Comparison of non-schistosomal colorectal cancer and schistosomal colorectal cancer

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Research

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Abstract

Aim: The purpose of this study was to compare clinicopathological features of patients with non-schistosomal and schistosomal colorectal cancer to explore the effect of schistosomasis on CRC patients` clinical outcomes.

Methods: 351 cases of CRC were retrospectively analyzed in this study. Survival curves were constructed by using the Kaplan-Meier method. Univariate and multivariate Cox proportional hazard regression models were performed to identify associations with outcome variables.

Results: Patients with schistosomiasis (CRC-S) were significantly older (Table 3, P<0.001) than patients without schistosomiasis (CRC-NS). However, there were no significant differences between CRC-S and CRC-NS patients in other clinicopathological features. Schistosomiasis were associated with adverse overall survival upon K-M analysis (P=0.0277). By univariate and multivariate analysis, as shown in Table 2, gender (P=0.003), TNM stage (P<0.001), schistosomiasis (P=0.025), lymphovascular invasion (P=0.030) and cancer node (P<0.001) were all independent predictors in the whole cohort. When patients were stratified according to clinical stage and lymph node metastasis state. Schistosomiasis was also an independent predictors in patients with stage Ⅰ-Ⅱ tumors and in patients with lymph node metastasis, but not in patients with stage Ⅲ-Ⅳ tumors and in patients without lymph node metastasis.

Conclusion: Schistosomiasis was significantly correlated with OS and it was an independent prognostic factor for OS in the whole cohort. When patients were stratified according to clinical stage and lymph node metastasis state, schistosomiasis was still an independent unfavorably prognosis factor for OS in patients with stage Ⅰ-Ⅱ tumors or patients with lymph node metastasis.

Introduction

Growing evidences have emerged in recent decades that inflammation is the root of many malignant tumors[1, 2]. As the fourth most common cancer and the second leading cause of cancer deaths in the world[3], colorectal cancer(CRC) represents a growing number of cancers that correlated with inflammation[1, 4, 5]. Schistosoma japonicum (*S.japonicum*) which is common in Southeast Asia[6], is regarded as a risk factor of CRC development[7]. Schistosomal infestation has been implicated in the aetiology of several human malignancies including bladder, liver, and colorectal cancer (CRC)[8, 9]. The prevalent view is that the sequestered eggs in the mucosa and submucosa incite a severe inflammatory reaction with cellular infiltration and consequent granuloma formation. This in turn leads to mucosal ulceration, microabscess formation, polyposis, and neoplastic transformation[10]. But the causal relationship between *S. japonicum* and CRC still remained controversial[11]. Some case reports and descriptive studies from Africa and the Middle East raised the possibility of an association between *S. japonicum* infestation and induction of CRC[12-14]. Nonetheless, the pathological evidence supporting this conclusion is rather weak. While some research demonstrated that *S. japonicum* infestation was unrelated with CRC[15].

In the 1950s, schistosomiasis was epidemic at a large scale in regions along the Yangtze River and in more than 400 counties in South China[16] . Because of the effective prevention and cure measures taken in China in recent years, schistosomiasis has been eliminated in most epidemic regions. However, its spread is not yet completely controlled and schistosomiasis occurs every year in a small number of people in the epidemic regions of China[17]. Qingpu District of Shanghai used to be one of the 10 areas with serious schistosomiasis epidemic in China[18], and problems of treatment and outcome of a large number of late schistosomiasis patients left over from history are still remaining. Therefore, detailed knowledge about schistosomiasis is necessary to improve the accuracy of clinical prognosis prediction and will shed light on improving our ability to prevention and control of schistosomiasis.

In the present study, we made a retrospective analysis of schistosomiasis and clinicopathologic characteristics in 137 schistosomiasis CRC patients (CRC-S) and 214 patients without schistosomiasis (CRC-NS) to investigate the effect of schistosomiasisn on CRC patients` clinical outcomes.

Materials And Methods

Patients and samples

A total of 351 colorectal cancer patients were enrolled in this retrospective study. All patients had undergone primary surgical resection at Qingpu Branch of Zhongshan Hospital affiliated to Fudan University, from January 2008 to August 2016. All of operations followed the principle: adequate resection margins, en bloc high ligation of the inferior mesenteric artery (IMA) and lymphadenectomy. All circumferential margins were cleared. The number of positive lymph nodes and total number of retrieved lymph nodes were recorded. The inpatient medical records and pathological reports were reviewed and the patients were followed up by telephone. Overall survival (OS) is

defined as the interval from the surgical operation date to the last follow-up or death caused by colorectal cancer. Inclusion criteria included (1) patients with colorectal cancer as primary focus; (2) none of these patients had received any prior anti-tumor therapy; (3) patients were diagnosed as adenocarcinoma by pathology after resection of colorectal cancer. Exclusion criteria included (1) Tis tumours; (2) patients who lacked complete information; (3) patients with synchronous malignancy; (4) patients with survival time less than one month.

Two expert pathologists reviewed HE-stained slides to determine the diagnosis and to restage the tumors according to the eighth edition of American Joint Committee on Cancer (AJCC).

