World Journal of Gastrointestinal Oncology

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World J Gastrointest Oncol 2022 March 15; 14(3): 734-745

DOI: 10.4251/wjgo.v14.i3.734

ISSN 1948-5204 (online)

SYSTEMATIC REVIEWS

Association of *Blastocystis hominis* with colorectal cancer: A systematic review of *in vitro* and *in vivo* evidences

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Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Farouk S

Received: May 17, 2021
Peer-review started: May 17, 2021
First decision: July 4, 2021
Revised: July 16, 2021
Accepted: February 22, 2022
Article in press: February 22, 2022
Published online: March 15, 2022

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Abstract

BACKGROUND

Recently, there have been several findings that showed intestinal colonisation of *Blastocystis hominis* (*Blastocystis*) as a risk factor to the worsening of colorectal cancer (CRC). However, studies have shown controversial results in the pathogenicity of *Blastocystis*.

AIM

To review systematically the evidence available on the association between CRC and *Blastocystis* and the prevalence of *Blastocystis* in CRC patients and to investigate cytopathic and immunological effects of *Blastocystis* in *in vitro* and *in vivo* studies.

METHODS

PRISMA guidelines were utilised in conducting this systematic review. Original

articles published before February 2, 2020 were included. PubMed, Science Direct, Scopus and Google scholar databases were searched. Manual searching was carried out to find articles missed during the online search.

RESULTS

Out of 12 studies selected for this systematic review, seven studies confirmed the prevalence of *Blastocystis* and found it to be between 2%-28% in CRC patients, whereby subtype 1 and subtype 3 were predominantly seen. A total of four studies employing *in vitro* human colorectal carcinoma cell line study models showed significant cytopathic and immunological effects of *Blastocystis*. In addition, one *in vivo* experimental animal model study showed that there was a significant effect of infection with *Blastocystis* on exacerbation of colorectal carcinogenesis.

CONCLUSION

Blastocystis is a commonly identified microorganism in CRC patients. These studies have provided supportive data that *Blastocystis* could exacerbate existing CRC *via* alteration in host immune response and increased oxidative damage. Future studies of CRC and *Blastocystis* should attempt to determine the various stages of CRC that are most likely to be associated with *Blastocystis* and its relationship with other intestinal bacteria.

Key Words: *Blastocystis hominis*; Colorectal cancer; Cytopathic effect; Immunological effect

Core Tip: Certain gut microorganisms are known to be important factors associated with initiation and development of colorectal cancer (CRC). However, data on the roles of parasites are vague and restricted. *Blastocystis hominis* (*Blastocystis*) is one of the most commonly recovered microorganisms in faecal specimens, and its widespread presence is found in CRC patients. This systematic review aims to quantify the studies published so far that revealed the association of *Blastocystis* and CRC. We sought to identify the prevalence of *Blastocystis* and its subtypes among CRC patients, *in vitro* studies using *Blastocystis* antigen and *in vivo* studies using animal models.

Citation: Kumarasamy V, Atroosh WM, Anbazhagan D, Abdalla MMI, Azzani M. Association of *Blastocystis hominis* with colorectal cancer: A systematic review of *in vitro* and *in vivo* evidences. *World J Gastrointest Oncol* 2022; 14(3): 734-745

URL: https://www.wjgnet.com/1948-5204/full/v14/i3/734.htm

DOI: https://dx.doi.org/10.4251/wjgo.v14.i3.734

INTRODUCTION

Blastocystis hominis (*Blastocystis*) is one of the most commonly recovered microorganisms in faecal specimens, and its widespread presence is found in colorectal cancer (CRC) patients[1]. Its distribution is known to be prevalent in both rural and urban areas[2]. This microorganism has been in discussion since early 1900s[3,4]; however, the taxonomic position of *Blastocystis* remains unanswered. *Blastocystis* treatment is often difficult due to its drug resistance and the failure of the host defenses to counter the infection[5]. Previous studies suggest that *Blastocystis* is a common and diverse element of microbiota in human host, as it has been highly prevalent in healthy individuals[6-8]. *Blastocystis* is commonly found in both patients with gastrointestinal symptoms and in healthy people widely across the world. More recently, researchers consider *Blastocystis* as an emerging zoonotic disease, and its pathogenic potential in human is somewhat controversial[9]. Although accumulating data suggest that *Blastocystis* is a pathogen, the pathogenic role in humans is still a matter of debate.

It is suggested that around 20% of cancer reported worldwide could have been due to infectious agents[10,11]. Viruses such as hepatitis B virus, human papilloma virus and Epstein-Barr virus have been associated with carcinogenesis. Various other bacteria also have been described previously to exacerbate cancer[12]. There are numerous epidemiological evidences that strongly support the fact that parasites can be a factor of various malignant tumours[13], but it is challenging to validate this relationship. Previously, a review article highlighted the correlation of various protozoan parasites including *Blastocystis* with carcinogenesis[13]. In addition, there was a case report in India that demonstrated a possible association of subtype 3 *Blastocystis* in the worsening of CRC[14].

