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Original Article

Detection of early squamous metaplasia in bladder biopsies of spinal cord injury patients by immunostaining for cytokeratin 14

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Study design: A prospective, immunohistochemical study of bladder biopsies taken from spinal cord injury (SCI) patients.
Objectives: To investigate whether cytokeratin 14 immunostaining may be useful to detect early squamous metaplasia in bladder biopsies from patients with SCI.
Setting: Southport, United Kingdom.
Methods: Biopsy of bladder mucosa was taken from adults with SCI, while they underwent an elective therapeutic procedure in the urinary tract. A total of, 54 biopsies, which showed transitional epithelium only with no evidence of squamous metaplasia on routine H&E staining, formed the study group. In all, 22 biopsies, which showed squamous metaplasia on routine H&E staining, acted as controls. All biopsies were benign with no evidence of dysplasia or malignancy. Immunohistochemical staining for cytokeratin 14 was performed on all biopsies in a single batch, using a standard avidin-biotin complex method.
Results: All control biopsies showed positive immunostaining for cytokeratin 14 in basal and parabasal cells in areas of squamous metaplasia. Of the 54 biopsies, which showed only transitional epithelium on H&E staining, immunohistochemistry for cytokeratin 14 showed no staining in 47 biopsies. The remaining seven biopsies showed positive immunostaining for cytokeratin 14 in the epithelium, in individual cells or clusters of basal cells, revealing unexpected early squamous metaplasia in these biopsies.
Conclusion: Immunostaining for cytokeratin 14 identifies an early phenotypic switch from transitional to squamous epithelium in bladder mucosa. Cytokeratin 14 staining is sufficiently sensitive to identify early squamous metaplasia, which is not yet evident on examination of routine H&E stained sections. This early identification may be of use in alerting physicians to change bladder management regimens to prevent predisposition to recurrent urinary infection and progression of squamous metaplasia. A cost/benefit analysis should be performed to assess the feasibility of routine cytokeratin 14 immunostaining of bladder biopsies from SCI patients.
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Keywords: cytokeratin 14; spinal cord injury; squamous metaplasia; urinary bladder biopsy

Introduction

Squamous metaplasia of bladder mucosa is frequently seen in spinal cord injury (SCI) patients with long-term indwelling urinary catheters, bladder calculi or recurrent urinary infection.[1] *In vitro* studies have shown that squamous differentiation of human urothelial cells (induced by retinoid deficiency in the growth medium) is accompanied by *de novo* expression of cytokeratin 14.[2] In a clinical setting, Harnden and Southgate[3] have suggested that cytokeratin 14 expression is a sensitive early marker of a switch to a squamous phenotype within urothelial tumours. These authors identified focal positive staining for cytokeratin 14 in 47% of transitional cell carcinomas of the bladder, which showed no morphological evidence of squamous differentiation on routine H&E staining.[3] We suggest that this method can be of clinical use when applied to biopsies of the non-neoplastic neuropathic bladder in patients with SCI.

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This article is dedicated to the memory of Mr John Ashcroft and Ms Debra Frodsham, who were patients of Regional Spinal Injuries Centre, Southport.

Methods

Bladder biopsy was taken from SCI patients while they were undergoing an elective therapeutic procedure in the urinary tract such as endoscopic lithotripsy of bladder stone insertion of a ureteric stent or diagnostic cystoscopy. The SCI patients were adults; they were registered with the Regional Spinal Injuries Centre, Southport, England and were not suffering from acute urinary infection. Written informed consent was obtained from the patients for taking bladder biopsy. The North Sefton Local Research Ethics Committee approved this study. Gentamicin was administered intravenously as a prophylactic for urinary infection, since SCI patients with neuropathic bladder are at risk for developing acute urinary infection after any procedure in the urinary tract. Cold cup biopsy of the bladder mucosa was taken from the trigone of the urinary bladder. The biopsy site was fulgurated with diathermy to achieve haemostasis. Indwelling urinary catheter drainage was maintained after the procedure, and all patients remained in hospital for at least 24 h. Biopsies were fixed in neutral buffered formaldehyde 4%, and then embedded in Paraplast. (Paraplast is a commercial paraffin wax incorporating plasticisers, used for embedding formalin-fixed tissues for histology.)

A total of 54 biopsies, which showed transitional epithelium only with no evidence of squamous metaplasia on routine H&E staining, formed the study group. In all, 22 biopsies, which showed squamous metaplasia on routine H&E staining, acted as controls. All biopsies were benign with no evidence of dysplasia or malignancy.

Immunohistochemical staining for cytokeratin 14 was performed on all biopsies in a single batch, using a standard ABC method. The mouse monoclonal antibody (catalogue number NCL-LL002, Novocastra Laboratories Ltd, UK), raised against a synthetic peptide of the extreme c-terminal end (the last 15 amino acids) of human keratin 14, was used. The working dilution was 1:25, with primary antibody incubation at 25°C for 60 min after pretreatment with proteinase-K. For each biopsy, a negative control was processed in parallel but omitting the primary antibody.

Results

All control biopsies showed positive immunostaining for cytokeratin 14 in basal and parabasal cells in areas of squamous metaplasia (Figure 1a). Where squamous and transitional epithelium were present in the same biopsy, a sharp cutoff was observed in immunostaining between positive squamous epithelium and negative transitional epithelium (Figure 1b).

