Morphometric and pathological Study of Urinary Bladder Carcinomas: Significance of Schistosomal Fibrosis.

Ali Hassan Al-Timimi   Mohammad Sabri Abdu-Razak*

Dept. of Pathology, College of Medicine, Babylon University,

* Dept. of Microbiology, College of Medicine, Babylon University,

Abstract

Objective measurement of microscopic features has been advocated for decades as a method to make more reproducible and "scientific" the practice of histopathology, but it is only recently that technical advances in computing have rendered this procedure suitable for diagnostic and prognostic determinations in surgical pathology.

Urinary bladder cancer is the third most common malignancy in Iraq. Heavy *Schistosoma haematobium* infection predisposes to bladder cancer which is usually of squamous cell type and accounts for about one quarter of deaths from this infection.We investigated the extent of fibrosis in forty urinary bladder carcinomas (15 Schistosomal associated SASCC and 25 Schistosomal non-associated transitional cell carcinoma SNATCC). The cases were subjected to quantitative assessment of their fibrosis by (1) colorimetric micromethod for collagen measurement (2) morphometric assessment of collagen by use of image analyzer.

The results obtained by both methods showed that SASCC were more fibrogenic than SNATCC and displaying more desmoplasia. It is concluded that the total amount of collagen in addition to the distribution pattern of the fibrotic process in schistosomal cases around the newly formed blood vessel and lymphatic both play a role in determination of the unique behavior of such neoplasm.

الخلاصة

المقياس الموضوعي للميزّاتِ المجهريةِ دعي لـه لعقودِ كطريقة لجَعْل عِلْمِ الأمراض النسجي أكثرِ قابلية للإنتاج و"علميةِ" للممارسةِ ، لكنَّـه فقطُ مـؤخراً أصـبح ذلك ممكنـا بسـبب التقـدِّمِ التقنِي فـي اسـتعمال الحاسـوب . حيث أعـادَ هـذا التطـور الأهميـة التشخيصية والتتبؤيه لهذه العملية في عِلْمِ الأمراض الجراحِي.

سرطان المثانةِ الخبيثُ هو السرطانُ الثالثُ الأكثر شيوعاً في العراق.والإصابة بالبلهارزيا الثقيلة تُهيِّئُ للإصابة بسرطانِ المثانةِ الذي عادتا ما يكون مِنْ نوعِ الخلية القشريةِ، وذلك يُفسِّرُ حوالي رُبْعَ نسبة الوفيّاتِ مِنْ هذه العدوى. تَحرّينَا في هذا البحث مدى

انتشار التنسج الليفي في أربعون ورم مثانةٍ سرطاني (١٥ ورم مثانةٍ سرطاني مِنْ نوعِ الخلية القشريةِ المصـاحب للإصابة البلهارزية و ٢٥ سرطان مثانة غيرا لمصاحب للإصابة البلهارزية). الحـالات أُخضِعتْ إلى التقييمِ الكمّي للترسبات التنسـج الليفي باستعمال(١) مقياسٍ الكلوريمتري لقياسِ الكولاجينِ (٢)   المقياسِ المورفومتري لقياسِ الكولاجينِ باستعمال محلّلٍ الصورةِ.

النَتائجُ التي حَصلناْ عليها بكلتي الطريقتين أظهرتا أنّ ا ورم المثانةِ السرطانية مِنْ نوعِ الخلية القشريةِ المصاحب للإصابة البلهارزية كَانتْ الترسبات الليفيـة النسيجية فيهـا أكثر مِنْ سرطان المثانة غير المصـاحب للإصابة البلهارزية. تم الاستنتاج بأنّ الكميةَ الكلّيَّةَ للكولاجينِ بالإضافة إلى نمطَ توزيعَ عمليةِ التليف النسيجية حول الوعاء الدموي المُشَكَّلِ حديثاً والأوعية اللمفاويةِ في حـالاتِ ا ورم المثانةِ المصاحب للإصابة البلهارزية كلتاهما له دور في تصميمِ السلوكِ الفريدِ لمثل هذه الأورام.