Detection of schistosome ova and assessment of tumor budding

Schistosome ova were observed in all of original HE-stained formalin fixed paraffin-embedded (FFPE) sections (usually 4-6 slides), which were examined at ×10 and ×40 magnification fields using a conventional light microscope by two pathologists who were blind to clinic data. The diagnosis of schistosomiasis was done by finding schistosome eggs in HE-stained slides.

Tumor budding was defined as the presence of de-differentiated single cells or small clusters of up to 5 cells at the invasive front of colorectal cancer[19]. To assess tumor budding in the 10-HPF method [20], the invasive front is first scanned at low magnification (×4-×10) to identify areas of highest budding density. Tumor buds are then counted under high magnification (×40) and the tumor budding count is reported. The evaluation of tumor budding was conducted by two pathologists who were blinded to clinic data. 5 tumor budding counts were used as breakthrough point. In brief, tumor bud counts greater than or equal to 5 were defined as high group, otherwise as low group.

Statistical analysis

The association between schistosomiasis and clinicopathological characteristics was evaluated by using the Chi square and Fisher's exact tests. Overall survival (OS) was defined as the time of surgery to death. Kaplan-Meier curves with log-rank tests were used to determine the prognostic significance for OS. Univariate and multivariate regression analyses were used to identify independent prognostic factors, and $P < 0.05$ was defined as the criterion for variable deletion when performing backward stepwise

selection. Statistical analyses were performed using SPSS 22.0 (SPSS Inc, Chicago, IL).

Results

Clinical characteristics in full cohort

A total of 351 surgically resected FFPE primary CRC samples were included in the study. In the whole cohort, 39.0% (137 out of 351) were infected with schistosoma (Figure 1A). The clinical and pathologic features of the cohort are summarized in Table 1. In the whole cohort, age of patients at diagnosis ranged from 33 to 91 years (median, 69 years) and were predominantly male (60.2%, 212 out of 351). By anatomic site, 27% tumors were in the rectum, 33% in left colon and 40% in right colon. Lymph node metastasis were observed in 40% of patients and 46% of patients were at late stage disease. While patients without lymph node metastasis were 60%. On the basis of the AJCC Staging Manual (seventh edition), there were very few highly differentiated cases in the follow-up data. Thus, highly differentiated and moderately differentiated cases were classified as "well differentiation", and classified poorly differentiated cases as "poor differentiation". 76% cases were well differentiated, and 24% were poorly differentiated. As shown in Table 1, lymphovascular invasion, perineura invasion, cancer node and tumor budding were prone to appear in patients with stage III-IV tumors or patients with lymph node metastasis. More poorly differentiated tumors and deeper tumor invasion depth were also mostly observed in patients with late tumor stage or patients with lymph node metastasis. The distribution trend of other clinicopathologic features, such as colonic perforation, ulceration, histological type were similar within different subgroups.

Survival analysis

The median follow-up times was 62.4 (1.25-134.4) months. During the follow up, there was 41.6% (146 out of 351) patients died. Mean and median times to overall survival were 62.54 and 62.85, respectively.

To investigate the association between schistosomiasis and clinical outcomes, we conducted Kaplan-Meier analysis according to schistosoma infection status. Result demonstrated that schistosoma infection was significantly associated with poor survival in total colorectal cancer patients (median survival time: 80.82 for CRC-S set; 119.20 for CRC-NS set. P=0.0277) (Figure 1B).

Further analysis was conducted to explore the effect of schistosoma infection on CRC patients with similar stage tumors. In stage □-□ set (N=192), a Kaplan-Meier (K-M) curve was plotted and found that schistosoma infection (40%) was uncorrelated with survival (*P*=0.5018) (Figure 2A). Nevertheless, in stage □-□ set (N=159), K-M analysis showed significant correlation between schistosoma infection and OS (*P*=0.0260) (Figure 2B).

In patients with lymph node metastasis (N=144), schistosoma infection was observed in 39% (56 out of 144) CRC patients and associated with poor survival (*P*=0.0249) (Figure 2C). In contrast, there was no statistically significant difference observed in OS between CRC-S and CRC-NS patients without lymph node metastasis (*P*=0.4005) (Figure 2D).

Univariate and multivariate analysis

The Cox proportional hazards model was used to determine factors that may influence OS of CRC patients. In the whole cohort, by univariate analysis and multivariate analysis (Table 2), gender (*P*=0.003), TNM stage (*P*□0.001), schistosomasis (*P*=0.025), lymphovascular invasion(*P*=0.030) and cancer node (*P*□0.001) were significantly independent predictors. Schistosomiasis was statistically significant associated with decreasing OS.

In late-stage (□-□) CRC patients(Table 2), gender (*P*=0.030), pathological T stage (*P*=0.12), tumor differentiation (*P*=0.016), schistosoma infection (*P*=0.008) and cancer node (*P*=0.004) were significantly independent prognostic factor for OS. While in early-stage (□-□), cancer node (*P*=0.007) was the only independent prognostic factor for OS in multivariate analysis.

