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Presence of schistosome eggs in lymph node predict unfavorable prognosis in schistosomal colorectal cancer

Weiyu Pan*, Jiaojiao Guo*, Jiali Li, Jieakesu Su, Xiaolei Zhang, Jia Liu, Chen Xu and Yingyong Hou

Objective: **The purpose of this study was to investigate the prognostic significance of schistosome eggs' location in schistosomal colorectal cancer (SCRC).**

Methods: **172 cases of SCRC were retrospectively analyzed. Patient clinicopathological parameters and survival rates were analyzed.**

Results: **There were 102 males and 70 females, the median age was 71 years (range, 44-91). All patients were followed, and the median time was 50.1 months (range, 1.0-79.7). There were 87 patients with PS1 (presence site 1, eggs deposited in the mucosa) and 85 patients with PS2 (presence site 2, eggs deposited in the muscularis propria or throughout the full thickness of the intestinal wall), 159 patients presented with eggs in cutting edge and 83 patients presented with eggs in lymph node (LN). Hepatic schistosomiasis was found in 27.3% of patients by imaging modalities and correlated to patients with PS2 ($P < 0.001$) and LNs' eggs ($P < 0.001$). Survival analyses showed that in stage III SCRC, eggs' presence in LN associated with worse DFS ($P = 0.004$) or marginally worse OS ($P = 0.056$), patients with PS2 had shorter OS ($P = 0.044$). Multivariate analyses revealed hepatic schistosomiasis was an independent prognostic factor for DFS and OS in stage III SCRC ($P = 0.001$, 0.002, respectively). In adjusted multivariate analysis, eggs' presence in LN was an independent prognostic factor for DFS in stage III SCRC ($P = 0.006$).**

Conclusions: **In stage III SCRC, eggs' presence in LN could predict poor prognosis and hepatic schistosomiasis was an independently unfavorable prognosis factor.**

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Keywords: colorectal cancer, prognosis, ***Schistosoma japonicum*****, schistosomiasis**

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Introduction

Colorectal cancer (CRC) remains the leading cause of cancer-related mortality worldwide despite advancements in tumor screening, early diagnosis, and curative resection (Siegel *et al*., 2020). There are many risk factors for CRC that have been identified, such as family history, unhealthy diet, fat, and excessive alcohol consumption (Siegel *et al*., 2020). In areas endemic to *Schistosoma japonicum*, such as China, intestinal schistosomiasis is even considered a risk factor for CRC (Dimmette *et al*., 1956; Liu *et al*., 2013; Chen, 2014).

Studies have shown that it is schistosome eggs, not the adult worms, induce the morbidity caused by schistosomiasis (Olveda *et al*., 2014). Adult *S. japonicum* inhabit the veins of its mammal host and lay eggs (Ross *et al*., 2002). The majority of eggs will be trapped in the liver and intestine (Chuah *et al*., 2014). The pathology of intestinal schistosomiasis is due to the eggs deposition and the continuous antigenic stimulation, which eventually induces chronic inflammation and polyp formation (Elbaz and Esmat, 2013). Schistosome eggs-related proteins has be evidenced that may promote CRC progression (Wu *et al*., 2020). Wang *et al.* (2016) found that the depositional site of eggs was significantly correlated with OS in 74 patients with schistosomal CRC (SCRC). The deposited eggs in lymph nodes (LNs) of intestinal schistosomiasis have been reported in previous case reports (Li *et al*., 2006; Wong *et al*., 2008). However, their pathological significance remains unclear. The prognostic significance of schistosome eggs warrants investigation in larger cohorts.

Here, we retrospectively analyzed the deepest depositional site of schistosome eggs in the intestinal wall, the presence of eggs in cutting edges, and in LNs to explore the relationship between schistosome eggs and prognosis.

Methods

Patients' data

All patients had undergone curative surgery at Zhongshan Hospital, affiliated to Fudan University from January

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2016 to December 2018. Clinicopathological data, including age, gender, tumor size, tumor location, differentiation, and lymphovascular or perineural invasion, were obtained from patients' medical and pathological records. Tumors were restaged according to the 8th edition of the American Joint Committee on Cancer (AJCC) staging system. Prior written informed consent was obtained from all patients, and the study was approved by the Ethics Committee of Zhongshan Hospital and complied with the ethical standards of the World Medical Association Declaration of Helsinki.

Inclusion and exclusion criteria

Inclusion criteria included the following: (1) patients underwent primary resection; (2) none of them received preoperative chemotherapy or radiation therapy; and (3) patients were diagnosed with SCRC by postoperative pathology findings. Exclusion criteria included the following: (1) Tis tumor; (2) patients who lacked complete information; (3) patients with distant metastases; (4) patients with synchronous malignancy; and (5) patients with family cancer syndromes. Finally, a total of 172 consecutive SCRC patients were included in the study.

Detection of schistosome eggs and assessment of hepatic schistosomiasis

Two pathologists who were blinded to clinical data observed schistosome eggs in original hematoxylin and eosin stained sections (usually 10–12 sections) of all patients under a light microscope, recording the deepest depositional site of eggs in the intestinal wall, the presence of eggs in cutting edges, and in LNs.

