The significance of histopathological diagnosis of toxoplasmosis
(Experimental acute acquired toxoplasmosis in mice)

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The clinical features of acquired toxoplasmosis are varying from asymptomatic lymphadenopathy to the lethal infection in the immunologically compromised host.

The histological changes are described in lymph nodes because of the commonest presenting sign. Although the typical histomorphologic features are described here, acquired toxoplasmosis is usually diagnosed serologically. On the other hand, despite its size and prominent morphological features, many pathologists appear hesitant to accept the characteristic histopathologic changes. Therefore, it should be remembered that it is not easy to make a differential diagnosis of toxoplasmosis from malignant lymphoma and some reactive lesions especially in the lymph nodes. For this purposes, the intraperitoneously infected mice have been searched not only for the presence of T.gondii. but also for the histopathologic changes either in the lymph nodes or in the other tissues.

We have presented the results of this study to call to the attention of both clinicians and pathologists that toxoplasmosis is more frequent than is recognised at present and because of the succesfull treatment with pyrimethamine and sulfonamides, effective diagnostic procedures should be considered even in clinical or morphological suspicion.
[Turk J Med Res 1992; 10(6):314-320]

Key Word*: Toxoplasma, Toxoplasmosis

Toxoplasmosis is not nearly as rare as formerly thought. In the United States, chronic asymptomatic form is estimated to be present in about 50 percent of the population (1,2). In Turkey, toxoplasmosis was first described in 1953 by Unat, Alyanak and Sahin (3). The exact prevalance of acquired toxoplasmosis is unknown but its range changed between 16.4% to 29.4% according to dye test and over the past 40 years few number of reports were published indicating the increasing frequency (4).

Although occurrences of toxoplasmosis are widespread, a little attention has been paid to the charasteristic nature of its histopathologic changes (5-7). Toxoplasmic lymphadenitis is the most frequently observed form and its differential diagnosis from much more serious conditions, especially the neoplastic lesions of the node may be quite hard on both clinical and pathological grounds (5,6,8-10).

Geliş Tarihi: 29.6.1992 **Kabul Tarihi:** 3.11.1992

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According to these reviews the histopathologic findings are sufficiently distinctive and correlate to a remarkable degree with the level of serological tests (5,6,11,12). However, cases with definite demonstration of pathogen, Toxoplasma gondii, are exceedingly rare. For this purpose, the peroxidase-antiperoxidase immuno-histochemical technique was employed to stain formalin fixed, paraffin embedded tissue sections and the immunofluorescence method was also used (13-15). But these methods need more detailed studies.

The present study, based on the histopathologic findings in mice with the diagnosis of acute acquired toxoplasmosis, showed effective invasion of nonphagocytic as well as phagocytic cells by T.gondii. The distinctive histopathologic changes are not only occured in lymph nodes, the parasites could also be seen in most such cases even in conventionally stained sections if pathologists gave more attention.

MATERIALS AND METHODS

In this study, 29 albino mice weighing 20-25gr were used. The strain used was the virulent strain otained from the Department of Microbiology, Anakara university-

Mice were injected intraperitoneally with peritoneal exudate obtained from mice that had been injected intraperitoneally with a similar exudate four days previously.

The exudate which was obtained from infected mice was diluted with sterile physiological saline to get 2-5 parasites per high power field. 0.3ml of this suspension was injected intraperitoneally into the mice.

Thi mice were observed daily. They were noted to be rather quiet and remain apart from their cage mates, and all died of toxoplasmosis within 4 or 9 days of inoculation.

Six mice were sacrificed as a control group.

At autopsy impression from the surfaces of the lungs, liver, spleen, kidneys and smears from peritoneal cavities were done and stained with both Giemsa and Papanicolaou's method.

The spleens were usually somewhat enlarged and some of them showed small gray-to-brown nodules scattered throughout the cut surface. Some liver contained multipl cysts due to Cysticercus fasciolaris. The other organs showed no abnormalities.

After cutting off the skull bones whole body was fixed in 10% formalin, The eyes and the brain were obtained after the fixation. After fixation all tissues were embedded in paraffin, cut at 4-5 u, and stained with Hematoxylin-Eosin and Giemsa stain. While further detailed studies are to be continued, here, we have decided to report the results of five of them which will be a precedent for others.

