The Effect of Toxoplasmosis on the Histology of a Mouse Small Intestine

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Abstract

Background: This study is concerned with the effect of Toxoplasmosis on the histology of a mouse small intestine. A group of 10 mice were examined by a light microscope. The mice were inoculated intraperitoneally with placental fluid containing Toxoplasma gondii parasite to induce infection. After 10-15 days of inoculation, the small intestines of the mice were examined histologically by a light microscope.

Patient and Method: Isolation of the Toxoplasma gondii parasite came from pregnant women who suffered from acute Toxoplasmosis which was proven by an ElISA test showing the presence of the IgM antibody against the parasite in their blood. These women were chosen as a source of the Toxoplasma gondii parasite. Placental fluid was taken from those women after abortion and inoculated intraperitoneally in the mice.

Result: Histological examination of the mice's small intestines showed the presence of Toxoplasma Gondii tachyzoites and bradyzoites invading all layers of the small intestine together with an intense inflammatory cellular infiltration, vasodilatation, congestion, hemorrhage and necrosis with the destruction of the villi, degeneration of the central lacteal inside the lamina propria of these villi. These changes indicated that the infection with the Toxoplasma Gondii parasite could affect all the organs in the body especially the small intestine.

Conclusion: Toxoplasma Gondii invade all the layers of the small intestine leading to the destruction and disturbance in their function.

Keywords: Toxoplasma gondii, mice intestines, Toxoplasmosis.

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Introduction

Toxoplasma gondii is a universally distributed pathogen that infects over one billion people worldwide.[1] Toxoplasma Gondii is a ubiquitous parasite of the phylum Apicomplexa, the largest and most important group of obligate parasite, which also includes the human pathogens Plasmodium (the cause of malaria) and Cryptosporidium.[2] Humans and other warm-blooded animals are often infected with Toxoplasma gondii through the ingestion of contaminated food and water.[3] Once ingested Toxoplasma gondii parasites penetrate the intestine and rapidly disseminate through all the organs of the body.[4]

Toxoplasma Gondii causes many changes when they invade the cell; those changes are particularly due to the DNA damage that is provoked by the infection.[5] Nearly all the internal organs and tissues will be affected after an

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infection with the Toxoplasma gondii parasite. This will lead to many pathological changes ranging from mild congestion to severe degeneration involving mainly the liver, spleen and pancreas.[6,7]

The wall of the small intestine consists of mucosa, submucosa, muscular layer and serosa. The mucosa of the small intestine exhibits specialized structural modifications that increase the cellular surface areas for the absorption of nutrients and fluid. These modifications include the plicae circulares, the villi, and the microvilli.

Intestinal glands (crypts of Lieberkuhn) are located between the villi throughout the small intestine. These glands open in the intestinal lumen at the base of the villi. The glands consist of stem cells, absorptive cells, goblet cells, paneth cells, and some enteroendocrine cells.

The main function of the small intestine is the digestion of the gastric contents and absorption of the nutrients into the blood capillaries and lymphatic lacteals.[8]

The natural infection with the Toxoplasma gondii parasite is acquired by the ingestion of the encysted organisms which are released in the digestive tract; they invade the intestinal tissues and then disseminated via the blood stream.[9]

In this study, an attempt was made to evaluate the effect of an infection of the Toxoplasma gondii parasite on the histology of a mouse small intestine.

Patients, Materials and Methods

Experimental Animals: 6-10 week old male mice were used for the experiment. Ten mice were isolated and maintained on a normal diet; they were inoculated intraperitoneally with placental fluid containing the Toxoplasma gondii parasite.