There are a few other systematic reviews on the interventional studies done on *Blastocystis*, but we did not find any systematic reviews on the association between *Blastocystis* and CRC. Therefore, this systematic review aimed to (1) identify prevalence of *Blastocystis* in CRC patients; (2) review *in vitro* colorectal carcinoma cell line study models on the cytopathic and immunological effects of *Blastocystis* antigens; and (3) review an *in vivo* experimental animal model study to investigate the effect of infection with *Blastocystis* on exacerbation of colorectal carcinogenesis.

MATERIALS AND METHODS

PRISMA guidelines were utilised in conducting this review[15].

Eligibility criteria

Original articles that reported the prevalence and association of *Blastocystis* subtypes with CRC patients, *in vitro* studies using *Blastocystis* antigen and an *in vivo* study using an animal model published before February 2, 2020 were included.

Exclusion

Articles that reported the association of *Blastocystis* with cancer in general without specific findings on its association with CRC, reviews papers, conference proceedings and case reports were excluded from this review.

Search

PubMed, Science Direct, Scopus and Google scholar databases were searched.

The various keywords used were: *Blastocystis* infection and CRC and their MESH terms and synonyms. Manual searching through reference lists of included journal articles was done to find the missed studies during online search.

Study selection

We identified 872 papers in the initial screening (Figure 1).

Identification

First, two authors developed a search strategy with different key words and their synonyms. All articles were moved to the Endnote X7 software (Clarivate Analytics, Philadelphia, PA, United States), and 146 duplicate papers were removed. Two authors independently went through the titles and abstracts of the remaining 726 papers. Subsequently, a total of 22 papers were retained for full text review. The eligibility of retained papers was evaluated by two other authors. Authors checked the reference lists of all included articles for any relevant studies that met this systematic review inclusion criteria and had not been found during the database searches.

After full review of the 22 papers, 12 were selected. All authors have completed data extraction and agreed eligibility of included papers (Figure 1).

Quality assessment

For *in vivo* studies, the quality of the studies was assessed using Quasi experimental appraisal tool[16], which contained nine questions. For *in vitro* studies, checklist of Systematic Review Center for Laboratory Animal Experimentation's Risk of Bias tool for assessing risk of bias as quality assessment was used[17]. The quality of all included studies was acceptable.

Data extraction

The data extracted included author-year, country, sampling, setting, methods, results and study conclusion (Table 1).

RESULTS

Prevalence of Blastocystis in CRC patients

Based on the seven reviewed articles, the prevalence of *Blastocystis sp.* in CRC patients were found to be between 2.8%-46.7%, whereby subtype 1 and subtype 3 were predominantly isolated.

A study by Esteghamati *et al*[35] evaluated the prevalence of *Blastocystis* in 85 cancer patients, including 39 CRC and 46 cancers outside gastrointestinal tract (COGT). In this study, *Blastocystis* was identified in 11/39 (28.2%) among CRC group. Another study in China by Zhang *et al*[18] showed the prevalence of *Blastocystis* in 4 among 49 (8.1%) patients with CRC. In another study conducted in Saudi Arabia, the prevalence of *Blastocystis* among CRC patients was 29.7%[19]. Subtype I was the

