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ORIGINAL PAPER

Intestinal helminths and protozoan infections in patients with colorectal cancer: prevalence and possible association with cancer pathogenesis

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Abstract

The purpose of the present study was to determine the prevalence of intestinal helminths and protozoa in colorectal cancer (CRC) patients and to evaluate the possible association between the prevalence and CRC pathogenesis. A total of 200 CRC patients and 200 residents of Tashkent, who had no complaints related to the gastrointestinal tract, were examined by triple coproscopy using a concentration method and estimations of protozoan infection intensity. Of the CRC patients tested, 144 were classified as T_{1}-$_{4}N_0M_0$ (without metastases) and 56 were classified as $T_{1–4}N_{1–2}M_{0–1}$ (with metastases). Parasitological examination was performed during CRC diagnosis before and after surgery and chemotherapy. A significantly higher prevalence of *Blastocystis sp.*, *Chilomastix mesnili*, *Jodamoeba butschlii*, and *Endolimax nana* was found in CRC patients than in the control population ($p < 0.0001$), amounting to 80, 20, 22.5, and 11.5%, respectively. The high prevalence of *Blastocystis* sp., as well as the patterns of infection intensity, was stable at all stages of examination. The ratio of the number of CRC patients with and without *Blastocystis sp.* in the $T_{1–4}N_0M_0$ and $T_{1–4}N_{1–2}M_{0–1}$ groups amounted to 3.3 and 7.0, respectively. The ratios for *C. mesnili*, *E. coli*, *J. butschlii*, and *E. nana* in both groups were 0.2 and 0.2, 0.07 and 0.07, 0.3 and 0.16, and 0.18 and 0.01, respectively. The prevalence of helminths and *Giardia lamblia* in CRC patients and the control population was not significantly different. Taken together, these data indicate a possible role for *Blastocystis* sp. in CRC pathogenesis. Diagnosis, treatment, and further observation of patients with *Blastocystis* sp. are necessary at all stages of CRC, including during diagnosis and before and after surgery and chemotherapy.

Keywords Colorectal cancer · *Blastocystis sp.* · Intestinal helminths · Protozoan infections · CRC

Introduction

Colorectal cancer (CRC) is the third-most frequent cancer in the world and one of the leading causes of cancer-related mortality. Causes of high mortality include a frequently asymptomatic course of disease in the early stages, with a consequently late diagnosis combined with decreased survival rates of approximately 10% in metastatic and late-stage tumors. The aggressive nature of this type of cancer is also important (Siegel et al. 2012; Favoriti et al. 2016; O'Connell et al. 2004). Another factor is the frequent failure of chemotherapy due to the development of drug resistance (Panczyk 2014). Genetic, environmental, and dietary factors have also been found to be involved in the development and progression of CRC (Triantafillidis et al. 2009). The most recent evidence suggests the involvement of the intestinal microbiota in the initiation and progression of disease (Coleman and Nunes 2016). The role of

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intestinal microbiota in gut physiology, as well as outside of the intestine, is well documented. The impacts of the intestinal microbiota on the maturation of the immune system, multiple metabolic pathways, and various digestive diseases, including CRC and inflammatory bowel diseases, among others, have been reported (Landman and Quevrain 2016). Although some microorganisms were found to be associated with carcinogenesis in CRC, including *Enterococcus spp*., *Helicobacter pylori*, enterotoxigenic *Bacteroides fragilis*, pathogenic *Escherichia coli*, *Clostridium difficile*, and *Fusobacterium nucleatum* (Fukugaiti et al. 2015; Yu et al. 2015; Wang and Huycke 2015), the prevalence of parasites and their association with carcinogenesis remain unclear. Helminths, including *Opisthorchis voiverrini*, *Clonorchis sinensis*, and *Schistosoma haematobium*, belong to the first group of biological carcinogens. This group also includes the human *papilloma* virus, the *hepatitis C* virus, and *Helicobacter pylori* (Brindley et al. 2015). Studies on the prevalence of *Ascaris lumbricoides* (*A. lumbricoides*), *Enterobius vermicularis* (*E. vermicularis*), and *Giardia lamblia* (*G. lamblia*) in CRC patients are limited by the description of individual cases of CRC with concomitant parasitosis, which results in them being considered unlikely to be involved in cancer pathogenesis (Peterson and Weidner 2011). Little is known about the protozoan portion of the microbiota, especially in CRC patients. A high frequency of *Blastocystis* sp. infection has been observed in immunocompromised individuals with hematological malignancies and other forms of cancer (Mohamed et al. 2017; Kumarasamy et al. 2017; Rasti et al. 2017; Yersal et al. 2016; Taşova et al. 2000; Devera et al. 1998). However, data on the prevalence of other intestinal protozoa in CRC patients is not available.

