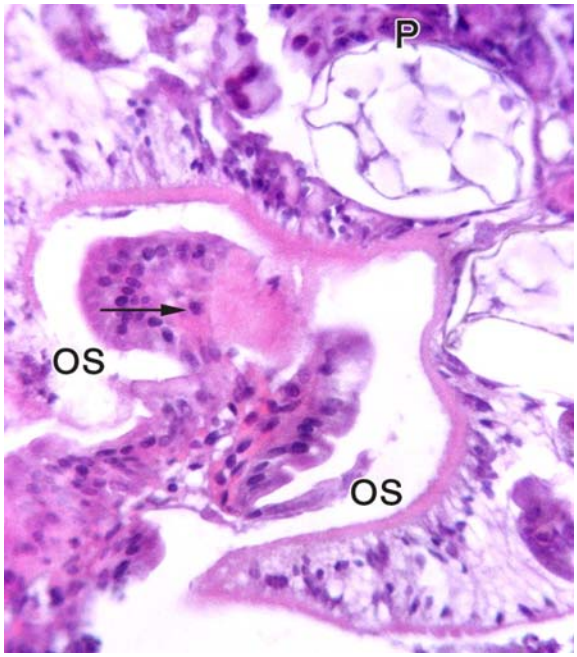

Fig. 8: Ph.J./U. T.S. of five days p.i. showing pinched villi with the oral sucker (OS) of the parasite (P). Degenerative changes are noted in the mucosal area (arrow) at contact with the parasite, HE stain (X400).

Furthermore, congestion of lymphatic and blood vessels in submucosa was frequently observed. Of interest, free floating red blood cells were observed in the stroma of infected intestine (Fig. 9-12). On the other hand, no further progress in the pathological figure was recorded at the seventh day p.i., which may imply that the recorded alteration were peaked at the 6th day p.i. under the mentioned conditions of the current study.

Detection of mRNA and semi-quantitative analysis of TGF-β expression:

On testing the viability of the RNA samples via the amplification of mouse GAPDH, it was found that all tissue samples from controls and infected individuals, except for negative control, presented detectable quantities of

GAPDH mRNA (453 base pair (bp) fragment) revealing an acceptable integrity to amplification as well as a successful first-strand cDNA preparation. This stable and consistent expression was used for the normalization of mRNA levels of TGF-β.

The levels of TGF-β mRNA were assessed by RT-PCR in jejunum/upper intestine specimens from *P. vivax*-infected (6 h p.i. up to 7 days) as well as non-infected control mice. RT-PCR product of TGF-β, analyzed by gel electrophoresis, gave the expected 336 bp given the selected primer pair. Meanwhile, there was no expression in the negative control samples (Fig. 13).

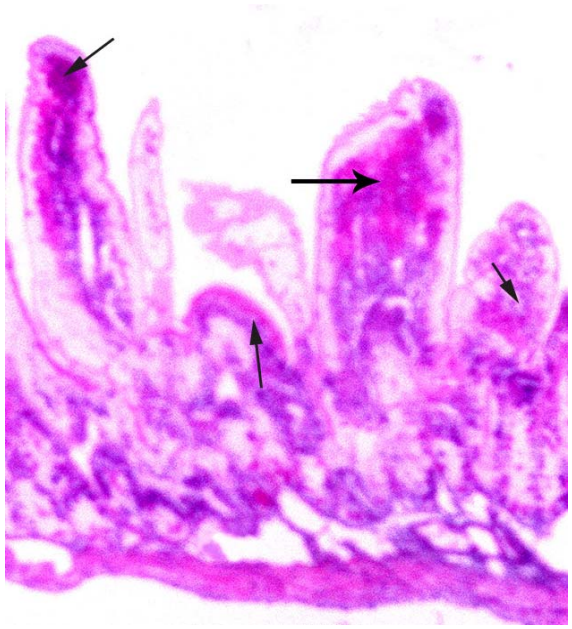

Fig. 9: Ph.J./U. T.S. of six days p.i. showing prominent sub mucosal hemorrhage (arrows) , HE stain (X40).

