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Original article

Haematobium eggs detection in human bladder cancer and sporocysts in snail vectors: Seven cases report and a review of the Burkina Faso literature

Détection des œufs de Haematobium dans le cancer de la vessie et des sporocystes dans les mollusques vecteurs : étude de sept cas et de la littérature au Burkina Faso

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ABSTRACT

Schistosoma haematobium plays a central role in the development of bladder cancer in Burkina Faso. The objective of this study was to determine the presence of *S. haematobium* in the bladder cancer and in vector snails. For the first time, formalin-fixed tissues embedded in paraffin were analyzed by immunohistochemistry and PCR. Molecular detection has resulted in 7/7 positive bladder cancer. Finally, as the snail vectors were positive. We suggest the use of molecular methods in the snail vectors for the detection of cysts and in cancerous tissues in larger studies.

Mots clés:
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RÉSUMÉ

Schistosoma haematobium joue un rôle central dans le développement du cancer de la vessie au Burkina Faso. L'objectif de cette étude était de déterminer la présence de *S. haematobium* dans le cancer de la vessie et dans les mollusques vecteurs. Pour la première fois, les tissus fixés au formol et inclus en paraffine ont été analysés par immunohistochimie et PCR. La détection moléculaire a permis d'obtenir 7/7 cancer de la vessie positive. Enfin, aussi les mollusques vecteurs ont été positifs. Nous suggérons l'utilisation des techniques moléculaires dans les mollusques vecteurs pour la détection des sporocystes et dans les tissus cancéreux dans des études approfondies.

1. Introduction

Schistosomiasis or bilharzia is a tropical parasitic disease caused by blood-welling fluke worms of the genus *Schistosoma* [1]. In Burkina Faso the main schistosomes infecting human beings are *Schistosoma haematobium*, transmitted by *Bulinus* snails and causing urinary schistosomiasis; and *Schistosoma mansoni*, which is transmitted by *Biomphalaria* snails and causes intestinal and hepatic schistosomiasis. *S. haematobium* inhabits the human vesical venous plexus, where female worms lay eggs. These eggs either pass into the urine or remain trapped in the tissues, particularly in the bladder and ureter walls, where they cause major pathological alterations of the bladder, ureter, and kidneys [2]. Pathological features of *S. haematobium* schistosomiasis includes: hematuria, scarring, calcification, squamous cell carcinoma and occasional embolic egg granulomas in brain or spinal cord [3,4]. Bladder cancer diagnosis and mortality associated are generally elevated in affected areas. In Burkina Faso the data of the distribution of the two *Schistosoma* species involved with man (*S. haematobium* and *S. mansoni*) confirm their endemicity and the focal transmission [5], with high predominance of *S. haematobium* [6]. *S. haematobium* infection was found prevalent throughout cohort schools in the country. Burkina Faso is the first country that achieved full national coverage with anti-helmintic treatment of more than 90% of the school-age population [7]. Children who benefited the most from treatment in terms of increased

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haemoglobin concentrations were those who had anaemia at baseline and those with highly positive micro-hematuria scores at baseline [8]. Counter-productive to national schistosomiasis control programme is the hydro-agricultural planning which turns out to be an amplifying factor of the *species* proliferation and parasitic flux host-parasite [5]. The molluscs species collected and identified in this country were: *Bulinus truncatus rohlfsi*, *B. senegalensis* and *B. globosus* for the urinary schistosomiasis [9]. *B. senegalensis* and *truncatus* were found naturally infested by *S. haematobium* [10]. In Burkina Faso the prevalence of *S. haematobium* was higher than 46%. Remarkable was that the 55% of the infected children presented at least one lesion of the bladder [11]. Approximately 50% of the tumours associated with schistosomiasis are squamous cell carcinoma; 10% are adenocarcinomas, and 40% are transitional cell carcinomas [12].

Microscopic examination of excreta (stool, urine) is the gold standard diagnostic test but requires the adult worms to be producing eggs; serological tests can diagnose less advanced infections [13]. Rectal or bladder biopsy for the identification of eggs may be performed if stool or urine are egg-negative but schistosomiasis is still suspected [14]. Because the eggs may not be detected in urine during chronic parasitation stages and cancer, the final diagnosis is based on the presence of granulomas or dysplastic cells and *Schistosoma* eggs in the submucosa of bladder biopsies. We were interested to test moleculars method in which *S. haematobium* eggs were detected by immunohistochemistry and polymerase chain reaction (PCR) in formalin-fixed and paraffin-embedded human bladder cancer or snail vectors.

2. Materials and methods

2.1. Collection of human and snail tissues

A total of seven vesicals carcinoma and ten control tissues were obtained from patients undergoing curative intent surgical resections at the S. Camille Medical Centre, Nanorò, Burkina Faso. For collaborative work-project macroscopic and microscopic analysis were performed in the 2nd Department of Pathology, Spedali Civili, University of Brescia, Italy. *Bulinus* snails were collected during 2010 from transmission site of Nanorò region of the Burkina Faso. Control uninfected snails, used for this study, were from Italy.

