A Microscopic Study, Mucosal Cells of Urinary Bladder in Streptozotocin Diabetic Rats with Parasite

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Abstract

It has been known that as a result of diabetes several diseases occur in organisms such as parasitic infection. This gives rise to inflammatory conditions associated with mast cells. For the purpose of observing mucosal cells in bladder tissue, streptozotocin diabetic rat bladders were examined with light and electron microscope. A microscopic study of serial sections revealed numerous mast cells in transitional epithelium and lamina propria of bladder mucosa. Mast cells seem to migrate from blood vessels through the transitional epithelium and the lamina propria. Mast cell population found in epithelial layer and lamina propria were observed to differ in characteristics as ultrastructural features. Intraepithelial mast cell cytoplasm were full of granules including both electron dense and lucent material however, mast cells in lamina propria with thin pseudopodia were observed in the process of degranulation. Eosinophil cells beside mast cells were observed in connective tissue of mucosa. These cells were full of electron dense granules in their cytoplasm. Cell interaction between eosinophil and macrophage was evident. Accumulation of mast and eosinophil cells in mucosa and also the cell and tissue interactions could be associated with diabetes and used as a criteria for the evaluation of parasitic (*Trichosomoides crassicauda)* bladder.

Key words: Diabetes, bladder, mast cell, eosinophil cell, light microscope, transmission electron microscope

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1. Introduction

It has been reported that urinary tract infections are associated with diabetes mellitus [1, 2, 3]. Urinary tract infections in patients who have diabetes include renal abscess, fungal infection, cystitis etc. [4]. Infective organisms such as *Escherichia coli, Candida albicans, Klebsiella pneumoniae* [5] also a number of parasites including worms cause urinary tract infections [6]. It is well known that streptozotocin induced rat model of diabetes mimics human with respect to bladder dysfunction [7, 8]. Mast cells play a role in hypersensitivity reactions and in certain inflammatory reactions [9,10]. Mast cells also participate in host resistance to parasites and infections [11]. Mast cells contain specific secretory granules that secrete and store chemical mediators [12] and can be activated by directly interacting with pathogen through pattern recognition receptors [13]. A urinary tract infection, cystitis, induced by Pseudorabies virus caused inflammation in a study of Jasmin et al. (2000) and concluded that inflammation is associated with proliferation and degranulation of mast cells [14]. Fiori et al. (1994) found that urinary bladders of rats treated with β- cell toxicants revealed presence of a large number of mast cells in the mucosal lamina propria and transitional epithelium [15]. Signs of inflammatory processes were not observed and the occurrence of this cell type might be due to regeneration of nerve terminals in diabetic neuropathy. It is known that eosinophil chemotaxis is most effective in defence area of parasitic larval schistosomes [16]. Regard to authors eosinophil cells appear to modulate

and neutralize the potentially deleterious effects of mast cell action. Eosinophils can regulate mast cell function in a paracrine manner as well. This type of interaction between eosinophil and mast cells can pave the way for chronic inflammatory responses [17].

In this assay we have examined the distribution and ultrastructural properties of mast cells and eosinophil cells in diabetic bladders with infection of *Trichosomoides crassicauda.*

2. Methods

2.1. Experimental Animals

This study was done with the permission of ethical commission of Ankara University, Ankara, Turkey. Young adult male Wistar rats weighting 200-210 gr were used. Their care were maintained in accordance with the institutional guidelines. Rats were kept in separate cages at room temperature, humidity of % 65 and 12:12h light: dark photoperiod. They were fed with standard laboratory chow and had free access to water. Animals were quarantined for ten days and then divided into groups of control (n: 10) and diabetic (n: 10).

2.2. Induction of Diabetes

Age matched control group were injected only citrate buffer. Diabetes mellitus was induced by a single intraperitoneal injection of 45 mg/kg body weight of streptozotocin *(Sigma)* dissolved in citrate buffer (0.1 M, pH 4.5). 48 h after injection plasma glucose concentrations were measured and >200mg/dl were accepted as diabetic.

2.3. Preparation for Microscopy

Rats were killed by excess sodium pentobarbitale anaesthesia on the 24th day of diabetes

induction. The urinary bladders were immediately dissected out, rinsed in sodium phosphate buffer. Tissues were sliced into pieces of 1-2 mm^3 and immersed in 2.5% glutaraldehyde buffered to pH 7.4 with 0.1 M sodium phosphate and kept for 2h. Samples were washed several times with sodium phosphate and post fixed in 1% osmium tetroxide solution for 1h. Tissues were dehydrated by graded ethanol series of 70-100 %, placed into propylene oxide and embedded in araldite [18]. Sections were cut with an ultramicrotome (*Leica*). Semi-thin sections were stained with toluidine blue-pyronin G and studied with light microscope *(Olympus).* Ultrathin sections were stained with uranyl acetate-lead citrate and examined with transmission electron microscope *(JEOL JEM 100 CXII)* at 80-100 kV in Electron Microscope Laboratory of Ankara University, Faculty of Science, Department of Biology.