All patients underwent abdominal imaging modalities before surgery, such as ultrasound, computed tomography (CT), and MRI. Diagnostic criteria of hepatic schistosomiasis refer to previous studies (Ross *et al*., 2002; Khanna and Sarin, 2014).

Follow-up

Follow-up principles referred to the Chinese guidelines for CRC. Patients were asked to come to the hospital for a follow-up clinic and the following examinations were done: physical examinations; serum carcinoembryonic antigen level; abdominal ultrasound every 3 months for 2 years, then every 6 months for 5 years, then every year after 5 years; chest/abdominal/pelvic CT scan every 6 months for 2 years, then every year after 2 years; and colonoscopy at 6 months after primary tumor resection, then every year for 5 years, then every 2 years after 5 years. Patients who did not report on time were followed up by trained study interviewers through telephone.

Recurrence included local recurrence and distant metastasis. Disease-free survival (DFS) was defined as the time from initial surgery to the first recurrence, metastasis, death (for any reason), or the last follow-up (12 December 2022). The overall survival (OS) was defined as the time between surgery and cancer-related death. During follow-up, 35 patients suffered from recurrence (local recurrence or distant metastases) and 32 patients died, including 15 deaths without evidence of recurrence.

Statistical analysis

The association between schistosome eggs and clinicopathologic parameters was evaluated by the Chi square and Fisher's exact tests. The Kaplan–Meier curves with log-rank tests were used to determine the prognostic significance for DFS and OS. The univariate and multivariate Cox proportional hazard regression analyses were used to identify the independent prognostic factors.

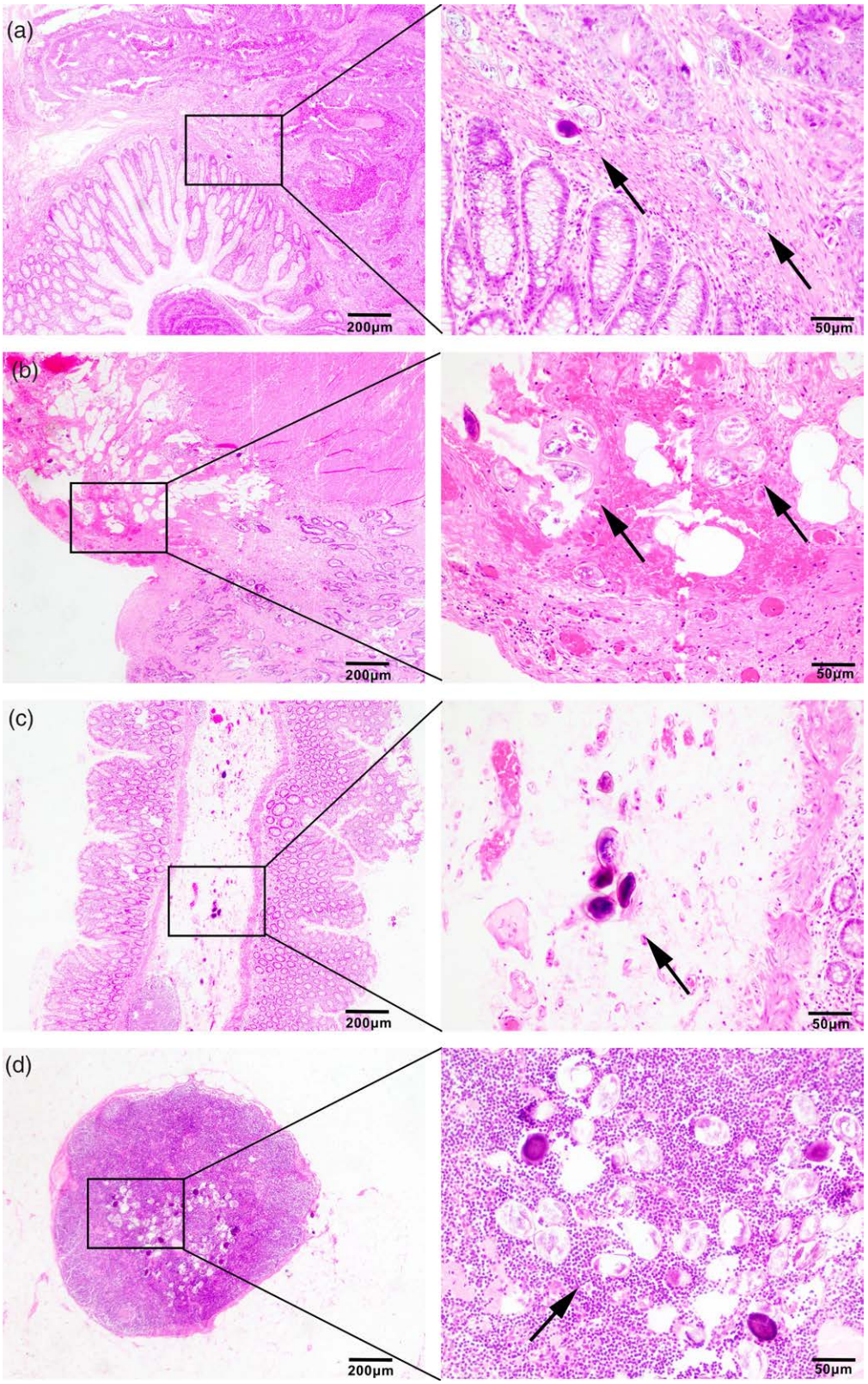
All statistical analyses were carried out using SPSS version 20.0 and GraphPad Prism 7.0. The *P*-value of less than 0.05 was considered to be statistically significant.