In patients with lymph node metastasis (Table 2), gender (*P*=0.026), pathological T stage (*P*=0.025), schistosoma infection (*P* =0.023) and cancer node (*P*=0.003) were independently prognostic factors. In patients without lymph node metastasis (Table 2), TNM stage (*P*□ 0.001) and tumor budding (*P*=0.014) but not schistosoma infection were associated with OS in multivariate analysis. These results further proved that schistosoma infection may have different effects on CRC patients` clinical outcomes, especially for patients with stage □-□ tumor and patients with lymph node metastasis.

Association of schistosomiasis with clinicopathological features

The relationship between schistosomiasis and clinicopathological features was shown in Table 3. Patients with schistosomiasis were significantly older than the patients without schistosomiasis (median age: 74.0 years vs 64.0 years, p□0.001). Clinical stage of patients with schistosomiasis and without schistosomiasis were similar (p=0.816). In the total cohort, the male/female ratio was also higher in CRC-S set (1.67 vs 1.43). Besides, in patients with lymph node metastasis, there were significant association between male sex and female sex (p□0.001). There were no significant differences of other clinicopathological characteristics between CRC-NS and CRC-S set.

In order to further investigate the effect of schistosomiasis on particular CRC population, we divided the whole cohort into different groups according to their clinical stage or the state of lymph node metastasis and further subgrouped into CRC-S and CRC-NS set based on schistosomiasis. Except age, there were no correlation between other clinicopathological features and schistosomiasis when compared between CRC-NS and CRC-S sets in different groups(Table 3).

Discussion

At present, there are sufficient evidence to conclude that *S. haematobium* has a role in causing some types of bladder cancer[21-23] and hepatocellular carcinoma[6, 10]. There is limited evidence suggesting that *S. japonicum* is possibly carcinogenic to humans leading to colorectal cancer.

In this study, results demonstrated that schistosomiasis was an independently unfavorable factor for OS (p=0.0260, Fig.1B; p=0.025,Table 2). These results indicated that schistosomiasis pay an important role in CRC progression and metastases. Shindo[24] reviewed 276 cases of large intestine cancer with schistosomiasis and found significant differences between carcinoma with schistosomiasis and ordinary carcinoma in symptoms, age, sex and histological findings, suggesting that schistosomiasis could induces the carcinoma. Ye et al.[25] reported that intestinal schistosomiasis was a risk factor for colorectal cancer and that the lesions caused by the disease might be considered precancerous. Liu et al.[26] reported that the history of colon schistosomiasis was a probable risk factor for the development of colorectal neoplasia, but only a few studies reported the clinicopathological characteristics and prognosis of patients with schistosomal colorectal cancer. This may be explained by reasons as follow. Firstly, there is little relevant clinical data in the medical literature, mostly limited to case reports, physicians know little about it[27, 28]. Secondly, since samples of schistosomiasis patients is too difficult to obtain, leading to small sample size and bias in data analysis.

Previous reports revealed that the development of CRC-S occurs in younger age group[29, 30]. It was opposite with our results. This might be explained by the reasons as follow. First, since effective prevention and control measures taken in China in 1983, the infection rate has decreased magnificently, which result in CRC-NS patients was in large quantity and relatively younger. Second, this disparity may be related to differences in hereditary factors, environmental carcinogens and so on. Our results showed that there is also a male predominance(61%, Table 1) in the cohort, although there was no significant difference between CRC-NS and CRC-S patients (Table 3). Given Qingpu district of Shanghai is previously a countryside, more males engage in farm work, and they may be at greater risk for exposure[31-33].

In the cohort, there were 22 (1.7%) patients were stage IV tumors, the survival time of them ranged from 1.25 months to 118 months. Although it is well known that stage IV tumors have a poor prognosis, as explained above, we want to investigate the impact of schistosoma on CRC in the complete process.

Schistosomiasis was statistically significant for OS in the univariate analysis and was an independent prognosis factor in multivariate analysis in the whole cohort (*P*=0.025, HR=1.458, 95% CI=1.049-2.027). When patients were stratified based on clinical stage or state of lymph node metastasis, schistosomiasis was also a significantly independent predictor, except in patients with stage Ⅳ tumor or without lymph node metastasis. Therefore, our observation indicated that schistosomiasis maybe a considerable risk for patients in different clinical stage, especially in late clinical stage. This conclusion may bright up the debate that schistosomiasis is a weak risk of colorectal cancer[5, 34, 35].

Our study had several limitations. First, because it was performed at a single institution, the university of the results may be low. Further work will be needed to validate present results. Second, patient selection bias was a possibility due to the nature of the retrospective study. Third, although we found a negative correlation between schistosomiasis and colorectal cancer outcomes, the precise functional roles of schistosomiasis in colorectal cancer progression and its underlying molecular mechanisms remain obscure. Chen et al. observed variable degree of colonic epithelial dysplasia in 60% of cases with *S. japonicum* colitis and regarded these changes as the transition on the way towards cancer development in schistosomal colonic disease[36]. A similar conclusion was drawn by Yu et al. from their studies on different types of schistosomal egg polyps[34]. All these results suggested the pro-tumor mechanisms of *S. japonicum* in tumor tissues. Therefore, further analysis about the functional roles of schistosoma infection and underlying molecular mechanisms need to be investigated. In addition, we did not sure any of these patients suffered from familial cancer syndromes, such as lynch syndrome. It was known that the proportion of patients with familial polyps and hereditary nonpolyposis colorectal cancer syndrome is higher in young patients (≤40 years old) [37, 38]. In our cohort, there were seven patients (0.02%) under 40 years old. However, further work will be continued to confirm this conclusion. Lastly, it was reported that schistosomiasis results from the host's immune response to schistosome eggs and the granulomatous reaction evoked by the antigens they secrete[39], and the process of granulomas formation will be accompanied by chronic inflammation[40, 41], which may induces the development of tumor. However, we could not provide evidence in this study, detection of inflammatory markers will be conducted to strengthen the hypothesis in further work.