3. Results

Our microscopic observations showed that four of the ten diabetic rats had parasitic infections. This case, *Trichosomoides crassicauda* infected diabetic bladder was reported in our previous paper [19]. The present study which is associated with accumulation of mucosal mast and eosinophil cells in diabetic and parasitic rat bladders was performed as a continuation of the published paper.

Light and electron microscopy of urinary bladders revealed a regular, characteristic transitional epithelium and lamina propria in control group. Mast cell in this group showed centrally-placed nucleus with typical homogenous granules on the subepithelial region between two adjacent transitional epithelial cells (Fig. 1).

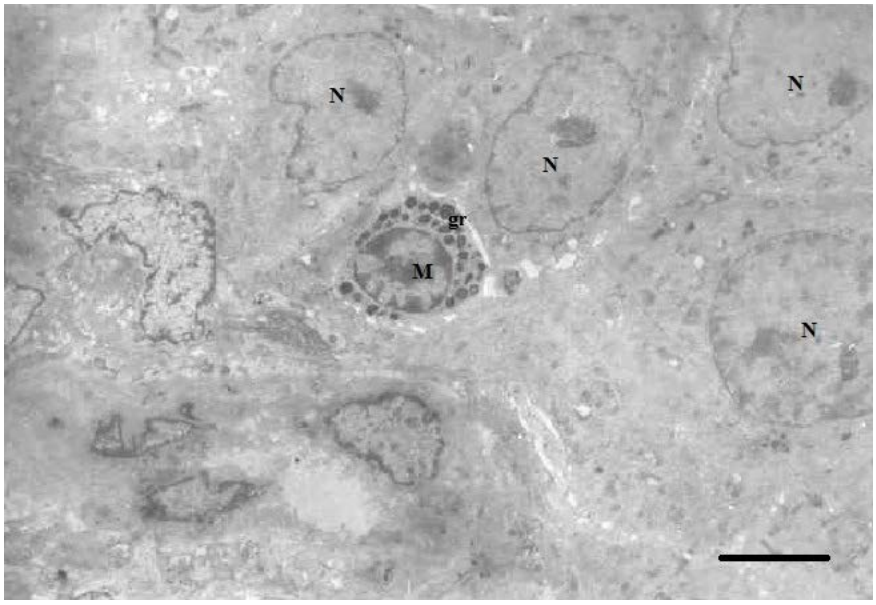

Figure 1. Typical mast cell (M) with granules (g) and epithelial cells in the urinary bladder of control group. Nuclei (N) of epithelial cells. Bar: 10.8µm.

Light microscopic findings of diabetic group demonstrated a significant increase in the mast cell number in the bladder mucosa of diabetic group compared with the control group (Fig. 2 and 3).

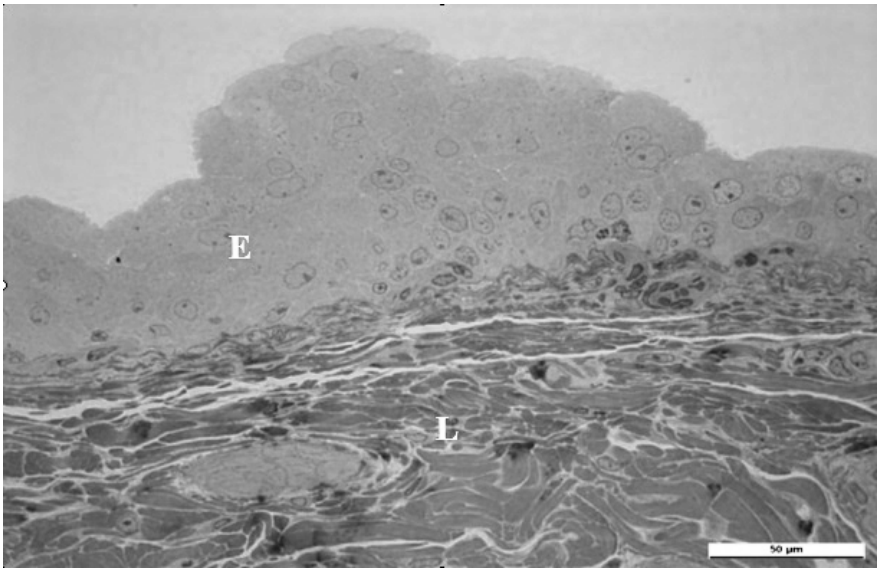

Figure 2. Mucosa of control group, no mast cells in transitional epithelium (E). Lamina propria (L). Bar: 50 µm