RESULTS

The lymph nodes, liver, spleen, lungs, kidney, suprarenal glands, pancreas, salivary glands, bowel, genital organs, muscle, adipose tissue, vessels, brain, **eye** and hearts of the mice in both control and test groups were searched histopathologically. None of the mice in control group showed toxoplasma. Congestion was also noted in almost all tissues of every mice.

The findings in the test group is outlined below according to the organs and tissues.

Lymph nodes: Histopathologic changes that occur in lymph nodes affected by T.gondii can be summarized as follows;

1. Focal distention of subcapsular sinuses by monocytoid cells,

2. Various amounts of necrosis ranged from cell to massive necrosis,

3. Multipl toxoplasma cysts and clusters of free tachyzoites.

The capsule was often infiltrated by lymphocytes and free tachyzoites streaming out into the surrounding fatty connective tissue. Follicular structures were not generally seen because of the necrosis or cysts. Cysts and free tachyzoites were present both in the areas of massive necrosis and some surviving nodal tissue. In these areas most lymphocytes were large lymphoid cells designated as antigenically stimulated cells (Fig 1,2). There were also numerous mitoses scattered through them. Organization of the necrosis was not seen.

T.gondii was observed in histiocytes in the cyst formation nevertheless many of them were found within the extracellular areas. While both cysts and tachyzoites were identified in all instances, diffuse necrosis were seen in omly 15 of 43 lymph nodes totally obtained from 5 mice.

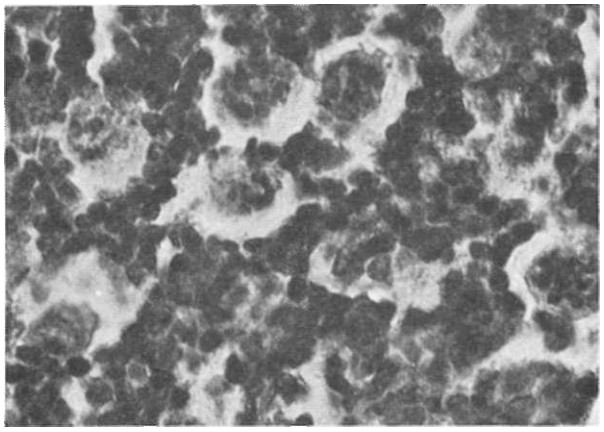

Figure 1. Toxoplasma cysts in a lymph node (H-E, X250).

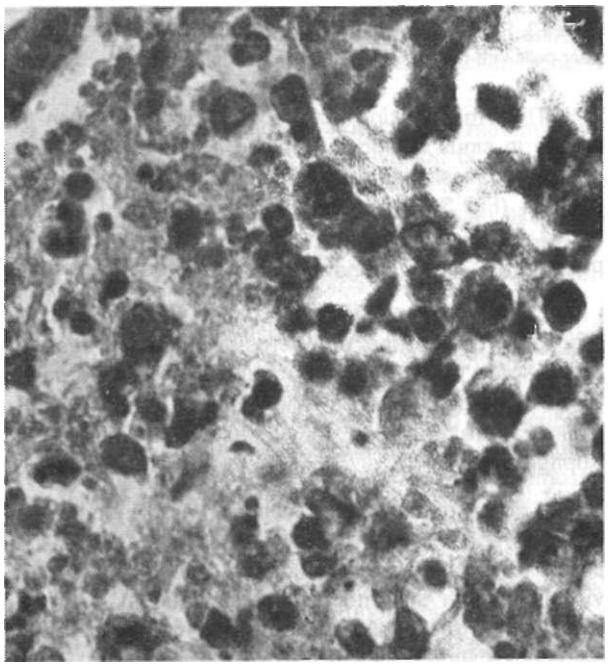

Figure 2. Free T.gondii tachyzoites and nuclear pleomorhism with necrosis in a lymph node (H-E, 250).

