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# ORIGINAL ARTICLE

# EVOLUTION OF COLORECTAL CANCER IN SCHISTOSOMIASIS

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**Aim:** *To define the clinico-pathologic character of schistosomiasis mansoni associated colorectal cancer (S.CRC) and the possible carcinogenic relation of Schistosoma mansoni (S.M).*
**Methods:** *This study included 176 patients with colorectal cancer associated with S.M. Their clinical database and surgical pathology sheets were documented with the detection of S.M on stool analysis, serologic tests, pathologic associated lesions and tumor P53 protein expression using immuno-cytochemical assay.*
**Results:** *Sixty eight patients (40%) with S.CRC were below 40 years with male predominance (1.8 – 1), distal CRC predominance in 109 patients (62%), mucinous type in 58 patients (33%), higher grades II, III in 136 patients (79%), with significant angio-invasion in 50 patients (30%), lymph vessels invasion in 50 patients (35%) and perineural invasion in 17 patients (10%), associates with poor immune response in 8 patients (5%), preceded with schistosomal lesions especially in patients with schistosomal colitis ≥ 10 years, associates with TP53 in 114 cases (65%) and presented at advanced stages in 99 cases (56%) with only hepatic metastasis in 28 cases (90%).*
**Conclusion:** *S.CRC is a special clinical entity that has an aggressive pathologic pattern, bad biologic behavior and the SM is implicated in SCRC progression.*

**Keywords:** *Bilharzial associated colorectal cancer, parasitic colitis.*

## INTRODUCTION

Colorectal cancer (CRC) is the 3rd common malignancy in men and the second in women that kills 550.000 patients annually.(1) In Egypt, it represents 3% of all malignant tumors,(2) and in Dakahlia governorate, it comprises 50.84% of all gastrointestinal malignancies.(3)

Significantly, 250 million people are infected with schistosomiasis, mainly in developing countries, resulting in 800.000 deaths per year.(4) In Egypt, SM is severely endemic since the early pharaonic times (3200 BC) especially around the Nile valley as the Dakahlia province (agricultural area, 4.850.000 citizens).(5) The prevalence of schistosomiasis mansoni (SM) is 36.4% in Lower Egypt(6) with a higher infection rates in children and young adolescents specially males.(7)

The p53 tumor suppressor gene (TP53) is commonly mutated in human cancers.(8,9) It is involved late in chromosomal instability model of CRC carcinogenesis(10,11) with specific mutation spectrum in CRC (fixed Transitions at C-P-G di-nucleotides).(12) It is involved in the tumor progression not the initiation(13) and it is a marker of aggressive biological behavior.(14-16)

Many papers have published about CRC association with Schistosoma Japonicum.(17,18) But no one has studied the risk for S.M- CRC association.

The aim of this study is to define the epidemiologic, clinico-pathologic and molecular characters of S.M associated colorectal cancer (S.CRC).

## PATIENTS AND METHODS

625 patients with CRC were identified retrospectively over the period from 1990 – 1999 and prospectively during 1999 – 2002 through Mansoura colorectal surgery unit (CRSU).

Only, 176 S.M associated CRC patients were included (S.CRC). They were 113 males and 63 females with a median age 45.2 years.

Their clinical database and surgical pathology sheet were documented.

All patients in our study were subjected to colectomy surgery either radical or palliative resection. Colectomy specimens were examined grossly for the tumor site, shape, size and other associated lesions. Representative sections from the tumor, surgical cut edge and draining lymph nodes were fixed in 10% formalin for 24-48 hours and paraffin blocks were prepared

Detection of Bilharzial affection was based on stool analysis, serologic tests, ultrasound on the liver and spleen and histo-pathologic features in the rectal snips or operative specimens.

Also, detection of tumor infiltrative lymphocytes (TIL), according to Adams and Morris (1997),[19] was identified. It was considered positive when lymphocytic infiltration occurred in ≥ 50% / high power field (HPF) or involving ≥ 50% of the tumor adjacent tissue interface significant in 20 HPF.

