Schistosomiasis and malignancy

L. B. LEMMER, P. J. FRIPP

Abstract **It is generally accepted that schistosomiasis, if not causative, is at least associated with malignancy. In this review, the epidemiology of schistosomiasis and bladder carcinoma, as well as the role of chronic bladder infection, are discussed together with known carcinogenic factors, possible abnormal vitamin metabolism and/or deficiencies and factors that influence conjugated carcinogens. Experimental evidence is briefly examined and recent work from the Far East on schistosomiasis and colon carcinoma reviewed.**

S Afr Med J 1994; **84:** 211-215.

It has been estimated that over 250 million people in the tropical and subtropical regions of the world suffer from schistosomiasis, also known as bilharziasis. The parasitic disease may cause morbidity that is protean in character, and have a mortality rate which is difficult, if not impossible, to calculate, especially in Third-World countries.

Of three major species of human schistosomes, *Schistosoma japonicum* is restricted to the Far East while *S. haematobium* is almost entirely restricted to the African continent and parts of Asia Minor and the Arabian peninsula. *S. mansoni* is found in Africa and tropical parts of the Western hemisphere.

Apart from the granulomatous reaction to the eggs, with its unique complications that may be seen in almost any organ, a causal relationship between parasitic infection and malignancy has been suggested. This association is more clearly defined in *S. haematobium* infection (the causative agent of urinary or bladder schistosomiasis) than in *S. mansoni* or *S. japonicum*, which generally inhabit the portal tract and involve the intestines and lungs.

Department of Anatomical Pathology, School of Pathology, South African Institute for Medical Research and University of the Witwatersrand, Johannesburg

L. B. LEMMER, M.B. CH.B., M.PRAX.MED., M.MED. (PATH.)

Department of Parasitology, Medical University of Southern Africa, Pretoria

P. J. FRIPP, C. BIOL., F.I. BIOL., SCI. NAT., PH.D.

Accepted 7 Jun 1993.

Bladder carcinoma

Historical background

Theodor Bilharz first found adult worms of the trematode, now known as *S. haematobium*, in the portal veins of a cadaver in Cairo in 1851.[1] As early as 1905, Goebel[2] noted the geographical similarity between endemic *S. haematobium* infection and the incidence of bladder carcinoma, later to be supported by Hashem[3] (1961), Gillman and Prates[4] (1962), Prates and Torres[5] (1965) and Brand[6] (1979), among others.

In 1911, Ferguson postulated a causal relationship between the parasite and bladder carcinoma.[7] Proof of this association has remained somewhat elusive, with proponents both for and against the argument; the latter perhaps are in the minority, especially in recent years when possible interactions between the parasite and urinary carcinogens have become known.

Schistosomal epidemiology and the incidence of bladder carcinoma

In populations not at risk for schistosomiasis, the peak incidence of bladder carcinoma occurs during the 6th decade of life; only 12% of cases occur in people under the age of 50 years.[8] In lower Egypt, a hyper-endemic region for *S. haematobium*, the mean age of patients with carcinoma of the bladder is 46 years,[9] however, with 73% of patients below the age of 50 years.[10]

The mean age of presentation with carcinoma of the bladder in those areas of Malawi endemic for *S. haematobium* is also lower (44,9 years) than in non-endemic areas;[11] similar patterns are seen in Zambia[12] where most of the patients were younger than 50 years.

