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Microscopic investigations in a diabetic rat urinary bladder infected with *Trichosomoides crassicauda*

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Abstract Rats are widely used laboratory animals and have several parasites. One of these are helminths, known not only to cause serious effects on the experimental results in healthy subjects, but also in subjects with heavy infections. One of the relatively pathologic helminth is *Trichosomoides crassicauda*, which lives in the nodules of the urinary bladder. It is known that diabetics are more prone to infections with several microorganisms. Observations in a diabetic rat bladder showed *T. crassicauda* eggs inside the transitional epithelium, and structural changes in the bladder epithelium were evident. Urinary-bladder tissues taken from streptozotocin-injected diabetic subjects and citrate buffer-injected control subjects were fixed, embedded in araldite and investigated under a light microscope. Distinct changes in the histological structure of a diabetic urinary bladder transitional epithelium were observed after*T. crassicauda* infection. Many papillomas were formed and the epithelial tissues were completely degenerated. In addition, electron microscopic examinations also revealed degeneration of the subepithelial tissues.

Keywords *Trichosomoides crassicauda*, rat, diabetes, urinary bladder, light microscope, transmission electron microscope

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Introduction

Rats are commonly used laboratory animals [1] and they have several parasites. *Trichosomoides crassicauda* is a helminth living in the urinary bladder of rats. This is a non-pathogenic hair-like worm, which has been reported both in laboratory and in wild rats [2,3]. The male parasite lives within the uterus of the females [4,5]. Mature females are 10–19 mm long and males are 1–3.5 mm long. The biology of the parasite is direct. Infection occurs when the eggs are digested. Transmission from the mother to the young ones has also been reported. The eggs are expelled into the urine and excreted. The larvae leaving the eggs settle in the organs of the urinary system and mature. As a result of the infection, the urine becomes bloody and bladder tumors and formation of bladder stones are seen according to the severity of the infection. Furthermore, the larvae are also known to cause eosinophilic granulomas [6,7].

The inner surface of the urinary bladder is covered with a transitional epithelium that consists of multiple layers of epithelial cells that can contract and expand [8]. Owing to the irritative effects of the parasites in the urinary bladder during maturation, the transitional epithelium degenerates, and as a consequence, regeneration occurs, thus resulting in the stratification of the epithelium, making it prone to secondary infections. Cystisis or hyperplasia can appear, and proliferative sites, epithelium-originated papilloma and inflammation may become visible [9]. Parasitic infections in the bladder can lead to granulomatous lesions. The presence of the parasite may also be responsible for the bladder cancer [10]. It is known that the presence of diabetes impairs several

aspects of a phagocyte function, including phagocytosis and intracellular killing of the microorganisms. Hyperglycemia reduces the oxidative killing capacity because of the increased glucose metabolism. Diabetics are strikingly prone to infections with several organisms. Some of the infections undoubtedly disturb the blood glucose control. Previous studies have shown a relationship between the level of blood glucose and carriage rates of microorganisms as well as the rate of infection [11]. In diabetics, the urinary bladder undergoes structural changes, e.g. lamina propria appears to be thickened and irregular [12].

It has been reported that hyperplasia occurs in the transitional epithelium infected with *T. crassicauda* cells containing single or multiple pycnotic nuclei. Their cytoplasm is fibrogranular in appearance, with few distinct organelles, and the luminal surfaces of the cells are not limited by the plasma membrane. However, no infection-related increase of inflammatory cells in the lamina propria has been observed [13].

The aim of this study was to observe the eggs of the parasite embedded in the transitional epithelium as well as the structural changes in the urinary bladder of diabetic rats infected with *T. crassicauda*.

Methods

Ethical approval

Institutional ethical approval for this experiment was granted by the Animal Experimentation Ethics Committee of Gazi University, Ankara.