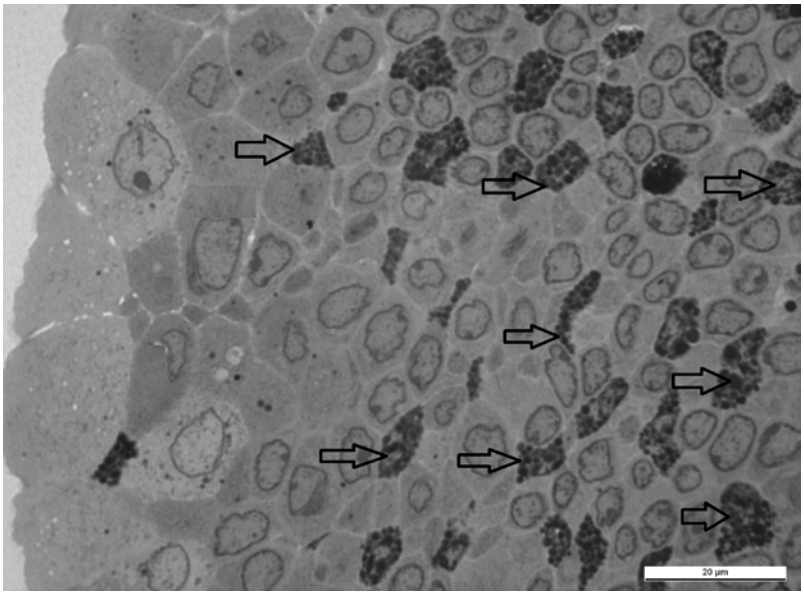

Figure 3. Micrograph depicting numerous mast cells (arrows) with densely packed granules accumulated to transitional epithelium in diabetic bladder. Bar: 20 µm

TEM observations also confirmed the acceleration of mast cell number both in transitional epithelium and lamina propria in a number of sections. A decrease in density of electron material in diabetic mast cells granules was readily visible (Fig. 4).

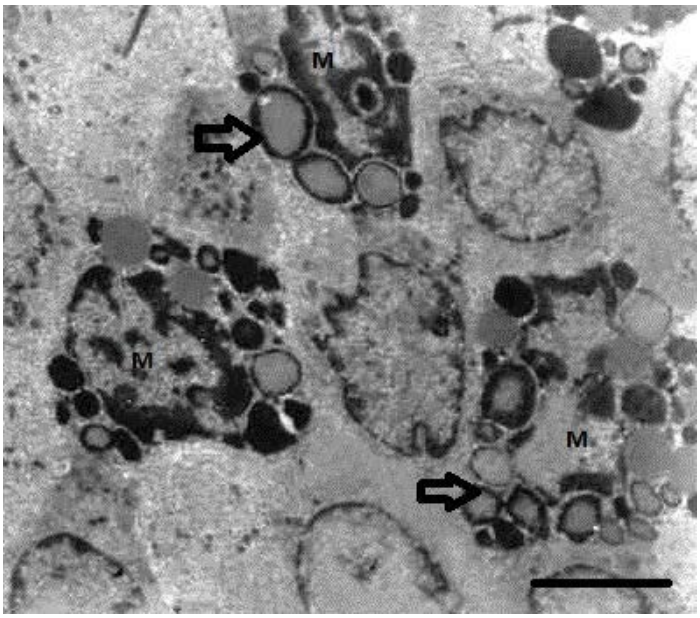

Figure 4. Intraepithelially localized mast cells (M) with monolobed nuclei in diabetic group. Loss of electron dense material in granules (arrow). Bar: 6.8µm

Intraepithelially localized mast cells in diabetic parasitic group were packed with granules varying in shape. These granules had lucent and dense electron material representing different granular patterns (Fig. 5).