Introduction

Objective measurement of microscopic features has been advocated for decades as a method to make more reproducible and "scientific" the practice of histopathology[1-8] .Traditionally the measurements have been made from photographs, from projected images, or by the use of eyepiece graticules[7-14]. Currently, semiautomatic or fully automated image analyzers are employed.[8,15]Most of the original contributions employing this technique have been in the evaluation of non-neoplastic diseases of skeletal muscle, peripheral nerve, small bowel, and bone.[1,2]

Carcinoma of Urinary bladder constitute 7.5% of all cancer in Iraq, with steady increase in incidence [16]. Heavy *Schistosoma haematobium* infection predisposes to bladder cancer which accounts for about one quarter of deaths from this infection and has peak incidence between 40-60 years. Unlike the usual bladder cancer, the schistosomiasis associated tumour is usually a well differentiated squamous cell carcinoma. It grows from the posterior of lateral wall and project in to the bladder lumen, forming a keratinizing mass, invading through the wall and later metastasize. It is a etiology is uncertain, neither the worms nor the eggs have been shown to produce any carcinogenic agent. Best explanation are: 1- chronic urinary schistosomiasis causes squamous metaplasia of the urothelium and predisposes to gram-negative bacterial cystitis, 2- bacteria (e-g: *Escherichia coli*) produce nitrosamines by break down of dietary nitrites and nitrates excreted in the urine, 3- the carcinogenic nitrosamines[17,18] act on the squamous epithelium as

initiating agents and 4- the persistence inflammation and epithelial irritation induced by the schistosomal infection act as promoting factors.

Schistosoma is also claimed to be responsible for the delayed metastatic spread of such tumours owing to dense fibrosis which possibly retards the spread of the neoplastic cells, thus despite the high stage and the histological type of the tumour. Lymph node metastasis was much lower than expected[19].

The aim of the present study was to investigate the extent of fibrosis in schistosomal associated and non schistosomial associated urinary bladder carcinoma using objective measurement of microscopic features.

Material and Methods

Thirty two urinary bladder carcinomas were studied at department of Pathology and department of Microbiology, College of Medicine, Babylon University. The cases were fixed in 10% formaldehyde solution, paraffin processed and cut at 4 and 10 micron thick sections. The cases included in the study comprised 12 cases of urinary bladder carcinoma associated with bilharziasis and 20 without evidence of bilharzial

infestation. All sections were stained with haematoxylin, phloxine and safran for routine evaluation. All cases were studied for :

(1) measurement of collagen by the dye-binding method [20,21].15μ thick sections were deparafinized and stained with a saturated solution of picric acid in distilled water containing 0.1% of fast green and 0.1% of sirius red (BDH chemical company Ltd.) left incubated in the dark chamber at room temperature for 2 hours. The sections were rinsed in distilled water for 15mins. and then transferred to test tube containing 1ml 0.1% NaOH in absolute methanol mixing the tube gently untill complete elution of the colour. Absorbance of the eluted colour was then read in spectrophotometer. Fast green absorbance at 605nm and sirius red at 540 nm for determination of non collagenous and collagenous proteins respectively.

(2) Histomorphometric measurement of fibrosis. The morphometric measurements were performed on sirius red stained 4μ sections with a light microscope, using a tracing device with a cursor placed on a digitizing plate connected to a disk computer, described previously[1-5,8].

The nuclear circumference was followed with the light diode, recordings made at x400 magnification. A morphometric program was used to measure the length of the circumference, the area of the nucleus (in mm^2), and the shape of the nucleus, expressed as a form factor. A circle has the form factor 1.0. Random measurements—that is, measurements of epithelial nuclei as they presented on screening of the slide regardless of subjective assessment— were performed in all cases. The total area and the area of fibrosis were drawn and evaluated. The degree of fibrosis was expressed as µg collagen/mm^2. The same methods were used throughout. The statistical methods used were Student's t test and the X^2 test. Level of significance was set at $p < 0.05$.

Results

Forty cases were studied, 15 cases were schistosomial associated squamous cell carcinoma of bladder and 25 cases were non schistosoma associated transitional cell carcinoma.