In summary, our observations support the prognostic roles of schistosomiasis and shed light on the adverse effects of schistosomiasis on CRC patients.

Declarations

Ethics approval and consent to participate:

This study was approved by the medical ethics committee of Fudan University (Ethical approval number 2019-017), in accordance with the Helsinki Declaration of 1975. Prior written informed consent was obtained from all patients.

Consent for publication：Written informed consent was obtained from each participant.

Availability of data and materials:

The datasets used and/or analyzed during the current study are available from the corresponding authors on reasonable request.

Conflict of interest: The authors declare that they have no competing interests.

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Authors' contributions

Weixia Wang contributed data analysis, manuscript editing, article revision and data supplement. Kui Lu and Limei Wang assessed all the dyeing slices. Hongyan Jing contributed to the research design, data analysis, and manuscript writing. Weiyu Pan, Sinian Huang, Yanchao Xu, Dacheng Bu, Meihong Cheng, Jing Liu, Jican Liu, Weidong Shen, Yingyi Zhang and Junxia Yao contributed to the data collection and perform experiments. Ting Zhu contributed to the data analysis and manuscript editing. All authors read and approved the final manuscript.

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Abbreviations

colorectal cancer=CRC; colorectal cancer patients with schistosomiasis =CRC-S; patients without schistosomiasis=CRC-NS; Schistosoma japonicum=*S. japonicum*; American Joint Committee on Cancer =AJCC; formalin fixed paraffin-embedded (FFPE); Overall survival =OS;

References

1. Mantovani, A., Cancer: Inflaming metastasis. Nature, 2009. **457**(7225): p. 36-7.
2. Hanahan, D. and R.A. Weinberg, Hallmarks of cancer: the next generation. Cell, 2011. **144**(5): p. 646-74.
3. Bray, F., et al., Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin, 2018. **68**(6): p. 394-424.
4. OE, H.S., et al., Colorectal carcinoma associated with schistosomiasis: a possible causal relationship. World J Surg Oncol, 2010. **8**: p. 68.
5. Chen, M.G., Assessment of morbidity due to Schistosoma japonicum infection in China. Infect Dis Poverty, 2014. **3**(1): p. 6.
6. Gryseels, B., et al., Human schistosomiasis. Lancet, 2006. **368**(9541): p. 1106-18.
7. Hamid, H.K.S., Schistosoma japonicum-Associated Colorectal Cancer: A Review. Am J Trop Med Hyg, 2019. **100**(3): p. 501-505.
8. Yosry, A., Schistosomiasis and neoplasia. Contrib Microbiol, 2006. **13**: p. 81-100.
9. Eder, B.V.A., et al., Sigmoid colon cancer due to Schistosomiasis. Infection, 2019. **47**(6): p. 1071-1072.
10. Ross, A.G., et al., Schistosomiasis. N Engl J Med, 2002. **346**(16): p. 1212-20.
11. Schwartz, E. and J. Rozenman, Schistosomiasis. N Engl J Med, 2002. **347**(10): p. 766-8; author reply 766-8.
12. Uthman, S., et al., Association of Schistosoma mansoni with colonic carcinoma. Am J Gastroenterol, 1991. **86**(9): p. 1283-4.
13. Al-Mashat, F., et al., Rectal cancer associated with schistosomiasis: report of two cases and review of the literature. Ann Saudi Med, 2001. **21**(1-2): p. 65-7.
14. Ameh, E.A. and P.T. Nmadu, Colorectal adenocarcinoma in children and adolescents: a report of 8 patients from Zaria, Nigeria. West Afr J Med, 2000. **19**(4): p. 273-6.
15. Cheever, A.W., et al., Schistosoma mansoni and S. haematobium infections in Egypt. III. Extrahepatic pathology. Am J Trop Med Hyg, 1978. **27**(1 Pt 1): p. 55-75.
16. McManus, D.P., et al., Conquering 'snail fever': schistosomiasis and its control in China. Expert Rev Anti Infect Ther, 2009. **7**(4): p. 473-85.
17. Zhu, R. and J.J.C.J.o.S.C. Xu, [Epidemic situation of oversea imported schistosomiasis in China and thinking about its prevention and control]. 2014. **26**(2): p. 111-114.
18. Yu, S.Z., [Schistosomiasis must be eradicated: a review of fighting schitosomiasis in Qingpu, Shanghai]. Zhonghua Liu Xing Bing Xue Za Zhi, 2016. **37**(7): p. 1044-6.
19. Koelzer, V.H., I. Zlobec, and A. Lugli, Tumor budding in colorectal cancer–ready for diagnostic practice? Hum Pathol, 2016. **47**(1): p. 4-19.
20. Horcic, M., et al., Tumor budding score based on 10 high-power fields is a promising basis for a standardized prognostic scoring system in stage II colorectal cancer. Hum Pathol, 2013. **44**(5): p. 697-705.
21. Elsebai, I., Parasites in the etiology of cancer–bilharziasis and bladder cancer. CA Cancer J Clin, 1977. **27**(2): p. 100-6.
22. Bedwani, R., et al., Schistosomiasis and the risk of bladder cancer in Alexandria, Egypt. Br J Cancer, 1998. **77**(7): p. 1186-9.
23. Barnett, R., Schistosomiasis. Lancet, 2018. **392**(10163): p. 2431.