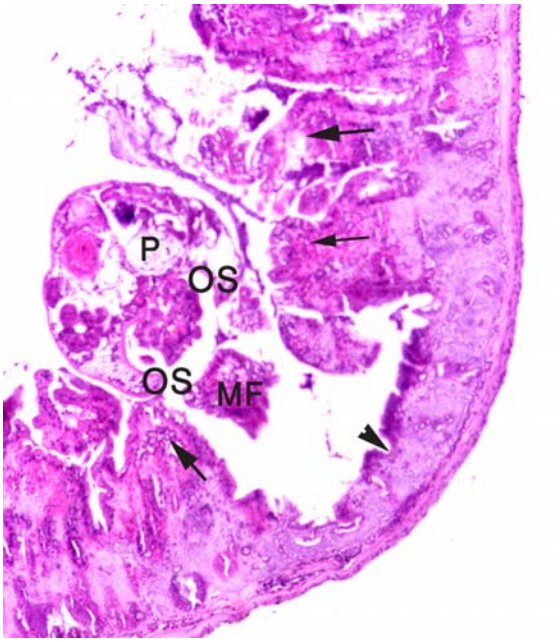

Fig. 10: Ph.J./U. T.S. of six days p.i. showing sub mucosal hemorrhage in the stroma (arrows) of the parasitized intestine as well as complete loss of villi (arrow head). Mucosal fragments (MF) are seen in the area of oral sucker (OS) of the parasite (P), HE stain (X40).

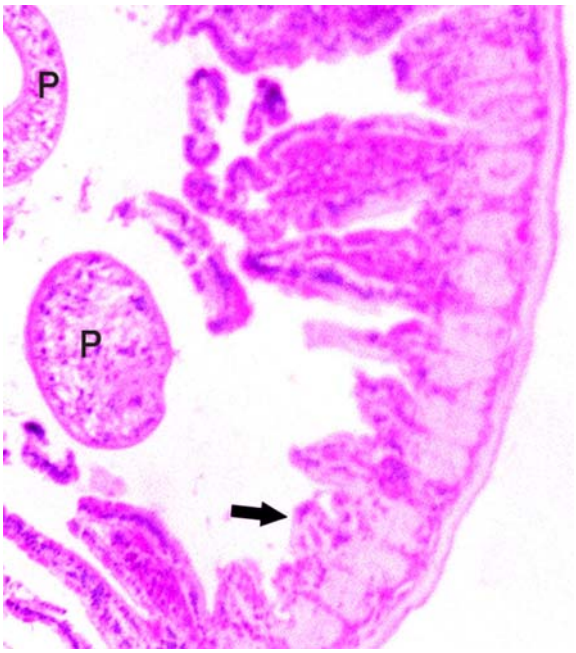

Fig. 11: Ph.J./U. T.S. of six days p.i. showing regeneration of lost villi (arrow), HE stain (X40).

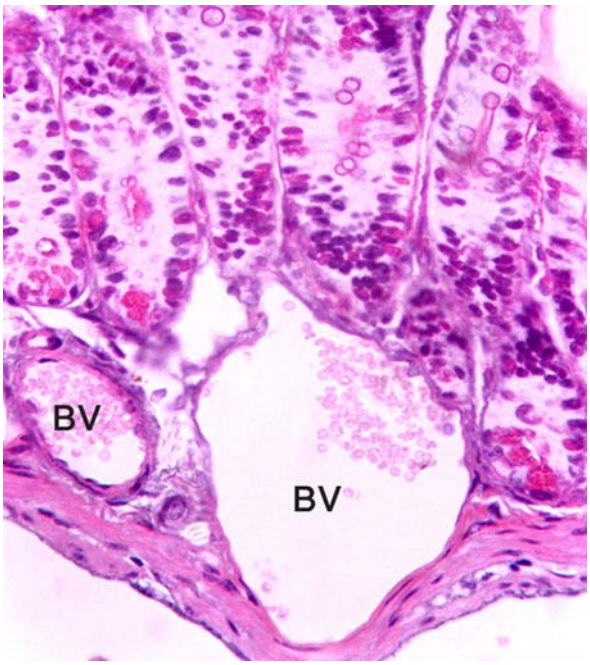

Fig. 12: Ph.J./U. T.S. of six days p.i. showing congestion of blood vessels (BV), HE stain (X400).