Table 1 Data extraction table

No.	Ref.	Country	Sampling	Setting	Method Please note: (1) Main outcomes assessed; (2) If the protocol is published; and (3) If risk of bias is reported	Main results	Conclusion
Prevalence studies							
1	Esteghamati *et al*[35], 2019	Tehran, Iran	Study design: cross-sectional. Study duration: July 2016 and November of 2017. Sample size (190): 80 patients (Primary Immunodeficiency), 85 (cancer patients) and 25 (organ transplant recipients)	3 hospitals in in Tehran, Iran	The aim of this study to determine the prevalence of intestinal parasites in 3 different groups of patients referred to 3 hospitals. Method used for parasite identification: Conventional methods, nested PCR and amplification of the *18S rRNA* gene	The prevalence of *Blastocystis* hominis among CRC patient was 13/39 (28.2%)	The prevalence of *Blastocystis hominis* was found high in cancer patients, especially CRC patients
2	Zhang *et al* [18], 2017	China	Sample size: 381 faecal specimens were collected from cancer patients including CRC. Study duration: 2016 to 2017	Tumor Hospitalof Harbin Medical University	The aim of this study to determine the prevalence and genotypes/subtypes-*Blastocystis* in CP and analysed for the *Blastocystis* by PCR amplifying and sequencing	Prevalence of *Blastocystis* was 4 (8.1%) among CRC patient	*Blastocystis* subtype 1 and 3 have been identified in humans and animals
3	Mohamed *et al* [19], 2017	Saudi Arabia	Total sample size: 218. Two groups of participants: (1) CP (138) of which 74 had CRC and 46 had cancers outside gastrointestinal tract; and (2) NCP (80). Exclusion criteria: (1) Patient started chemotherapy regime; and (2) Receiving any anti-parasitic medication. Study duration: 2013-2015	King Abdulla Medical city (KAMC), Makkah	Case control study design: Aim, to determine the prevalence of *Blastocystis* among CRC patients compared to patients who had cancers outside gastrointestinal tract and control group. Obtained *Blastocystis* isolates were grouped into 2 categories (A and C), then subtyped into 3 various subtypes; subtype-I, subtype-II and subtype-V	Prevalence of *Blastocystis* among CRC = 22 (29%). *Blastocystis* infection frequency was significantly different between CP group and NC group. There was a higher probability of *Blastocystis sp*. among CP. Subtype I was the common subtype among CRC patients (54.5%). Interestingly, an association risk between *Blastocystis* subtype 1 with a greater risk of association in CRC group	The study revealed a probable association between subtype 1 of *Blastocystis* and CRC
4	Toychiev *et al* [20], 2018	Uzbekistan	A total sample of 400 participants, two groups of participants: (1) 200 CRC patients; and (2) 200 of Tashkent residents (without any gastrointestinal tract complaints). Exclusion criteria: (1) patient had problems with stool sample collection; and (2) received any treatment 2–3 wk before the study. Study duration: 2015-2017	Research Institute of Epidemiology, Microbiology and Infectious Diseases and the Research Center of Oncology, Tashkent, Uzbekistan, during the period	Study design: Prospective cohort: Prevalence of some parasites including *Blastocystis sp*. in CRC patients before and after surgery and chemotherapy compared to control group. Methods: "3 stool samples for parasitological examination were taken at 2-d intervals during CRC diagnosis before and after surgery and chemotherapy"	A significantly higher prevalence of protozoa was found in CRC patients than in control population "the prevalence of *Blastocystis* in CRC patients is 4 times as high as in the control population. The overall prevalence of *Blastocystis sp*. was 2.8% and was higher than the other protozoa"	Data revealed a potential role for *Blastocystis sp*. in CRC pathogenesis
5	Kumarasamy *et al*[21], 2014	Malaysia	Sample size: 425 patients who go through diagnostic colonoscopy. faecal samples and colonic washouts were obtained from 221 control patients and 204 patients	University of Malaya Medical Centre	To determine the *Blastocystis* genotype present by comparing the prevalence using colonic washouts and faecal samples PCR and standard stool culture. Both techniques were used to detect *Blastocystis* from control and	The prevalence of *Blastocystis* was 15.29% (65/425). "Colonic washouts and faecal samples showed 12.24% (n = 52) and 5.65% (n = 24) of *Blastocystis* infection respectively". A total of 43 individuals were positive for *Blastocystis* in CRC patients and	*Blastocystis sp*. is common in CRC patients. Subtype 3 is the most common genotype in the infected individuals