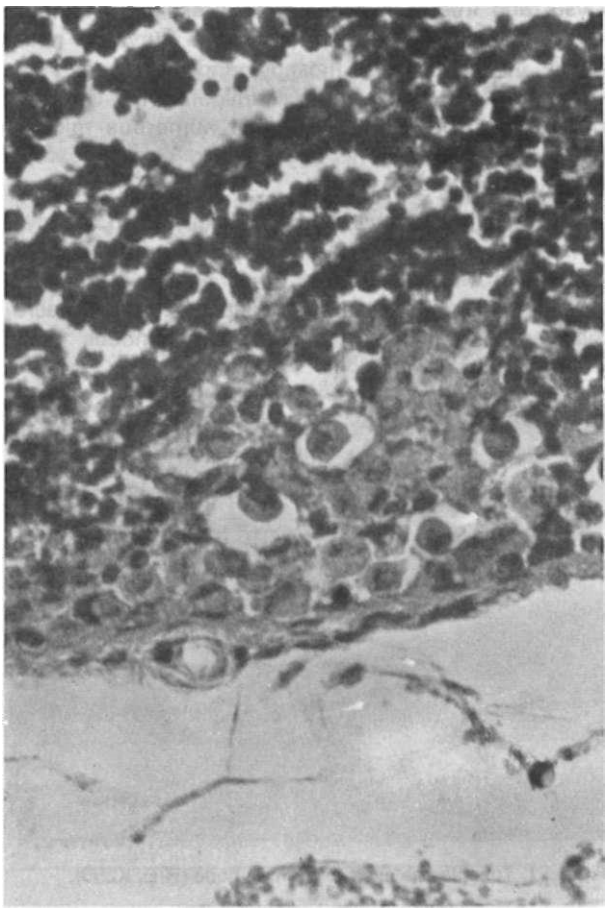

Figure 3. Subcapsular sinus is filled and distended by mononuclear cells in a lymph node (H-E, X50).

The other striking feature was focal or diffuse increase in sinus histiocytes having abundant pale cytoplasm and vesicular nuclei especially filling peripheral sinuses (Fig 3). This finding was seen in 11 of 43 nodes, but in only 5 of them showed excessive hyperplasa.

Liver: All liver tissues of 5 mice were invaded by T.gondii. Focal areas of necrosis, slight increase of portal areas with mainly mononuclear cells were seen. Some liver cells were swollen with a granular cytoplasm. There was also an increase in the number of Kupffer cells.

T.gondii demonstrated in the areas of necrosis in, between and inside the liver cells scattered over the tissue (Fig 4). The morphology of infected liver cells visualised both the encysted or included at least two or more free tachyzoites within a host cell vacuole, the pseudocyst form. None of the liver cell necrossi, cysts and free tachyzoites were surrounded by inflammatory reaction.

Spleen: Every spleen from 5 mice were also invaded by T.gondii. Various amounts of necrosis and free tachyzoites in these necrotic areas were seen. Parasitized cells and cyst formation were seen, but it was a less common finding.

Lungs: The parasites invaded alveolar cells especially located at the peripheric areas of the lung. There was also marked infiltration of parasites throughout the pleura. Except one case only a few cysts were found in the alveolar cells and some polymorphonuclear leucocytes in the septal spaces, there wasn't any interstitial infiltration by inflammatory cells. On one occasion a cyst was found in the alveolar space which could only be demonstrated by Giemsa stain despite exhaustive study of skip sections (Fig 5).

Kidneys: Free toxoplasmas and cysts were seen in every fibrous capsula and surrounding perirenal adipose tissue. But only a few organisms were found in one kidney of mice which were located in its tubular cells.

Suprarenal glands: Cysts and free organisms invaded both capsular and subcapsular areas of the gland in every mice of 5.

Pancreas: Both peripheral and septal fibrous tissue were infiltrated with toxoplasma but the density of the parasites varied and two of the 5 mice showed foci of necrosis and paranchimal invasion.

Salivary glands: Numerous sections from all glands of five mice revealed mild capsular invasion. Three of them showed occasional free tachyzoites in their paranchimal cells.