Studies from Iraq based on the records of the Baghdad Tumour Registry for the years 1976 - 1982 confirmed bladder carcinoma as the second most common malignancy recorded. The phenomenon was more prominent in areas endemic for urinary schistosomiasis where an even higher incidence was reported. These areas border Kuwait, where bladder carcinoma is the third most common malignant tumour reported in men.[13] It has further been shown that carcinoma of the bladder is the commonest malignancy seen in Egypt with prevalence rates varying between 11% and 40%.[10,14,15]

Since they are more likely to enter infested water in the course of their work, rural Egyptian men are more often exposed to cercarial challenge than women and it is to be expected that the incidence of bladder tumours in men will therefore be higher than in women. This is indeed the case and a male/female ratio of 5:1 has been recorded.[16,17]

It has also been shown that the topographic distribution of carcinoma of the bladder associated with schistosomiasis clearly differs from the distribution of cryptogenic or aromatic amine-induced cancers seen in Western countries. In the latter group, most cancers occupy the most dependent trigonal region of the bladder, while in the schistosome-associated group, the trigone is rarely affected.[18-20]

A definite association also exists between the presence of eggs and carcinoma of the bladder, especially squamous carcinoma. Data from Egypt have shown that 82,49% of bladder tumours were associated with schistosomiasis,[21] while in Malawi 67,1% of bladder tumours contained schistosome eggs.[11] A massive 94% of bladder tumours diagnosed in Zambia were found to contain *S. haematobium* eggs. Of the latter tumour group, 72% were squamous carcinomas, 18% transitional carcinomas and 10% adenocarcinomas or undifferentiated carcinomas.[12] Figures from southern Iraq showed schistosome eggs to be present in 70% of squamous carcinomas, 17,1% of transitional carcinomas and 12,8% of adenocarcinomas and anaplastic tumours.[22] Reports from King Edward VIII Hospital in Durban indicated a 61% association between chronic urinary schistosomiasis and malignant bladder disease in black patients.[23]

It thus becomes evident that the ratio between transitional cell carcinoma and squamous carcinoma (together the largest percentage of bladder malignancies) clearly differs if one compares tumour incidence in areas endemic for bladder schistosomiasis with non-bilharzial regions. A published series from Egypt showed squamous carcinomas to be most common with ratios of 70:25:5 between squamous carcinoma, transitional cell carcinoma and adenocarcinoma.[18] This contrasted sharply with squamous carcinoma, transitional carcinoma and adenocarcinoma ratios of 5:94:1 respectively, reported in Western countries.[24,25] Similar changes in the ratios of bladder tumours were also reported from Malawi[11] and Zimbabwe[26] although squamous carcinomas seemed to occur less frequently in Iraq (53,8% in one series[13] and 48,8% in another[22]).

Obviously not all bladder malignancies in endemic schistosomiasis areas are associated with the parasite; indeed, in 17,6% of bladder cancers in Egypt no eggs of *S. haematobium* were found.[21] These cases, however, represented either patients without schistosomiasis from non-bilharzial areas, patients with mild schistosomal disease or in whom the disease had burnt itself out, or those patients who had been successfully treated and cured.[21] Of paramount importance is the fact that in the treated group, tumours presented at a later age; patient ages were thus comparable with those in industrialised countries, as opposed to the younger age (46 years) reported from Egypt.[9]

Although the incidence of bladder malignancies, notably squamous carcinoma, is thus much higher in areas endemic for schistosomiasis, subtle differences occur within these endemic areas. Fripp and Keen[27] reported a higher prevalence in the Shangaan tribes of Mozambique and eastern Transvaal, especially in women (the sex incidence approaching and exceeding that in men), when compared with different ethnic groups of the northern Transvaal. Egg load, naturally occurring carcinogens, dietary factors and cultural or behavioural patterns may possibly explain these differences, although the nature of the dietary factors remains elusive. One dietary factor that has been shown to be associated with a raised incidence of bladder cancer in Ugandans, is 3-hydroxyanthranilic acid. The source of this tryptophan derivative is the Matoke plantain, a staple food of some of the inhabitants. It should, however, be noted that this particular dietary factor is of importance in non-endemic *S. haematobium* areas.[28]

Dietary factors cannot, however, be dismissed and will be discussed later. Other factors also play a role in bladder cancer induction, and the urinary tract infections that almost always coexist with urinary schistosomiasis have to be considered.