Tumor (TP53) protein-expression detection was performed by using 3-step immuno-cytochemical assay (I.C.A) for formalin embedded, paraffin fixed tumor specimens. The primary antibody is the mouse IgG monoclonal antibody (MAb) (clone 1801 from Biogenex San Ramon, CA) that detects both the wild-type and mutant p53 proteins and works on formalin fixed tissues. While the secondary antibody is the biotylanted one (Vectastain Lite ABC Kit from Vector Laboratories, Burlingaine, CA) followed by staining of Antigen-Antibody complex using 3. 3 diaminobenzedine stain (DAB) and finally interpretation for staining involves:

a. Diffuse positive: ≥ 30% positive tumor cells,

b. Focal positive: < 30% positive tumor cells,

c. Negative: No stain

## RESULTS

In the period from 1990 – 2002, a combined retrospective and prospective study on 176 patients with S.CRC (Fig 1) was performed through Mansoura colorectal surgery unit (CRSU). It was found that about 40% of patients with S.CRC were below 40 years, median age 45.2 years, (range 20 – 80 years) and male to female ratio 1.8 – 1 Table 1.

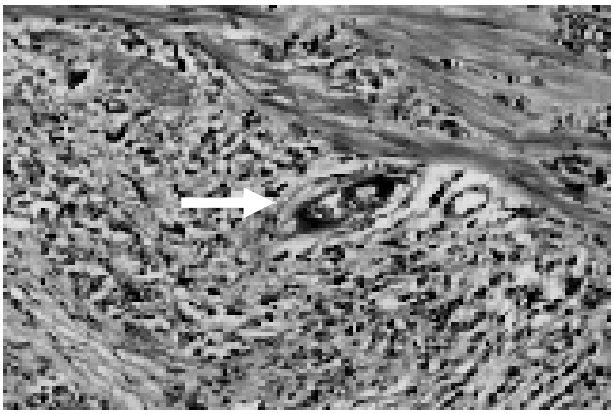

*Fig 1. Schistosomal associated (Bilharzial ova), high grade adenocarcinoma, The arrow demonstrates the bilharzial ovum within the tumor cells*
*Hx & Eosin staining (Mag. X 100)*

**Table 1. Age and sex of the studied group.**

| | No | % |
|---|---|---|
| **Age group** | | |
| ≤ 20 | 4 | 2% |
| > 20 - ≤ 40 | 64 | 38% |
| > 40 - ≤ 60 | 80 | 45% |
| > 60 | 28 | 15% |
| **Sex** | | |
| Male | 113 | 64.2% |
| Female | 63 | 35.8% |
| Median age: 45.2 years<br>Range: 20 – 80 years | | ≤ 40 years: 68 (40%)<br>> 40 years: 108 (60%) |

Table 2 showed rectosigmoid predilection in 109 patients (62%), mainly rectal in 67 patients (38%) and synchronous lesions in 5 patients (3%). The ulcerative form was present in 95 patients (54%) that far exceeded other macroscopic forms with linitis plastica in 10 patients (6%). The salient histologic type was the adenocarcinoma variant in 110 patients (62.5%) but the mucinous type, whether primary in 14 patients (8%) or secondary in 44 patients (25%), formed only 34%, and their cytologic grading defined as GI, II, III were 33%, 41% and 36% respectively. The early

metastatic invasion signs were prominent, as 50 patients (30%) had vascular emboli, 62 patients (35%) had lymph vessel invasion and 17 patients (10%) had perineural invasion associated with poor host immune response. Only 8 patients (5%) had significant tumor infiltrative lymphocytes. The Dukes' staging revealed advanced tumor staging in 99 patients (56%) as the nodal status was involved in 99 patients (56%) with superimposed distant metastasis in 31 patients (18%), mainly hepatic in 28 patients (90%) of them.

**Table 2. Patient pathologic data.**

| | No | % |
|---|---|---|
| **Tumor site** | | |
| Rectum | 67 | 38 |
| Sigmoid | 42 | 24 |
| Caecum | 9 | 5 |
| Others | 53 | 30 |
| Combined | 5 | 3 |
| **Macroscopic type** | | |
| Ulcerative | 95 | 54 |
| Cauliflower | 44 | 25 |
| Stenotic | 27 | 15 |
| Diffuse | 10 | 6 |
| **Histologic type** | | |
| Adenocarcinoma | 110 | 62.5 |
| Mucinous | 44 | 25 |
| Signet ring | 14 | 8 |
| Others | 8 | 4.5 |
| **Cytologic grade** | | |
| I | 40 | 33 |
| II | 73 | 41 |
| III | 63 | 36 |
| **Tumor microenvironment** | | |
| Vascular emboli | 50 | 30 |
| Lymph vessel invasion | 62 | 35 |
| Perineural invasion | 17 | 10 |
| **Positive host immune response tumor lymphocytic infiltration** | 8 | 5 |
| **Dukes staging** | | |
| A | 7 | 4 |
| B | 70 | 40 |
| C | 68 | 38 |
| D | 31 | 18 |
| **Metastasis** | | |
| Liver | 28 | 90 |
| Lung + Liver | 3 | 9 |
| Bone | 1 | 1 |

Patients were presented electively with bleeding in 105 patients (60%) and/or constipation in 109 patients (62%) but 17 patients (9.8%) had emergent forms [12 patients (7%) with obstruction and 5 patients (3%) with perforation]. The duration of symptoms before diagnosis was ≤ 6 months in 79 patients (45%) and > 2 years in 17 patients (10%).