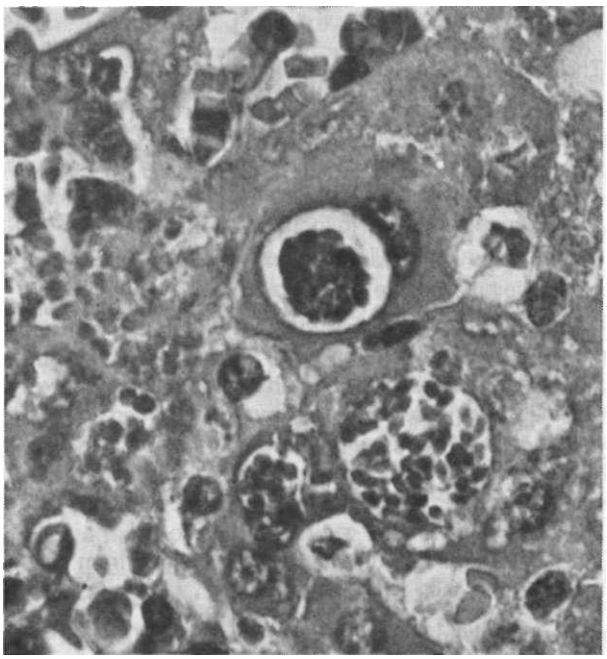

Figure 4. Cyst, pseudocyst and free tachyzoites of T.çondii in degenerated hepatocytes (H-E, X250).

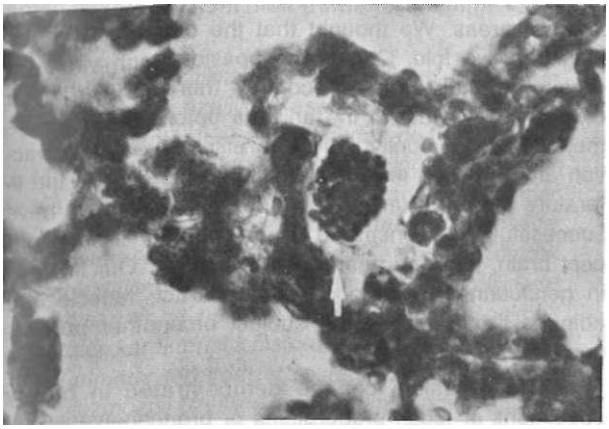

Figure 5. Toxoplasma cyst in the alveolar space of a lung (Giemsa, X250).

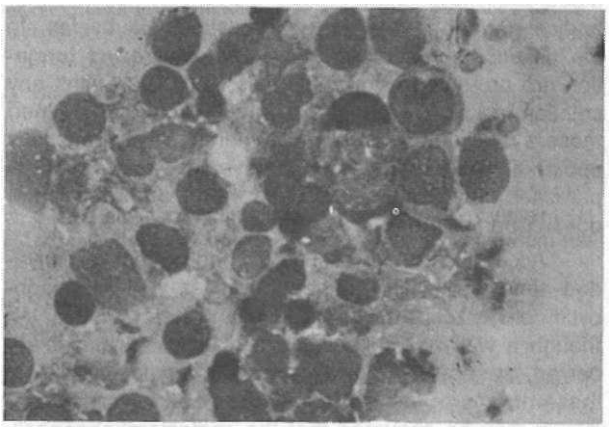

Figure **6.** T.gondii in a touch smear of a spieen (Giemsa, X250).

Bowel: On microscopic examination, sections were made from the Peyer's patches, the serosal surfaces, lymphoid tissues and the epithelium were all invaded by the parasite.

Genital organs: In the presented study 2 of the 5 mice were male. While the organisms were found only in the peritesticular and periepididymal structures, in females they were also found in endometrium, myometrium, ovaries and in their surrounding fibroadipose tissues.

Muscle: The necropsy specimen was taken from the upper parts of the lower extremities. The histopathologic changes consist of areas of some necrotic muscle fibers surrounded by free organisms.

Adipose tissue: These tissues were obtained especially from surrounding genital organs. Patchy necrosis of fat were encountered, cysts and free parasites were seen in both necrotic foci and between intact cells around them.

Vessels: Cysts and free tachyzoites were seen in some large vessel walls. Only few of them were located in the lumen.

Although numerous serial sections from the brain, eye and hean were made, Toxoplasma was not recovered from either the brain and eye or myocardium.

Both Giemsa and Papanicolaou-stained impression smears from the surfaces of the liver, lung, kidney and spleen revealed many parasites both in enlarged cells and free (Fig 6).