Of the 54 biopsies, which showed only transitional epithelium on H&E staining, immunohistochemistry for cytokeratin 14 showed no staining in 47 biopsies. (Figure 1c). The remaining seven biopsies showed positive immunostaining for cytokeratin 14. Immunostaining was seen either in individual cells in the basal layer (Figure 1d) or in clusters of cells in the basal layers of the epithelium. (Figure 1e). In one of these seven biopsies, positive immunostaining was observed in scattered cells in the superficial layer of the epithelium in addition to positive staining of cells in the basal layer. (Figure 1f). Positive immunostaining for cytokeratin 14 in transitional epithelium indicated a phenotypic switch towards squamous differentiation. The seven patients, in whom bladder biopsy showed positive staining for cytokeratin 14, are being followed up closely to assess progression of squamous differentiation in the vesical epithelium.

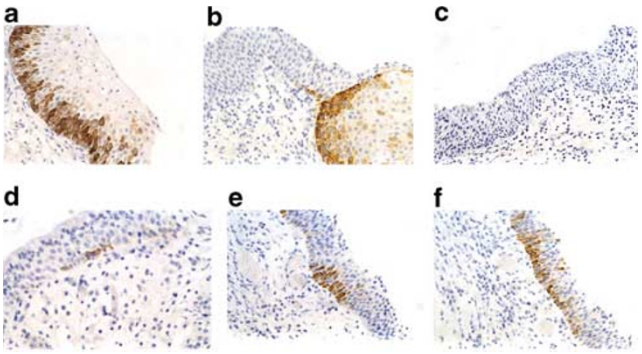

Figure 1 (**a**) Control bladder biopsy from SCI patient with long-term indwelling urinary catheter shows positive immunostaining for cytokeratin 14 in basal and parabasal cells within an area of squamous metaplasia. (**b**) Control bladder biopsy from a SCI patient shows positive immunostaining for cytokeratin 14 in metaplastic squamous epithelium (right) and absent immunostaining for cytokeratin 14 in native transitional epithelium (left). (**c**) Negative immunostaining for cytokeratin 14 in transitional cell epithelium in bladder biopsy from a SCI patient with ventilator-dependent tetraplegia. (**d**) Occasional basal cells show positive immunostaining for cytokeratin 14, indicating the earliest evidence of a phenotypic switch towards squamous differentiation. (**e**) Positive immunostaining for cytokeratin 14 in clusters of basal cells, indicating a rather more advanced stage of squamous differentiation than in (**d**). (**f**) Bladder biopsy from an SCI patient showing scattered positive immunostaining for cytokeratin 14 in superficial as well as basal layer cell

Discussion

Cytokeratins are a class of intermediate filaments, which form part of the cytoskeleton of epithelial cells. In all, 20 subtypes of cytokeratins, defined by their molecular weight and isoelectric point, are currently recognised and are specific for different types of epithelia, in which they can be identified by type-specific antibodies. Cytokeratin 14 is a marker of nonkeratinising (stratified and nonstratified) squamous epithelium.[4] Harnden and Southgate[3] have shown that cytokeratin 14 immunostaining can identify the earliest stages of squamous metaplasia in transitional cell carcinomas of the bladder, even if not identifiable morphologically on routine H&E staining.[3] In this current study, we have shown similar findings in non-neoplastic epithelium in bladder biopsies

from patients with SCI. This information may be valuable to the treating physician, by alerting them to the presence of early complications of the neuropathic bladder; changes in management may prevent progression of the metaplasia, and may help to prevent more serious complications such as the development of squamous carcinoma. For example, finding early squamous metaplasia in a routine biopsy from a SCI patient with an indwelling urinary catheter may provide the impetus to change to a regimen of intermittent catheterisation, assuming that this is both feasible and acceptable to the patient. Alternatively, detection of early squamous metaplasia in a patient using penile sheath draining may suggest to the physician that incomplete bladder emptying is causing recurrent urinary tract infections, prompting a need for supplementary catheterisations.[5] Intermittent catheterisation should ideally be combined with anticholinergic agents such as oxybutynin to achieve complete, low pressure, bladder emptying.

We, therefore, believe that bladder biopsy and routine cytokeratin 14 immunostaining can provide valuable information about the milieu of the urothelium in the neuropathic bladder of SCI patients at a microscopic level. Implementing a policy of routine immunohistochemical staining would, of course, have financial implications for the histopathology laboratory. However, squamous cell carcinoma of the bladder in SCI patients is usually high grade at diagnosis and often stage pT3a or worse; chemotherapy and radiotherapy are largely ineffective.[6] Therefore, we believe that trials should be performed to assess the cost effectiveness of the approach, which we advocate. Such trials should specifically investigate whether changes in bladder management, in response to immunohistochemical identification of the earliest stages of squamous metaplasia, can decrease morbidity in SCI patients in terms of prevention of (1) urinary infection and (2) bladder cancer. Squamous metaplasia can predispose to recurrent urinary infections.[7] Squamous metaplasia is commonly associated (17–25%) with squamous cell carcinoma of the bladder.[8] The epithelial changes (metaplasia) are thought to be the aetiological factor for the high incidence of bladder cancer (mainly squamous cell carcinoma or transitional cell carcinoma with squamous cell elements) in SCI patients. Yalla[9] encountered SCI patients with squamous metaplasia suddenly progressing to invasive squamous cell carcinoma and therefore recommended active surveillance with cytology, cystoscopy and urothelial biopsies even after eliminating potential risk factors, such as indwelling catheters, bladder stones and chronic sepsis.

Conclusion

Cytokeratin 14 immunostaining identified early squamous metaplasia, which was not identifiable on routine H&E staining, in seven out of 54 bladder biopsies from patients with SCI. This early identification may be of use in alerting physicians to change bladder management regimens to prevent predisposition to recurrent urinary infection and progression of squamous metaplasia.

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