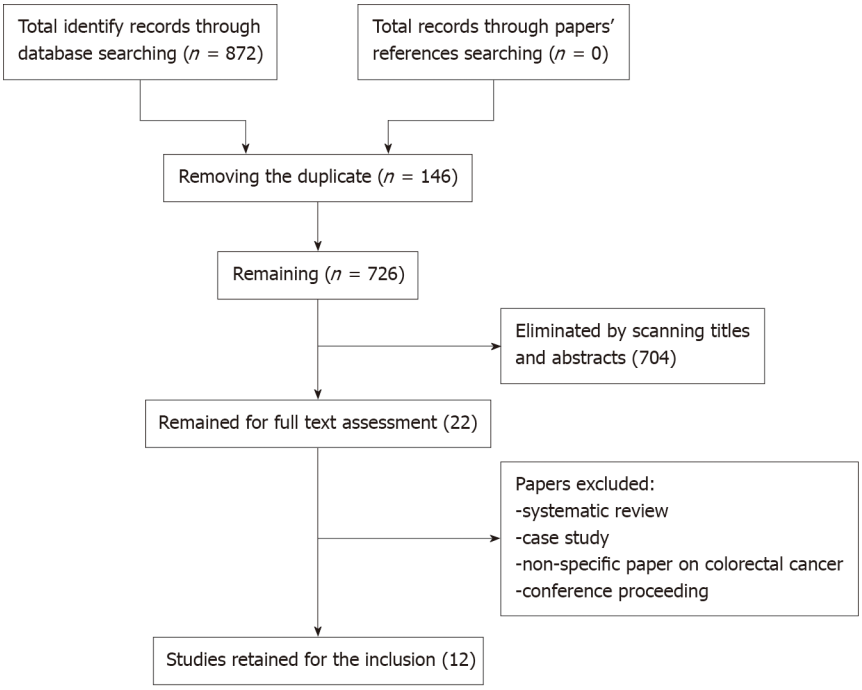
			with CRC. Study duration: 2010 and 2012		patients with CRC.	was significantly higher compared to control group. Subtype 3 was predominant compared to other subtypes. It was significantly higher in CRC group as compared with control group	
6	Chandramathi *et al*[1], 2012	Malaysia	Stool samples were obtained from 46 and 15 breast cancer and CRC patients, respectively	Department of Parasitology, Faculty of Medicine, University of Malaya	Aim: To investigate whether intestinal parasites can be an opportunistic infection in breast cancer and CRC patients who are undergoing chemotherapy treatment. Molecular detection of microsporidia species was done using a PCR technique. The presence of *Blastocystis hominis* was further confirmed by culturing stool samples	This study found that 7 out of 15 CRC patients were positive *Blastocystis* in various chemotherapy cycles accounting for 46.7%	*Blastocystis hominis* and microsporidia could appear as opportunistic infections during chemotherapy treatment of CP. This infection may diminish the efficacy of chemotherapy treatments and consequently advance the progression of cancer
7	Majeed *et al* [22], 2019	Iraq	116 faecal specimens with *Blastocystis* and *Helicobacter pylori* infection, 15 biopsy specimens from CRC patients	Middle Technical University/Baghdad 1st Feb 2018-15th June 2018	Faecal specimens were screened for *Blastocystis* and *Helicobacter pylori*. Direct DNA sequencing was done to evaluate mutations in CRC-associated molecular pathways	Prevalence of *Blastocystis* infection statistically insignificant in various age groups. Prevalence of *Blastocystis* infection was more in females [females 29 (46.9 %), males 22(43.1%)]. Prevalence of mixed infection (*Blastocystis* and *Helicobacter pylori*) was 27 (23.32%)	Prevalence of *Blastocystis* infection was more in females. *KRAS* and *TP53* gene mutation was observed in the CRC patients with mixed infection (*Blastocystis* and *H. pylori*)
In vitro studies							
8	Chandramathi *et al*[7], 2010	Malaysia	*In vitro* study model. PBMCs were isolated from blood collected from healthy persons. Solubilised antigen of *Blastocystis* isolate was obtained from a human subject. Human colorectal carcinoma cell line, HCT116, was used	University of Malaya, Kuala Lumpur	Effect solubilised antigen of *Blastocystis* on the HCT116 proliferation quantified. Gene expressions of certain genes in HCT116 and PBMCs evaluated *via* real-time reverse transcription PCR. PBMCs were isolated from blood using Histopaque technique. Cell proliferations were measured using MTT assay	Increased number of PBMCs/ HCT116 cells observed with *Blastocystis* antigen. *IFN-γ* and *TNF-α* were downregulated and *IL-6*, *IL-8* and *NF-κB*, *p53* were upregulated in the PBMCs treated with the antigen. *IFN-γ* was downregulated and *IL-6* and *NF-κB* was upregulated in HCT116 cells	Solubilised antigen of *Blastocystis* could facilitate increased number of PBMCs/ HCT116 cell and has the ability to downregulate immune cell responses
9	Chan *et al*[23], 2012	Malaysia	*In vitro* study model. Solubilised antigen of *Blastocystis* isolate was obtained from symptomatic and asymptomatic human subject. HCT116 was used	University of Malaya, Kuala Lumpur	Effects of solubilised antigen of *Blastocystis* isolate was obtained from symptomatic and asymptomatic human subject on HCT116. Gene expressions of certain genes in HCT116 and PBMCs evaluated *via* real-time reverse transcription PCR	Increased number of HCT116 cells observed with symptomatic *Blastocystis* antigen. Th2 cytokines/ *CTSB* were upregulated in HCT116. *NF-κB* was observed upregulated in HCT116 exposed to symptomatic *Blastocystis* antigen	Solubilized antigen *of Blastocystis* from symptomatic individual was more virulent than that in asymptomatic. Higher inflammatory reaction and increased proliferation of cancer cells was observed
10	Kumarasamy *et al*[24], 2013	Malaysia,	*In vitro* study model using HCT116 treated with solubilised *Blastocystis* antigen from 5 *Blastocystis* subtypes	University Malaya research Lab	*In vitro* study. HCT116 treated with solubilised antigen from *Blastocystis*. Following Assays: Proliferation of the cell line, HCT116 on exposure to different *Blastocystis* subtypes; Gene expression profile of apoptotic genes like *p53* and *CTSB*; Transcription factor gene expression profile	*Blastocystis* subtypes (5) increased the proliferation of HCT116, especially subtype 3. *Blastocystis* antigen caused the upregulation of Th2 and Th1 cytokines, and downregulation of *IFN-γ* and *p53* in HCT116 cells. *Blastocystis* antigen caused a higher stimulation of gene expression of *CTSB* and *TGF-β* genes	Infection with *Blastocystis* caused exacerbation of existing colon cancer cells. The effect may be due to weakening of the cellular immune response and dysregulation of *IFN-γ* and *p53* expression. Infection with *Blastocystis* subtype 3 has a higher pathogenic potential