The role of cystitis in schistosome-infected bladders

A report from Egypt stated that males between the ages of 10 and 25 years infected with *S. haematobium*, consistently had a load of 10^3 - 10^5 bacterial organisms per millilitre of urine.[29]

In another study, the incidence of bacteriuria in boys between 5 and 16 years of age in endemic regions of Egypt was found to be ten times greater than in boys from non-endemic areas.[30]

In Western populations, most bladder infections are monocultures of *Escherichia coli* (88%) or *Proteus* spp. (10%), the remainder divided between a range of organisms.[31] In Egypt,[32] *E. coli* was the most common cause of bacterial infection in bladders affected by schistosomiasis, although mixed cultures, in which anaerobes as well as *E. coli* occurred, often predominated.[31-33]

Bacterial infections in schistosomal bladders seldom, if ever, clear up totally and this constant presence of predominantly mixed infections may act synergistically on naturally occurring carcinogens in the urine.[31] A statistically significant higher incidence of bladder carcinoma in quadriplegics and paraplegics has been reported; these patients have chronic bladder infections due to stasis, often involving multiple organisms.[33] Stasis of urine as a result of the many complications of bladder schistosomiasis, as well as the fact that eggs, whether live, dead or calcified, will act as foreign bodies thereby promoting bacterial infections, have to be considered.

Carcinogens, metabolites, chronic bacterial infections and schistosomiasis

In 1983, Hicks[29] postulated a multistep process in the induction of schistosomal bladder cancers that involved genotoxic and non-genotoxic stages. Small amounts of nitrosamines, many of the derivatives that have been shown to be carcinogenic to a wide range of vertebrates, are formed endogenously by nitrosation of ingested or metabolically derived secondary and tertiary amines. There is therefore a significant and constant, albeit low, concentration of carcinogens present in human urine. Furthermore, urinary bacteria can produce large quantities of nitroso compounds over a wide urinary pH range since many bacterial species are able to reduce diet-derived nitrate to nitrite, followed by the formation of *N*-nitrosamines through nitrosation of amine precursors.[34] This will clearly augment the action of the already extant small quantities of naturally occurring free nitrosamines in the urine.

E. coli and *Proteus* spp. are good examples of bacteria able to nitrosate amine precursors, and high levels of urinary *N*-nitrosamines can therefore be present in urinary infections caused by these species. Although metabolic production of locally reactive compounds is not seen in monocultures of these organisms, this is unfortunately not true in mixed infections, especially where both aerobic and anaerobic strains coexist.[31]

Various *N*-nitroso compounds are known to be bladder carcinogens in rodents and dogs. These include *N*-nitrosomethylurea (MNU), *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine (BBN) and *N*-nitrosomethyl-dodecylamine (NMDCA).[35-37] In 1986, Higgy[38] showed convincingly that BBN which is formed during urinary bacterial infections, induces the development of carcinoma of the bladder in rats.

Data from the Qalyub area in Egypt, which is endemic for *S. haematobium*, showed that relatively healthy young men, who would be at risk for developing bladder carcinoma 20 - 30 years later, had elevated concentrations of *N*-nitroso substances in their urine. Figures of 13 - 32 µg/l in individuals without *S. haematobium* infections, 21 - 36 µg/l in those infected with *S. haematobium* and levels higher than 42 µg/l when more than 10^5 nitrate-reducing organisms per millilitre urine were present, were given.[29] The level of *N*-nitroso compounds therefore seems to reflect the presence and metabolic activity of nitrate-reducing organisms that commonly contribute to chronic bladder infections, which are known to occur with greater frequency in the presence of *S. haematobium*.