In summary, Toxoplasma was found in microscopical sections of the lymph nodes, liver, spleen, lungs, kidneys, adrenal glands, pancreas, salivary glands, skletal muscle, bowel, genital organs of male and females of the experimentally infected mice.

DISCUSSION

Toxoplasmosis is a common, mostly asymptomatic infection caused by a protozoan, Toxoplasma gondii. In man, serelogic data indicate that there is increasing prevalence of serologic reactions with increasing age and little or no difference in prevalence between sexes (16,17). There are considerable geographic differences. Colder regions and high areas or hot, dry areas have less human infection than warm, moist regions (11,16,18,19).

In contrast to other obligate intracellular protozoal parasites, T.gondii has an unusually broad intermediate host spectrum (20). However, only cat and the other members of the felidae serve as a definitive host (1,21). Actually, it has been demonstrated that rats and sheep lacked Toxoplasma antibody on Pacific islands where cats are absent (22).

Human infections arise directly from oocysts shed by cats or indirectly by eating undercooked meat of infected animals containing various stages of parasites. Infection of the fetus follows maternal infection acquired by either route (9, 21,23).

T.gondii invades almost all nucleated mammalian cells (20,24). According to Werk, invasion of host cells by toxoplasma is a complex mechanism in which both phagocytosis of target cell and active invasion of parasite participate (20). Chemical factors and membrane composition seem to be important for the entrance of the parasite (25-30).

Toxoplasmosis is usually fatal in the acute stage in mice (31,32). When experimenting with Toxoplasma, a few, sometimes even a single one will suffice to kill the mice (33). In the acute stage following an incubation period varying in length, parasites spread via the blood and lymph channels (31). Infection occurs most commonly by the respiratory route. In the naturally occuring disease primary involvement is the nasopharyngeal lympoid tissue. Indeed cervical lymph nodes are commonly affected without sore throat (6,7,34-37).

The tachyzoites and bradyzoites are parasitic stages. While the tachyzoites multiply rapidly and destroy the cells, slowly multiplying organisms, the bradyzoites, coexist with the host cells for weeks, even years (21). Proliferating parasites often produce focal necrotic lesions especially in the liver, lung, spleen,

heart, brain and other organs (23,38). When the antibodies appear in the serum parasitemia subsides. In the tissues, parasites in a cyst or pseudocyst formation are protected from antibodies (31). There isn't any cellular reaction around the intact cycts (23). When these cysts rupture localized hypersensitivity reactions occur. In the central nervous system proliferation of parasites may continue because the blood-brain barrier doesn't permit the entrance of antibodies (21,31,38).

According to our results T.gondii effectively invaded almost all cells, nonphagocytic as well as phagocytic cells, except the brain, eye and heart tissues. Although it was not possible to test all of the tissue received, we couldn't discover even a single parasite in these organs of the five mice inoculated with the parasite. It may be assumed that the cholesterol/phospholipid content of the membrane of these cells is different and it may also be attributed to their mitotic activity which is related to their level of specialization. It is clear that neurons and cardiac myofibrils are nondividing (permanent) cells.

Both liver and spleen are invaded early in the acute infection. Toxoplasma has been isolated from the lung and myocardium in fatal cases as well. Parasitized cells have been found although it is difficult to see them in tissue sections in early stages of infection. But while these lesions heal the presence of cysts without any cellular reaction around them may be noted (5,14,31,38). Toxoplasma also invades the skletal muscle, some endocrine glands such as suprarenal and pituitary glands (38). Although cases of hepatitis, pneumonitis, myocarditis and meningoencephalitis have been reported, the most common acquired form is lymphadenitis with periadenitis (10,21,39).

In the presented study both hepatic, splenic and lymph node involvement is the most striking finding.

The diagnostic histomorphologic features of lymphadenitis has been described (5,6). Marked follicular hyperplasia, some epitheloid cells and distension of sinuses by monocytoid cells are the triad of the disease. However this triad is not always present in all cases, besides the cellular pleomorphism make it difficult to distinguish from malignant lymphoma. Furthermore, the presence of T.gondii in such lymph nodes is extremely rare (9,10,40,41).