11	Ahmed *et al* [25], 2019	Cairo, Egypt	Seven *Blastocystis* isolates were from stools specimen from patients with early diagnosed CRC (Oncology and Surgery and Colonoscopy unit) of a Hospital in Egypt. The different groups were: Group I (GI), 12 isolates from infected non-CRC; Group II (GII), 6 from infected symptomatic patients and Group III (GIII), 6 from infected non-symptomatic carriers	Department of Parasitology lab, Faculty of Medicine, Ain Shams University, Cairo, Egypt	Aim: To investigate some phenotypic characters like the surface ultrastructure, protein profiles and protease activity of *Blastocystis* from three different clinical groups. Techniques performed: Scanning electron microscopy to study morphology of the organism; SDS-PAGE to analyse the *Blastocystis* protein profiles and their protease activities	Observations: All CRC *Blastocystis* isolates showed a very rough intensely folded surface when compared to less rough and smooth surface of isolates from symptomatic and asymptomatic and non-CRC isolates; SDS-PAGE showed presence of 2 protein bands of 230 and 32 KDa in 42.9% of *Blastocystis* CRC isolates and these proteins were absent in Non-CRC isolates. When the protease activity of the parasite was tested, no significant difference existed between isolates of the three groups	There was significant difference in the surface structure and the protein profiles between different clinical isolates of *Blastocystis*. Differences indicate that it may be: (1) secondary to the altered gut environment in the presence of CRC or (2) indicators of a different pathogenic potential of the parasite in inducing malignancy
In Vivo studies							
12	Kumarasamy *et al*[26], 2017	Malaysia	Different specimens collected: Blood, urine, faecal samples and gastrointestinal tract sections from 24 male Wistar rats. Age of the rats: 3 wk. Weight of each rat: Average of 65 g/rat	University Malaya research Lab	*In vivo* experimental study. Aim: To investigate the effect of infection with *Blastocystis* cyst on exacerbation of carcinogenesis. Twenty-four rats divided into different groups for the study (4 groups, 6 rats each): Control group, AOM group, group inoculated with *Blastocystis* cyst, the group inoculated with *Blastocystis* cyst and AOM injection. Body weights recorded once a week. Rat faecal samples screened for presence of *Blastocystis* post-inoculation. Histopathological assessment of the rat colon for aberrant crypts. Urine and blood samples assessed for oxidative stress	Observations: lower body weight showed by *Blastocystis* infected rats than rats infected with *Blastocystis* and injected with AOM ($P < 0.05$). Stools from AOM-rats with *Blastocystis* infection were softer and watery compared to the AOM-rats without *Blastocystis* infection. *Blastocystis* was present in the stool of all infected rats from Day 3 to 7 post-inoculation. All the rats injected with AOM developed numerous abnormal, hyperplastic colonic crypts. Co-administration of *Blastocystis* cyst showed a 1.6-fold increase in the number of crypts when compared with control rats treated with AOM only. Two of the co-*Blastocystis* infected AOM-rats were found to have adenomas. Major dysplasia and presence of hyperplastic aberrant crypts were observed in rats injected with AOM and co-infected with *Blastocystis*	*Blastocystis* infection considerably enhanced the AOM-induced carcinogenesis because of the oxidative damage of the intestinal epithelium

AOM: Azoxymethane; CP: Cancer patients; CRC: Colorectal cancer; CTSB: Cathepsin B; IFN: Interferon; IL: Interleukin; NCP: Non cancer patients; NF-kB: Nuclear factor kappa B; PBMCs: Peripheral blood mononuclear cells; PCR: Polymerase chain reaction; SDS-PAGE: Sodium dodecyl sulphate–polyacrylamide gel electrophoresis.

predominant (54.5%) among CRC patients, while subtype II was predominant (43.7%) among COGT patients[19]. Higher prevalence of intestinal helminths and protozoa was observed in CRC patients than in the control population in a study conducted in Uzbekistan. The prevalence of *Blastocystis* in CRC patients was four times higher than that in the control population. The overall prevalence of *Blastocystis* (2.8%) was significantly higher than the other protozoa[20]. In a study by Kumarasamy *et al*[21] in Malaysia, among 221 control patients and 204 CRC patients with colorectal malignancies, the overall prevalence of *Blastocystis* infection was 15.29% (65/425). A total of 43 (21.08%) samples were positive for *Blastocystis* infection in CRC patients and was significantly higher compared to normal individuals (n = 22, 9.95%, $P < 0.01$). Subtype 3 was present at higher levels compared to other subtypes detected in both groups and was significantly higher in CRC patients as compared with control patients[21].

