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Ultrastructural Alterations of Inflammatory Cells in Bladder Cystitis Versus Bladder Carcinoma

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ABSTRACT

Chronic inflammatory cystitis, caused either by chemical irritants or bacterial, viral and parasitic infections, has long been known to play a crucial role in the development of bladder cancer. However, two opposing actions have been suggested to be produced by inflammation as it may either enhance cancer development or destroy malignant cells. An ultrastructural comparative study between inflammatory cells in bladder cystitis and bladder cancer is conducted in a trial to verify the anti-tumour and tumorigenic role played by such inflammatory cells during the development of tumor on top of cystitis. Urine cytology samples were obtained from three groups of patients, three patients each: bladder cystitis, transitional cell carcinoma [TCC] and squamous cell carcinoma [SqCC] and examined by electronmicroscopy using the agarose cell block technique. Most inflammatory cells seen in bladder cystitis (with predominance of granulocytes) revealed features of activity as indicated by presence of intracytoplasmic phagocytic vacuoles, cytoplasmic villous projections and hypogranularity (denoting a degranulation process). However, in cancerous conditions, inflammatory cells showed two main entities with distinct morphological features . Some cells appeared with reactive features similar to those of cystitis and others represented abnormal forms with marginated and condensed nuclear chromatin, bizarre-shaped granules, disrupted cytoplasm, abnormal undefined inclusions, intense vacuolation and occasional phagocytic vacuoles. It is hence suggested that, in cases of bladder cancer, the inflammatory cells with reactive features could be assigned to a protective antibacterial (similar to cases of uncomplicated cystitis) or tumoricidal /tumoristatic action. The distorted population, on the other hand, could be assigned to a different function with a tumorigenic action. Therefore the appearance of the latter population in the urine of patients suffering from cystitis could be regarded as an early warning sign to the urine cytologist for cancer development.

Key Words: *Bladder cancer - Bladder cystitis - Urine cytology - Ultrastructure - Inflammatory cells.*

INTRODUCTION

In Egypt, bladder carcinomas account for about 25-50% of all malignancies **[1-3]**. They are regularly associated with schistosomiasis that results in chronic inflammation in which infiltrating cells such as neutrophils, macrophages and eosinophils are sources of mediators (Oxygen radicals, cytokines…etc) known to induce DNA damage of the bladder epithelium **[1]**. However, other factors are implicated in carcinogenesis in these patients e.g. smoking, bacterial bladder infections or exposure to environmental carcinogens **[4]**.

Hence an intricate relationship exists between inflammatory reaction and cancer development (carcinogenesis). In fact, inflammation may produce two opposing effects depending on the state of activation of inflammatory cells. It may either enhance cancer development or may destroy malignant cells (tumoricidal action) or arrest their growth (tumorstatic action).

This concept has been evidenced by many in vivo and in vitro studies dealing with various cancers. Macrophages and neutrophils are regarded as components of inflammatory infiltrates commonly observed in stroma of tumors. They seem to play a dualistic role in human development either producing cytotoxic effects or enhancing tumor growth **[5-11]**.

The significance of natural killer cell and lymphocyte infiltrates in cancer was related to lysis and killing of tumor cells (12; cited by 13).

On the basis of such association of inflammation with bladder cancer, the pursuit of inflammatory cells in various bladder lesions could provide a valuable predictive marker for development, growth, invasiveness and aggressiveness of bladder carcinomas.

However, morphology of inflammatory infiltrates, histopathologically notable in tumors, cannot distinguish inflammatory cells that cause tumor destruction from those that promote tumor growth **[13]**.

On the other hand, electronmicroscopy (EM) in urine cytology by showing more details in the cellular infiltrates could demonstrate the definite differential morphological features of this double action.

It is aimed in the present work to use the alternative non-invasive urine cytology technique for electronmicroscopic examination of exfoliated inflammatory cells in urine samples of both bladder cystitis and bladder cancer. The inflammatory cell ultrastructural alterations in bladder cystitis as compared to urothelial cancers could be of value in verifying their differential role in both conditions. They could also be important in deciding the therapeutic effectiveness of eliminating the chronic irritation in reducing the incidence of bladder cancer.