The purpose of the present study was to determine the prevalence of intestinal helminths and protozoa in CRC patients, including the identification of changes in the microbiota of patients with and without metastases before and after surgery and chemotherapy and the evaluation of their possible association with CRC pathogenesis.

Materials and methods

This prospective cohort study was conducted on the basis of the Research Institute of Epidemiology, Microbiology and Infectious Diseases and the Research Center of Oncology, Tashkent, Uzbekistan, during the period from January 2015 to January 2017.

Patients eligible for inclusion were adults with a confirmed diagnosis of CRC and residents of Tashkent without any complaints relating to the gastrointestinal tract (the control population), matched by gender and age to the patients with CRC. We excluded patients if they had problems with stool sample collection, received any treatment 2–3 weeks before the study, refused surgery, or refused to participate due to a depressive condition. Patients were also excluded if they had concomitant diseases associated with the gastrointestinal tract, if follow-up was impossible, or if they were unwilling and/or unable to provide written informed consent.

The diagnosis of CRC was based on the results of clinical examination, endoscopy, histology, X-ray, and laboratory data, according to the International Classification of Diseases proposed by the *American Joint Committee on Cancer* (AJCC) with the application of TNM criteria for diagnosis. Descriptors of TNM are as follows: T (primary tumor) is the degree of tumor spread into the layers of the intestine; N is the absence of metastases or the number of lymph nodes with metastases; and M is the absence or presence of remote metastases (Edge and Compton 2010). Of the patients included in this study, 144 patients belonged to the $T_{1–4}N_0M_0$ (without metastases) group and 56 patients belonged to the $T_{1–4}N_{1–2}M_{0–1}$ (with metastases) group.

All of the CRC patients were admitted to and were operated on at the Research Center of Oncology. A total of 200 patients hospitalized in the coloproctology department were examined before surgery and 100 patients were examined after surgery. In addition, 20 CRC patients hospitalized in the chemotherapy department were examined before and after surgery and chemotherapy. The control population included 200 residents of Tashkent without any complaints relating to the gastrointestinal tract who applied to the clinic in the Research Institute of Epidemiology, Microbiology and Infectious Diseases for prophylactic medical examination.

All of the patients underwent resection of the affected parts of the intestine with anastomosis. Chemotherapy of CRC patients was conducted according to the FOLFOX (folinic acid (leucovorin), fluouracil, oxaliplatin) chemotherapy regimen.

Collection of stool samples

Three stool samples for parasitological examination were taken at 2-day intervals from CRC patients before and after surgery after a complete course of chemotherapy and from control individuals. Stool samples were collected in individual containers, containing 5 ml of Turdiev's preservative for conservation and staining of protozoan cysts and worm eggs for up to a year. The Turdiev's preservative includes the following: 80 ml of 0.2% aqueous solution of sodium nitrite, 10 ml of formaldehyde, 2 ml of glycerin, 8 ml of Lugol's solution, and 250 ml of distilled water.

Stool samples for the detection *C. parvum* (*Cryptosporidium parvum*) were collected in empty individual containers no more than 1 h before microscopy.

Stool sample examination

The parasitological diagnosis was performed by triple coproscopy using a formalin-ethyl acetate concentration

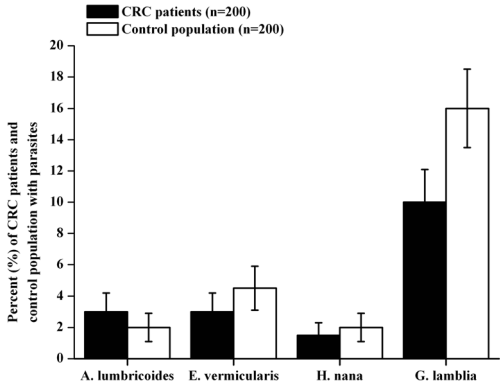

Fig. 1 Prevalence of pathogenic intestinal parasites. The data show the percentage of intestinal parasites in CRC patients ($n = 200$) and the control population ($n = 200$)

technique (Truant et al. 1981) and an iodine-stained smear. The intensity of protozoa was estimated by the number of protozoa in the field of view (ocular × 10, objective × 40) in iodine-stained smears before the application of the formalin-ethyl acetate concentration technique. The number of protozoa was calculated for at least 10 fields of view. Categories of 1–2, 3–4, and 5–6 microorganisms in a field of view were considered low, mean, and high infection intensities, respectively. *C. parvum* was detected using a modified Ziehl-Neelsen method.