On semi-quantitative analysis, TGF-β was amplified weakly in control individuals and there was no evidence for increment in its mRNA level in 6 and 12 h. However, significant difference in the expression of TGF-β could be observed at day 2 p.i. and onwards.

The expression of TGF-β peaked after 6 day p.i. with ~ 8.5 folds up-regulation compared to non-infected controls, while no further increase in its level was detected on day 7 p.i. (Fig. 13). Along with, TGF-β/GAPDH mRNA ratio was significantly increased during the course of *P. vivax* infection compared with those in the control non-infected individuals (P<.05) (Fig. 14).

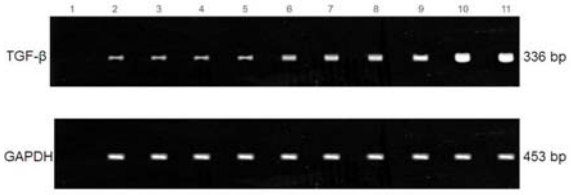

Fig. 13: Expression of TGF-β mRNA in the small intestine of BALB/c mice. Electrophoresis of RT-PCR products of TGF-β (336 bp) and GAPDH (453 bp) mRNA was performed in ethidium bromide-stained agarose gel (1.5%). Shown are amplicons; Lane 1: negative control, Lane 2: non-infected control, Lane 3: 6 h p.i., Lane 4: 12 h p.i., Lanes 5-11: 1, 2, 3, 4, 5, 6 and 7 day p.i., respectively.

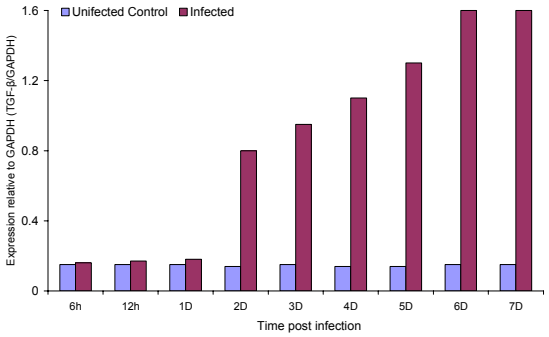

Fig. 14: RT-PCR product signal intensity expressed as a ratio of TGF-β to GAPDH optical density values. The expression of GAPDH was constant and thus served as internal control to assess variations in gene expression. Shown are comparisons of the relative intensity in BALB/c infected mice to non-infected control. (h: hour, D: Day).

DISCUSSION:

The present study reveals that as for the locality of *P. vivax* in host, the parasite harboured the jejunum/upper intestine of the final host. This result is in agreement with those reported by Khalil (1987) and Amer (1992) for the same parasite. The duodenum as well as the upper part of the small intestine are considered as preferred biological niche for many trematodes such as *Echinostoma caproni* (Isaacson *et al*., 1989), *E. hortense* (Lee *et al*., 1990; Kim *et al*., 2000), *Fibricola seoulensis* (Lee *et al*., 1985; Huh *et al.*, 1988) and *Gymnophalloides seo* (Chai *et al.*, 2001). *P. vivax* was found to be inserted deeply at the interspaces of the villi, between the intestinal villi, while the parasite was never seen invading the crypt region.