24. Shindo, K., Significance of Schistosomiasis japonica in the development of cancer of the large intestine: report of a case and review of the literature. Dis Colon Rectum, 1976. **19**(5): p. 460-9.
25. Ye, C., et al., Endoscopic characteristics and causes of misdiagnosis of intestinal schistosomiasis. Mol Med Rep, 2013. **8**(4): p. 1089-93.
26. Liu, W., et al., Schistosomiasis combined with colorectal carcinoma diagnosed based on endoscopic findings and clinicopathological characteristics: a report on 32 cases. Asian Pac J Cancer Prev, 2013. **14**(8): p. 4839-42.
27. Issa, I., M. Osman, and G. Aftimos, Schistosomiasis manifesting as a colon polyp: a case report. J Med Case Rep, 2014. **8**: p. 331.
28. Li, W.C., Z.G. Pan, and Y.H. Sun, Sigmoid colonic carcinoma associated with deposited ova of Schistosoma japonicum: a case report. World J Gastroenterol, 2006. **12**(37): p. 6077-9.
29. Xu, Z. and D.L. Su, Schistosoma japonicum and colorectal cancer: an epidemiological study in the People's Republic of China. Int J Cancer, 1984. **34**(3): p. 315-8.
30. El-Bolkainy, M.N., et al., The impact of schistosomiasis on the pathology of bladder carcinoma. Cancer, 1981. **48**(12): p. 2643-8.
31. Madbouly, K.M., et al., Colorectal cancer in a population with endemic Schistosoma mansoni: is this an at-risk population? Int J Colorectal Dis, 2007. **22**(2): p. 175-81.
32. Xiao, G., et al., Analysis of risk factors and changing trends the infection rate of intestinal schistosomiasis caused by S. japonicum from 2005 to 2014 in Lushan city. Parasitol Int, 2018. **67**(6): p. 751-758.
33. Wang, Z., et al., Comparison of the clinicopathological features and prognoses of patients with schistosomal and nonschistosomal colorectal cancer. Oncol Lett, 2020. **19**(3): p. 2375-2383.
34. Yu, X.R., et al., Histological classification of schistosomal egg induced polyps of colon and their clinical significance. An analysis of 272 cases. Chin Med J (Engl), 1991. **104**(1): p. 64-70.
35. Ojo, O.S., W.O. Odesanmi, and O.O. Akinola, The surgical pathology of colorectal carcinomas in Nigerians. Trop Gastroenterol, 1992. **13**(2): p. 64-9.
36. Chen, M.C., et al., Colorectal cancer and schistosomiasis. Lancet, 1981. **1**(8227): p. 971-3.
37. Yantiss, R.K., et al., Clinical, pathologic, and molecular features of early-onset colorectal carcinoma. Am J Surg Pathol, 2009. **33**(4): p. 572-82.
38. Antelo, M., et al., A high degree of LINE-1 hypomethylation is a unique feature of early-onset colorectal cancer. PLoS One, 2012. **7**(9): p. e45357.
39. Boros, D.L. and K.S. Warren, Delayed hypersensitivity-type granuloma formation and dermal reaction induced and elicited by a soluble factor isolated from Schistosoma mansoni eggs. J Exp Med, 1970. **132**(3): p. 488-507.
40. Peterson, W.P. and F. Von Lichtenberg, Studies on granuloma formation. IV. In vivo antigenicity of schistosome egg antigen in lung tissue. J Immunol, 1965. **95**(5): p. 959-65.
41. Colley, D.G. and W.E. Secor, Immunology of human schistosomiasis. Parasite Immunol, 2014. **36**(8): p. 347-57.