Animals and experimental protocol

Young male adult Wistar albino rats ($n = 20$) weighing 200–220 g were obtained from Animal Laboratories of Gazi University, Ankara. They were kept in separate plastic cages at room temperature in a 12-h light–dark cycle and fed with standard laboratory chow and tap water ad libitum. The rats were randomly divided into two groups each of 10 rats. Control rats received only an injection of citrate buffer (1 ml). Diabetes was induced by an intraperitoneal injection of streptozotocin (STZ; Sigma, USA) (45 mg kg^{-1} body weight) in 1 ml of 0.1 M citrate buffer. Rats with fasting blood glucose levels exceeding 200 mg dl^{-1} at 48 h after STZ injection were considered diabetic subjects. The animals were sacrificed by excess sodium pentobarbital anesthesia. The urinary bladders were immediately dissected out and rinsed in a sodium phosphate buffer. Tissues were sliced into pieces of 1–2 m^3 and fixed in 2.5% glutaraldehyde buffered to a pH of 7.4 with 0.1 M sodium phosphate. The samples were washed several times with sodium phosphate and post-fixed in 1% osmium tetroxide for 1 h. The tissues were dehydrated in serial concentrations of ethanol, placed in propylene oxide and embedded in araldite.

Light microscope

The araldite blocks were cut with an ultramicrotome (OM 2, Reichert, Germany) into semi-thin sections. The sections were stained with toluidine blue-pyronin G and studied with a light microscope (Olympus, Japan).

Transmission electron microscope

Thin sections (600 nm) were mounted on copper grids (200 mesh) and stained with uranyl acetate and lead citrate for examination under a transmission electron microscope (JEOL JEM 100CXII).

Results

Control group

The urinary bladder epithelium was 3–4 cells deep with cuboidal or columnar basal cells, intermediate cells and superficial squamous cells. The basal cells were attached by half desmosomes or attachment plates on their basal membranes to a basement membrane, which separated the epithelium from the lamina propria. Light and electron microscopy of the urinary bladders revealed a regular, characteristic transitional epithelium and lamina propria in the control group (Figs. 1 and 2).

The number of transitional epithelium cell layers varied based on whether the organ was empty or full. In the investigated area, 3–5 cell layers of the epithelium were observed. The epithelium facing the lumen was found to be a part of the superficial cells. The semi-thin sections stained with toluidine blue-pyronin G revealed that the surface of the cells was stained darker than the other cell layers

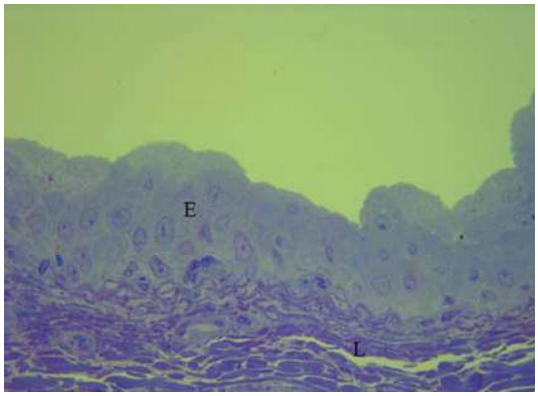

Fig. 1. Control-group urinary bladder. Transitional epithelium (E) and lamina propria (L). Magnification: × 400.

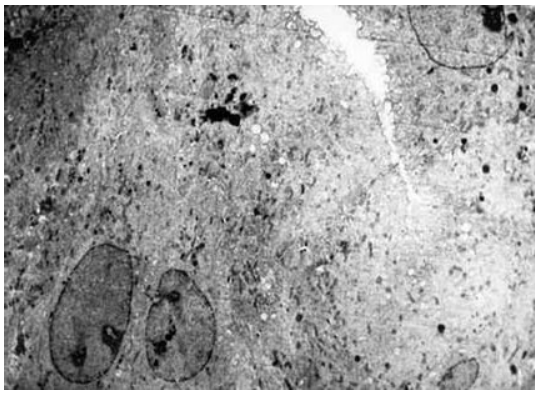

Fig. 2. Control-group superficial cells of transitional epithelium with two nuclei. Magnification: × 1900.

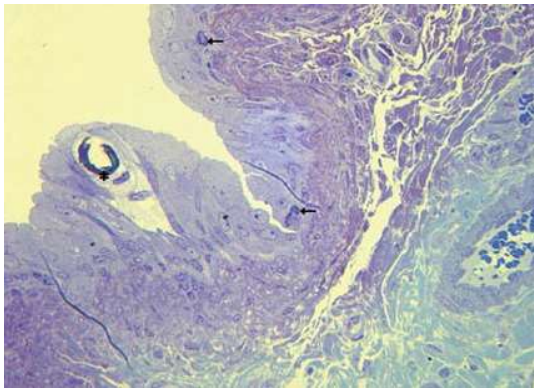

Fig. 3. *T. crassicauda* eggs embedded in transitional epithelium (asterisks) in diabetic group. Mast cells in epithelium (left arrow). Magnification: × 200.