The results of the present study indicate that *P. vivax*–induced histopathological alterations appeared as early as one day p.i. The intensity of the alteration increased progressively up to six day p.i. and extended to the deeper layers of the intestine. The mucosal changes were in the form of shortening, thickening and blunting of the villi. By the time, villous fusion and crypt hyperplasia were pronounced. With the progression of infection, the parasite induced stromal changes such as capillary congestion, lymphatic dilatation and inflammatory cells infiltration. Sub mucosal oedemas as well as haemorrhage were occasionally seen. Furthermore, the submucosa became thicker than that of the non-infected controls. Similar mucosal and stromal changes were seen in the upper part of small intestine of rats and cats infected with *Mrtagoinmus yokogawai* (Chai, 1979; Lee, 1981), in rats and mice infected with *Fibricola seoulensis* (Lee *et al.*, 1985; Huh *et al.*, 1988), in mice infected with *pygidiopsis summa*, in rats infected with *Centrocestus armatus* (Hong *et al.*, 1997), and in mice infected with *Gymnophalloides seoi* (Chai *et al.*, 2001).

In addition, comparable histopathological findings were observed in other intestinal parasitosis including trichinosis in mice (Manson–Smith *et al*., 1979), nipostrongylosis in rats (Symons, 1976) and *Isospora belli* infection in man (Liebman *et al.*, 1980). More or less similar pathologic alterations were recorded in other intestinal situations such as malabsorrtion syndromes (Shiner and Barkin, 1985). The recorded pathological changes may be related to the effect of the parasite itself, and/or to the host response. *P. vivax* has a well developed adhesive organ as well as spiny topography (Khalil and Helal, 1992) which may have a direct role in the pathogenesis of the parasite due to the resultant irritation to the host mucosa. In this regard, it is claimed that rhythmic movement of the anterior body and/or rotation of the digenean trematodes result in abrasion of host tissues at the contact areas of micro–niche as well as mechanical pressure on the neighbouring villi (Jang *et al*., 1985; Lee *et al*., 1985; Chai *et al*., 2001). Chai (1979) suggested the possibility of pressure atrophy due to increased amount of gases and mucus in the intestinal lumen of the parasitized intestine. The present results indicated that the parasite was frequently seen entrapping the villi and/or engulfing tissue debris. It is postulated that the mechanically destroyed tissues of the host were ingested through the oral cavity of the parasite (Lee *et al*., 1985; Chai *et al*., 2001). Khalil (1987) reported that *P. vivax* produces eggs just 6 days after infection. The early gravitation of the parasite

may impose the necessity for rich supply of nutrient, therefore it is plausible to find the parasite pinching out the villi and devouring the epithelia tissue.

Regarding the immune response of the host, it is established that *P. vivax* has the potency to trigger the cellular and humoral immune responses of the host. Helal *et al*. (1998) recorded tremendous influx of mast cells and eosinophils in the intestine of rats infected with this parasite. Furthermore, mice infected with the same parasite were able to mount strong local and systemic immune responses (Amer, 2005). In view of the fact that the molecular pathology of intestinal infection is generally studied in the context of specific mechanisms and to infer a profound insight in the association between pathological and immunological aspects in *P. vivax* infected mice, the level of mRNA expression of the cytokine TGF-β was evaluated. The results presented here verify, for the first time, this association. *P. vivax* infected mice had high TGF-β levels when compared to non-infected control individuals. This up-regulation progressively continued during the examined course of infection until the sixth day p.i. This is consistent with the finding of previous studies using experimental models in response to different parasitic infection such as lambs to *Trichostrongylus colubriformis* (Paalangara *et al*., 2003) and mice infected with *Echinococcus granulosus* (de-la-Pena *et al*., 2002), *Schistosoma japonicum* (Zhang *et al*., 2004), and *Heligmosomoides polygyrus* (Su *et al*., 2005). TGF-β is a multifunctional growth factor that plays a crucial role in several physiological and pathological conditions (Blobe *et al*. 2000) as well as in preventing the severe pathology of parasitic infection (Omer *et al*., 2003). It is also a key regulator in the maintenance of immunological homeostasis in the gastrointestinal tract (Groux and Powrie, 1999). TGF-β has important chemotactic and anabolic actions on fibroblasts involved in tissue repair, where it stimulates their production of critical components of the extracellular matrix, such as collagen and fibronectin, as well as proteoglycans. On the other hand, it inhibits the action of proteolytic enzymes that destroy newly formed connective tissues. TGF-β is also known to inhibit intestinal epithelial cell proliferation, a process that may inhibit re-epithelialisation after surface injury (Souza *et al*., 1997). Babyatsky *et al*. (1996) showed that TGF-β may play a role in promoting healing of the overlying epithelium by modulating epithelial cell restitution, which serves to re-establish surface continuity after mucosal injury. Similarly, Beck *et al*. (2003) using transgenic mice, demonstrated that TGF-β mediates intestinal healing and susceptibility to injury both *in vitro* and *in vivo*, through epithelial cells. Likewise, studies in rats treated with TGF-β have shown that the healing of acetic acid-induced ulcers is accelerated with excessive deposition of extracellular matrix and scarring (Ernst *et al*., 1996; Shih *et al*., 2005).