Tables

Table 1: Clinicopathological characteristics of the CRC cohort

Characteristics	All patients (N=351)		Patients with stage I-II disease (N=192)		Patients with stage III-IV disease(N=159)		Patients with LNM (N=144)		Patients without LNM (N=207)	
	N	%	N	%	N	%	N	%	N	%
Age(≥60ys)	83	*24*	46	*24*	37	*23*	34	*24*	49	*23*
Gender(Male)	214	*61*	118	*61*	65	*59*	71	*49*	123	*57*
Tumor location										
Rectum	94	*27*	50	*26*	44	*28*	37	*26*	57	*28*
Left colon	115	*33*	61	*32*	54	*34*	51	*35*	64	*31*
Right colon	142	*40*	81	*42*	61	*38*	56	*39*	86	*41*
Tumor size(≥5cm)	174	*50*	94	*50*	80	*49.7*	71	*49*	103	*48*
Differentiation										
Well diff.	267	*76*	165	*86*	102	*65*	93	*65*	173	*82*
Poor diff.	84	*24*	27	*14*	57	*35*	51	*35*	36	*18*
Lymphovascular invasion (positive)	122	*35*	46	*24*	76	*48*	68	*47*	54	*26*
Nervous invasion (positive)	31	*1.0*	12	*6.0*	19	*12*	18	*12.5*	13	*6*
Cancer node(≥2 nodes)	42	*1.2*	1.0	*0.0*	41	*26*	35	*24*	7	*3*
Colonic perforation (Yes)	13	*0.4*	8	*4.0*	5	*3.0*	4	*3.0*	9	*4.0*
Tummor budding (≥5 cells)	219	*62*	99	*52*	120	*75*	110	*79*	109	*53*
Ulceration (Yes)	149	*42*	79	*41*	70	*44*	64	*44*	85	*41*
Histological type										
Adenocarcinoma	311	*89*	173	*90*	138	*87*	124	*86*	187	*90*
Mucinous/SRCC	40	*11*	19	*10*	21	*13*	20	*14*	20	*10*
Pathological T stage										
T1-2	80	*23*	65	*34*	15	*9*	14	*10*	63	*31*
T3-4	271	*77*	127	*66*	144	*91*	130	*90*	146	*69*
Lymph node metastasis										
No	207	*60*	189	*98*	18	*12*	----	----	----	----
Yes	144	*40*	3	*2*	141	*88*	----	----	----	----
TNM stage										
I+II	190	*54*	----	----	----	----	3	*2*	192	*92*
III+ IV	161	*46*	----	----	----	----	141	*98*	17	*8*
schistosomiasis	137	*39*	76	*40*	61	*38*	56	*39*	81	*40*

----:Data is not applicable; Abbreviation: N=Number

Table2. Univariate and multivariate Cox regression of clinicopathological for overall survival

Variable	All patients		Patients with stage I-II disease		Patients with stage III-IV disease		Patients with LNM		Patients without LNM	
	P	HR(95%CI)	P	HR(95%CI)	P	HR(95%CI)	P	HR(95%CI)	P	HR(95%CI)
Univariate analysis										
Age (≥60ys)	0.010	1.759(1.142-2.708)	0.009	3.413(1.355-8.597)	0.244	1.343(0.818-2.205)	0.116	1.533(0.900-2.611)	0.024	0.424(0.201-0.894)
Gender (male/female)	0.008	1.602(1.129-2.271)	0.046	1.897(1.010-3.564)	0.018	1.670(1.093-2.553)	0.020	1.714(1.089-2.697)	0.103	1.584(0.912-2.752)
Tumor size(5cm)	0.913	1.018(0.728-1.400)	0.735	0.909(0.523-1.580)	0.480	1.157(0.772-1.735)	0.233	1.297(0.846-1.987)	0.435	0.816(0.491-1.358)
Tumor site										
Rectum		Refer		Refer		Refer		Refer	Refer	
Left colon	0.908	1.025(0.676-1.553)	0.670	1.168(0.572-2.385)	0.926	0.975(0.572-1.663)	0.926	0.975(0.572-1.663)	0.942	1.025(0.527-1.995)
Right colon	0.464	0.859 (0.572-1.290)	0.860	0.939 (0.467-1.888)	0.249	0.728 (0.425-1.249)	0.249	0.728 (0.425-1.249)	0.967	1.013 (0.531-1.865)
Pathological T stage	＜0.001	2.591(1.562-4.297)	0.633	1.158(0.634-2.116)	0.007	6.803(1.675-27.633)	0.010	6.323(1.554-25.722)	0.275	1.385(0.541-1.486)
Lymph node metastasis	＜0.001	2.802(2.012-3.902)	0.041	4.410(1.063-18.289)	0.558	0.828(0.441-1.556)	—	—	—	—
TNM stage	＜0.001	3.197(2.271-4.501)	—	—	—	—	0.827	0.855(0.210-3.481)	＜0.001	4.275(2.203-8.298)
Differentiation	＜0.001	1.889(1.334-2.674)	0.833	1.084(0.510-2.307)	0.019	1.083(1.083-2.466)	0.126	1.407(0.909-2.177)	0.074	1.742(0.957-3.169)
Lymphovascular invasion	＜0.001	3.251(1.987-5.318)	0.163	1.538 (0.840-2.815)	0.011	1.702 (1.132-2.559)	0.019	1.670(1.088-2.561)	0.036	1.782(1.039-3.056)
Nervous invasion	0.140	1.497(0.876-2.559)	0.281	1.759 (0.630-4.909)	0. 866	1.056 (0.562-1.985)	0.943	0.976(0.503-1.893)	0.176	1.888(0.752-4.741)
Cancer node	＜0.001	4.006(2.686-5.973)	0.007	16.949(2.188-131.284)	＜0.001	2.178(1.410-3.366)	＜0.001	0.723(1.479-3.693)	0.001	5.338(1.907-14.943)
Colonic perforation	0.541	0.700(0.223-2.198)	0.824	1.174(0.285-4.829)	0.768	0.809(0.198-3.303)	0.461	0.475(0.066-3.428)	0.512	1.475(0.462-4.711)
Tummor budding	＜0.001	2.028(1.400-2.938)	0.043	1.812(1.019-3.221)	0.163	1.423(0.867-2.336)	0.234	0.723(0.424-1.232)	0.023	1.849(1.087-3.146)
Schistosomiasis	0.044	1.399(1.009-1.940)	0.474	1.225(0.703-2.132)	0.011	1.699(1.128-2.560)	0.019	1.674(1.087-2.577)	0.428	1.229(0.738-2.049)
Ulceration	0.624	0.9205(0.660-1.282)	0.362	0.766(0.4313-1.360)	0.971	1.008(0.670-1.514)	0.649	1.104(0.721-1.690)	0.189	0.698(0.408-1.194)
Histological type	0.921	1.025(0.626-1.680)	0.797	0.886(0.352-2.230)	0.870	0.952(0.529-1.713)	0.708	0.889(0.482-1.641)	0.902	0.948(0.408-2.206)
Multivariate analysis										
Gender	0.003	1.676(1.178-2.384)	—	—	0.030	1.601(1.047-2.450)	0.026	1.675(1.064-2.639)	—	—
Pathological T stage	—	—	—	—	0.012	6.042(1.476-24.729)	0.025	5.040(1.228-20.678)	—	—
TNM stage	＜0.001	0.389(0.267-0.567)	—	—	—	—	—	—	＜0.001	4.507(2.303-8.818)
Lymph node metastasis	—	—								
Tumor budding	—	—							0.014	0.513(0.301-0.876)
Differentiation	—	—	—	—	0.016	1.677(1.101-2.555)				
Schistosomiasis	0.025	1.458(1.049-2.027)	—	—	0.008	1.743(1.153-2.635)	0.023	1.648(1.070-2.538)	—	—
Lymphovascular invasion	0.030	1.461(1.036-2.060)	—	—	—	—	—	—	—	—
Cancer node	＜0.001	2.256(1.461-3.483)	0.007	16.8587(2.176-130.580)	0.004	1.911(1.230-2.969)	0.003	2.005(1.267-3.175)	—	—