MATERIAL AND METHODS

Patients:

Three groups of patients (three in each) were selected: A group with bladder cystitis (diagnosed by urine analysis showing 100 pus cells/HPF); a second group with transitional cell carcinoma (TCC) and third group with squamous cell carcinoma (SqCC) (as diagnosed by histopathological examination of cystoscopic biopsy).

Specimen Preparation:

Voided urine was centrifuged at 1500 rpm for 10 min., supernatant decanted and the deposit fixed in 2% glutaraldehyde in cacodylate buffer for one hour and after centrifugation processed for agarcyto cell block formation.

Agarcyto Cell Block Preparation:

This was performed according to a technique first reported by Kerstens et al. **[14]** for diagnosis of cervical cancer and modified by Mansy **[15]** to suit urine cytology samples.

The fixed cells were centrifuged for 10 minutes at 2000 rpm, supernatant decanted and deposit transferred to 1.5ml Eppendorf tube and spun down. The deposit was resuspended in 1ml of 2% liquid agarose at 65ºC and concentrated by centrifugation in the agarose which is solidified at 4ºC for 30 minutes. The other agarose block was processed for EM examination where the specimen was refixed in buffered glutaraldehyde for two hours after being sectioned into small pieces (1x1 mm in size) and processed according to Clement et al. **[16]**. After rincing, the specimen was postfixed in osmium tetroxide (1% in cacodylate buffer) for one hour at 4ºC, dehydrated in ascending grades of alcohol, substituted in a mixture of Epon and absolute alcohol in equal volumes and lastly infiltrated in three baths of epon resin. The specimen was then embedded in a mixture of Epon resin and tri-dimethylamino ethyl phenol accelerator (DMP30) in capsules. Polymerization then follows in an oven for 12 hours at 37ºC and then for 2 or 3 days at 60ºC. Ultrathin sections were prepared and examined by Philips electron microscope (EM 208S).

RESULTS

The urine cytology of the majority of bladder cystitis cases showed abundant neutrophils, some phagocytic mononuclear cells, many bacteria (intra- and extracellular) and some urothelial cells. The phagocytic cells appeared scattered and actively engulfing bacterial particles (Fig. 1). Neutrophils showed nuclei with fine chromatin (Figs. 2,3). The cytoplasm was of normal density and amount but showed many projections (Fig. 3). Intracytoplasmic granules displayed the normal heterogenous structure with variation in size, shape and density with predominance of large electron-dense primary granules (Fig. 2). Frequent phagocytic vacuoles were apparent with or without bacterial contents. No abnormal inclusions were present. Occasional apoptotic and degenerating neutrophils were encountered.

Patients with transitional cell carcinoma had a more aggressive cellular infiltration in their urine cytology with many malignant degenerated urothelial cells and neutrophils but fewer bacterial particles. Most neutrophils appeared in aggregates or adherent to the tumor cells (Fig. 4,5). The phenomenon of phagocytosis was less

prominent than in cystitis cases. Many degenerated neutrophils were encountered with membrane lysis and extracellular release of granules (Fig. 6). A detailed examination of the neutrophil infiltrate revealed many cells with gross cellular abnormalities, viz. chromatin margination or condensation, bizarre-shaped granules, disrupted cytoplasm, abnormal undefined inclusions, intense vacuolation and occasional phagocytic vacuoles containing debris (Figs. 6,7). Some other less distorted cells are encountered showing fine nuclear chromatin but their granules were more or less bizarre and the cytoplasm was slightly vacuolated (Figs. 4,5).

Most patients with SqCC presented with hematuria and so their urine contained many red blood cells. Malignant urothelial cells and eosinophils were rarely seen. Unlike TCC, eosinophils in SqCC urine cytology constituted the main component of the inflammatory infiltrate. Most of these cells appeared degenerated with disintegrated nuclei, lysed membrane and cellular fragmentation. Intact cells showed bizarre-shaped granules in their cytoplasm (Fig. 8).