The investigators who performed the parasitological diagnoses did not have access to any information about the individuals under examination. All information was blinded.

Statistical analysis

Data analysis was performed with the program Origin 6.1 (OriginLab, Northampton, MA). The results are expressed as mean ± standard error (SEM) for continuous variables and numbers (percentages) for categorical data. Data were analyzed by odds ratios (OR), 95% confidence intervals (CI) of the mean, and Pearson Chi-square tests for numerical variables. A p value < 0.05 was considered statistically significant.

Data availability All data generated or analyzed during this study are included in this published article and its supplementary information files.

Results

Demographic and clinical characteristics of participants

The ages of the CRC patients were within the range of 19–88 years old, with the mean age of patients being 52 years old. A total of 132 patients (60%) were males and 88 (40%) were females. Individuals in the control group were matched by gender and age to the CRC patients.

Table 1 Prevalence of *Blastocystis* sp., *E. dispar*, and *E. coli* in CRC patients (no./%)

Group under study	The number of individuals with the following:											
	Blastocystis sp.				*E. dispar*				*E. coli*			
	The total number of patients with protozoa	The number of protozoa in a field of view			The total number of patients with protozoa	The number of protozoa in a field of view			The total number of patients with protozoa	The number of protozoa in a field of view		
		1–2	3–4	5–6		1–2	3–4	5–6		1–2	3–4	5–6
Patients with CRC ($n = 200$)	160/80 ± 2.8 **	43/26.8 ± 3.5**	99/61.8 ± 3.8**	18/11.25 ± 2.4	2/1 ± 0.7	1/50 ± 35	1/50 ± 35	–	14/7.0 ± 1.8 *	12/85.7 ± 9.3	2/14.2 ± 9.3	–
Control population ($n = 200$)	36/18.0 ± 2.7	28/77.7 ± 6.9	8/22.2 ± 6.9	–	1/0.5 ± 0.4	1/100	–	–	38/19.0 ± 2.7	38/100	–	–

Note: significant difference in comparison with the control population: *$p = 0.0003$; **$p < 0.0001$

Table 2 Prevalence of *C. mesnili*, *J. butschlii*, and *E. nana* in CRC patients (no./%)

Group under study	The number of individuals with the following:											
	C. mesnili				*J. butschlii*				*E. nana*			
	The total number of patients with protozoa	The number of protozoa in a field of view			The total number of patients with protozoa	The number of protozoa in a field of view			The total number of patients with protozoa	The number of protozoa in a field of view		
		1–2	3–4	5–6		1–2	3–4	5–6		1–2	3–4	5–6
Patients with CRC ($n = 200$)	40/20 ± 2.8 **	38/95 ± 3.4	2/5 ± 3.4	–	45/22.5 ± 2.9 ***	43/96 ± 3.0	2/4 ± 3.0	–	23/11.5 ± 2.25 *	23/100	–	–
Control population ($n = 200$)	6/3.0 ± 1.2	6/100	–	–	12/6.0 ± 1.6	12/100	–	–	6/3.0 ± 1.2	6/100	–	–

Note: significant difference in comparison with the control population: *$p = 0.001$; **$p < 0.0001$

Of the 200 CRC patients, the anatomical localization of CRC presented as rectal cancer in 80 cases (40%), anal canal cancer in 42 cases (21%), cancer of the sigmoid intestine in 30 cases (15%), cancer of the caecum and ascending colon in 22 cases (11%), cancer of the transverse colon in 10 cases (5%), cancer of the descending colon in 8 cases (4%), and cancer of the rectosigmoid colon in 8 cases (4%). An additional group, including 20 CRC patients examined before surgery and chemotherapy, presented as rectal cancer in 13 cases (65%), anal canal cancer in 6 cases (30%), and cancer of the sigmoid intestine in 1 case (5%).

According to World Health Organization's Classification of Tumors (Hamilton and Aaltonen 2000), histological data allowed us to identify the forms of CRC: adenocarcinoma, mucinous adenocarcinoma, and squamous cell carcinoma were diagnosed in 93, 6, and 1% of cases, respectively.