Pathological features (fig. 1-4)

Carcinoma of bladder were found anywhere in the bladder. The location most seen, in order of frequently was as follows: lateral walls, 37%; posterior wall, 18%; trigone, 12%; neck 11%; ureteric orifices, 10%; dome, 8%; and anterior wall, 4%. When located around the ureteral orifices, they might produce partial or complete blockage of one or both ureters, with resulting hydronephrosis and pyelonephritis.The pattern of growth were either exophytic or endophytic, or a combination of both. When exophytic, the tumor might adopt a papillary configuration (with central fibrovascular cores) or a solid (nodular) appearance . Stromal invasion by the tumor proceeds in two stages: invasion of the lamina propria and invasion of the muscle layer. These tumors were mostly poorly differentiated and had nearly always invaded the muscle at the time of diagnosis.

Several cytoarchitectural variations of transitional cell carcinoma were seen. Foci of glandular metaplasia were common, usually in the form of intracytoplasmic mucin-containing vacuoles.. Similarly, many otherwise typical transitional cell carcinomas (especially grade III and grade IV lesions) show foci of squamous differentiation. These tumors were regarded as of transitional

origin and clearly separated from pure squamous cell carcinomas.

Squamous cell carcinoma was seen on a background of chronic cystitis with marked squamous metaplasia and chronic infection with schistosomiasis and excessive deposition of Fibrosis. Some squamous cell carcinomas of the bladder probably represented metaplastic changes in tumors that were originally of transitional cell type. Grossly, these tumors were large, ulcerated, and necrotic .