Another study was designed to investigate the emergence of *Blastocystis* and Microsporidia infections in breast and CRC patients undergoing chemotherapy treatment. This study found that 7 out of 15 CRC



DOI: 10.4251/wjgo.v14.i3.734

Figure 1 Search strategy.

patients were positive *Blastocystis* in various chemotherapy cycles, accounting for 46.7%. However, the researchers did not mention whether the isolate was from the same patients in different cycles[1]. In a study carried out in Iraq, stool samples from 116 patients with *Blastocystis* and *H. pylori* infections were investigated. Fifteen tissue samples of CRC were taken from 15 suspected patients out of 116 infected cases, and it was shown that the infection with *Blastocystis* and *H. pylori* was associated with pathological gene mutation in the CRC patients[22].

In vitro colorectal carcinoma cell line studies on the cytopathic and immunological effects of Blastocystis antigen

Three of the reviewed articles used *in vitro* study models and observed considerable cytopathic and immunological effects induced by the solubilised antigen of *Blastocystis* to the human colorectal carcinoma cell line[7,23,24]. These findings speculated that *Blastocystis* infection may enhance the proliferation, invasiveness and metastatic properties of CRC cells. One study investigated some phenotypic characteristics of *Blastocystis* isolated from CRC patients[25].

The three *in vitro* model studies used the human colorectal carcinoma cell line HCT116 and the *Blastocystis* isolated from a human subject[7,23,24]. One of the studies demonstrated the cytopathic effect of *Blastocystis* antigen on peripheral blood mononuclear cells. This study findings showed increased cell proliferations in *Blastocystis* antigen-stimulated HCT116 cell-lines, which suggested that the infection by *Blastocystis* may facilitate the growth of colon cancer cells[7]. Another *in vitro* study showed that the five subtypes of *Blastocystis* significantly increased the proliferation of HCT116, especially subtype 3. *Blastocystis* antigen caused the upregulation of T helper (Th)2 and Th1 cytokine gene expressions, and downregulation of interferon gamma and *p53* gene expressions in HCT116 cells. In addition, *Blastocystis* antigen caused a significantly higher stimulation of Cathepsin B (CTSB) and Transforming growth factor beta (TGF-β) gene expression, which indicates the pathogenic potential of this protozoan[24]. Another study showed an increase in cell proliferation in HCT116 cells inoculated with the symptomatic *Blastocystis* antigen. Gene expression studies carried out in this research also showed a significant upregulation of Th2 cytokines, which indicates the parasites' potential in weakening the cellular immune response[23]. HCT116 cells exposed to symptomatic and asymptomatic *Blastocystis* antigen caused a significant upregulation of *CTSB*, which led to the postulation that the *Blastocystis* antigen may enhance the invasive and metastasis properties of CRC[23].

Another study sought to investigate some phenotypic characteristics such as the surface ultrastructure, protein profiles and protease activity of *Blastocystis* isolated from three different clinical groups: CRC patients, non-CRC symptomatic and asymptomatic infected persons. This study showed the presence of two protein bands of 230 and 32 KDa in 42.9% of *Blastocystis* CRC isolates with their complete absence from non-CRC isolates. There was no significant difference in the protease activity of the protein among isolates of the three groups, CRC and Non-CRC *Blastocystis* isolates[25].

In vivo experimental animal model to investigate the effects of infection with Blastocystis on exacerbation of colorectal carcinogenesis

An animal model study compared the effects of *Blastocystis* infected rats and rats infected with *Blastocystis* co-administered with Azoxymethane (AOM), a potent carcinogen. This finding showed that the co-administration of *Blastocystis* cyst resulted in a 1.6-fold increase in the number of colonic crypts when compared with control rats treated with AOM only. Two of the co-*Blastocystis* infected AOM rats were found to have adenomas. Major dysplasia and the presence of hyperplastic aberrant crypts were also observed in rats injected with AOM and co-infected with *Blastocystis*[26].

DISCUSSION

Blastocystis is one of the most common gut microorganisms found in healthy individuals[27]. Besides being associated with a healthy gut microbiota[27,28], *Blastocystis* infection is also known to be opportunistic in immunocompromised patients[29]. CRC is the third most common cancer diagnosed worldwide and one of the major causes of cancer-associated fatality[30]. The reason for high mortality is due to the asymptomatic progression of the disease that usually results in late diagnosis[31,32]. Certain gut microorganisms are known to be one of the important factors that had been associated with initiation and development of CRC[33]. However, data on the roles of parasites are vague and restricted. Various findings have been reported regarding the association between *Blastocystis* among CRC patients, whereby positive association was shown in all the studies[1,23,24]. Therefore, the aim of this systematic review was to quantify the studies published so far that revealed the direct association of *Blastocystis* and CRC. This paper outlines the results of a systematic review to evaluate the prevalence of *Blastocystis* in CRC patients, *in vitro* studies using *Blastocystis* antigen and an *in vivo* study using animal models.