Also in Egypt, the urine of 2 379 individuals from a rural area were tested for the presence or absence of nitrites. Of this group of people, 5,6% infected with *S. haematobium* tested positive for nitrites, with a higher frequency (25%) seen in patients with active symptomatic schistosomiasis. Of those individuals with bladder carcinoma associated with schistosomiasis, 66,2% tested positive for nitrites. Of the total group whose urine tested positive for nitrites, 133 also had dysplastic changes of the bladder epithelium.[39]

Since many bacteria, especially *E. coli*, contain the enzyme nitrate reductase, it is not surprising that higher nitrite levels are present in severe bacterial infections.[32]

Bacterial nitrate reductase is, however, not the only enzyme involved in carcinogenesis of bladder tumours. Beta-glucuronidase, which splits glucuronide conjugates formed in the liver, plays an important role in activating naturally occurring or dietary carcinogens in the bladder. Beta-glucuronidase has many sources: it is present in blood leucocytes, occurs free in plasma and is filtered though the glomerulus. It is also present in the epithelial cells of both ureters and bladder.[40] Urothelial cells damaged by the passage of schistosome eggs and released into the lumen should therefore make a significant contribution to the high levels of the enzyme. This has definitely been shown, since the diurnal variation in urinary egg output parallels a similar variation in β-glucuronidase levels. The excretion pattern of the enzyme was lost following successful treatment of patients.[41] It has further been shown that bacteria, notably *E. coli*, are another source of the enzyme, and higher levels of β-glucuronidase activity have been detected in schistosomal bladders infected by this organism.[32] The pH of the urine may, however, reduce the activity of the bacterial enzyme.[40]

Levels of the carcinogen, 3-hydroxyanthranilic acid (3-OHAA), which is present in low quantities in normal urine, were found to be elevated in random urine specimens from donors living in rural subsistence economy areas of the Transvaal, where bladder cancer occurred with high frequency.[42] The exact origin of the dietary source in different areas of the Transvaal is still unknown, although 3-OHAA, as has already been discussed, plays a role in certain areas of Uganda.[28] Since a deficiency of vitamin B_6 (pyridoxine) is also associated with high excretion levels of 3-OHAA, the urines of the Transvaal donors were subjected to kynurenine analysis. The kynurenine levels were low, however, disproving a possible vitamin B_6 deficiency in this 'high-risk' group of patients. However, since levels of free 3-OHAA were much higher than bound 3-OHAA when compared with the urine of people living in areas where no bladder cancers had been reported, it was concluded that β-glucuronidase was responsible for the hydrolysis of the conjugate in this population.[42] The raised levels of the enzyme in response to the higher concentration of pre-carcinogenic substrate may therefore explain the differences in bladder cancer incidence as seen in different areas of the Transvaal where schistosomiasis of the bladder is endemic.

Patients suffering from *S. mansoni* infection, where not only the colon but segments of small bowel may also be affected, have higher levels of urinary kynurenine, indicating a vitamin B_6 deficiency that is probably a result of malabsorption.[43] Bladder cancer patients also have high urinary levels of kynurenine.[43] Some kynurenine metabolites are in themselves carcinogenic and it is therefore possible that a vitamin B_6 deficiency with consequent high secretory levels of kynurenine derivatives in the urine will augment the action of other carcinogenic factors already present in the bladder, but only if the latter are unconjugated.

Whether other vitamin deficiencies or the abnormal metabolism of certain vitamins play a role in carcinogenesis of the bladder is to a great extent still unclear. Fujimake[44] showed that albino rats fed a vitamin A-deficient diet developed carcinoma of the stomach. Vitamin A analogues, such as 13-cis-retinoic acid, inhibit the incidence and extent of pre- and neoplastic lesions of the urinary bladder in experimental animals,[45] and oral retinoids retard the growth of bladder papillomas and may accelerate the disappearance of these lesions.[46] Reports from Egypt have shown that patients with schistosomiasis of the bladder, as well as those with squamous carcinoma of the bladder associated with schistosomiasis, had reduced levels of vitamin A compared with normal controls.[47] As vitamin A is synthesised in the liver, and as the liver is often the target in schistosomiasis, even in mixed infections, the connection seems to be obvious. However, these patients also suffered from β-carotene deficiencies since many of them came from poor socio-economic backgrounds.