Intestinal macro- and microbiota in CRC patients

Data on the parasitic portion of the detected intestinal macro- and microbiota were divided into two groups. The first group included pathogenic parasites (helminths, *G. lamblia*, and *C. parvum*), and the second group included *Blastocystis* sp., protozoa with debated pathogenicity and commensal protozoan species, including *Entamoeba coli* (*E. coli*), *Endolimax nana* (*E. nana*), *Entamoeba dispar* (*E. dispar*), *Chilomastix mesnili* (*C. mesnili*), and *Jodamoeba butschlii* (*J. butschlii*).

The prevalence of *A. lumbricoides*, *E. vermicularis*, *Hymenolepis nana* (*H. nana*), and *G. lamblia* in CRC patients amounted to 3 ± 1.2, 3 ± 1.2, 1.5 ± 0.8, and 10 ± 2.1%, respectively, and to 2 ± 0.9, 4.5 ± 1.4, 2 ± 0.9, and 16 ± 2.5%, respectively, in the control population. *C. parvum* was not detected in the stool samples of CRC patients or the control population. The prevalence of pathogenic intestinal parasites in CRC patients and the control population is shown in Fig. 1.

The overall prevalence of helminths in CRC patients (7.5 ± 1.86%) was not significantly different from their prevalence in the control individuals (8.5 ± 1.96%) (OR: 0.8728; 95% CI: 0.4232 to 1.7999; $p = 0.708$). Figure 1 shows that *G. lamblia* was the most prevalent parasite in both groups, but the difference between the patients and control individuals was insignificant. Mixed pathogenic intestinal parasite infections were not found in CRC patients or in the controls.

We observed a significantly higher prevalence of *Blastocystis* sp. in CRC patients. We found the prevalence of *Blastocystis* sp. in CRC patients to be four times as high as in the control population (OR: 18.2222; 95% CI: 11.0503 to 30.0489; $p < 0.0001$) (Table 1). Moreover, high-intensity infections were observed only in patients with CRC (11.25%). The frequency of mean-intensity *Blastocystis* sp. infections was significantly higher than in the control population (61.8 ± 3.8 and 22.2 ± 6.9%, respectively; $p < 0.0001$). The prevalence of *E. dispar* was low in both groups, at 1.0 ± 0.7% in

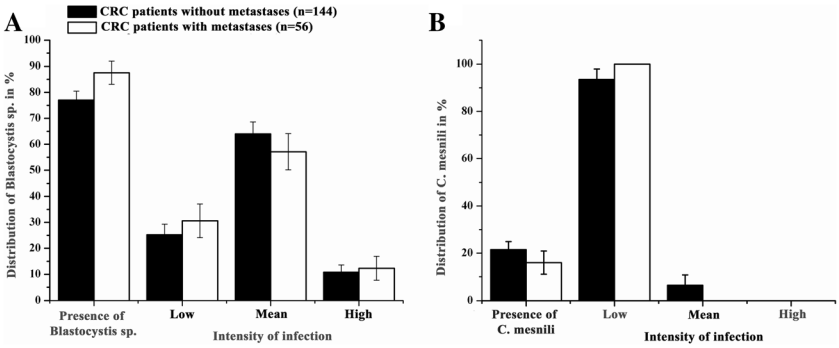

Fig. 2 Distribution of *Blastocystis* sp. and *C. mesnili* infections in CRC patients without and with metastases. **a** Presence and intensity of *Blastocystis* sp. infection in CRC patients with ($n = 56$) and without ($n = 144$) metastases, represented as a percentage. **b** Presence and intensity of *C. mesnili* infection in CRC patients with ($n = 56$) and without ($n = 144$) metastases, represented as a percentage

CRC patients and 0.5 ± 0.4% in the control population (OR: 2.0101; 95% CI: 0.1808 to 22.3479; $p < 0.5$). The percentage of individuals with *E. coli* was significantly lower in CRC patients (7.0 ± 1.85%) than in the control population (19.0 ± 2.7%) (OR: 0.3209; 95% CI: 0.1679 to 0.6134; $p = 0.0003$). Additionally, unlike the controls, mean-intensity infections were observed in only 14.2% of CRC patients. The overall prevalence of *Blastocystis* sp. (80.0 ± 2.8%) was significantly higher than the prevalence of *E. dispar* (1.0 ± 0.7%) and *E. coli* (7.0 ± 1.85%) ($p < 0.0001$) (Table 1).