The morphologic schistosomal pathologic changes associated with S.CRC were in the form of microscopic tubulo-villous adenoma in 15 patients (9%), hyperplastic lesions in 84 patients (48%), Cryptitis in 21 patients (11.93%), gland erosion in 56 patients (32%) with increased mucin in 70 patients (39.77%) and S.M eggs in 136 patients (77%) Table 3.

**Table 3. Schistosomal morphologic pathologic data in the studied groups and their duration.**

| | No | % |
|---|---|---|
| **Mucosal changes** | | |
| Cryptitis | 21 | 11.93 |
| Increased mucin | 70 | 39.77 |
| Gland erosion | 56 | 32 |
| Hyperplastic lesions | 84 | 48 |
| **Cryptitis with decreased mucin** | 22 | 13 |
| **Microscopic tubulo-villous adenoma with hyperplasia** | 15 | 9 |
| **Bilharzial ova** | 136 | 77 |
| **Duration of symptoms** | | |
| ≤ 10 years colitis | 49 | 28 |
| > 10 years colitis | 127 | 72 |

The TP53 protein expression was detected in 114 patients (65%) of S.CRC Table 4, (Fig 2, 3).

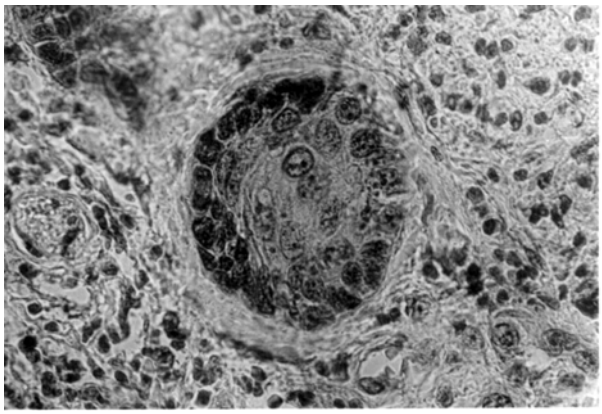

*Fig 2. TP53 positive, schistosomal colorectal cancer. DAB stain (X 400)*

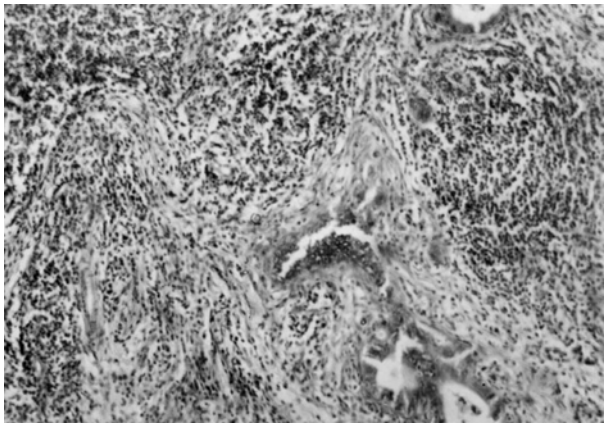

*Fig 3. TP53 negative, schistosomal colorectal cancer. DAB stain (X 400)*

**Table 4. Tumor (TP53) protein expression.**

| | No | % |
|---|---|---|
| **TP53** | | |
| +ve | 114 | 65% |
| -ve | 62 | 35% |

## DISCUSSION

Peculiar to CRC prevalence in developing countries, in this study, about 40% of patients with S.CRC were below the age of 40 years as reported by Abou-Zeid et al. (2002) & Mansour et al. (2002).[2,20] . And since 90% of CRC are environmental and 10% are genetic,[21,22,23] so, the likely factors are schistosomal exposure as 21% of young children have schistosoma mansoni,[7] westernization of Egyptian diet (likely to affect the young group)[24,25] or caused by uncommon gene mutation that has weak penetration and expression i.e. inherited predisposition without family clustering as Soliman et al. (1998)[26] found reduced expression of mismatch repair genes in Egyptian CRC.