In conclusion, the data from the present study highly postulated an association between the observed pathological alterations in mice infected with the intestinal trematode *P. vivax* and the up-regulation of TGF-β, which might imply that it is a critical determinant of host resistance to prohemistomumiasis in mice. Although these data agree with the overwhelming studies evaluating the role of this multifunctional cytokine, participation of other host immune response elicited to *P. vivax* infection in induction of the aforementioned pathological alterations requires further investigations.

REFERENCES:

Amer, S. (1992): Immunological studies on a mammalian host after infection with two trematode species. M.Sc. Thesis, Fac. Sci. Tanta Univ.

Amer, S. (2005): Effect of dietary zinc levels: examination of the effect of Zn^{++} supplementation on the host immune responses to *Prohemistomum vivax* infection. J. Egypt. Ger. Soc. Zool., 48(A): 105-124.

Babyatsky, M. W.; Rossiter, G.; Podolsky, D. K. (1996): Expression of transforming growth factors α and β in colonic mucosa in inflammatory bowel disease. Gastroenterology, 110: 975-984.

Beck, P. L.; Rosenberg, I. M.; Xavier, R. J.; Koh, T.; Wong, J. F. and Podolsky, D. K. (2003): Transforming growth factor-β mediates intestinal healing and susceptibility to injury *in vitro* and *in vivo* through epithelial cells. Am. J. Pathol., 162 (2): 597-608.

Blobe, G. C.; Schiemann, W. P. and Lodish, H. F. (2000): Role of transforming growth factor beta in human disease. New Engl. J. Med. 342: 1350-1358.

Chai, J. (1979): Study on *Metagonimus yokogawai* (Katsurada, 1912) in Korea. V. Intestinal pathology in experimentally infected albino rats. Seoul J. Med., 20: 104-117.

Chai, J.; Lee, H.; Hong, S.; Yoo, J.; Guk, S.; Seo, M.; Choi, M. and Lee, S. (2001): Intestinal histopathology and *in situ* postures of *Gymnophalloides seoi* in experimentally infected mice. Korean J. Parasitol., 39(1): 31-41.

Dinarello, C. A. (2000): Proinflammatory Cytokines. Chest, 118: 503-508.

Drury, R. A. B.; Wallington, E. A. and Cameron, S. R. (1976): Carleton's histological technique. 4th Ed. Oxford Univ. Press, Ely House London W.I.

EL-Naffar, M. and Hassan I. (1985): Role played by fish in transmitting some termatodes of dogs and cats at Aswan province, A. R. Egypt Assiut Vet. Med. J., 14 (27): 157- 167.

Else, K.J. and Finkelman, F. D. (1998): Intestinal nematode parasites, cytokines and effector mechanisms. Int. J. Parasitol., 28 (8): 1145-1158.