DISCUSSION

The functional relationship between inflammation and cancer has long been established in many studies. Moreover, a dualistic role of inflammatory cells has been assumed **[1,2,5,7, 11,10,17,18,19]**. Although inflammation may enhance cancer development by helping to add mutations or support malignant growth, inflammation may also destroy malignant cells or arrest their growth. Investigators **[20]** explained these opposing actions by pointing out that intense inflammation leading to destruction of the initiated cells could decrease tumor development but that nondestructive inflammatory responses might promote tumor growth.

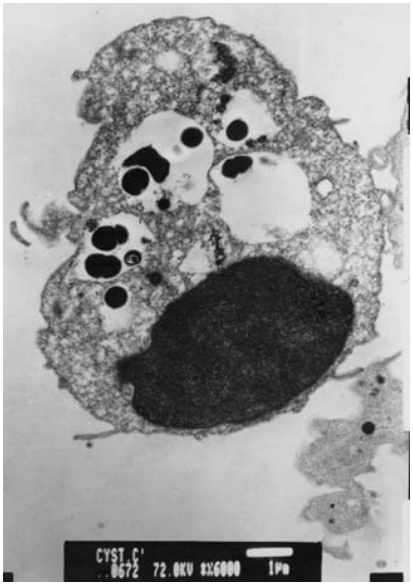

Fig. (1): A phagocytic cell from a case of bladder cystitis engulfing many bacterial cocci enclosed in large phagocytic vacuoles in the cytoplasm X6000.

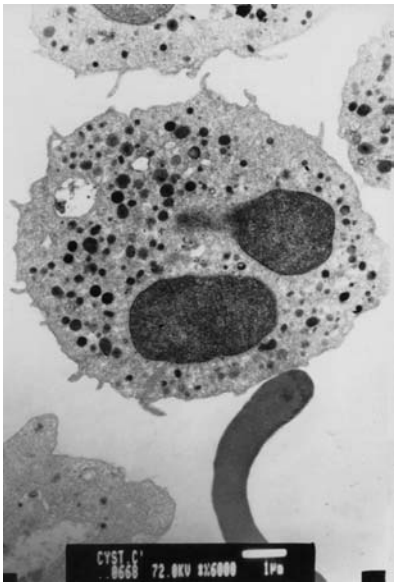

Fig. (2): Urine cytology from a case of bladder cystitis showing a neutrophil with bilobed nucleus and relatively fine chromatin. The cytoplasm appears studded with rounded electron-dense primary granules over-numbering the less dense small elongated secondary granules. Few phagocytic vacuoles are also encountered and the surface membrane shows projections X6000.

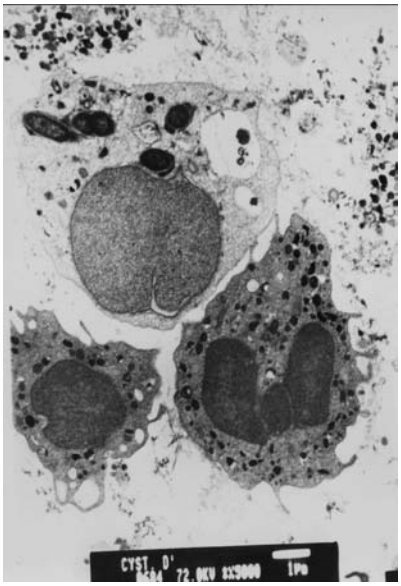

Fig. (3): Two neutrophils in a bladder cystitis case juxtaposed to a urothelial cell. They display phagocytic vacuoles, many granules and cytoplasmic projections X5000.

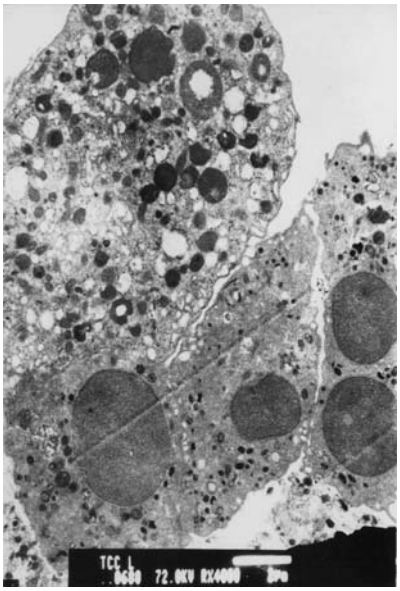

Fig. (4): A photomicrograph showing a collection of adherent neutrophils from a patient with TCC (grade II) showing nuclei with fine chromatin and few scattered bizarre-shaped granules. A nearby malignant cell (k) is also encountered X4000.