C. mesnili infection rates in CRC patients (20.0 ± 2.8%) and controls (3.0 ± 1.2%) were also found to be significantly different (OR: 8.0833; 95% CI: 3.3420 to 19.5514; $p < 0.0001$). Mean-intensity infections (3–4 in a field of view) were only found in CRC patients. The same pattern was also detected in *J. butschlii* infection, in which protozoa were found in CRC patients (22.5 ± 2.9%) 3.5 times as frequently as in the controls (6.0 ± 1.6%) (OR: 4.5484; 95% CI: 2.3243 to 8.9007; $p < 0.0001$). Similarly, mean-intensity infections were only observed in CRC patients. The prevalence of *E. nana* in CRC patients (11.5 ± 2.25%) was also significantly higher than in controls (3.0 ± 1.2%) (OR: 4.2015; 95% CI: 1.6722 to 10.5565; $p = 0.001$), but the intensities of the infection in both groups were low. The overall prevalence of *Blastocystis* sp. (80.0 ± 2.8%) was significantly higher than the prevalence of *C. mesnili* (20.0 ± 2.8%), *J. butschlii* (22.5 ± 2.9%), and *E. nana* (11.5 ± 2.25%) ($p < 0.0001$) (Table 2).

Prevalence and intensity of *Blastocystis* sp. and other protozoa at various stages of CRC

The distribution of protozoa in patients with and without metastases was of special interest because it may indicate a possible association of a particular species with cancer progression.

The distributions of *Blastocystis* sp. and *C. mesnili* in CRC patients at various stages of disease are shown in Fig. 2.

In total, *Blastocystis* sp. were identified in 77.0 ± 3.5% of $T_{1–4}N_0M_0$ and 87.5 ± 4.4% $T_{1–4}N_{1–2}M_{0–1}$ patients (OR: 0.4622; 95% CI: 0.1916 to 1.1147; $p = 0.08$). The number of $T_{1–4}N_{1–2}M_{0–1}$ patients with *Blastocystis* sp. infection of low, mean, and high intensity was not statistically different from the number of $T_{1–4}N_0M_0$ patients with these characteristics.

Quite a different distribution was observed for the commensal protozoa, *C. mesnili*. In total, *C. mesnili* was found in 21.5 ± 3.4% of $T_{1–4}N_0M_0$ and 16.0 ± 4.9% $T_{1–4}N_{1–2}M_{0–1}$ patients (OR: 1.4326; 95% CI: 0.6333 to 3.2411; $p = 0.1$) (Fig. 2). Low-intensity *C. mesnili* infections were detected in 93.5 and 100.0% of $T_{1–4}N_0M_0$ and $T_{1–4}N_{1–2}M_{0–1}$ patients, respectively. Mean-intensity infections were detected in only 6.4%

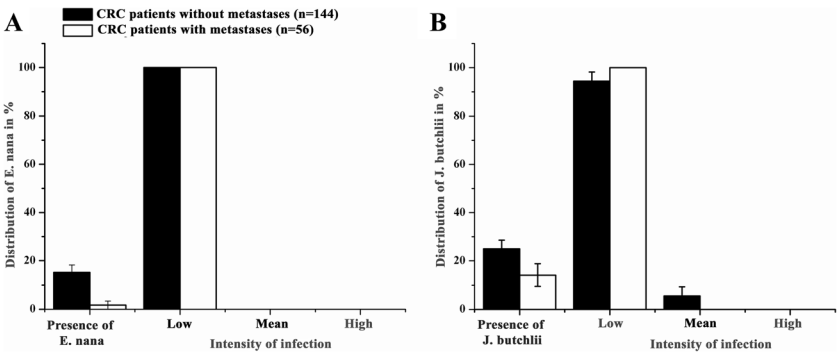

Fig. 3 Distribution of *E. nana* (**a**) and *J. butschlii* (**b**) infection (%) in CRC patients without and with metastases. **a** Presence and intensity of *E. nana* infection in CRC patients with ($n = 56$) and without ($n = 144$) metastases, represented as a percentage. **b** Presence and intensity of *J. butschlii* infection in CRC with ($n = 56$) and without ($n = 144$) metastases, represented as a percentage

Table 3 *Blastocystis* sp. in CRC patients before and after surgery (no./%)