Ernst, H.; Konturek, P. C.; Brzozowski, T.; Konturek, S. J. and Hahn, E. G. (1996): Subserosal

application of transforming growth factor-beta 1 in rats with chronic gastric ulcers: effect on gastric ulcer healing and blood flow. J. Physiol. Pharmacol., 47:443-454.

Finkelman, F. D.; Shea-Donohue, T. and Goldhill, J. (1997): Cytokine regulation of host defense against parasitic gastrointestinal nematodes: lessons from studies with rodent models. Ann. Rev. Immunol., 15: 505-533.

Groux, H. and Powrie, F. (1999): Regulatory T cells and inflammatory bowel disease. Immunology Today, 20: 442-446.

Helal, I.; Agamy, E. and Amer, S. (1998): Interaction between *Toxocara vitulorum* (Nematoda) and *Prohemistomum vivax* (Trematoda). J. Med. Res. Inst., 19(4): 166-182.

Hong, S.; Han, J.; Park, C. and Kang, S. (1997): Intestinal pathologic findings at early stage infection by *Centrocestus armatus* in albino rats. Korean J. Parasitol., 35(2): 135- 138.

Huh, S; Chai, J.; Hong, S. and Lee, S. (1988): Clinical and histopathological findings in mice heavily infected with *Fibricola seoulensis*. Korean J. Parasitol., 26(1): 45-53.

Isaacson, A.; Huffman, J. and Fried, B. (1989): Infectivity, growth, development and pathology of *Echinostoma caproni* (Trematoda) in the golden hamster. Int. J. Parasitol., 19(8): 943-944.

Jang, Y.; Kang, S.; Kim, S. and Cho, S. (1985): *In situ* posture of anterior body of *Metagonimus yokogawai* in experimentally infected dog. Korean J. Parasitol., 23(2): 203-213.

Khalil, A. (1987): Biochemical and pathological changes in *Clarias lazera* (catfish) naturally infected with metacercariae parasites in Gharbia province. PhD. Thesis, Fac. Sci. Tanta Univ.

Khalil, A. and Helal, I. (1992): Scanning electron microscopy of the tegumental surface of adult *Prohemistomum vivax* (Sonsino, 1892) Azim, 1933 (Trematoda: Cythocotylidae). J. Egypt. Ger. Soc. Zool., 7: 459-473.

Khalil, A.; Helal, I.; Mansour, M. and Gabr, H. (1998): Host-induced morphological variations in the digenetic trematode *Prohemistomoum vivax* (Sonsino, 1892) Azim, 1933. J. Egypt. Ger. Soc. Zool., 25(D): 91-113.

Kim, I.; IM, J.; Lee, K. and Pyang, Y. (2000): Muscosal mast cell responses in the small intestine of rates infected with *Echinostoma hortense*. Korean J. Parasitol., 38(3): 139-143.

Knight, P. A.; Wright, S. H.; Lawrence, C. E.; Paterson, Y. Y. and Miller, H. R. P. (2000): Delayed expulsion of the nematode *Trichinella spiralis* in mice lacking the mucosal mast cell-specific granule chymase, mouse mast cell protease-1. J. Exp. Med., 192: 1849-1856.

Lee, J.; Chi, J.; Lee, S. and Cho, S. (1981): Study on the pathology of metagonimiasis in experimentally infected cat intestine. Korean J. Parasitol., 19(2): 109-129.

Lee, S.; Yoo, B.; Hong, S.; Chai, J.; Seo, B. and Chi, J. (1985): A histopathological study on the intestine of mice and rats experimentally infected by *Fibricola seoulensis*. Korean J. Parasitol., 23(1): 58-72.

Lee, S.; Nah, T.; Sohn, W.; Kho, W.; Hong, S. and Chai, J. (1990): Chronological observation of intestinal lesions of rats experimentally infected with *Echinostoma hortense*. Korean J. Parasditol., 28: 45-52.