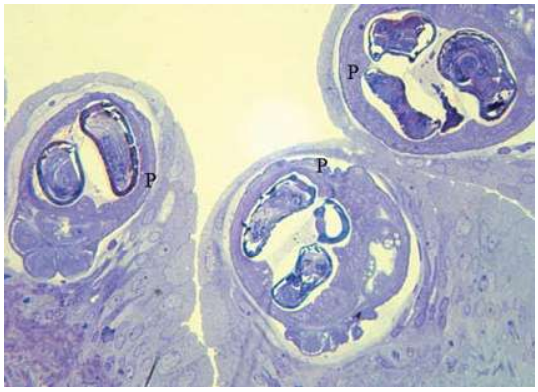

Fig. 4. Parasite eggs embedded within the papilloma (P). Magnification: ×1000.

(Fig. 1). A search for the superficial cells in the epithelium revealed a few cells in the middle layer, which were with two nuclei (Fig. 2). In addition, the surface of the cells facing the lumen of the superficial bladder cell membrane was very irregular with prominent interdigitations.

Diabetic group

A section of the parasite eggs embedded in the transitional epithelium of the urinary bladder within a papillitis was observed under a light microscope. Diabetic bladder with papillitis revealed an irregular lamina propria, and the mast cells were rarely seen near the papilla (Fig. 3).

Light microscopic findings of the diabetic group revealed a disruption in the shape of the epithelial cells and differences in the placement and in the number of the cells. Transitional epithelium displayed a reduction or an increment in the number of cell layers due to the formation of papilloma. On examining the sectioned material, more than one papilloma were visible (Fig. 4).

Transmission electron microscopic observations confirmed the presence of parasite eggs. Some of the *T. crassicauda* eggs were localized intraepithelially, while others were found subepithelially (Fig. 5). The fine structure of the transitional epithelium was subtle and severely altered in appearance.

The cytoplasm of the infected bladder epithelium was fibrogranular, and except the cell nucleus, no other distinct organelle was observed. Owing to the formation of papilloma, the transitional epithelium was degenerated and changes in the ultrastructure were evident. Furthermore, in most of the sections, the transitional epithelium was degenerated and vacuole formation was evident. The plasma membrane facing the lumen of the bladder was found to possess several microvilli (Fig. 6). The ultrastructure of the parasite eggs was elliptical in shape with

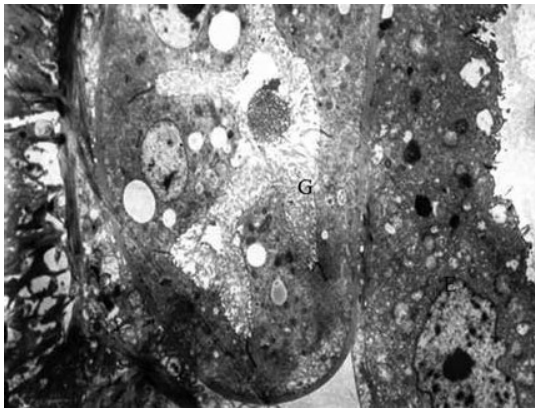

Fig. 5. Subepithelial localized *T. crassicauda* egg (G); transitional epithelium (E). Magnification: ×2900.

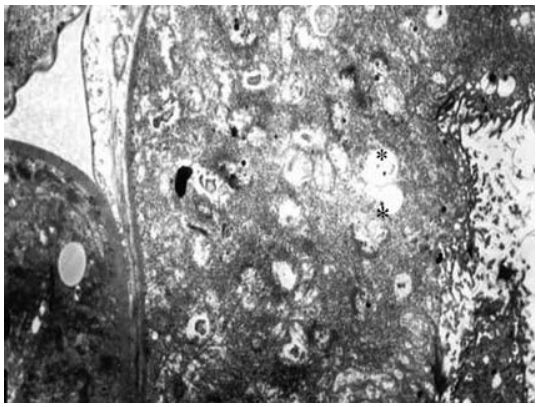

Fig. 6. Degeneration and vacuole (asterisks) formation in transitional epithelium. Magnification: ×4800.

distinct margins and revealed round granules that may possibly be lipid droplets (Fig. 7).