Group under examination	The total number of patients with *Blastocystis sp.*	The number of protozoa in a field of view		
		1–2	3–4	5–6
CRC patients before surgery ($n = 100$)	78/78 ± 4.14	14/17.9 ± 4.3	53/67.9 ± 5.2	11/14.6 ± 3.9
CRC patients after surgery ($n = 100$)	79/79 ± 4.07	12/15.1 ± 4.0	53/67.0 ± 5.2	14/17.7 ± 4.2

of $T_{1–4}N_0M_0$ patients. There were also significant differences between the prevalence of *Blastocystis* sp. and *C. mesnili* in $T_{1–4}N_0M_0$ and $T_{1–4}N_{1–2}M_{0–1}$ patients ($p < 0.001$).

Figure 3 demonstrates the distributions of *E. nana* and *J. butschlii* infections.

E. nana and *J. butschlii* were found in 15.3–25.0% of $T_{1–4}N_0M_0$ and 1.8–14.3% of $T_{1–4}N_{1–2}M_{0–1}$ patients, respectively (OR: 9.918; 95% CI: 1.3037 to 75.4545; $p = 0.007$ and OR: 2.0; 95% CI: 0.865 to 4.6242; $p = 0.1$). Low-intensity infections with *E. nana* and *J. butschlii* were detected in 100.0–94.4% of $T_{1–4}N_0M_0$ and in 100.0–100.0% of $T_{1–4}N_{1–2}M_{0–1}$ patients, respectively. Mean-intensity *J. butschlii* infections were observed in only 5.6% of patients without metastases (Fig. 3). The patterns of distribution for both protozoa at various CRC stages were analogous to those of *C. mesnili*, with values that were significantly different from the prevalence of *Blastocystis* sp. in $T_{1–4}N_0M_0$ and $T_{1–4}N_{1–2}M_{0–1}$ patients ($p < 0.0001$).

A complete analysis of the distribution of *E. dispar* and *E. coli* by CRC stage could not be carried out due to the low prevalence of both protozoa (1% for *E. dispar* and 7.0% for *E. coli*).

The frequency and intensity of *Blastocystis* sp. infection among $T_{1–4}N_0M_0$ and $T_{1–4}N_{1–2}M_{0–1}$ CRC patients were predominant compared to *C. mesnili*, *J. butschlii, E. nana*, and *E. coli*. The ratios of the number of CRC patients with *Blastocystis* sp. to the number of patients without it for the $T_{1–4}N_0M_0$ and $T_{1–4}N_{1–2}M_{0–1}$ groups were 3.3 and 7.0, respectively. For *C. mesnili*, this index for both groups was 0.2. These indices were 0.3 and 0.16, respectively, for *J. butschlii*, 0.18 and 0.01 for *E. nana*, and 0.07 in both groups for *E. coli*.

Protozoa in CRC patients before and after surgery

The distribution patterns of *Blastocystis* sp. and the commensals, *C. mesnili*, *J. butschlii*, and *E. nana*, in$T_{1–4}N_0M_0$ and $T_{1–4}N_{1–2}M_{0–1}$ CRC patients provided a basis for the analysis of only *Blastocystis* sp. before and after surgery. On the 3rd–5th days after surgery, stool samples were collected from 100 of the 200 CRC patients in the main group. The results are shown in Table 3.

Table 3 demonstrates that tumor removal does not significantly impact the frequency or intensity of *Blastocystis* sp. infection (OR: 0.9425; 95% CI: 0.4799 to 1.8508; $p = 0.86$). Spontaneous elimination of *Blastocystis* sp. was not observed.

Protozoa in patients with CRC before and after surgery and chemotherapy

All 20 patients included in this portion of the study were negative for helminths and *G. lamblia*. The total number of CRC patients with *Blastocystis* sp. after surgery and chemotherapy was stable (75.0%) and differed significantly from the corresponding estimate in the control population (18.0%) (OR: 13.6667; 95% CI: 4.6667 to 40.0234; $p < 0.0001$). The number of patients after chemotherapy with high-intensity infections (26.6%) was also unchanged. After chemotherapy, the number of the patients with low-intensity infections was increased, and the number of patients with mean-intensity infections was decreased, but these changes were not significant in both cases (Table 4).

