Value of the innovated technique of agarose embedding media in increasing the diagnostic sensitivity of cytology for malignancy

Soheir S. Mansy, PhD¹; Mahmoud A. Abbas, MSc¹; Tarek M. Amin, MD²
Electron Microscopy Research Department(Pathology)¹, Urology Research Department², Theodor Bilharz Research Institute, Imbaba Guiza, Egypt

Abstract
The non invasive cytological screening process for malignancy assists the early diagnosis of diseases and thereby enables effective treatment. Proper handling of cells during processing prior to their light or electron microscopic examination represents an important challenge for diagnostic accuracy. The authors of the present work innovated a technique for the processing of cell in suspension simultaneously for light and electron microscopic examination with the prospect to improve the sensitivity of cytological diagnosis for malignancy. In this maneuver, they used agarose gel which is characterized by its low melting point as embedding media for cell sediment. This was associated with adjustment of the used fixative and processing media. The consolidated cell block is divided into halves. Half processed for light microscopic examination and the performance of any needed immunostaining technique. The other half was processed for electron microscopic examination.

The authors have investigated the value of this technique in processing urine samples concomitantly for light and electron microscopic examination. The material of this pilot study consisted of 45 voided urine samples. Diagnosis of the bladder lesions was confirmed by the examination of the corresponding cystoscopic bladder biopsy. Also, urine sample from two patients subjected to follow up after transurethral resection of primary tumor for superficial bladder cancer and with no cystoscopic apparent bladder mass was involved in the study.
The result proved to be promising. The sensitivity of the agarose block technique for the diagnosis of bladder lesions was 90% with 82.4% negative predictive value versus 70% and 57.1% respectively for the routine Papanicolaou stained urine smears. Also, electron microscopic examination of the prepared ultrathin sections highlighted new cytomorphological diagnostic criteria which distinguished successfully low grade urothelial cell carcinomas from dysplastic lesions. Moreover, this study focused attention on the importance of electron microscopy as a diagnostic instrument in controversial conditions and in forecasting tumor recurrence in those patients under postoperative follow up.

**Key word:** cytology – urothelial cell carcinoma- electron microscopy- cytological technique

**Introduction:**

Cytological screening process assists the early diagnosis of diseases prior to the development of symptoms and thereby enables effective treatment [1]. Proper handling of cell in suspension for light microscopic and ultrastructural examination represents an important challenge for accurate diagnosis. Initially, cytological diagnosis was assessed on smears made from the sediment of centrifuged samples. Subsequently, methods included thin membrane filtration, cytocentrifugation, agar block formation were assessed [2,3]. The aim of this work is to use agarose gel as an embedding media for cell sediment and to process the consolidated block for simultaneous examination using light and electron microscopy with the prospect to enhance the quality of diagnosis. The hypothesis of this technique relayed on the low melting point of agarose which can allow a uniform cellular sedimentation before its consolidation. Also, this study
evaluated the use of this technique in the processing of exfoliated urothelial cell in urine and its effectiveness in the diagnosis of low grade bladder carcinoma.

**Material & Methods.**

The material of this study consisted of 45 voided urine samples collected from the outpatient clinic and urology department of the Theodor Bilharz Research Institute hospital. Moreover, the harvested urine sample from two patients underwent transurethral resection of primary tumor (TUR-T) for superficial bladder cancer and had no cystoscopic apparent bladder mass, was included in this study. Cystoscopic bladder biopsies were taken from the enrolled patients and were considered the reference for accurate diagnosis. Informed consent was obtained from all patients according to the rules of Declaration of Helsinki. Urine samples were processed for the performance of Papanicolaou-stained urine smears, agarose cell block paraffin sections stained with hematoxylin & eosin, as well as, electron microscopy-contrasted ultrathin sections. The preparation of agarose cell block included the fixation of urothelial cell sediment in buffered glutaraldehyde for one hour prior to the embedding of cells in the melted agarose gel. Agarose cell block is divided longitudinally into halves. Half is fixed in 10% buffered formalin and processed for the production of paraffin block. The other half is sectioned into tiny pieces and processed for electron microscopic examination.
**Results**
The mean quantitative number of urothelial cells in Pap-stained smear from cases of acute cystitis and malignant cases was $4.1 \pm 1.42$ and $16.7 \pm 7.9$ respectively in comparison with $7.3 \pm 1.6$ and $22.8 \pm 9.7$ ($p < 0.05$) in the corresponding urine samples processed using agarose cell block technique, paraffin embedded, and stained with hematoxylin and eosin. The sensitivity of Pap-stained urine smears for the diagnosis of malignant bladder lesions was 70% versus 90% in agarose cell block prepared samples and examined with light microscopy. On the other hand, malignant cells were detected in 62.5% of the low grade lesions in Pap-stained smears versus 87.5% in agarose cell block paraffin-embedded hematoxylin and eosin-stained sections.

In the present work, electron microscopic examination of corresponding urine samples examined by light microscopy add important new findings which allowed the discrimination between low grade urothelial cell carcinoma (UCC) and dysplastic bladder lesions. The nucleus of low-grade UCC had a characteristic voluminous appearance and showed cellular processes extending nearly from the entire cellular circumference, and an increase in cytoplasmic lysosomes (Fig1&2).
In conclusion, The application of the agarose cell block technique in processing urine samples proved to be effective in increasing sensitivity of urine cytology and opens a new prospect for cytomorphological study. Its application in other centers is recommended to test the reproducibility of the obtained results.

References


Fig 1 & 2 - Electron micrographs of low grade urothelial cell carcinoma show the characteristic irregular size voluminous nucleus highlighted in the present study.