—:Data is non-significant ; Abbreviation: CI=confidence interval; HR=Hazard ratio; P < 0.05 was defined as the criterion for variable deletion when performing backward stepwise selection.

Table3. The association between clinicopathological characteristics and schistosomiasis in CRC cohort

Characteristic			All patients		P	Stage I–II disease patients		P	Stage III–IV disease patients		P	With lymph node metastasis patients		P	Without lymph node metastasis patients		P
			CRC-NS (N=214)	CRC-S (N=137)		CRC-NS (N=116)	CRC-S (N=76)		CRC-NS (N=98)	CRC-S (N=61)		CRC-NS (N=88)	CRC-S (N=56)		CRC-NS (N=126)	CRC-S (N=81)	
Age(60ys)					0.001			0.001			0.001			0.001			0.001
		60	78	5		41	5		37	0		34	0		44	5	
		≥60	136	132		75	71		61	61		54	56		82	76	
Gender					0.467			0.695			0.520			0.001			0.588
		Male	126	86		70	48		42	23		35	36		53	31	
		Female	88	51		46	28		56	38		53	20		73	50	
Tumor site					0.274			0.750			0.829			0.806			0.030
		Rectum	57	37		31	19		26	18		22	15		35	22	
		Left colon	64	51		29	31		35	19		33	18		31	33	
		Right colon	93	49		56	25		37	24		33	23		60	26	
Tumor size					0.985			0.597			0.581			0.372			0.443
		5cm	106	68		55	39		51	29		46	25		60	43	
		≥5cm	108	69		61	37		47	32		42	31		66	38	
Differentiation					0.570			0.577			0.700			0.952			0.417
Well diff.	165	102	101	64		64	38		57	36		108	66				
Poor diff.	49	35	15	12		34	23		31	20		18	15				
Lymphovascula invasion					0.909			0.732			1.000			1.000			1.000
		Negative	138	90		87	59		51	32		46	30		92	60	
		Positive	76	47		29	17		47	29		42	26		34	21	
Nervous invasion					1.000			0.766			1.000			1.000			1.000
Negative	194	125	108	72		86	54		78	49		117	76				
Positive	20	12	8	4		11	7		10	7		9	5				
Cancer node					0.867			0.998			0.710			1.000			1.000
		≤2 nodes	189	120		115	76		74	44		67	42		122	78	
		2 nodes	25	17		1	0		24	17		21	14		4	3	
Colonic perforation					0.966			0.482			0.373			0.299			0.487
		No	206	132		110	74		96	58		87	53		119	79	
		Yes	8	5		6	2		2	3		1	3		7	2	
Tumor budding					0.652			0.184			0.454			0.841			0.393
5 cells	83	49	61	32		22	17		20	14		63	35				
≥5 cells	131	88	55	44		76	44		68	42		63	46				
Histological type					0.731			0.470			0.590			1.000			0.633
		Adenocarcinoma	191	120		106	67		85	53		76	48		115	72	
		Mucinous/SRCC	23	17		10	9		13	8		12	8		11	9	
Ulceration					0.740			0.881			0.774			0.495			1.000
		No	125	77		69	44		56	33		51	29		74	48	
		Yes	89	60		47	32		42	28		37	27		52	33	
Pathological T stage					0.562			0.395			0.891			0.749			0.388
	T1-2	I+ II	51	29		42	23		9	6		8	6		43	23	
	T3-4	III	163	108		74	53		89	55		80	50		83	58	
Lymph node metastasis					0.883			0.823			0.641						----
		No	126	81		114	75		12	6		0	0		----	----	
		Yes	88	56		2	1		86	55		88	56		----	----	
TNM stage					0.816			----			----			0.842			0.379
I+II	116	76	----	----		----	----		2	1		112	75				
III+ IV	98	61	----	----		----	----		86	55		14	6				