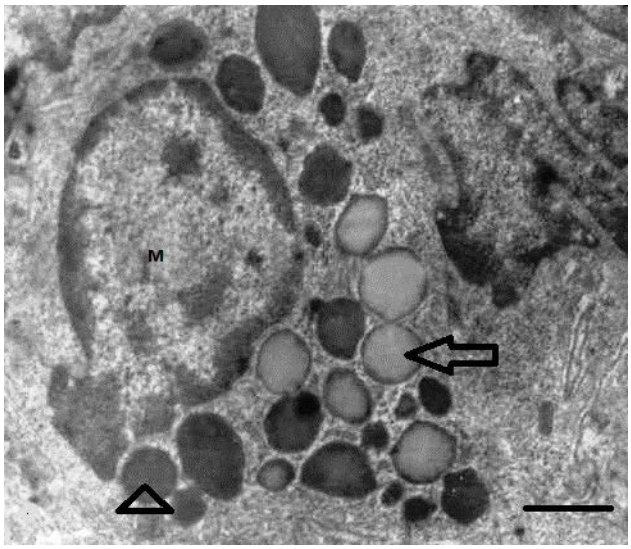

Figure 5. Diabetic bladder epithelial tissue frequently showed mast cells with monolobed nuclei (M) and cytoplasmic granules which have electron-lucent (arrow) and electron-dense (arrow head) material. Bar: 20 μm

The most distinctive feature seems to be the decrease in electron density of granules in mast cells of diabetic group. It is evident that granules retained their membranes and remained in place in the cytoplasm. Granule to granule fusion was not observed in any sections. Most granules revealed a round and oval shape. Large and small granules fused with the cell membrane of mast cell in the lamina propria of diabetic bladder give the appearance of an elaborate degranulation (Fig 6).

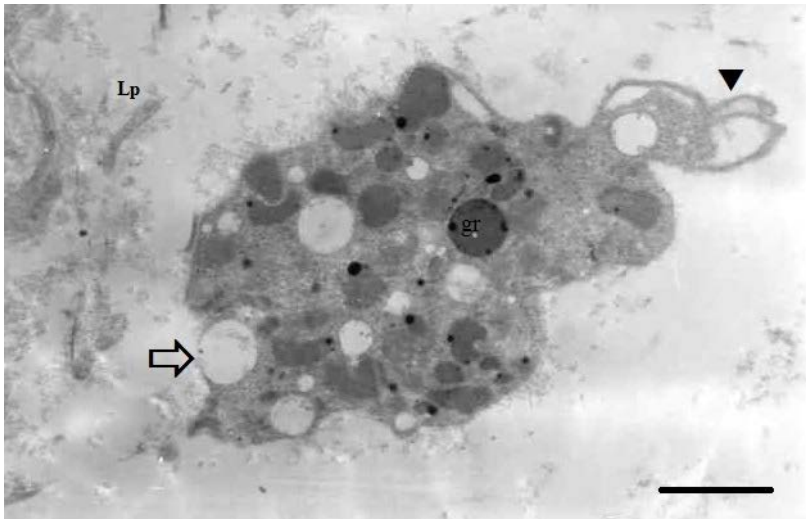

Figure 6. Mast cell in the lamina propria (Lp) of diabetic bladder, mast cell surface architecture existing narrow, elongated folds (arrow head); presence of cytoplasmic granules (gr) variable in shape and density and revealed degranulation (arrow).

Bar: 2.7µm

Surface architecture of mast cells existing narrow, elongated folds in the same figure were remarkable. Mast cells in diabetic bladders displayed degranulation in different sections. Degranulating mast cells exhibited electron lucent vesicules from small to large in size. Finger-like protrusions were projected outward from the surface of mast cells as well. Mast cell and eosinophil cells were apparently observed to locate around the capillaries and full of granules in their cytoplasm of diabetic and parasitic rat group bladders (Fig 7).

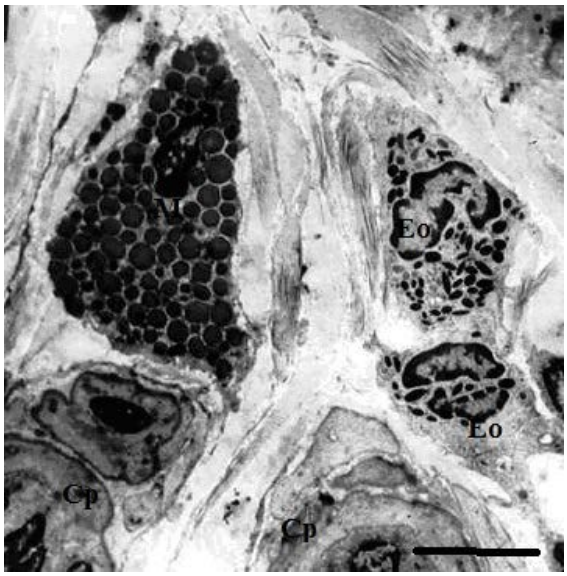

Figure 7. Mast cell (M) and eosinophil cells (Eo) full of granules around the capillaries (Cp). Bar: 6.8 µm

Eosinophil cell of diabetic parasitic group had numerous and electron dense granules and observed to be in close contact with macrophage in another section of rat bladder (Fig 8).