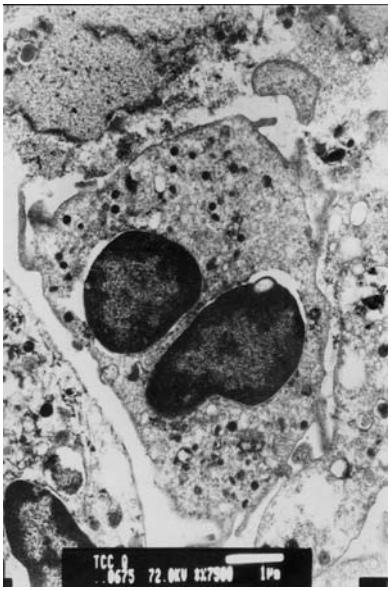

Fig. (5): A photomicrograph of a neutrophil from urine cytology of a patient with TCC revealing a bilobed mature nucleus. The cytoplasm contains few granules and some vacuoles. Some projections are seen extending towards nearby cells X7500.

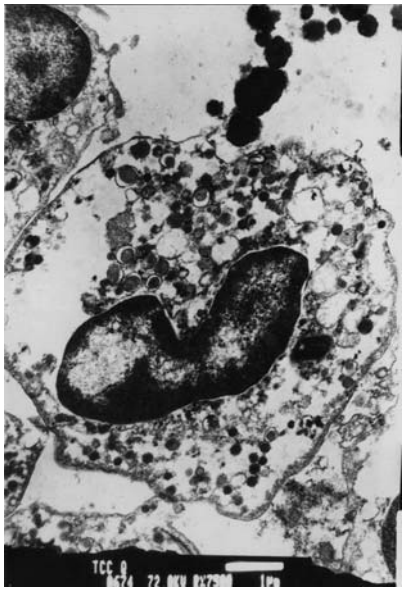

Fig. (6): A phagocytic cell in urine cytology of a TCC patient showing disintegrated cytoplasm and including many vacuoles and distorted granules X7500.

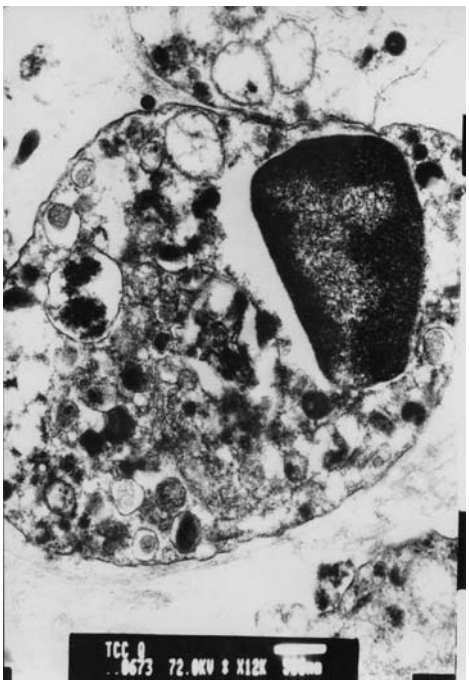

Fig. (7): A photomicrograph of a phagocytic cell in urine of a patient with TCC (stage II). The nucleus appears mature but unilobular with condensed marginal chromatin and central less dense euchromatin. The disrupted cytoplasm shows markedly distorted granules. Engulfed particles are seen in large phagocytic vacuoles X12,000.

