PREVALENCE OF CRYPTOsporidium SP. IN PATIENTS WITH COLORECTAL CANCER*

Violetta Sulżyc-Bielicka1, Lidia Kołodziejczyk2, Sylwia Jaczewska, Dariusz Bielicki3, Józef Kładny4, Krzysztof Safranow5

Department of Clinical Oncology, Pomeranian Medical University
Kierownik: dr hab. V. Sulżyc-Bielicka
Chair and Department of Biology and Medical Parasitology, Pomeranian Medical University
Kierownik: prof. dr hab. E. Kalisińska
Department of Gastroenterology, Pomeranian Medical University
Kierownik: prof. dr hab. T. Starzyńska
Department of General and Oncological Surgery, Pomeranian Medical University
Kierownik: prof. dr hab. J. Kładny
Biochemistry and Medical Chemistry
Kierownik: prof. dr hab. D. Chlubek

Parasitic protozoans of the Cryptosporidium genus are intracellular intestinal parasites of mammals, causing cryptosporidiosis. Clinically, cryptosporidiosis manifests as chronic diarrhoea. Individuals with immune disorders, including those with neoplasms, are at risk of symptomatic invasion.

The aim of the study was the evaluation of Cryptosporidium sp. prevalence in patients with diagnosed colorectal cancer.

Material and methods. The studied group encompassed 87 patients with diagnosed colorectal cancer, undergoing surgery at the Department of General and Oncological Surgery, Pomeranian Medical University, in the years 2009-2010. Immunoenzymatic tests for Cryptosporidium sp. on faeces samples were performed with the use of commercial test kit, ProSpecT®Cryptosporidium Microplate Assay (Remel Inc).

Results. The presence of Cryptosporidium sp. was found in 12.6% of studied patients with colorectal cancer. The performed statistical analysis did not reveal any correlation between Cryptosporidium sp. infection and gender, age, neoplasm advancement stage as per Astler-Coller scale, neoplasm differentiation grade, or neoplastic tumour localisation in relation to the splenic flexure.

Conclusions. There was found high prevalence of Cryptosporidium sp. in patients with colorectal cancer. It was comparable to the prevalence reported for patients with immune deficiency.

Keywords: Cryptosporidium, colorectal cancer

Protozoans of the Cryptosporidium genus are intracellular intestinal parasites common in humans and animals. The Cryptosporidium genus was first described in 1907 by Tyzzer, following the identification of C. muris in mice, while the first report of human cryptosporidiosis comes from 1976 (1). To date, there have been over 20 species of this genus distinguished (2), among from which the cause of human cryptosporidiosis are mainly C. hominis (previously C. parvum, genotype 1) and C. parvum (previously C. parvum, genotype 2). It is estimated that poor sanitary conditions cause 250-500 million C. parvum infections annu-
ally in African, Asian and Latin American developing countries (3). The Cryptosporidium sp. infection in healthy individuals is asymptomatic or results in transient spontaneously retreating aqueous and mucous diarrhoea. Cryptosporidium, as an opportunistic parasite, constitutes a threat for individuals with acquired or congenital immune deficiency. Neonates and small children, in whom the immune system is not sufficiently developed yet, the elderly, HIV carriers and patients post organ transplantation or chemotherapy in neoplastic diseases are at risk of symptomatic invasion. In the above groups of patients, due to chronic diarrhoea, water and electrolyte disorders, and absorption and nutrition disorders may develop, leading even to patient’s death in extreme cases (4). In patients with AIDS, cryptosporidiosis, along with toxoplasmosis and pneumocystosis, constitutes a direct threat to life. The prevalence of Cryptosporidium sp. infections in patients with AIDS is estimated at over 40% (5, 6). In the above group of patients, there is observed the highest percentage of fatal cryptosporidiosis cases (7, 8, 9). In Poland, Cryptosporidium infection was found in 6.7%-12.8% of HIV carriers (10, 11).

Secondary immunodeficiency may also occur in patients with diagnosed neoplasm. Recent reports indicate the potential involvement of Cryptosporidium parvum in colorectal cancer carcinogenesis (12-15). It appears justified to perform studies evaluating the prevalence of Cryptosporidium infection in patients with diagnosed colorectal cancer prior to the initiation of oncological treatment.

**MATERIAL AND METHODS**

The study was conducted on a group of 87 patients with colorectal cancer undergoing surgery at the Department of General and Oncological Surgery, Pomeranian Medical University, in the years 2009-2010. The clinical and pathological data are presented in tab. 1.

Parasitological faeces testing was performed in patients with histopathologically confirmed diagnosis of colorectal cancer at the time of their admission to the Department for elective surgery. None of the patients had previously received chemotherapy. In the group of patients with rectal cancer, 9 had patients received preoperative radiation therapy. Cryptosporidium sp. infection was not found in any of the patients treated with preoperative radiation therapy, therefore, those patients were not excluded from the analysis.