Table 1 Characteristics of 172 patients with schistosomal colorectal cancer

Characteristics	All patients ($n = 172$)	
	No.	%
Age		
<60	12	7.0
≥60	160	93.0
Gender		
Male	102	59.3
Female	70	40.7
Tumor size		
<5 cm	101	58.7
≥5 cm	71	41.3
Tumor location		
Rectum	70	40.7
Left colon	61	35.5
Right colon	41	23.8
Histology subtypes		
Adenocarcinoma	118	68.6
Mucinous/SRCC	54	31.4
Histology grade		
Well/moderate	140	81.4
Poor	32	18.6
Lymphovascular invasion		
Absent	118	68.6
Present	54	31.4
Perineural invasion		
Absent	110	64.0
Present	62	36.0
pT stage		
T1	0	0.0
T2	35	20.3
T3	109	63.4
T4	28	16.3
pN stage		
N0	103	59.9
N1	49	28.5
N2	20	11.6
pTNM stage		
Stage I	25	14.5
Stage II	85	49.4
Stage III	62	36.0
Hepatic schistosomiasis		
Absent	125	72.7
Present	47	27.3

pN stage, pathological N stage; pT stage, pathological T stage; pTNM stage, pathological TNM stage.

Fig. 1



(a) Schistosome eggs (arrow) were deposited in mucosa. (b) Eggs (arrow) are deposited in subserosa. (c) Eggs (arrow) were found in cutting edges. (d) Eggs (arrow) are found in LN. LN, lymph node.

Table 2 Association of schistosome eggs with clinicopathologic characteristics

	Presence site of schistosome eggs			Schistosome eggs in cutting edge			Schistosoma eggs in LN		
	PS1, n (%)	PS2, n (%)	*P*-value	Neg, n (%)	Pos, n (%)	*P*-value	Neg, n (%)	Pos, n (%)	*P*-value
Gender			0.421			0.029			0.945
Male	49 (56.3)	53 (62.4)		4 (30.8)	98 (61.6)		53 (59.6)	49 (59.0)	
Female	38 (43.7)	32 (37.6)		9 (69.2)	61 (38.4)		36 (40.4)	34 (41.0)	
Tumor location			0.063			<0.001			0.089
Rectum	30 (34.5)	40 (47.1)		3 (23.1)	67 (42.1)		31 (34.8)	39 (47.0)	
Left colon	30 (34.5)	31 (36.5)		0 (0.0)	61 (38.4)		31 (34.8)	30 (36.1)	
Right colon	27 (31.0)	14 (16.5)		10 (76.9)	31 (19.5)		27 (30.3)	14 (16.9)	
Perineural invasion			0.043			0.535			0.327
Absent	62 (71.3)	48 (56.5)		8 (7.3)	102 (92.7)		60 (67.4)	50 (60.2)	
Present	25 (28.7)	37 (43.5)		5 (8.1)	57 (91.9)		29 (32.6)	33 (39.8)	
Hepatic schistosomiasis			<0.001			0.257			<0.001
Absent	75 (86.2)	50 (58.8)		11 (84.6)	114 (71.7)		77 (86.5)	48 (57.8)	
Present	12 (13.8)	35 (41.2)		2 (15.4)	45 (28.3)		12 (13.5)	35 (42.2)	

LN, lymph node; PS1, presence site 1, eggs deposited in the mucosa; PS2, presence site 2, eggs deposited in the muscularis propria or throughout the full thickness of the intestinal wall.

Results

Patient characteristics

The clinicopathological characteristics of the study cohort were summarized in Table 1. In brief, the median age at diagnosis was 71 years (range, 44–91). The patients comprised 59.3% male and 40.7% female. A total of 41.3% of tumors were larger than 5 cm in maximum diameter. By anatomic site, 40.7% of tumors were in the rectum, 35.5% in the left colon, and 23.8% in the right colon. The majority of tumors were adenocarcinomas (68.6%), with well or moderate differentiation (81.4%). 31.4% and 36.0% of tumors presented with lymphovascular invasion and perineural invasion, respectively. According to the AJCC staging system (8th edition), 14.5% of patients had stage I disease, 49.4% had stage II disease, and 36.0% had stage III disease. Hepatic schistosomiasis was found in 27.3% of patients.