Quantitative assessment of fibrosis and collagen content

The two methods (the dye-binding and the histomorphometry) were similar in their estimation of collagen content irrespectively of the sample size. Schistosomal associated squamous cell carcinoma (SASCC) contained the highest amount of collagen in the malignant group being 105.55 μg col/mg protein and 0.09μg col/mm^2 compared to schistosomal non-associated transitional cell carcinomas 61.48 μg col/mg protein and 0.051 μg col/mm^2.
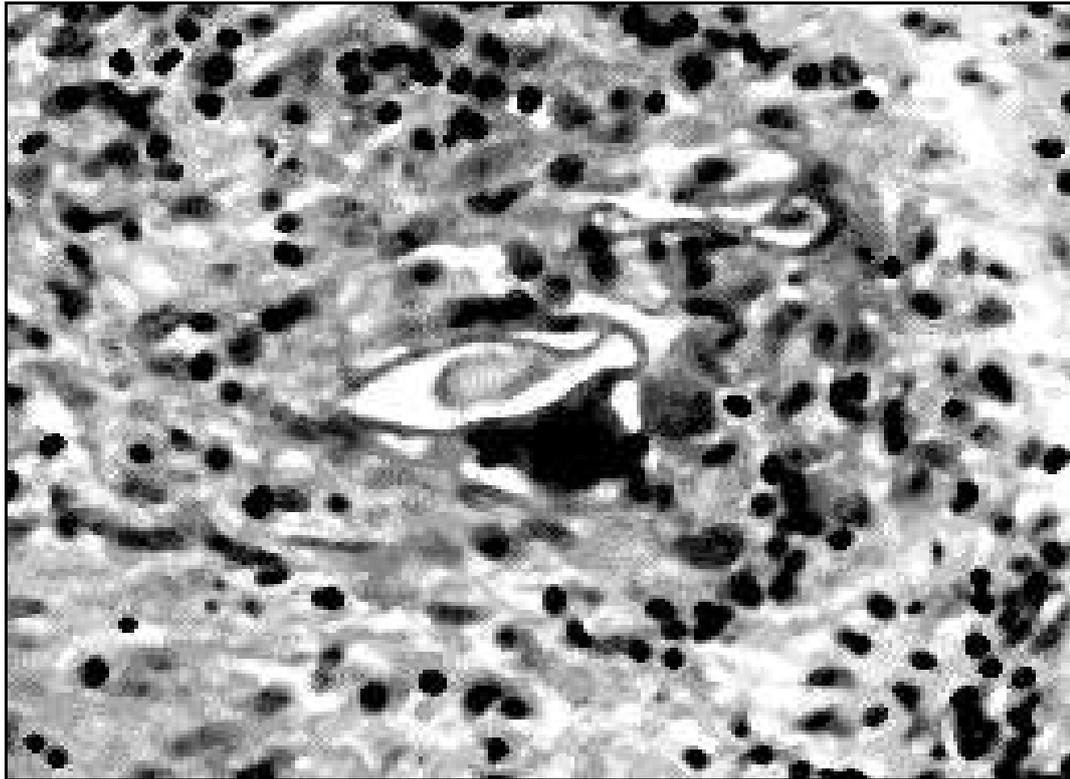
Figure1 Schistosomial associated urinary bladder carcinoma showing Schistosomial ova deposition.
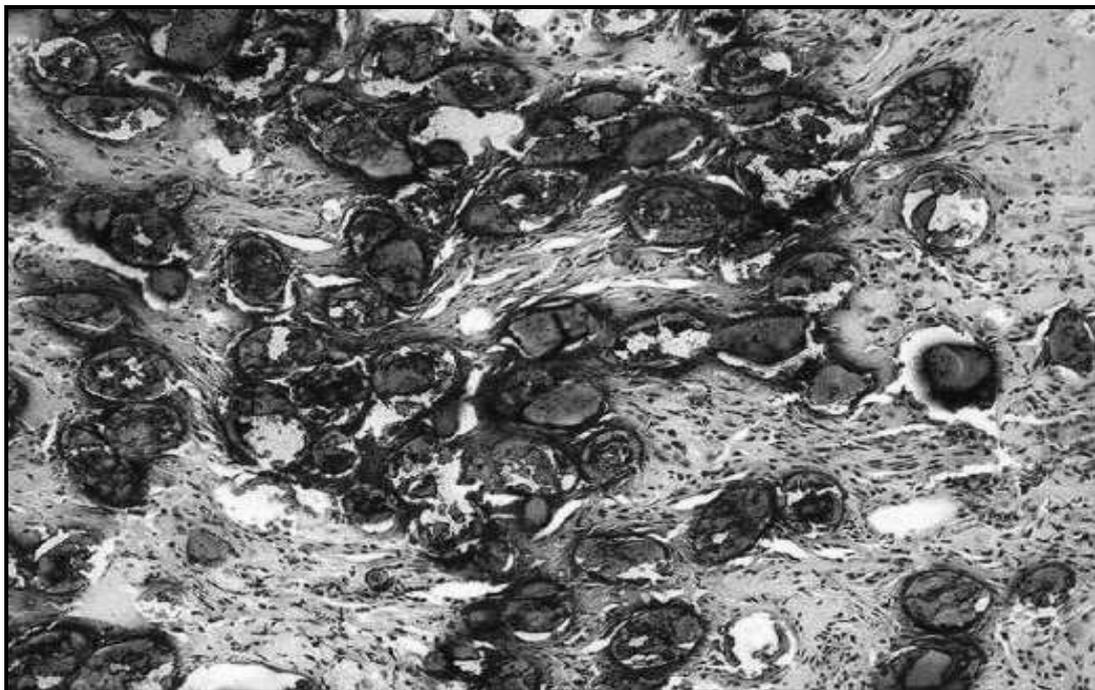