It therefore seems possible that vitamins A and B_6 could play protective roles in preventing carcinogenesis since patients with leukaemia, Hodgkin's disease and carcinoma of the pancreas have also been shown to have low levels of vitamin A.[48]

Abnormal tryptophan metabolism has long been suspected of playing at least a partial role in bladder carcinogenesis. Dyer and Morris[49] found that, when given supplements of tryptophan, rats on a diet containing 2-acetylaminofluorine excreted grossly increased amounts of xanthurenic acid, kynurenic acid and 3-hydroxykynurenine. Morris[50] furthermore observed that rats on a pyridoxine-deficient diet developed altered tryptophan metabolism, whereas mice exposed to 3-hydroxykynurenine showed a very high incidence of bladder tumours.[51]

Abnormal tryptophan metabolism was also observed in patients with schistosome-associated bladder cancer when challenged with 2 g of L-tryptophan; after this they showed a significant increase in the secretion of kynurenic acid, acetylkynurenine, kynurenine and 3-hydroxykynurenine. It would seem, however, that tryptophan metabolites require a local abnormality such as a foreign body in the bladder to function as promoter[52,53] and urinary schistosomiasis could be a highly likely candidate although, as already mentioned, kynurenine metabolites in themselves are carcinogenic.

Proline has a regulating effect on fibroblast proliferation, and occurs in high levels in schistosome eggs that are known to elicit a very strong fibroblastic reaction. Indeed, the eggs will induce collagen synthesis *in vitro*.[54] It has been postulated that a change in the stromal envi-

ronment of tumours is necessary before tumour infiltration can take place. Since the proline diffuses across the egg shell into the environment surrounding the eggs, changes in stromal integrity or altered interaction between stroma and epithelium could possibly promote tumour formation or infiltration. Parallels exist between schistosomiasis and asbestosis, since both conditions are known to elicit a very strong fibroblastic reaction and both are associated with malignant transformation of the epithelia in question.[55]

Experimental evidence

Hicks *et al.*[29,56] showed that the *N*-nitroso compound, BBN, when fed to baboons infected with *S. haematobium*, induced bladder tumour formation. Uninfected control baboons fed on BBN had not developed tumours more than 2 years after exposure, whereas baboons infected with *S. haematobium* but receiving no BBN, only developed the usual schistosomal polyps and hyperplastic lesions. It therefore seems highly likely that bladder schistosomiasis acts as a proliferative stimulus in the already altered epithelium of carcinogen-exposed bladders.

A possible causal relationship was also experimentally shown when Kuntz *et al.*[57] infected a Talapoin and a Capuchin monkey percutaneously with cercariae of an Iranian strain of *S. haematobium*. Papillary transitional cell carcinomas of the bladder were present in the Talapoin monkey when it died 21 weeks after exposure and similar tumours were observed in the Capuchin monkey, which died 56 weeks after infection. The infected bladders also contained areas of squamous metaplasia, cystitis glandularis and polypoid schistosomal cystitis. This in itself does not meet with the criteria for a controlled animal model experiment and further evidence is needed.

Colorectal carcinoma

Colonic tissue as well as small intestine may primarily be infected with both *S. mansoni* and *S. japonicum*. *S. mansoni* is found throughout many parts of Africa and mixed infections with *S. haematobium* are well known.

A raised incidence of colorectal cancer in association with schistosomiasis due to *S. mansoni* has not been detected in Africa, although regenerative polyps (pseudopolyps) and inflammatory polyps are common. However, a different situation seems to exist in mainland China where *S. japonicum* causes colorectal schistosomiasis.