Characteristic to SCRC, this study has revealed male predominance in line with Hayne et al., 2001[1] although McDermott (1999)[27] found equal sex distribution. That difference in our agricultural area is not hormonal dependent[28,29] but speculative to more prevalence of schistosomal infection in males suffering more exposure.

Coincide with schistosoma mansoni distribution (venous radicles), the SCRC subsite distribution revealed distal CRC predominance.[2,30,31] Since the tumor site may be related to specific risky factors affecting that anatomical segment of colorectum.[32] So, S.M could be a specific risky factor in CRC carcinogenesis.

Pathologically, as all ulcerative lesions were preceded by cauliflower variant[33] and the ulcerative variant comprised the majority in this study, while microscopically, the mucinous variant in SCRC comprised one third of cases. This is favorable with the national reports of Abou-Zeid et al. (2002),[2] but exceeded both the local ratios reported by Khafagy et al. (2000)[34] (21.5%) and the internationally reported ratio (15%).[35] Furthermore, in the adenocarcinoma variant, the higher grades formed the majority despite Morson and Dawson (1990)[36] found the opposite. These salient features reflect the aggressive tumor pattern.

Early metastatic invasive signs (vascular emboli VE, lymph vessel invasion LI and perineural invasion PI collectively VELIPI) were prominent in this study (16.8%, 16.2%, 10% respectively) in contrast to Pages et al. (2005)[37] modest ratios. That is a bad tumor microenvironment associated with poor disease free and over all survival.

Critically, the host immune response, as reflected by the tumor infiltrative lymphocytes, proved to be infrequent in contrast to Kapoor et al. (2005),[38] that might be related to CRC and S mansoni association that induced T helper2 polarized immune response.[39] That immune incompetence produces immune-editing (select tumor cells that resist immune surveillance). SO, S mansoni (poor host immune response) plays a critical role in CRC persistence, progression and decreased challenge for both clearance and curitherapy.

Conventional SCRC staging found a significant proportion with advanced Dukes as published before,[40] (more than 50% had positive lymph nodes and fourth the cases had distant metastasis). That advanced disease predominance is likely to be due to aggressive pathologic and bad biologic tumor behavior (tumor microenvironment-host immune response), rectal predominance, lack of primary health services or all.

Constantly, the SCRC secondaries were hepatic which might be owed to the local down regulated intra-hepatic immune response caused by S mansoni ovi-deposition.[41-43]

Although Mokhtar (1998), and Soliman et al. (2001)[44,45] defined CRC as de novo carcinogenesis, we found dysplastic changes at the edge of SCRC especially ≥ 10 years colitis supporting the adenoma carcinoma sequence. Whether the malignant growth overruns the dysplastic changes or just parallism, it is to be studied on epidemiologic, immune cytochemical (ras-oncogene) or histochemical (mucin), ultra-structural features and flow cytometry.

Mostly related to sampling variability, only 10% of SCRC presented with abdominal emergency despite Abou-Zeid (2002); Ayuub (2002)[2,46] found it about 20%. But it is logic

to S mansoni symptomatology and its endemicity as it was also reported by others.[6] A significant patient delay > 1 year in 30% of patients with SCRC was related to patient factors (Negligence – ignorance), primary health services and hospital management inadequacies.

On molecular basis, this study found a close association between TP53 mutation and S.CRC (65%).[47] This is going with other reports for S.Japonicum CRC (59.1%)[48] and from the same Egyptian endemic area (60%)[14] Compared with (47.2% of non S.CRC) western reports.[49] That close association supports the notion of S.CRC bad biological behavior related to TP53 mutation. Furthermore, Zhang et al. (1988)[47] suggested that the clastogen of TP53 is S.Japonicum derived and since others[48] found cross reactivity and antigenic community among different schistosomiasis species, so, SM derived molecules inactivate P53 resulting in S.CRC progression. Hence, SM is implicated indirectly in CRC progression. Yet Osada et al. (2005)[50] denied worm and egg extracts mutagenicity.

Hence, CRC is a special clinical entity with young age predilection, male predominance, distal colorectum prevalence and presents at advanced stage.

Pathologically SCRC is preceded by morphologic dysplastic changes, aggressive both pathologic variants and tumor micro-environment, and elicits poor host immune response.

Molecular basis revealed TP53 protein mutation expression.

In support of S mansoni carcinogenicity; epidemiologically (young age predilection, male and distal CRC predominance), pathologically (dysplastic morphologic precursors) and on molecular basis, (TP53 protein expression).

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