Figure 2 Schistosomial associated urinary bladder carcinoma with heavy Schistosoma haematobium infection.
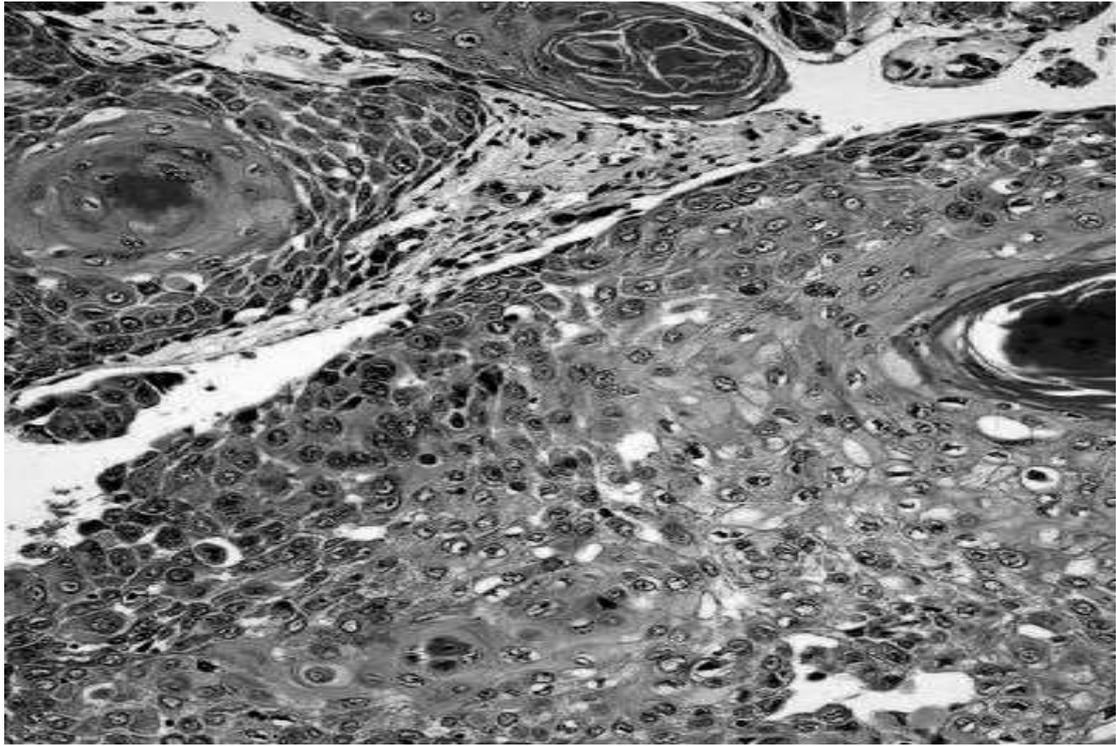
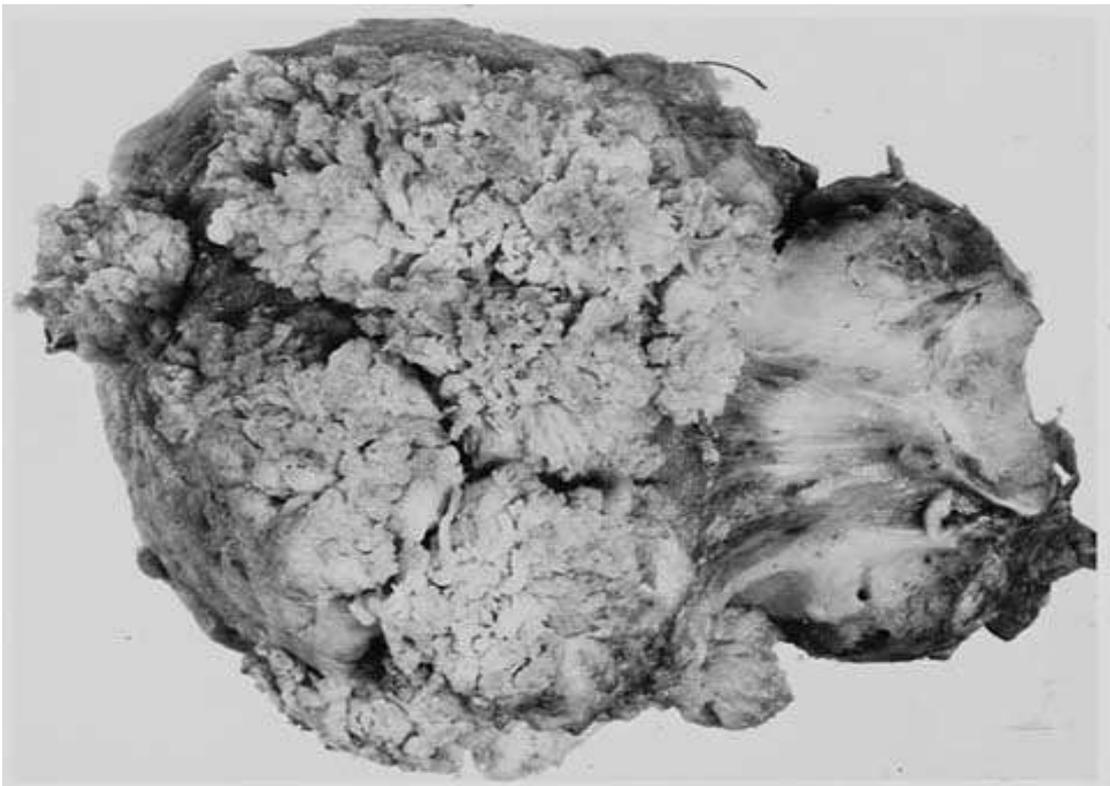**Figure3** Grade 1 squamous cell carcinoma of bladder.