The correlation of schistosome eggs with clinicopathological characteristics

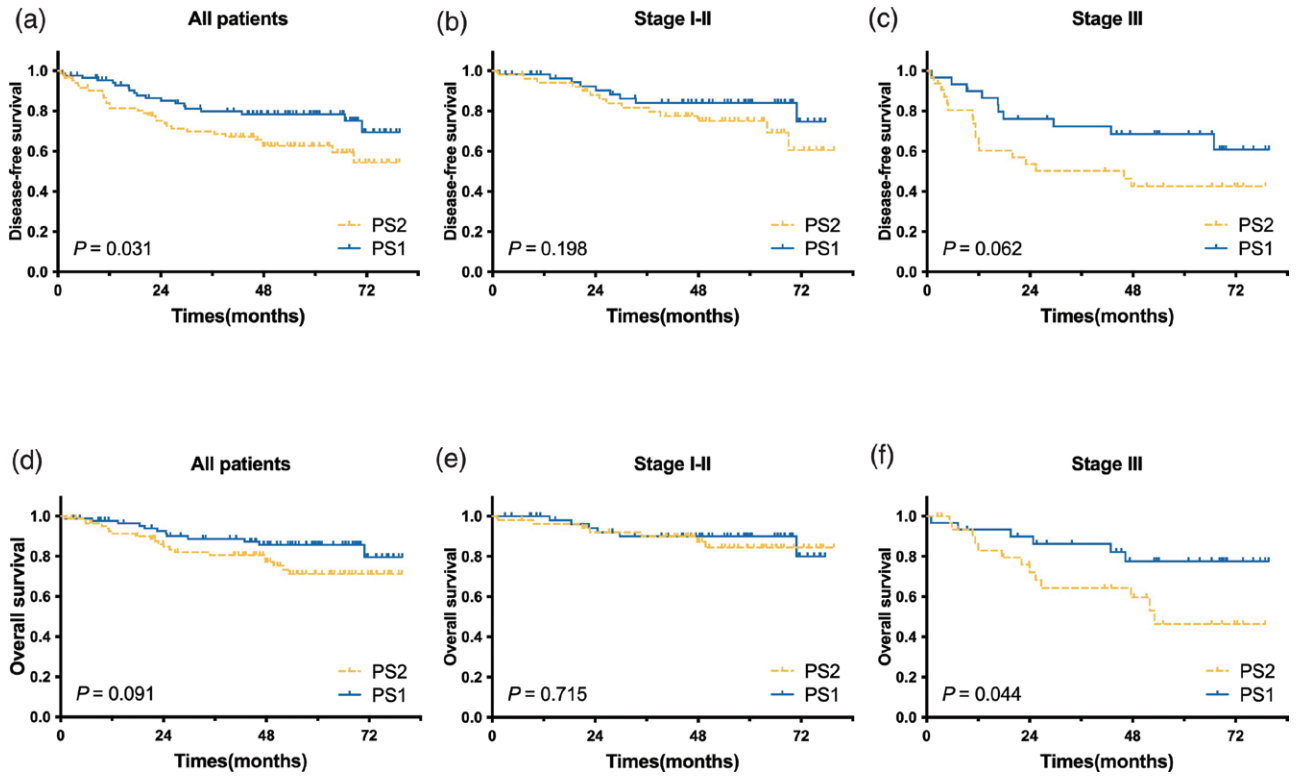
According to the deepest depositional site of schistosome eggs in the intestinal wall, we divided patients into PS1 group (presence site 1, eggs deposited in the mucosa, Fig. 1a) and PS2 group (presence site 2, eggs deposited in the muscularis propria or throughout the full thickness of the intestinal wall, Fig. 1b). It was regarded as positive when eggs were present in the distal or proximal cutting edge (Fig. 1c) and in one or more LNs (Fig. 1d). In this study, there were 87 patients in PS1 group and 85 patients in PS2 group; 159 patients had positive cutting edge and 83 patients had positive LN by pathological examination.

As shown in Table 2, PS2 group was significantly associated with perineural invasion ($P = 0.043$). Patients with PS2 were more likely to combine hepatic schistosomiasis than patients with PS1 ($P < 0.001$). The correlation analysis between positive cutting edge and clinicopathologic characteristics showed that the majority of male patients had positive cutting edge compared with female patients ($P = 0.029$). Patients with positive LN were more likely to combine hepatic schistosomiasis than those with negative LN ($P < 0.001$).

Survival analyses based on the deepest depositional site of schistosome eggs in the intestinal wall

The median follow-up was 50.1 months (range, 1.0–79.7). The Kaplan–Meier survival analysis showed that DFS was significantly different between PS1 group and PS2 group ($P = 0.031$, Fig. 2a). Further analysis based on the clinical stage found that patients with PS2 tended to show worse DFS in stage III, but did not achieve statistical significance ($P = 0.062$, Fig. 2c). OS was not significantly different between PS1 group and PS2 group ($P = 0.091$, Fig. 2d). Further analysis based on the clinical stage found that patients with PS2 had significantly worse OS in stage III ($P = 0.044$, Fig. 2f).

Fig. 2



Prognostic significance of the presence site of eggs in SCRC patients. (a) DFS of all patients. (b) DFS of patients with stage I–II SCRC. (c) DFS of patients with stage III SCRC. (d) OS of all patients. (e) OS of patients with stage I–II SCRC. (f) OS of patients with stage III SCRC. DFS, disease-free survival; OS, overall survival; SCRC, schistosomal colorectal cancer.