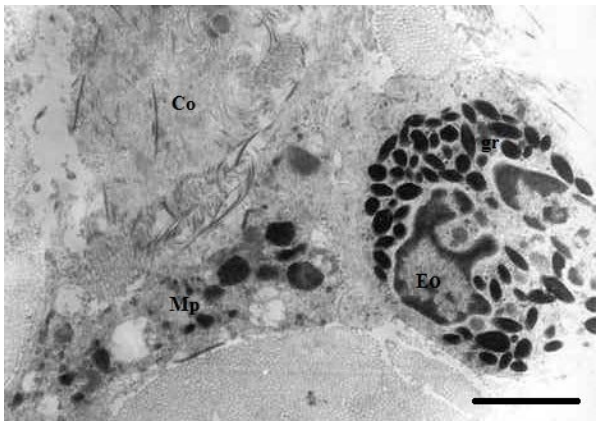

Figure 8. Eosinophil cell (Eo) full of granules (gr), in close contact with macrophage (Mp). Collagen fibres (C). Bar: 6.8 μm

These results represented a marked difference among the mast cells of control and diabetic and parasitic samples.

4. Discussion

The present study revealed that mast cells play a defensive role in diabetic and parasitic mucosal bladder of rats. Since mast cells proliferate and release bioactive chemical mediators in many conditions like allergy, neuro inflammatory diseases and parasitic response, the increase in mast cell number can be interpreted for such situations [20]. In addition, mast cell activation was shown to occur in conditions associated with tissue fibrosis by Howard et al. (2001)[21]. We also determined activated mast cells in our study. Rizk et al. (2006) was studied female rat bladders with streptozotocin diabetes [2]. They pointed out that a number of mast cell were accumulated to intraepithelial area suggesting inflammation. Also diabetic parasitic urinary bladder showed transepithelial migration of

mast cells. Increased mast cell number in streptozotocin and alloxan diabetic bladders were observed by Fiori et al. (1994) in a light microscopic study but inflammatory processes were not seen in any sections [15]. It was proposed that increased frequency of mast cells was admitted as an evidence associated with complications of diabetes. Similar data were obtained in urinary bladder sections of cystitis patients and intraepithelial distribution of mast cells were seen [22]. The present study is in agreement with report of Aldenborg et al. (1986) that transepithelial migration of mast cells was also observed in our study.

Mast cells are known to release some mediators that play different roles in different pathological conditions. Sometimes in case of activation mast cells undergo ultrastructural alterations and appearance of their electron dense granules changes [20]. Mucosal mast cell increase and activation without typical exocytosis in mucosa and submucosa were also seen in studies with non-ulcerative cystitis [23]. It was reported that damaged or dysfunctional urothelial cells produce cytokines and these stimulate proliferation and activation of mast cells. Our observations in diabetic bladders were consistent with this finding. In electron microscopic observations we also demonstrated degranulation of mast cells. It is suggested that the degranulation was piecemeal degranulation because within the same cell altered granules like empty containers and normal resting granules were seen together [24]. Ozsoy and Gul's study (2005) reported mast cells had granules with electron lucent material in gingiva of diabetic rats [10]. Additionally, mast cells located around capillaries were seen by the authors. In fact, present study is parallel with the report of Ozsoy and Gul (2005) who inscribed mast cells around capillaries.

As known, mast cells function together with leukocytes in pathologic and allergic conditions. Rizk et al. (2006) reported that mast cells and leucocytes were located in stroma of streptozotocin diabetic urinary bladder [2]. Mast cells together with eosinophil leukocytes located around capillaries was another finding of our study. According to Cross and

Mercer (2002), eosinophils are indicated at the sites of mast activity attracted from nearby blood vessels to the region by factors released during mast cell activation due to parasitic infection [16]. This report constitutes a strong evidence for our study.

In conclusion, our study revealed a defensive reaction of mast cells together with eosinophil cells against diabetic and parasitic infection. Furthermore, changes in ultrastructural alterations in mast cells were found in the mucosa of diabetic and parasitic bladder. We believe that our data will shed light on the further studies.

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