Isolation of the Toxoplasma Gondii Parasite

Pregnant women infected by an acute Toxoplasmosis and their infection was proved by an ELISA test showing the presence of the IgM antibody against the parasite in their blood were chosen as a source of the Toxoplasma gondii parasite. After an abortion, the placentae were taken from those women and pieces of placental tissue about 30-50 gm in weight were taken, cut into small pieces and grinded using a pestle and mortar. This was followed by placing them in a homogenizer added with 0.9% normal saline and then by adding the enzyme trypsin (0.25% in Nacl solution). This mixture was then placed in an incubator for one hour. Filtration of this mixture was made using filter paper to remove large pieces of tissue, followed by placing the mixture in a centrifuge for 10 minutes. Then a phosphate buffer saline (PBS) was added to the precipitate with a normal saline solution to prevent bacterial contamination. A solution of normal saline containing 1000 IU of penicillin and 100mg of streptomycin was also added to the above solution.[10]

Route of Infection: Intraperitoneal inoculations of 0.5-1 ml. of the placental tissue suspension were made for the mice. The mouse inoculation with placenta fluid containing congenital Toxoplasmosis was regarded as the best diagnostic way.[10] After about 10-15 days, the mice were scarified and the small intestines were taken for further study.

Preparation of Tissues for Histological Analysis: Pieces of a small intestine were kept for a few days in a 10% neutral buffered formalin solution. Then the tissues were cut into 1 cm thick slices, dehydrated by alcohol, cleared with xylol and finally embedded in paraffin made into blocks.

Using a Reichert Rotary microtome, serial paraffin sections of μm were made and the sections were then stained with Harries Hematoxylin and Eosin and Giemsa stain.[11]

Results

The examination of the small intestine revealed marked histological changes. The presence of the

Toxoplasma gondii parasite (tachyzoites and bradyzoites) was distributed throughout the small intestine invading all layers of the mucosa. Furthermore, the tachyzoites of *Toxoplasma gondii* were maximally accumulated in the submucosa, fig.(1), causing many changes including decomposition and destruction of the villi, fig.(2); an inflammatory reaction characterized by congestion, dilated blood vessels, the presences of the inflammatory cells infiltration in all layers of the small intestine and necrosis were noticed, figs.(3,4,5), in addition to the destruction of the central lacteal inside the lamina propria of the villi, fig. (6).

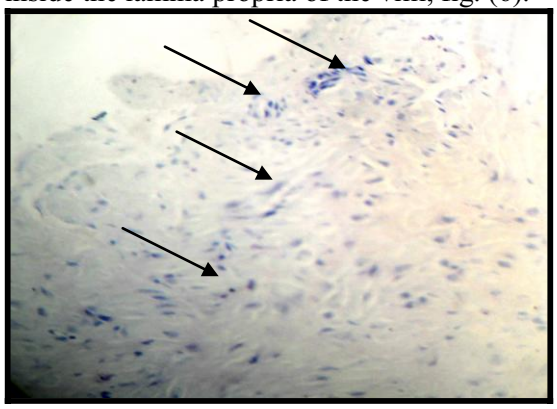

Fig (1): Photomicrograph of the submucosa in the small intestine of a mouse infected with Toxoplasma gondii parasites showing the tachyzoites and bradyzoites (arrowheads), Giemsa stain 400x.

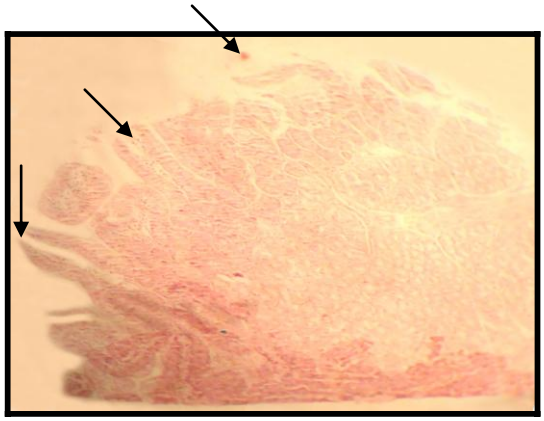

Fig (2): Photomicrograph of a mouse small intestine infected with Toxoplasma gondii parasites showing decomposition and destruction of the villi (arrowheads), H&E 100x.