Survival analyses based on the presence status of schistosome eggs in cutting edge

The Kaplan–Meier survival analysis showed that DFS of patients with positive cutting edge were not significantly different from that of patients with negative cutting edge ($P = 0.492$, Fig. 3a). Further analysis based on the clinical stage found no significant difference in DFS between the two groups (Fig. 3b and c). There was also no correlation between positive cutting edge and OS (Fig. 3d–f).

Survival analyses based on the presence status of schistosome eggs in lymph node

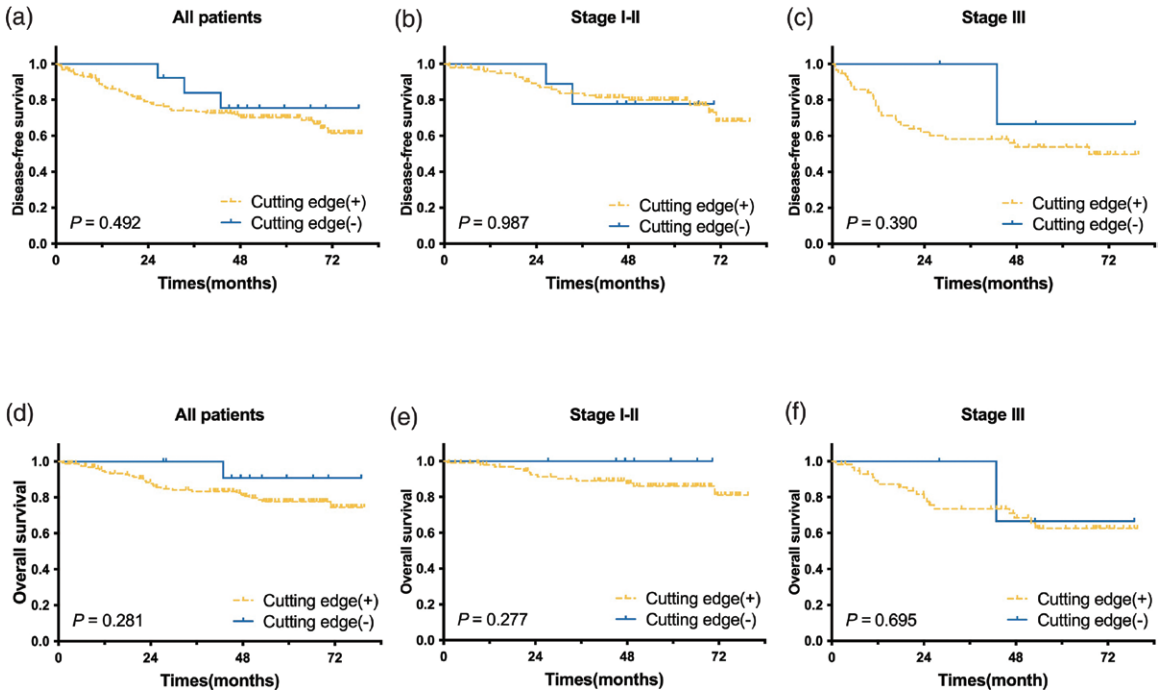
The Kaplan–Meier survival analysis showed that DFS was not significantly associated with the presence of eggs in LN ($P = 0.214$, Fig. 4a). But further analysis based on the clinical stage found that patients with positive LN had worse DFS in stage III ($P = 0.004$, Fig. 4c). Patients with positive LN also tended to show worse OS in stage III, while having only marginally significance ($P = 0.056$, Fig. 4f).

Univariate and multivariate analyses

Univariate and multivariate analyses of DFS and OS were performed using prognostic factors, such as known clinicopathologic factors, presence site of eggs in the intestinal wall, presence status of eggs in cutting edge, as well as in LN. As shown in Table 3, univariate analysis revealed that the conventional clinicopathologic factors, including lymphovascular invasion ($P = 0.007$), perineural invasion ($P = 0.001$), and presence site of eggs ($P = 0.034$), were significant prognostic factors for DFS. In multivariate analysis, only perineural invasion was independently prognostic for DFS ($P = 0.001$). In stage III SCRC patients, perineural invasion ($P = 0.023$) and hepatic schistosomiasis ($P = 0.001$) were significantly independent prognostic factors for DFS in multivariate analysis, while in stage I–II, no independent prognostic factor was identified. Considering that hepatic schistosomiasis was closely related to schistosome eggs (Table 2), adjusted multivariate analysis was performed after excluding the variable of hepatic schistosomiasis. The presence of eggs in LN emerged as an independent poor predictor for DFS in stage III SCRC ($P = 0.006$).