Ming-Chai *et al.*[58] studied a series of 454 colectomy specimens with carcinoma, of which 289 cases (63,6%) were associated with *S. japonicum*. The non-schistosomal group of tumours showed, apart from the carcinoma, other expected 'Western' lesions usually associated with carcinoma of the bowel, such as adenomatous polyps and villous adenomas. The schistosome group of tumours consisted of a large number of *multicentric* carcinomas as well as an array of associated lesions including:

1. Transitional changes adjacent to tumour foci consisting of an increase in mucosal thickness, glandular hyperplasia with elongated irregular tubuli; frequently branching in character, immature cells with stratification in the upper two-thirds of the crypt and also nuclear atypia — all indicative of a definite shift in the proliferative cell zone.
2. Pseudopolyps with or without atypia.
3. Diminutive polyps, approximately 4 mm in diameter, with stratification of the epithelium and hyperplastic features.
4. Inverted polyps, in which isolated islands of hyperplastic epithelium, situated deep in the muscularis mucosae, were connected to the overlying mucosa.
5. Frank denudation of epithelial layers.

The changes seen in association with multicentric carcinoma and schistosomiasis occurred in patients with a history of colitic symptoms lasting at least 10 years. The parallel with ulcerative colitis is clear.

Later, it was pointed out that dysplastic lesions of the colonic mucosa, which are usually diffuse, may also occur in 'flat' epithelium and need not only be seen in the pseudopolyps, polyps and other lesions as described above.[59]

The mean age of presentation of patients with schistosome-associated colorectal cancer was earlier (36,9 years) than that of other colon carcinoma patients (46,7 years), which stresses the difference in the disease profiles.[60]

In a study on people older than 30 years, Xinru Yu *et al.*[61] correlated polyps of the colon associated with schistosome eggs with factors such as: (*i*) atypical hyperplasia; (*ii*) production of carcino-embryonic antigen (CEA); (*iii*) sialomucin production in crypts as an expression of atypical change;[62] and (*iv*) crypt length in the polyps indicative of hyperplastic change.

In order to accomplish this correlation, the 'egg polyps' were classified as being either of the: (*i*) *fibrous type* (FT), where the fibrous stroma comprised more than one-third of the entire lesion, with fibrous tissue seen mainly in the submucosa; (*ii*) *mixed type* (MT) where the fibrous stroma comprised less than one-third but more than one-sixth of the lesion; or (*iii*) *epithelial proliferative type* (ET) where the fibrous stroma comprised less than one-sixth of the entire lesion.

Crypt length was measured from the basement membrane at the bottom of the crypt up to the apex of the surface epithelium.

Schistosome eggs were seen in 320 of the 754 biopsies. Of the positive biopsy specimens, 272 were used in the study.

After the 272 'egg polyps' had been classified, 81 were found to be FT, 91 MT and 97 ET. Crypt lengths varied, with the longest measurements seen in the ET type. Sialomucin production was increased in the majority of the ET types and in some of the MT types, while atypical hyperplasia varied from 2,5% in the FT type to 40,4% in the MT type, and 64,9% in the ET type. CEA positivity was demonstrated only in the ET polyps, as the staining was weak in surface membranes of the epithelial cells.

As controls, transitional zones in the vicinity of known carcinomas as well as adenomatous lesions were examined. These showed similar features except that sialomucins were also detected in the cytoplasm of some cells in adenomatous polyps.

It was therefore concluded that histological classification of egg polyps may point to pre-neoplastic or precursor lesions, since the FT polyps were dominated by fibrous stroma covered by inactive, sometimes even atrophic epithelium, while on the other hand, ET polyps were covered with hyperplastic, atypical epithelium with immunohistochemical and other features of malignancy.

It should be noted that the rate of occurrence of schistosomal egg polyps in this group of patients was high (42,5%) and that the majority of lesions were of the ET type which, however, occurred more frequently in patients over the age of 60 years.

In South Africa, schistosome eggs from both *S. haematobium* and *S. mansoni* are sometimes seen in association with malignant lesions of the colon, and polypoid lesions with ova also occur. This obviously may only be a coincidental finding, but clarity on the matter will only be reached once studies similar to and comparable with those from China have been performed.

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