E. dispar was not found in any patients before and after chemotherapy. However, this may be due to the small number of patients and relatively low prevalence of this protozoa in the groups under study. Before chemotherapy, *E. coli* was

Table 4 Prevalence of *Blastocystis* sp. in CRC patients before and after surgery and chemotherapy (no./%)

Group under study	The number of patients with *Blastocystis* sp.			
	The total number of patients with protozoa	The number of protozoa in a field of view		
		1–2	3–4	5–6
CRC patients before surgery and chemotherapy ($n = 20$)	15/75 ± 9.6	2/13.3 ± 8.7	9/60 ± 12.6	4/26.6 ± 11.4
CRC patients after surgery and chemotherapy ($n = 20$)	15/75 ± 9.6	3/20 ± 10.3	8/40 ± 12.6	4/26.6 ± 11.4

Table 5 Prevalence of *E. coli, C. mesnili*, *J. butschlii*, and *E. nana* in CRC patients before and after surgery and chemotherapy (no./%)

Group under study	The total number of patients with:			
	E. coli	*C. mesnili*	*J. butschlii*	*E. nana*
CRC patients before surgery and chemotherapy ($n = 20$)	1/6.6 ± 5.5	4/20.0 ± 8.9	2/10 ± 6.7	1/5 ± 4.8
CRC patients after surgery and chemotherapy ($n = 20$)	–	3/15 ± 7.9	3/15 ± 7.9	1/5 ± 4.8

detected in one patient (5.0 ± 4.8%), and after chemotherapy, it was not detected in any patients. The prevalence of *E. nana* was unchanged after chemotherapy (5 ± 4.8%). The tendencies of opposite character in the prevalence of *C. mesnili* and *J. butschlii* were observed: decrease and increase, respectively; however, the changes were insignificant (Table 5).

Discussion

The role of the intestinal microbiota is accepted as an important component of the initiation and development of CRC; however, studies investigating the parasitic portion of the microbiota are limited and contradictory. Helminths are known to induce type 2 immune responses that mediate a potent host protective response (Maizels et al. 2012). Helminths also promote an alternative regulatory program, which can further dampen Th1 and cytotoxic T lymphocyte responses (Aranzamendi et al. 2012; Massacand et al. 2009; Walsh et al. 2009), and this shift from a Th1 to a Th2 response can contribute to the growth of CRC (Hou et al. 2013). IL-6 and IL-17 play important roles during giardiasis (Lopez-Romero et al. 2015). Parasite-induced immunomodulation has not been shown to influence the course of CRC, although IL-6 and IL-10 have been shown to participate in both the initiation and progression of cancer (Landskron et al. 2014). A high number of infiltrating Th1-lymphocytes in the microenvironment of CRC is associated with improved prognosis in patients (Ling et al. 2015), while both local and systemic production of IL-17 is associated with CRC progression (Sharp et al. 2017). In our study, the prevalence of helminths (*A. lumbricoides*, *E. vermicularis*, *H. nana*) and *G. lamblia* in CRC patients was similar to that in the control population, which indicates that they are not associated with CRC initiation or progression. There are few studies available on the role of protozoa in the pathogenesis of CRC. Significantly higher incidences of *Blastocystis* sp. infection were found in CRC patients with adenocarcinoma, but not in patients with colorectal adenoma or patients with normal colonic epithelium (Steer 2007). Previous studies have reported a high level of *Blastocystis* sp., primarily of subtype 3, in CRC patients (Kumarasamy et al. 2014). The potential influence of *Blastocystis* sp. subtype 1 on CRC has also been suggested (Mohamed et al. 2017). Our data demonstrated that *Blastocystis* sp., *C. mesnili*, *J. butschlii*, and *E. nana* infections were more prevalent in CRC patients than in the control population, with prevalence rates of 80.0 ± 2.8, 20.0 ± 2.8, 22.5 ± 2.9, and 11.5 ± 2.25%, respectively ($p < 0.005$). *Blastocystis* sp. had the highest prevalence, in addition to displaying high-intensity infections only in CRC patients. This suggests an opportunistic pathophysiology of *Blastocystis* sp., *C. mesnili*, *J. butschlii*, and *E. nana* due to the unusual immunosuppression associated with cancer (Buquéa et al. 2015). Additionally, *Blastocystis* sp. were predominant in $T_{1–4}N_0M_0$ and $T_{1–4}N_{1–2}M_{0–1}$ patients, and the ratio of $T_{1–4}N_{1–2}M_{0–1}$ patients with *Blastocystis* sp. to those without was more than two times as high as that in $T_{1–4}N_0M_0$ patients, which was distinct from the commensals. The high frequency of *Blastocystis* sp. in $T_{1–4}N_0M_0$ and $T_{1–4}N_{1–2}M_{0–1}$ patients and opposite character of the ratio of patients with protozoa and without them in infection with *Blastocystis* sp. and commensals points to the possible association of *Blastocystis* sp. with carcinogenesis. To our knowledge, this is the first comparison of the prevalence of *Blastocystis* sp., *C. mesnili*, *J. butschlii*, *E. nana*, and *E. coli* during various stages of CRC.