According to our data necrosis, subcapsular monocytoid cells and both cysts and free forms of made parasite made our triad.

The presence of necrosis is not a common finding in human beings. Such necrosis are described as many freshly necrotic cells located in the germinal centers. Thus, the karyorrhectic particles of nuclear debris which have engulfed by macrophages may be confused with parasites. Anyway T.gondii may also be confused with nuclear debris (6,10).

The tachyzoites rapidly multiply and destroy the host cells. Thus focal lesions progressively become larger until the development of immunity. Immunity is generally effective (42).

In our study, generally we have observed large necrotic areas. We thought that the cause of this finding has two fold. One is the possible high virulence of our T.gondii strain. Secondly, this high virulence caused early death of the animals before the development of the immunity. There is also no cellular reaction around these lesions. Although according to the literature the parasite is rarely seen in tissues, we have successfully demonstrated T.gondii in all tissues except brain, eye and heart of all five mice. Our findings in genitourinary system, alimentary tract, muscle and adipose tissue will be the subject of another publication.

The organism can be demonstrated in biopsy specimens or touch preperations or bronchoalveolar lavage fluid even in the sputum when they are stained with Hematoxylen-Eosin, Giemsa, Papanicolaou methods or with immunohistochemical techniques whether using fluorescent or peroxidase labels (6-8,12,14,15, 21,37,38,43). But I think toxoplasma is easily recognizable with histological or cytological examination of conventionally stained specimens when we take care of it. Such methods are not always available in many laboratories or even in countries.

Although the diagnosis of toxoplasmosis usually is established with spesific serologic tests, there are cases which showed no demonstrable antibodies even when a number of toxoplasmas were demonstrated in the tissue. The poor antibody response of the host and the poor antigenic stimuli by the parasite may be the reason. In addition, these tests may be normal in early stages of the disease (37). It should also be remembered that because of the prevalence of antibodies in the general population, the definitive diagnosis of an adult as toxoplasmosis becomes much more difficult.

Millions of dollars are needed each year to care for victims of congenital toxoplasmosis in the United States as pointed out by Remington (2). The histopathologic findings of toxoplasmic lymphadenitis may be difficult to distinguish from lymphoma. The treatment of these two disorders is different and the treatment of lymphoma may exacerbate a dormant toxoplasmosis. Likewise toxoplasmosis may be treated succesfully with pyrimethamine and sulfonamides. Therefore, histopathologic interpretation is very important before diagnostic confirmation of toxoplasmosis with serologic tests.

ACKNOWLEDGMENTS

I am grateful to Prof.Dr.Gülendame Saygı, Department of Microbiology, Medical School of Cumhuriyet University, Sivas, for useful discussion and encouragement to perform this study. I thank Ass.Prof.Dr.Semra Özçelik for her technical helps and Prof.Dr.Kürşat Altıntaş, Department of Microbiology, Medical School of Ankara University, Ankara, for supplying the T.gondii strain used in the study.

Toksoplazmozisin histopatolojik tanısının önemi

İmmün yetmezlikti hastalarda edinsel toksoplazmozisin klinik özellikleri asemptomatik lenfadenopatiden letal enfeksiyona kadar değişir. En sık rastlanan bulgu olduğu için lenf düğümündeki histolojik değişiklikler tanımlandı. Burada her ne kadar tipik histomorfolojik özellikler tanımlanmışsada edinsel toksoplazmozis genellikle serolojik olarak teşhir edilir. Diğer bir deyişle morfolojik özelliklerin boyutlarına ve belirgin olmasına rağmen pekçok patolog karakteristik histopatolojik değişiklikleri kabul etmede tereddüt etmektedir. Bu yüzden, toksoplazmozisi özellikle lenf düğümlerinde olan malign lenfoma ve bazı reaktif lezyonlardan ayırtetmenin kolay olmadığı unutulmamalıdır. Bu amaçla intraperitoneal olarak enfekte edilen fareler T. gondii varlığı ve lenf düğümleri ile diğer dokulardaki histopatolojik değişiklikler araştırıldı. •

[Türk Tıp Araştırma 1992; 10(6):314-320]

Anahtar Kelimeler:Toxoplasma, Toksoplazmozis

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