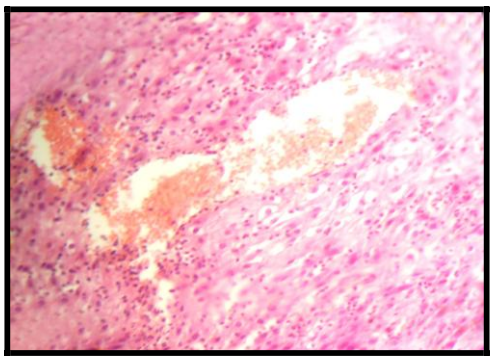

Fig (3): Photomicrograph of a mouse small intestine infected with Toxoplasma gondii parasites showing congestion and dilated blood vessels, H&E 400x.

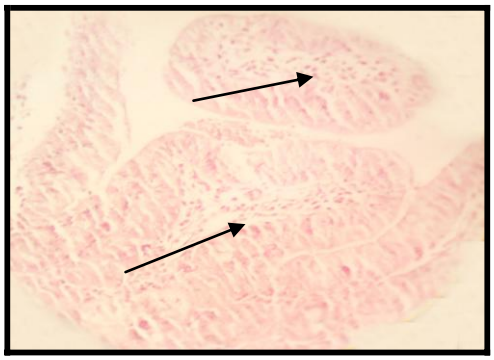

Fig (4): Photomicrograph of a mouse small intestine infected with Toxoplasma gondii parasites showing inflammatory cell infiltration in the lamina propria of the villi (arrowhead), H&E stain 400x.

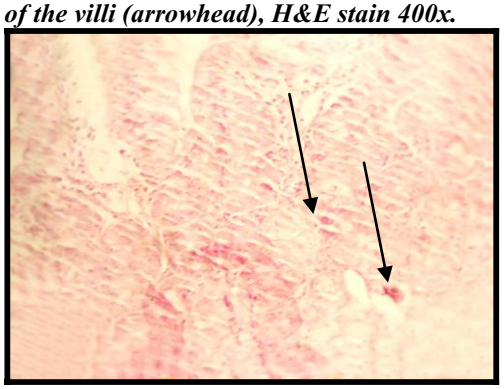

Fig (5): Photomicrograph of a mouse small intestine infected with Toxoplasma gondii parasites showing necrosis in the cells of intestinal gland (arrowheads), H&E 400x.

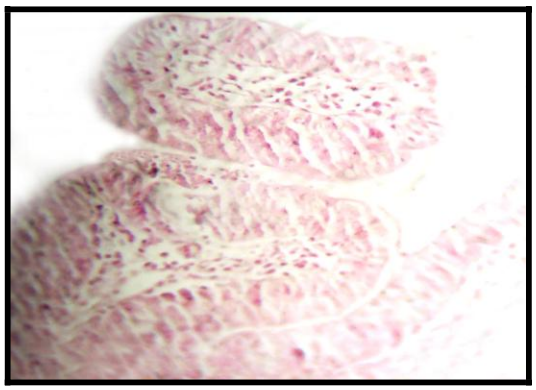

Fig (6): Photomicrograph of a mouse small intestine infected with Toxoplasma gondii parasites showing the absence of the central lacteal from the lamina propria of the villi, H&E 400x.

Discussion

Toxoplasmosis remains a serious disease. In the many studies that have been carried out on the *Toxoplasma gondii* parasite and its effect on tissue histology, the small intestine remains one of the most important organs affected by this infection and a preferable site for the growth and multiplication of the *Toxoplasma gondii* parasite.[5,6]

In the experimental models of infection with the *Toxoplasma gondii* parasite, acute mortality can result either directly from a failure to control the parasite number and the toxin produced from the parasite or indirectly due to an excessive immunological response against the infection. The natural infection with the *Toxoplasma gondii* parasite is acquired by the ingestion of the encysted organisms which are released in the digestive tract. They then invade the intestinal tissues and disseminate via the blood stream.[9]

In this study, many histological changes were observed in the small intestines of mice infected with the *Toxoplasma gondii* parasite.