Several research groups have reported the association of *Blastocystis* sp. with carcinogenesis. For instance, Chandramathi et al. showed that solubilized antigen from *Blastocystis* sp. had the ability to facilitate the growth of human CRC HCT116 cells and cause a more intensive inflammatory reaction (Chandramathi and Suresh 2010; Chan et al. 2012). These reports support the results of our study demonstrating that infection with *Blastocystis* sp. is associated with CRC and may exacerbate or even initiate CRC.

No studies on the influence of surgery on the protozoan portion of the microbiota currently exist. However, there are references to obturation due to carcinoma causing a high frequency and intensity of *Blastocystis* sp. infection in CRC patients, as well as the spontaneous elimination of infection after surgery (Horiki et al. 1999). In our study, the patients before surgery and after removal of the obstruction showed a stable frequency and intensity of *Blastocystis* sp. infection, although an increase in the infection frequency and intensity may have been expected due to the influence of surgery and anesthesia (Hogan et al. 2011).

In contrast to the findings of Chandramathi et al. (2012), which reported *Blastocystis* sp. and *Microsporidia* infections only during the intermediate chemotherapy cycle, we found

that the total number of CRC patients with *Blastocystis* sp. after surgery and chemotherapy was stable (75.0%) and differed significantly compared to the control population (18.0%) ($p < 0.0001$). It is clear that the treatment of *Blastocystis* sp. infection in CRC patients is of great importance. Nitazoxanide appears to be the best choice therapeutic preparation due to the combination of anti-*Blastocystis* and anticancer activities (Müller et al. 2008; Senkowski et al. 2015).

The prevalence of *E. dispar* was low in CRC patients (1.0%) and the control population (0.5%). A peculiar situation is observed with *E. coli* belonging to commensals. Our finding of an inverse association of *E. coli* with CRC was unexpected. The incidence of *E. coli* in CRC patients was 2.7 times less than in the control population. The significance of this association is unclear and raises questions about the possible relationship between *E. coli* and the conditions in the intestine mediated by CRC**.** Antagonism between *Blastocystis* sp. and *E. coli* may also be anticipated.

Conclusion

In this work, we systematically studied the prevalence of helminths and protozoa in CRC patients. Our results show that *A. lumbricoides*, *E. vermicularis*, *H. nana*, and *G. lamblia* are not associated with CRC pathogenesis. A high prevalence and intensity of *Blastocystis* sp. infection at various stages of CRC, a significantly higher ratio of the number of patients with *Blastocystis sp.* to without in $T_{1–4}N_{1–2}M_1$ patients in comparison with $T_{1–4}N_0M_0$ patients, and the stability of the infection after surgery and chemotherapy strongly suggests an association of *Blastocystis* sp. with CRC pathogenesis. Such an association was not found for the commensals, *C. mesnili*, *J. butschlii*, *E. nana*, *E. coli*, and *E. dispar*.

The monitoring of *Blastocystis* sp. is recommended upon the diagnosis of CRC, at peri- and postoperative periods, during chemotherapy and during all periods of observation. This problem requires further investigations, including the identification of *Blastocystis* subtypes and the evaluation of the susceptibility of various *Blastocystis* subtypes to metronidazole and nitazoxanide. Improved efficiency of eradication and the ability to decrease the intensity of *Blastocystis* sp. disease will increase the survival of CRC patients.

Authors' contributions

SO, SA, BN: designed study, wrote the paper; AT, NB, AI, ND: performed research, analyzed data.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethics statement The study was approved by the Medical Ethics Committee of the Ministry of Health of the Republic of Uzbekistan in accordance with the Declaration of Helsinki (World Medical Association 2013). The trial is registered at the US National Institutes of Health (ClinicalTrials.gov) #NCT03173001. Both informed and written consents were obtained from the patients and controls.

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