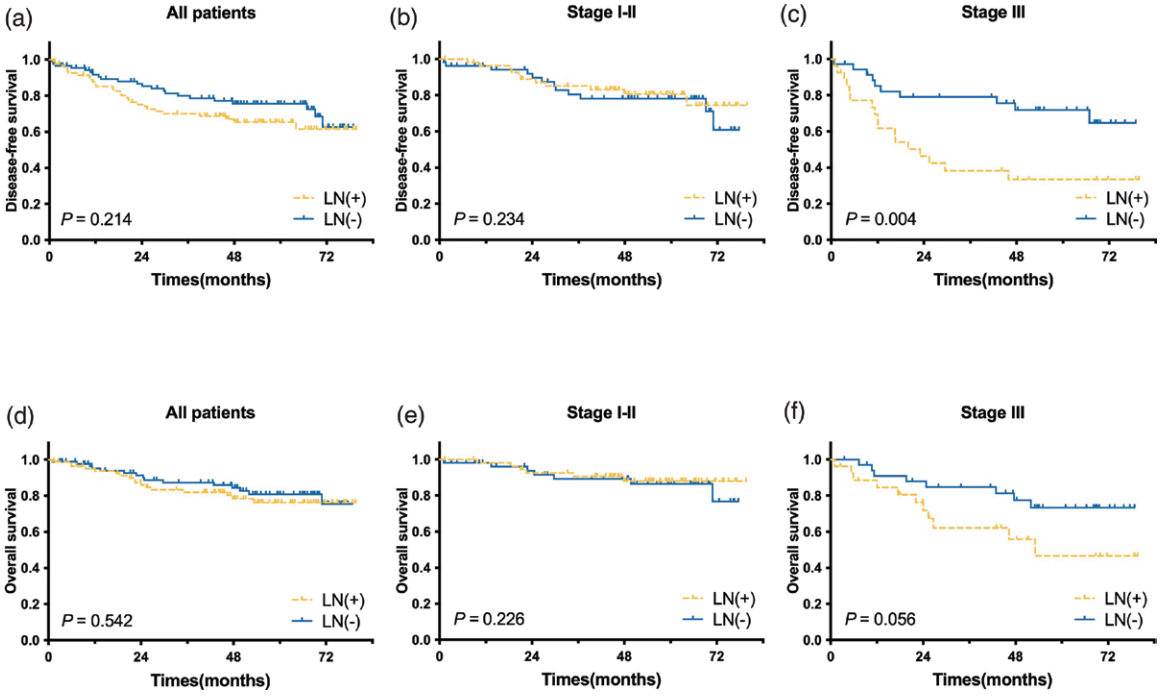
For OS (Table 4), the following factors were regarded as significant predictive by univariate analysis: histology grade ($P = 0.018$), lymphovascular invasion ($P = 0.039$), perineural invasion ($P = 0.003$). In multivariate analysis, histology grade ($P = 0.027$) and perineural invasion ($P = 0.005$) were independently prognostic for OS. In stage III SCRC patients, perineural invasion ($P = 0.043$)

Fig. 3



Prognostic significance of the presence of eggs in cutting edge in SCRC patients. (a) DFS of all patients. (b) DFS of patients with stage I–II SCRC. (c) DFS of patients with stage III SCRC. (d) OS of all patients. (e) OS of patients with stage I–II SCRC. (f) OS of patients with stage III SCRC. DFS, disease-free survival; OS, overall survival; SCRC, schistosomal colorectal cancer.

Fig. 4



Prognostic significance of the presence of eggs in LN in SCRC patients. (a) DFS of all patients. (b) DFS of patients with stage I–II SCRC. (c) DFS of patients with stage III SCRC. (d) OS of all patients. (e) OS of patients with stage I–II SCRC. (f) OS of patients with stage III SCRC. DFS, disease-free survival; LN, lymph node; OS, overall survival; SCRC, schistosomal colorectal cancer.

Table 3 Univariate and multivariate Cox proportional hazard regression analysis for disease-free survival among patients with schistosomal colorectal cancer