Lee, S.; Yoo, B.; Hong, S.; Chai, J.; Seo B. and Chi, J. (1989): A histopathological study on the intestine of mice and rats experimentally infected by *Fibricola seoulensis*. Korean J. Parasitol., 23(1): 58-72.

Letterio, J. J. (2000): Murine models define the role of TGF-beta as a master regulator of immune cell function. Cytokine Growth Factor Rev., 11: 81-87.

Letterio, J. J. and Roberts, A. B. (1998): Regulation of immune responses by TGF-ß. Ann. Rev. Immunol., 16: 137–161.

Liebman, W.; Thaler, M.; Delorimier, A.; Brandborg, L. and Goodman, J. (1980): Intractable diarrhoea of infancy due to intestinal coccidiosis. Gastroenterology, 78: 579-584.

Manson-Smith, D.; Bruce, R. and Parrott, D. (1979): Villous atrophy and expulsion of intestinal *Trichinella spiralis* are mediated by T cells. Immunology, 47: 285-292.

Nasr, M. (1941): The occurrence of *Prohemistomum vivax* infection, with description of the parasite. Lab. Med. Prog., 2: 135-149.

Omer, F. M.; Kurtzhals, J. A. and Riley, E. M. (2000): Maintaining the immunological balance in parasitic infections: a role for TGF-beta. Parasitology Today, 16:18-23.

Onah, D. N. and Nawa, Y. (2000): Mucosal immunity against parasitic gastrointestinal nematodes. Korean J. Parasitol., 38(4): 209-236.

Paalangara, R.; McClure, S. and McCullagh, P. (2003): Intestinal exposure to a parasite antigen in utero depresses cellular and cytokine responses of the mucosal immune system. Vet. Immunol. Immunopathol., 20; 93(3-4):91-105.

De-La-Pena, M. C.; Ramos-Solis, S.; Barbosa-Cisneros, O.; Rodriguez-Padilla, C.; Tavizon-Garcia, P. and Herrera-Esparza, R. (2002): *Echinococcus granulosus* down regulates the hepatic expression of inflammatory cytokines IL-6 and TNF alpha in BALB/c mice. Parasite, 9 (4): 351-356.

Seo, B.; Cheong, S.; Chai, J.; Lee, S. and Lee, J. (1986): Histopathology of small intestine of rats and mice experimentally infected with *Pygidiopsis summa*. Korean J. Parasito., 27: 125-134.

Shalaby, S. (1988): Parasitological observations on *Prohemistomum* sp., encysted metacercariae (Trematoda) in Nile fishes. II. Experimental animals. Alex. J. Vet. Sci., 4(1): 243-251.

Sharmin, S.; Shimizu, Y.; Hagiwara, A.; Hirayama, K. and Koyama, A. (2004): *Staphylococcus aureus* antigens induce IgA-type glomerulonephritis in Balb/c mice. J. Nephol., 17: 504-511.

Shiner, M. and Barkin, J. (1985): Structural changes in the malabsorption syndromes. In "Bockus Gastroenterology, Beek, J. E. (4th Ed.)". Saunders, W.B. Philadelphia, PP. 1681- 1699.

Shih, S. C.; Tseng, K. W.; Lin, S. C.; Kao, C. R. and Chou, S. Y. (2005): Expression patterns of transforming growth factor-beta and its receptors in gastric mucosa of patients with refractory gastric ulcer. World J. Gastroenterol., 7(11): 136-141.

Shull, M. M.; Ormsby, I. and Kier, A. B. (1992): Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. Nature, 359: 693-699.

Souza, R. F.; Lei, J. and Yin, J. (1997): A transforming growth factor β1 receptor type II mutation in ulcerative colitis-associated neoplasms. Gastroenterology, 112: 40-45.

Su, Z.; Segura, M.; Morgan, K.; Loredo-Osti, J. C. and Stevenson, M. M. (2005): Impairment of protective immunity to blood-stage malaria by concurrent nematode infection. Infect. Immun., 73(6): 3531-9.

Symon, L. (1976): Malabsorption, pathophysiology of parasitic infection. (Soulsby, E. Ed.) Academic Press, New York.