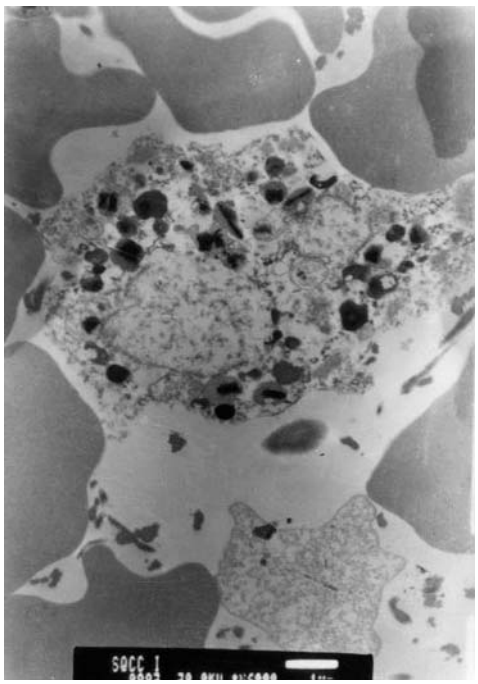

Fig. (8): A photomicrograph of urine cytology of a patient with SqCC showing many red cells and an eosinophil (Eo) with a disintegrated nucleus and disrupted cytoplasm studded with many distorted granules at various stages of degranulation. The cytoplasmic membrane appears lysed in many regions X6000.

The EM examination of the urine cytology in the present work has revealed quite distinguishing characteristics of the inflammatory infiltrates on comparing bladder cystitis with bladder carcinomas. In bladder cystitis, neutrophils show an activated reactive status as demonstrated by internalized bacterial particles in phagocytic vacuoles, many villous projections and cytoplasmic hypogranularity indicative of a degranulation process (Figs. 1,2,3). Marmont et al. **[21]**, by electronmicroscopic analysis of the cells fixed during phagocytosis, have shown that neutrophils incubated with Escherichia coli depicted numerous microorganisms within phagocytic vacuoles and the cell was almost completely degranulated.

In the urine of transitional cell carcinoma cases, the main inflammatory cell component was the neutrophil as with cases of cystitis. However, neutrophils show more propensity to aggregation as well as degeneration (Figs. 4,5,6, 7). The morphology also varies since some cells were demonstrated having reactive features similar to those of cystitits though with less phagocytic capacity as indicated by decreased intracytoplasmic phagolysosomes (Figs. 4,5). Other cells represented an abnormal form with marginated condensed chromatin, disrupted vacuolated cytoplasm including distorted granules and abnormal inclusions (Figs. 6,7).

It is suggested that the cells with reactive features could be assigned to a protective function either against a superimposed bacterial infection (similar to cases of uncomplicated cystitis) or against the tumor cell itself (tumoricidal or tumoristatic action). The distorted population with bizarre-shaped granules and inclusions and weak phagocytic ability could be assigned to a different function such as supporting tumor growth as assumed by the in vitro and in vivo function studies. Dallegri and Ottonello **[7]** have reported a neutrophil destructive action on human and urine tumor cells. This was supported in vivo by an IL4-induced neutrophil tumoricidal effect **[8]**. Furthermore, neutrophils may attract antigen-presenting cells to the inflammatory site generating tumor-specific T-cell response by the host **[18,19]**. Other lines of evidence, however have suggested a growth enhancing effect of neutrophils on cancer cells as granulocyte infiltration occurred in proximity to highly proliferative areas of the growth in murine squamous cell carcinomas **[6,9]**.

The main inflammatory cell associated with squamous cell carcinoma (SqCC) was the eosinophil. This could be explained by the fact that SqCC is common among patients with schistosoma infestation **[22]**. However, the eosinophil exhibits degenerative changes in the nucleus and cytoplasm (Fig. 8). Also Bizarre-shaped granules are demonstrated (Fig. 8). These changes are different from those encountered in the inflammatory eosinophilic reactions described in other conditions as allergy or parasitic infestations **[23,24]**. Hence, they could be regarded as tumor growth promoters rather than reactive antitumor effectors.

In conclusion, the distinguishable morphological alterations encountered in inflammatory cells associated with tumors but absent in bladder cystitis, though could not be diagnostic, yet could act as a warning sign to the urine cytologist when starting to appear in the urine of patients suffering from chronic irritation or inflammation of the bladder epithelium.

Further EM investigations of the features exhibited by the inflammatory cells in premalignant states and in various grades of bladder cell carcinoma could be of predictive value in tumor aggressiveness and invasiveness.

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