The presence of the parasite inside and outside the vessels of the small intestine indicated that it is one of the important organs involved and affected during the parasitaemia stage of the infection.[10]

The *Toxoplasma gondii* parasite undergoing endo-enteric development in the small intestine affect the structures of the lining cells in the epithelium of the villi which was related to the parasite distribution within the small intestine.[12]

This is also recorded by other researchers and some relate the disturbance in the small intestine function and necrosis due to DNA damage in the lining cells of the epithelium and the cells of the intestinal glands which are provoked by the infection of Toxoplasmosis.[5]

Another important histological finding was that vasodilatation was responsible for the increased vascular perfusion in order to prepare the way for the inflammatory infiltrate to enter the inflamed tissue. Vasodialation induced by the relaxation of the smooth muscles in the blood vessels wall results in the increase in blood flow. There is normally a balance between fluid leaving vascular spaces and fluid re-entering the system. Inflammation shifts this balance, and causes an accumulation of fluid into the extravascular compartment.[13]

Microscopical examination also revealed that the intense infiltration of the mucosa with the mixed inflammatory cells, mainly the lymphocytes and plasma cells, is one of the principle purposes in inflammation. This observation was also noted by other researchers [9,14] who found that chronic inflammatory cells are normally not present in the tissues unless there is an infection in which a chronic inflammatory process takes place within the vascular layers. These cells leave the blood and the enter the tissues as a part of the immunological response of the mice to the infection of the *Toxoplasma gondii* parasite. The lymphocytes usually accumulate somewhat later during the inflammatory process. Their presence in large numbers indicates a continuous presence of antigens and thus suggests an established infection.

Another important histological finding is the decomposition and distortion in the shape of the villi and in the structure of the mucosa through the wall of the small intestine caused by the

parasitic invasion within the small intestine.[14]

This could be also related to the immunological reaction that occurs in the wall of the intestine as observed by other researchers [15] who observed that the destruction of the mucosa of the small intestine in the *Toxoplasma gondii* infection is related to the immunological reaction induced by the T-lymphocyte cells rather than by the tachyzoites and bradyzoites itself. The absence of the central lacteal within the lamina propria of the villi in Toxoplasmosis was not observed by other researchers. This finding is important to the function of the small intestine affecting the absorption of fats.

In conclusion, this study suggests that there is an inflammatory reaction induced by an infection with *Toxoplasma gondii* which is manifested by the presence of the tachyzoites, inside and outside the vessels, followed by the accumulation of inflammatory cells which suggests an important role of these cells in the host inflammatory response against the *Toxoplasma gondii* parasite.

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تأثير داء المقوسات في أنسجة أمعاء الفئران الدقيقة

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الملخص

مقدمة: تبحث الدراسة الراهنة في تأثير داء المقوسات في أنسجة أمعاء الفئران الصغيرة؛ حيث تكوّنت عينتها من 10 فئران، درست بوساطة المجهر الضوئي. تم تلقيح الفئران بسائل يحوي على المقوسة الغوندية الطفيلية للحث على العدوى. وبعد 10-15 يوما من التلقيح، تم فحص أمعاء هذه الفئران الدقيقة بوساطة المجهر الضوئي.

الطرق: ثبتت عزلة الطفيل جونديايي التوكسوبلازما في نساء حوامل يعانين داء المقوسات الحاد والعدوى، وعن طريق اختبار الإليزا تبين وجود الضد IgM ضد الطفيل في دمائهم.

النتائج: أظهر الفحص النسيجي لأمعاء الفئران الصغيرة وجود جونديايي التوكسوبلازما تغزو جميع طبقات الأمعاء الدقيقة مع توسع الأوعية، والازدحام، والنزف، ونخر مع تدمير الزغابات، وأشارت هذه التغييرات إلى أن الإصابة بطفيل جونديايي التوكسوبلازما يمكن أن يؤثر في جميع أجهزة الجسم وخاصة في الأمعاء الدقيقة.

الخلاصة: تغزو جونديايي التوكسوبلازما جميع طبقات الأمعاء الدقيقة، مما يؤدي إلى دمار وظيفتها واضطرابها.

الكلمات الدالة: جونديايي التوكسوبلازما، أمعاء الفئران، جونديايي التوكسوبلازما.