Variable	All patients		Patients with stage I–II disease		Patients with stage III disease	
	P-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)
Univariate analysis						
Age (< 60 years)	0.438	1.750 (0.425–7.202)	0.650	1.591 (0.214–11.811)	0.549	1.842 (0.250–13.578)
Gender (male/female)	0.059	1.714 (0.980–2.998)	0.232	1.657 (0.724–3.791)	0.168	1.710 (0.798–3.663)
Tumor size (5 cm)	0.376	0.770 (0.432–1.372)	0.153	0.523 (0.215–1.273)	0.685	1.173 (0.544–2.527)
Tumor location						
Rectum	Reference		Reference		Reference	
Left colon	0.733	0.893 (0.466–1.712)	0.860	0.915 (0.341–2.458)	0.805	0.896 (0.377–2.132)
Right colon	0.686	1.154 (0.577–2.305)	0.548	1.354 (0.504–3.638)	0.830	1.113 (0.417–2.968)
Histology subtypes	0.855	1.057 (0.583–1.916)	0.507	1.328 (0.574–3.071)	0.969	0.983 (0.415–2.327)
Histology grade	0.119	1.676 (0.875–3.209)	0.180	1.975 (0.730–5.342)	0.648	1.222 (0.517–2.891)
Lymphovascular invasion	0.007	2.167 (1.238–3.795)	0.108	2.083 (0.851–5.100)	0.233	1.588 (0.743–3.395)
Perineural invasion	0.001	2.693 (1.537–4.719)	0.074	2.155 (0.927–5.011)	0.037	2.350 (1.051–5.255)
pT stage						
T2	Reference		Reference		Reference	
T3	0.061	2.444 (0.959–6.230)	0.200	2.223 (0.655–7.549)	0.238	2.412 (0.559–10.401)
T4	0.102	2.491 (0.834–7.439)	0.966	0.962 (0.160–5.769)	0.081	4.059 (0.842–19.579)
pN stage						
N0	Reference		Reference		-	
N1	0.010	2.209 (1.205–4.051)	0.625	0.607 (0.082–4.506)	Reference	
N2	0.058	2.189 (0.974–4.919)	-		0.714	0.857 (0.375–1.959)
pTNM stage						
Stage I	Reference		-		-	
Stage II	0.279	1.955 (0.581–6.580)	-		-	
Stage III	0.015	4.377 (1.327–14.437)	-		-	
Hepatic schistosomiasis	0.338	1.345 (0.733–2.467)	0.410	0.659 (0.244–1.777)	0.002	3.630 (1.625–8.108)
Presence site of schistosome eggs	0.034	1.857 (1.048–3.289)	0.204	1.723 (0.745–3.985)	0.067	2.079 (0.950–4.548)
Schistosome eggs in cutting edge	0.495	1.502 (0.467–4.830)	0.987	0.988 (0.231–4.234)	0.403	2.344 (0.318–17.288)
Schistosome eggs in LN	0.216	1.423 (0.814–2.490)	0.629	0.817 (0.360–1.855)	0.006	2.993 (1.364–6.568)
Multivariate analysis						
Perineural invasion	0.001	2.693 (1.537–4.719)	-		0.023	2.554 (1.135–5.747)
Hepatic schistosomiasis	-		-		0.001	3.948 (1.748–8.917)
Multivariate analysisa						
Perineural invasion	0.001	2.693 (1.537–4.719)	-		0.037	2.356 (1.052–5.278)
Schistosome eggs in LN	-		-		0.006	3.000 (1.365–6.592)

CI, confidence interval; HR, hazard ratio; LN, lymph node.
[a]Multivariate analysis, after adjustment for hepatic schistosomiasis-variable.

and hepatic schistosomiasis ($P = 0.002$) were significantly independent prognostic factors for OS in multivariate analysis, while in stage I–II, no independent prognostic factor was identified. After adjustment for hepatic schistosomiasis-variable, no independent prognostic factor was identified in stage I–II or stage III SCRC. Owing to most stage I–II SCRC patients had positive cutting edge (101 out of 110) and most stage III SCRC patients' age were over 60 years old (58 out of 62), presence status of eggs in cutting edge and age were not included in univariate analysis.

Discussion

Three main species of schistosomiasis infect humans, *S. mansoni*, *S. haematobium*, and *S. japonicum*. *S. haematobium* has been classified as a definite carcinogen to humans (Group I carcinogen), and *S. japonicum* is classified as a probable human carcinogen (Group 2B carcinogen) (Vennervald and Polman, 2009). *S. japonicum* is the main human pathogenic specie causing intestinal schistosomiasis, settle in the mesenteric circulation of the intestines and excrete eggs in the feces (Schwartz and Fallon, 2018). Eggs are transferred from the mesenteric vessels to the intestinal lumen through the serosa, muscularis, epithelium, and mucosa (Moore and Sandground, 1956; Fan and Kang, 2003). It is generally accepted that the deposition of eggs will lead to chronic inflammatory and oxidative stress, which are responsible for the role of schistosomiasis in carcinogenesis (Elbaz and Esmat, 2013; van Tong *et al*., 2017). However, it is unclear and difficult to assess which layer eggs are mainly located (i.e. serosa, submucosa, or mucosa) during this process of continuous passage of eggs (Costain *et al*., 2018).

In this study, we collected 172 SCRC patients and observed the relationship between the deepest deposition of eggs in the intestinal wall and the prognosis, and found that the deeper the deposition of eggs, the worse the prognosis of SCRC patients. This conclusion was consistent with the previous study (Wang *et al*., 2016). In addition, we firstly found that patients with eggs in LN had a worse prognosis, especially in stage III patients.

We speculate that when patients continue to be infected with schistosomiasis for a longer period, the eggs will be deposited deeper in the intestinal wall and spread through the LN, resulting in a large number of eggs trapped in other tissues such as liver, causing more complications, and consequently exacerbated host worse prognosis. In

Table 4 Univariate and multivariate Cox proportional hazard regression analysis for OS among patients with schistosomal colorectal cancer