Figure 4 Grade II transitional cell carcinoma involving large portion of bladder.

Discussion

Transitional cell carcinoma comprises about 90% of all primary tumors of this organ. As for most other carcinomas, its development seems dependent on a combination of genetic and environmental factors.[22-36] Among the latter, chemical factors are thought to be of great importance.[32-36] Bladder tumors are more common in industrial areas (especially in those associated with petrochemicals), and their incidence is increased with exposure to cigarette smoke and arylamines.[22-33] Auerbach et al.[23] have shown a sharp correlation between smoking habits and the occurrence of nuclear atypia in the transitional epithelium, complementing the epidemiologic evidence of a dose-response of cigarette smoking and urinary bladder carcinoma.

Other environmental factors include aniline dyes (particularly benzidine and beta-naphthylamine),[35-35] auramines, phenacetin, and cyclophosphamide.[28,35] It has been postulated that urinary tryptophan metabolites may be the endogenous counterparts of the carcinogenic dyes.[24] Schistosoma hematobium is also thought to be pathogenetically related to transitional cell (and squamous cell) carcinoma of the bladder, being that the greatest concentration of carcinoma of this organ occurs in areas of the world infested by this parasite[28].

Fibrosis represents an excessive deposition of connective tissue, which impaired normal function of organ tissue by replacing specialized cells with fibrous tissue [36]. However such fibrosis limit the spread or extension of the injurious agent as in dense fibrosis around infection or tumors [8].

In Iraq squamous cell carcinoma of urinary bladder are associated with schistosomal infection. Tissue fibrosis in schistosomiasis is largely responsible for the important morbidity that results from infection with schistosomiasis. Egg deposited in the tissue induce a chronic inflammatory granulomatous response, that is the hallmark of infection and precede the onset of adjacent tissue fibrosis [8].

In the present study the SASCC of bladder displayed more collagen than SNATCC. This is attributed to the increased collagen production associated with schistosomiasis. Tissue fibrosis in schistosomiasis is largely responsible for the important morbidity that results from infection with schistosoma. Eggs desposite in the

tissue induce a chronic inflammatory granulomatous responses, that is the hallmark of infection and precede the onset of adjacent tissue fibrosis [37]. The bilharzial granulomatous reaction present mainly in lamina propria of urinary bladder are composed of macrophages, lymphocytes, esinophils, neutrophils, fibroblast and occasional foreign body giant cells. Evidence is obtained that activated granuloma macrophage and the eggs themselves are direct source of a fibrogenic activity [38], indirect evidence also suggest that granuloma lymphocytes are additional source of such activity [39, 40]. In schistosomiasis the excess collagen synthesis has been attributed to the secretion of various factors that have been shown in vitro to stimulate both collagen and fibronectin synthesis by fibroblastic cells [39].

Studies on isolated egg granulomas have demonstrated in vitro that such biochemical active molecules not only stimulate fibroblast proliferation and collagen synthesis [39-41], but also inhibit contraction of collagen lattices[42] secrete prostaglandin [43]and inhibit macrophage migration [44]. Despite the fact that malignant tumours are inherently fibrogenic to different extents, it have been expected that such tumours in presence of schistosomal infection would thereby elicit more total collagen than the non-schistosomial associated malignant tumour. It is postulated that the limited tendency of schistosoma associated advanced stage squamous cell carcinoma to lymphatic and blood stream spread to be the result of bilharzial fibrosis[45]. Several investigators [43,44,46] considered the tissue reaction to schistosoma eggs in the wall of bladder, pelvic lymphatic and regional lymph nodes as an important limiting factors against neoplastic spread, despite the advanced stage of disease.

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