Out of the data extracted from 12 studies relevant to this topic, all the studies showed positive association between *Blastocystis* and CRC. Prevalence studies, *in vitro* investigations and *in vivo* studies were used to evaluate the pathogenicity of *Blastocystis* with CRC.

The global prevalence of *Blastocystis* infection ranged from 1.5%-20% in developed countries, which was much less than that in developing countries, which was 30%-50%[34]. Based on our review, the prevalence of *Blastocystis* infection in CRC patients ranged between 2.8%-46.7%. It has been widely reported in the world, in developing countries such as Iran, China, Saudi Arabia, Uzbekistan and Malaysia[18-20,24,35]. The first demonstration of *Blastocystis* infection in Iran was reported in 11 CRC patients in Tehran province[35]. In Malaysia, a total of 43 samples were positive for *Blastocystis* from 204 CRC patients[21]. This study utilised colonic washout in addition to stool sample to recover the parasites. Subtype 3 *Blastocystis* was detected predominantly as compared to other subtypes[21]. Some of these findings highlighted the high prevalence of certain subtypes of *Blastocystis* among these patients [19,21]. A previous study showed that subtype 1 was the most common genotype identified (54.5%) among CRC patients[19]. DNA was extracted from *Blastocystis* cultures *via* conventional method and subtyped using multiplex polymerase chain reaction (PCR) with restriction fragment length polymorphism and sequence-tagged site primers-based PCR. In another study, subtype 3 was predominant compared to other subtypes found in both CRC patients and healthy individuals[21]. Subtype 3 is speculated as the most pathogenic subtype in symptomatic individuals[36]. Some researchers have attributed subtype 3 to be more pathogenic compared to other subtypes[37,38]. *Blastocystis* was initially screened *via in vitro* culture and conventional PCR using stool samples and colonic washouts. The presence of *Blastocystis* infection in CRC patients could be contributed by various reasons including health status. For instance, positive cases were more likely in patients with gastrointestinal symptoms compared to healthy individuals[18]. Besides, *Blastocystis* was also identified in higher frequency in immunosuppressed CRC patients who were undergoing chemotherapy treatment[1,18].

A total of three *in vitro* studies were carried out using colorectal carcinoma cell line models to study cytopathic and immunological effects of *Blastocystis* antigen[7,23,24]. Some of the research studies suggested that solubilised antigen of *Blastocystis* could facilitate the exacerbation of CRC cells, HCT116 [7,23]. In another study by Kumarasamy *et al*[24], *Blastocystis* subtype 3 stimulated significantly higher *CTSB* and *TGF-β* gene expression in HCT116, which indicates the pathogenic potential of this protozoan. Result of *in vitro* studies that were performed in Malaysia were similar[7,23,24].

Blastocystis is commonly found in both patients with gastrointestinal symptoms and in healthy people widely across the world. More recently, researchers consider *Blastocystis* as an emerging zoonotic disease, and its pathogenic potential in human is unclear[9]. The pathogenic potential of *Blastocystis* was widely debated, as they are found in both symptomatic and asymptomatic patients. The significant expression of nuclear factor kappa light chain enhancer of activated B cells was observed in HCT116 exposed to *Blastocystis* antigen isolated from individuals with gastrointestinal symptoms, but such observations were not found when the colon cells treated with *Blastocystis* antigen isolated from asymptomatic individuals. This finding shows the potential pathogenicity of symptomatic *Blastocystis* in CRC patients[23]. Similarly, HCT116 cells exposed to symptomatic and asymptomatic *Blastocystis*

antigen caused a significant upregulation of *CTSB*. These gene expression findings lead to a postulation that the *Blastocystis* antigen may enhance the invasive and metastasis properties of CRC[23]. Besides, proliferation of HCT116 when exposed with *Blastocystis* antigen could be a result of higher levels of interleukin (IL)-6 and IL-8 expression[23].

Another study revealed that solubilised antigen isolated from subtype 2 and 3 isolates introduced to colon cancer cells showed significant IL-8 and IL-6 expression[24]. The production of inflammatory cytokines such as IL-8 together with reactive oxygen species could contribute to the pathogenesis of cancer[38]. In a few studies conducted previously, IL-6 expression was associated with proliferation of colon carcinoma[39,40]. Besides that, subtype 3 *Blastocystis* also triggered positive expression of *CTSB* in cancer cells. A previous study showed that *CTSB* expression is significant in CRC patients[41].