Variable	All patients		Patients with stage I–II disease		Patients with stage III disease	
	P-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)
Univariate analysis						
Age (< 60 years)	0.463	2.107 (0.287–15.440)	0.845	0.815 (0.106–6.276)	-	
Gender (male/female)	0.291	1.457 (0.725–2.927)	0.865	1.103 (0.358–3.399)	0.256	1.687 (0.684–4.163)
Tumor size (5 cm)	0.915	0.962 (0.475–1.948)	0.877	1.090 (0.366–3.249)	0.879	0.930 (0.366–2.363)
Tumor location						
Rectum	Reference		Reference		Reference	
Left colon	0.495	0.747 (0.323–1.726)	0.44	0.579 (0.145–2.316)	0.787	0.864 (0.299–2.496)
Right colon	0.654	1.211 (0.524–2.799)	0.833	1.146 (0.323–4.062)	0.549	1.407 (0.460–4.302)
Histology subtypes	0.777	0.895 (0.414–1.934)	0.702	1.244 (0.407–3.802)	0.75	0.835 (0.276–2.528)
Histology grade	0.018	2.465 (1.166–5.214)	0.067	3.029 (0.926–9.903)	0.224	1.826 (0.691–4.823)
Lymphovascular invasion	0.039	2.091 (1.039–4.208)	0.676	1.317 (0.361–4.799)	0.204	1.807 (0.726–4.500)
Perineural invasion	0.003	2.865 (1.418–5.786)	0.201	2.084 (0.677–6.416)	0.069	2.460 (0.931–6.497)
pT stage						
T2	Reference		Reference		Reference	
T3	0.061	3.978 (0.940–16.837)	0.221	3.609 (0.462–28.207)	0.216	3.607 (0.473–27.488)
T4	0.076	4.251 (0.858–21.066)	0.366	3.023 (0.274–33.356)	0.151	5.005 (0.555–45.125)
pN stage						
N0	Reference		Reference		-	
N1	0.007	2.824 (1.321–6.037)	0.911	1.124 (0.146–8.649)	Reference	
N2	0.072	2.605 (0.917–7.397)	-		0.683	0.808 (0.291–2.246)
pTNM stage						
Stage I	Reference		-		-	
Stage II	0.229	3.494 (0.454–26.876)	-		-	
Stage III	0.034	8.767 (1.173–65.516)	-		-	
Hepatic schistosomiasis	0.884	1.062 (0.476–2.367)	0.122	0.200 (0.026–1.540)	0.003	4.465 (1.671–11.933)
Presence site of schistosome eggs	0.095	1.839 (0.899–3.762)	0.712	1.228 (0.413–3.654)	0.051	2.627 (0.995–6.939)
Schistosome eggs in cutting edge	0.302	2.854 (0.389–20.925)	-		0.696	1.494 (1.199–11.212)
Schistosome eggs in LN	0.542	1.241 (0.620–2.487)	0.632	0.766 (0.257–2.282)	0.062	2.396 (0.957–6.000)
Multivariate analysis						
Histology grade	0.027	2.335 (1.103–4.943)	-		-	
Perineural invasion	0.005	2.771 (1.371–5.598)	-		0.043	2.744 (1.031–7.305)
Hepatic schistosomiasis	-		-		0.002	4.967 (1.836–13.432)
Multivariate analysisa						
Histology grade	0.027	2.335 (1.103–4.943)	-		-	
Perineural invasion	0.005	2.771 (1.371–5.598)	-		-	

CI, confidence interval; HR, hazard ratio; LN, lymph node.
[a]Multivariate analysis, after adjustment for hepatic schistosomiasis-variable.

our study, we also found that the deeper presence site of eggs and the presence of eggs in LN were associated with hepatic schistosomiasis, and hepatic schistosomiasis was an independent risk factor. These findings also provide some support for our speculation.

The presence of eggs in LN was significantly associated with shorter survival in univariate analysis, but not significant in the initial multivariate analysis. Due to the close association between the presence of eggs in LN and hepatic schistosomiasis, the prognostic significance of the presence of eggs in LN in multivariate analysis might be obscured. Therefore, we re-performed multivariate analysis after excluding hepatic schistosomiasis and found the presence of eggs in LN was an independent predictor in stage III SCRC. This implies that hepatic schistosomiasis was a stronger independent prognostic factor than the presence of eggs in LN. During the chronic infection stage of schistosomiasis, some eggs are deposited in the liver, causing hepatic granulomatous inflammation (Cheever *et al.*, 1994). Some infected patients develop hepatic fibrosis, and portal hypertension, which may result in gastrointestinal bleeding, hepatic encephalopathy, and liver failure (Schwartz and Fallon, 2018). Therefore, to improve the prognosis of SCRC patients, the adverse effects of hepatic schistosomiasis should not be ignored. Pathologists are suggested to indicate the presence of eggs in LN in the pathology report when observing them in histological slides.

Our study has several limitations. First, our dataset did not collect information on some possible prognostic factors, such as treatment variables and lifestyle. Different surgical and chemotherapy regimens and unhealthy lifestyles such as smoking and excessive alcohol consumption are known to affect survival outcomes. Second, this study is a single-institution retrospective study with a small sample size, and the uniformity of the results may be low. We will further expand the sample size and conduct a multi-institution study to verify the conclusions. Third, this study found a negative correlation between the presence of eggs and SCRC outcomes, but the exact functional role of the presence of eggs in SCRC progression and the underlying molecular mechanisms remain unclear. Therefore, further analysis of the potential carcinogenic mechanism of schistosome eggs is required.

Conclusions

In this study, our study first found the presence of schistosome eggs in LN was an unfavorable factor for prognosis in SCRC. Hepatic schistosomiasis was an independent poor prognostic factor for stage III SCRC patients.

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Conflicts of interest

There are no conflicts of interest.

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