----:Data is not applicable; Abbreviation: N=Number. The association between schistosomiasis and clinicopathological characteristics was evaluated by using the Chi square and Fisher's exact tests.

Figures

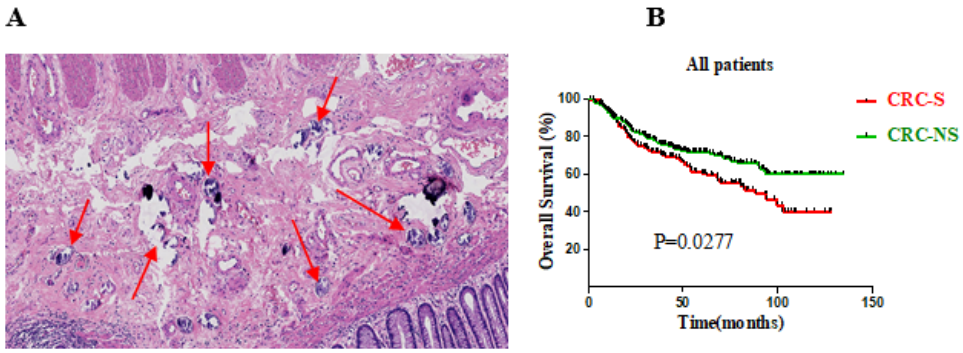

Figure 1

A. Typical sample of schistosomiasis-associated colorectal cancer, the red arrows indicate schistosome ova (HE, ×100); B. Kaplan-Meier analysis of overall survival in whole CRC cohort according to schistosoma infection status. P value was calculated by log-rank test.

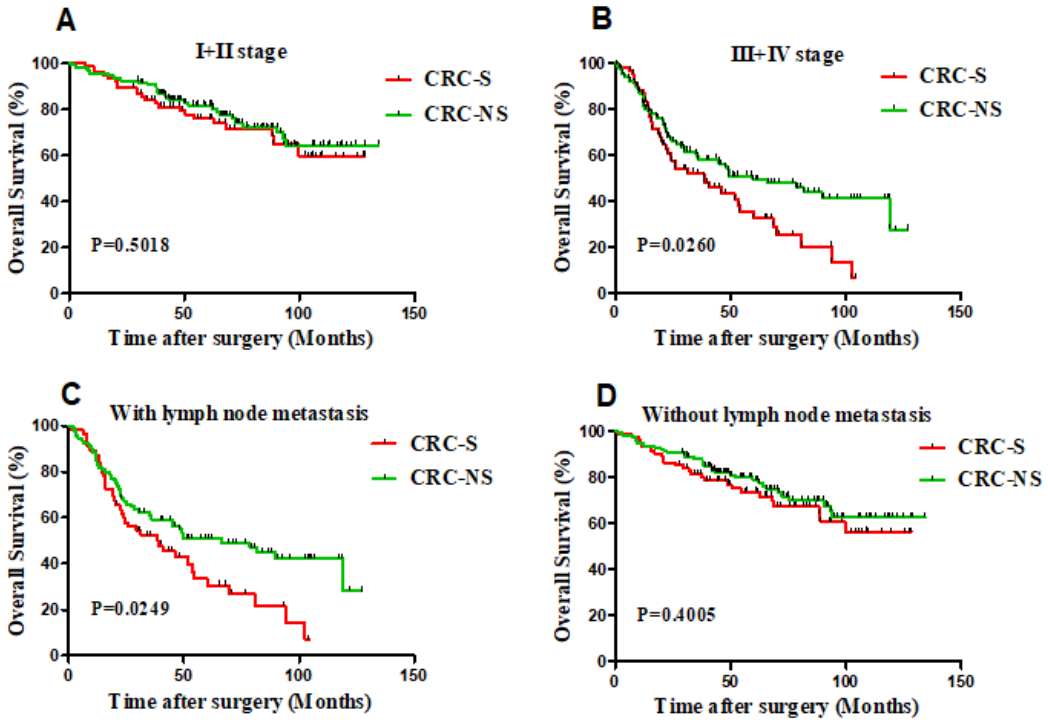

Figure 2

Kaplan-Meier analysis of overall survival in stratified CRC. A. Patients with stage □-□ tumors (N=192, P=0.5018); B. Patients with stage □-□ tumors (N=159, P =0.0260); C. Patients with lymph node metastasis (N=144, P=0.0249); and D. patients without lymph node metastasis (N=207, P =0.4005); P value was calculated by log-rank test.