Immunoenzymatic testing for Cryptosporidium sp. on faeces samples was performed with the use of commercial test kit, ProSpecT® Cryptosporidium Microplate Assay (Remel Inc).

**Statistical Analysis**

The correlation between Cryptosporidium sp. infection and nominal variables was analysed with the use of chi-squared test or Fisher’s exact test, and the age – with the Mann-Whitney U test. The statistical significance threshold was set at p < 0.05. Calculations were performed with the use of STATISTICA 10 software.

**RESULTS**

The presence of Cryptosporidium sp. was found in 12.6% of studied patients. In 7 out of the 11 infected individuals, cancer was localised in the rectum (63.6% positive tests), in 2 of them – in the sigmoid colon, and in 1 – in
the ascending and transverse colon. The performed statistical analysis did not reveal any correlation between the infection and patient’s gender, age, neoplasm advancement stage as per the Astler-Coller scale, neoplasm differentiation grade, or neoplastic tumour localisation in relation to the splenic flexure. The prevalence of Cryptosporidium sp. infection was twice higher in rectal tumours (16.7%) as compared with colon cancer (8.9%), however, the above difference was not statistically significant. The analysis of Cryptosporidium sp. infection prevalence in relation to the cancer differentiation grade indicated twice higher prevalence of this infection in G1+G2 cancers as compared with G3 cancers (14.9% vs. 7.1%), but the above difference was not statistically significant either.

In addition, microscopic faeces examination revealed the presence of Giardia lamblia in 1 individual, Entamoeba coli in 1 individual, and Blastocystis hominis in 9 patients.

**DISCUSSION**

Since 2001, cryptosporidiosis has been on the list of infections and infectious diseases subject to statutory reporting obligation in Poland. However, the first cases of cryptosporidiosis were not included in epidemiological reports of NIH until 2008 (16). Only few laboratories perform testing for cryptosporidiosis, therefore, knowledge on the prevalence of this parasitosis in humans is incomplete. In Polish studies on children with primary immunodeficiency, there have been found C. hominis, C. meleagridis and C. parvum, while in the studied immunocompetent individuals or patients with secondary immunosuppression, infection with C. parvum only has been observed (17).

The present study results, similarly as those in earlier publications (18), indicate high prevalence (12.6% of studied patients) of Cryptosporidium infection in patients with colorectal cancer, while studies conducted by Sreedharan and colleagues (19) and Radrapatna and colleagues (20) on larger populations of patients with diagnosed neoplasms at different sites have demonstrated markedly lower infection rates (1.3% and 0.3%, respectively). The above differences may stem from our selection of patients with colorectal cancer and from different diagnostic methods used. It should be noted that the studies conducted by Certad and colleagues (12-15) have suggested a correlation between Cryptosporidium parvum infection and colorectal cancer carcinogenesis. Certad and colleagues (12) have demonstrated on an animal model a correlation between cryptosporidiosis and the development of gastrointestinal adenomatous polyps or adenocarcinomas. In addition, the above authors have described a case of massive cryptosporidiosis in a patient post bone marrow transplantation (15). The infection of SCID mice with a C. parvum strain isolated from the above patient have caused the development of gastrointestinal adenocarcinomas and biliary tract cancer. Cryptosporidium has been found in the neoplastic tissue of infected mice (15).

Of note in the present study was the twice higher prevalence of Cryptosporidium sp. infection in patients with rectal cancer as compared with those with colon cancer, although the above difference was not statistically significant. However, such results may stem from the small size of the studied group.

It should be emphasised that viral, bacterial and parasitic infections may affect carcinogenesis (21). It is estimated that in over 20% of cases worldwide neoplasm development is associated with a viral, bacterial or parasitic infection (22). Recently, there has been demonstrated a correlation between Helicobacter pylori infection and gastric cancer development, HBV and HCV infection and hepatocellular carcinoma development, and HPV infection and cervical cancer development (23). Parasites may act as carcinogens as well (24). There has been evidenced a correlation between Schistosoma haematobium infection and squamous cell bladder cancer development. The International Agency for Research on Cancer (IARC) has also deemed Opisthorchis viverrini and Clonorchis sinensis infections to be carcinogenic factors in the development of biliary tract cancer (24). Those chronic parasitoses may affect apoptosis and DNA repair disturbances (25, 26).

In summary, the high prevalence of Cryptosporidium sp. in patients with colorectal cancer described in the present publication is comparable to that reported for patients with immunodeficiencies. The obtained results justify the conduct of studies evaluating the association between Cryptosporidium infection and carcinogenesis of this neoplasm.
REFERENCES