Only one study was conducted to investigate the *in vivo* effect of *Blastocystis* in Wistar rats. In parallel with *in vitro* studies, an *in vivo* study showed similar findings on the possible role of *Blastocystis* to exacerbate CRC. The results demonstrated that *Blastocystis* may cause damage to the intestinal mucosal layer and result in increased crypts formation. Furthermore, an increased oxidative stress was also observed in these rats. There have been numerous animal models of human CRC and animal model of tumour carried out *via* quantification of aberrant crypt foci[42,43]. This allows the study of gut microbiome and its role in pathogenesis. In humans, there are many potential pathogenic and non-pathogenic gut microbial infections, and various animal models have been used for such studies. Aberrant crypt foci are known as putative precancerous lesions of the colon in both animal models and humans[44,45]. Even though various studies associating *Blastocystis* and CRC were carried out *via in vitro* model using colon cancer cells, this study utilised animal model to bridge between *in vitro* findings in the laboratory and studies in humans. As such, this extensive *in vivo* study showed that *Blastocystis* had a major impact on normal intestinal function in Wistar rats resulting in damage to the intestinal mucosal layer and inducing oxidative stress, which caused increase in crypts formation in AOM-treated rat models. The study establishes that *Blastocystis* is a pathogen, and there is a need to screen cancer patients for harbouring this parasite.

This systematic review has some limitations. According to these investigations, a greater prevalence of *Blastocystis* was found in CRC patients, but the question whether increased prevalence of *Blastocystis* could be linked with increased high risk to CRC is unclear. The studies discussed in this review did not highlight the association of *Blastocystis* according to cancer stages, and it was unclear if *Blastocystis* itself could result in the initiation of malignancy as *Blastocystis* acquisition alone is insufficient for cancer development. Even though strong association between *Blastocystis* and CRC is apparent, some questions remain unanswered. Therefore, we propose future studies should focus on the pathogenicity of *Blastocystis* in various stages of CRC by concentrating on the molecular pathways involved in tumorigenesis.

CONCLUSION

In conclusion, according to various recent studies, *Blastocystis* is one of the most commonly identified microorganisms in CRC patients, whereby subtype 1 and subtype 3 were predominantly isolated. It is apparent in most cases that the prevalence is higher in developing countries compared to developed countries. These studies have provided supportive data that *Blastocystis* could exacerbate existing CRC *via* alteration in host immune response and increased oxidative damage. An *in vivo* study well-established that *Blastocystis* infections resulted in tissue damage from host inflammatory responses that may predispose the host towards neoplasm exacerbation. Upregulation of gene expression responsible for proinflammatory cytokines and downregulation of apoptotic genes was observed in *in vitro* studies. Through continued research in *Blastocystis* and CRC, we may discover new findings as well as develop new effective means of prevention. Future studies of CRC and *Blastocystis* should attempt to determine the various stages of CRC that are most likely to be associated with *Blastocystis* and its association with other intestinal bacteria. In addition, future *in vivo* studies should evaluate exposure to various subtypes of *Blastocystis*.

ARTICLE HIGHLIGHTS

Research background

Intestinal colonisation of *Blastocystis hominis* (*Blastocystis*) as a risk factor to the worsening of colorectal cancer (CRC).

Research motivation

There has been an increase in the prevalence of *Blastocystis* in CRC patients. Besides, various *in vitro* and *in vivo* studies have highlighted *Blastocystis* as an important risk factor for the worsening of CRC.

Research objectives
To perform a systematic review on all evidence on the association between CRC and *Blastocystis*.

Research methods
A systematic review of the literature was performed by searching PubMed, Science Direct, Scopus and Google scholar databases up to February 2020.

Research results
Out of 12 studies selected for this systematic review, seven studies have confirmed the prevalence of *Blastocystis*. A total of four studies employing *in vitro* human colorectal carcinoma cell line study models showed significant cytopathic and immunological effects of *Blastocystis*. One *in vivo* experimental animal model study showed that there was a significant effect of infection with *Blastocystis* on exacerbation of colorectal carcinogenesis.

Research conclusions
Blastocystis is a commonly identified microorganisms in CRC patients. These studies have provided supportive data that *Blastocystis* could exacerbate existing CRC *via* alteration in host immune response and increased oxidative damage.

Research perspectives
Future studies of CRC and *Blastocystis* should attempt to determine the various stages of CRC that are most likely to be associated with *Blastocystis* and its association with other intestinal diseases.

FOOTNOTES

Author contributions: Azzani M designed the research, performed the literature search and extracted the data; Kumarasamy V wrote the discussion and extracted the data; Atroosh WM wrote the methodology and extracted the data; Anbazhagan D wrote the results and extracted the data; Abdalla M wrote the introduction and extracted the data; All authors read and approved the final manuscript.

Conflict-of-interest statement: Dr. Azzani has nothing to disclose.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

Country/Territory of origin: Malaysia

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S-Editor: Chang KL
L-Editor: Filipodia
P-Editor: Chang KL

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