2.2. Histopathology

The human and snail samples were fixed in formalin solution (dilution 4%) and embedded in paraffin for histopathological processing. Tissue sections were stained with haematoxylin-eosin (H&E) for conventional histology and identify *Schistosoma* eggs. Moreover H&E-stained or Alcian-blue (pH 2.5) labelled slides from snails were examined.

2.3. Immunohistochemistry

The intracellular levels of Cytokeratin (CK) and Ki-67 proteins were investigated. Sections from tissue blocks of bladder cancer were immunostained with the following antibodies:

- Ki-67 clone MIB-1 (used 1:50) and Anti-human Cytokeratin clone MNF116 (used 1:200) from Dako, Copenhagen, Denmark, according to standard protocols;
- Anti-*Schistosoma* monoclonal antibody 291-5D5-A was a kind gift by Dr Cornelis H. Hokke (Department of Parasitology, Leiden University Medical Center, The Netherlands).

Immunohistochemistry analysis was performed on 4 μm sections of archival formalin-fixed, paraffin-embedded tissues were placed on slides. After deparaffinization and hydratation, heat-induced retrieval using water bath pre-heat at 98 °C and citrate buffer (0.5 M, pH 6), was applied. All samples were processed using an alkaline phosphatise-anti-alkaline phosphatise (APAAP) method. The Dako REAL™ Detection System, Alkaline Phosphatase/RED, Rabbit/Mouse kit (Dako, Copenhagen, Denmark) was used. The specificity of the antibody and the method were tested by the following control: (a) sections from human uninfected tissues were staining; (b) sections from one snail that was collected and stained in Brescia (Italy); (c) sections incubation within primary antibody. The title of Ab 291-5D5-A was determined by Enzyme Linked Immunoassay by Nibbeling et al. [15].

2.4. DNA extraction and Dra I - Polymerase chain reaction (PCR) method for S. haematobium detection

DNA was obtained according to standard proteinase K digestion of three sections of 10 μm macro-dissected and deparaffinised. The Dra I-PCR method [16] was applied to amplify the 121 base pairs (bp) tandem repeat DNA of the *S. haematobium* eggs. In order to test the specificity of the PCR products we used: (a) the amplification from tissue area containing the *Schistosoma* eggs; (b) the amplification from negative tissue for *Schistosoma* eggs; (c) the Dra I digestion of all the PCRs; (d) the sequencing of the PCR products; (e) the PCR for the detection of *S. mansoni*, carried out according to Pontes et al. [17]. The sensibility curve of the Dra I-PCR method was applied with 30 ng, 30 pg and 30 fg of the recombinant DNA.

3. Results

Seven *Schistosome*-related bladder cancers from Burkina Faso were analysed and the pathologic characteristics of the tumours were shown in Table 1. Infiltrating squamous cell carcinoma (SCC) were diagnosed, and keratinization was present with different degree. All neoplastic samples were immunoreactive for cytokeratin, this confirming the squamous phenotype. Ki-67 antigen was expressed in three of seven cases.

All cases showed chronic inflammatory stroma contained ovoid structures compatible with trematoda eggs. In all cases of bladder cancer infected strong 291-5d5 expression was demonstrated, clearly distinguishing the eggs from neoplastic tissue. The immunoreaction was restricted to ovoid formations correspondent to *Schistosoma* eggs, and with a fine details of *Schistosoma* miracidial nuclei (Fig. 1a).

To verify the presence of a specific DNA for the *S. haematobium* in the bladder cancer, genomic DNA from all specimens were amplified by specific Dra I-PCR. An intense and specific band of 121 base pairs and a smear were present in the samples with *Schistosoma* eggs. The absence of any bands in the negative controls, produced with DNA from tissue without eggs, or with an amplification specific for *S. mansoni*, confirmed the specificity of the results (Fig. 1b). The specificity of the results was further confirmed by the Dra I restriction analysis, a PCR of negative tissues

Table 1
Histopatology of SCC[a] cases.

Cases	Keratinization[b]	Cytokeratin[d]	Ki-67[d]	Flogosis[c]	Ovoid formation (compatible nematoda eggs)[c]
1	3+	1+	1+	1+	3+
2	3+	1+	1+	3+	3+
3	1+	2+	2+	2+	2+
4	1+	3+	2+	3+	2+
5	3+	3+	2+	3+	2+
6	1+	1+	1+	2+	2+
7	2+	1+	1+	1+	3+

[a] SCC: squamous cell carcinoma.
[b] Keratinization: 3+: strong; 2+: middle; 1+: occasional.
[c] Flogosis and nematoda eggs quantity: 1+: rich; 2+: middle; 3+: occasional.
[d] Cytokeratin, Ki-67: 3+: strong; 2+: middle; 1+: occasional positivity.