Taipale, J.; Saharinen, J. and Keski-Oja, J. (1998): Extracellular matrix associated transforming growth

factor-beta: role in cancer cell growth and invasion. Adv. Cancer Res., 75: 87 -134.

Tominaga, K.; Arakawa, T.; Kim, S.; Iwao, H. and Kobayashi, K. (1997): Increased expression of transforming growth factor-beta1 during gastric ulcer healing in rats. Dig. Dis. Sci., 42: 616-625.

Zhang, B. B.; Jiao, Y. W.; Cai, W. M.; Tao, J. and Liu R. H. (2004): Influence of interferon gamma treatment on expression of TGF-beta1 and its receptors in liver fibrosis of mice with *Schistosomiasis japonica*. Bing Za Zhi., 22(6): 340-343.

دراسة التغيرات النسيجيه والجزيئيه على أمعاء الفئران المصابه معمليا بطفيل بروهيمستوميوم فيفاكس "تريماتودا: سياثوكوتيليدى"

مجدى السيد محفوظ – نبيله موسى ميره – سعيد عماد الدين عامر

قسم العلوم البيولوجيه - كلية التربيه - جامعة طنطا- فرع كفر الشيخ

أظهرت نتائج الفحص الميكروسكوبى حدوث انضغاط وأحيانا تآكل فى الطبقة الطلائية المبطنة للأمعاء خاصة فى مكان وجود الطفيل. ولقد ظهر تشوه فى الخملات فى صورة قصر فى طولها مع تقدم الإصابه. كما لوحظ اختفاء بعض الخملات فى مكان وجود الطفيل وكذلك حدوث تضخم فى بعض التجاويف مع ظهور الخلايا الالتهابيه فى الصفيحة الأساسيه الداعمة (lamina propria) للفئران المصابه وقد وصل إلى ذروته مع نهاية مدة الإصابه. وفى نفس الوقت كانت نتائج التحليل الجزيئى متماشية مع نتائج التغيرات النسيجيه. ولقد ثبت أن مستوى التغير الجينى للسيتوكاين TGF-β كان عاديا فى بداية العدوى وازداد ارتفاعا خلال فترة العدوى بالمقارنة بمجموعة الفئران الضابطه. ولقد دعمت هذه النتائج دور TGF-β خلال عدوى طفيل البروهيمستوميوم فيفاكس. وقد تم مناقشة نتائج البحث فى ضوء نتائج الأبحاث المتاحه فى هذا المجال. لدراسة التغيرات المرضيه والجزيئية المحتمله فى الأمعاء الدقيقة للفئران خلال الإصابة بطفيل بروهيمستوميوم فيفاكس، تم إحداث العدوى معمليا عن طريق الفم لعدد 30 فأر بمعدل 150 ميتاسركاريا لكل منها. تم أخذ مجموعات من الفئران المصابه (3 فئران لكل مجموعه) وقتلها وتم تشريحها للفحص النسيجى بعد 6 و 12 ساعه من الإصابه ثم يوميا حتى اليوم السابع بعد الإصابه وكذلك من المجموعة الضابطه. بعد ذلك تم تجهيز عينات نسيجيه، وذلك من الجزء العلوى للأمعاء الدقيقه للفئران لصباغتها بالهيماتوكسلين والإيوسين. ومن ناحية أخرى تم أخذ عينات نسيجيه من نفس المناطق السابق ذكرها وتم استخلاص الحامض النووى الريبوزى وعمل نسخ عكسى له للحصول على الحامض النووى الديؤكسى الريبوزى، ثم تم عمل تحليل كمى عن طريق تفاعل البلمره "PCR" لجين السيتوكاين (TGF-β) لتحديد مستواه خلال فترة الإصابه.

المحكمون:

ا.د. فتحى عبدالعاطى عبدالغفار – علوم القاهرة

ا.د. ابراهيم بكر هلال - علوم طنطا