Discussion

Several parasite species are known today and most of them are helminths. *T. crassicauda* is one of the parasites infecting the urinary system. Parasitic infections can be treated with different antihelmintics [14].

Examinations of the rat bladder by a scanning electron microscope revealed several worms that were buried in the mucus, thus infecting the bladder surface [15,16]. Furthermore, immature eggs and embryonated larvae were evident. However, our examinations by a transmission electron microscope exhibited only eggs embedded in the epithelium and submucosa, causing a complete epithelial erosion and vacuolization.

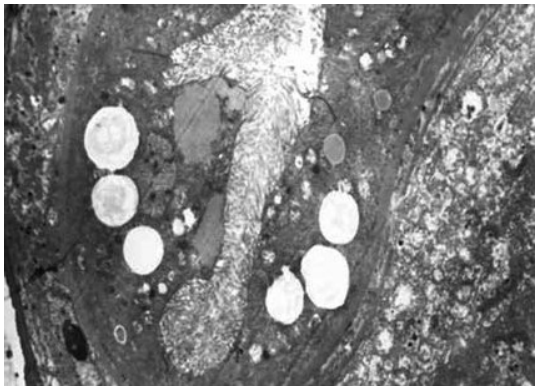

Fig. 7. Ultrastructure of a *T. crassicauda* egg. Magnification: ×3600.

Infectious papillomatosis occurs due to *T. crassicauda* infection in rats that are used for most of the experimental animal studies. Papillomas seen in ureters, renal pelvic cavity and urinary bladder are considered benign tumors and should be kept in mind while making evaluations [17]. Furthermore, in a cancer study by Chapman [10], it was reported that rats infected with this parasite revealed infectious papillomas in kidneys, ureters and urinary bladder.

Avcıoğlu [9] concluded that the results of the experiments with *T. crassicauda*-infected rats may be incorrect. During maturation of the parasites, the urinary bladder is irritated and the transitional epithelium degenerates, and as a consequence, regeneration occurs. Thus, the epithelium becomes stratified and is also prone to secondary infections. In addition, cystisis or hyperplasia can also appear, and proliferative sites, epithelium-originated papilloma and inflammation may become visible.

Antonakopoulos *et al.* [13] studied the urinary bladders of rats infected with *T. crassicauda* using a light microscope, a scanning electron microscope and a transmission electron microscope. The bladder epithelium of rats revealed a diffused, mild, flat hyperplasia, 4–6 cells thick. The anterior parts of the adult female worms were embedded in tunnels within the hyperplastic epithelium with the posterior portions of the parasites lying free in the bladder lumen. The hyperplastic epithelial cells forming the inner layer of the tunnel wall, adjacent to the parasite, showed degenerative changes. These cells contained single or multiple pycnotic nuclei. Their cytoplasm was fibrogranular in

appearance, with few distinct organelles, and the luminal surfaces of the cells were not limited by plasma membranes. In addition, the mast cells were also seen in the epithelium. However, there was no infection-related increase in the number or type of inflammatory cells in the lamina propria. Furthermore, as there was no significant chronic inflammatory response to the worms, it was suggested that the avascular hyperplastic epithelium of the bladder is an immunologically 'protected site' for the mature female *T. crassicauda* [13]. Our observations were also similar to these findings.

The epithelium in the diabetic bladder appeared degenerated and the cell membranes were not significant. Furthermore, the epithelium was vacuolized in appearance and reshaped according to the presence of the parasite eggs.

Concluding remarks

It is a fact that diabetes leads to the weakening of the immune system and hence, the onset of parasitic infections is easier. Based on this situation, we observed changes in the infected diabetic rat bladder epithelium. In our experiments, the diabetic bladder revealed several parasite eggs buried in the epithelial and subepithelial regions. Furthermore, the urothelium was degenerated in appearance, but showed no signs of inflammation.

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