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# *Full Length Research Article*

## THE INVESTIGATION OF CRYPTOSPORIDIOSIS DURING CHEMOTHERAPY IN CANCER PATIENTS

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**ABSTRACT**

*Cryptosporidium* spp.is a parasite more common in immunocompromised individuals and causes a symptomatic enteritis. In this study, we were aimed to investigation of relationship between the process of chemotherapy and incidence of *Cryptosporidium* spp. in cancer patients. The stool samples were obtainedfrom 57 cancer patientsduring chemotherapy atthe Oncology Center of Cumhuriyet University Hospital and also from 65 healty person as control groups. Personal characteristicsandclinical data of paDCERFVtientswere writtentoa questionnaire such as how long they havereceived chemotherapy. All samples were examined for *Cryptosporidium* spp. by Direct Fluoresan Antibody (DFA).The Cryptosporidium antigen was determined17.5% by DFA in 57 cancer patients.Also, antigen was determined6.1% by DFA in control group ($\chi^2$=3.87 p<0.05). The relationship between the length of the chemotherapy of cancer patients and incidance of Cryptosporidiosis was determined to be statistically significant. Chemotherapy treatment process of individuals who were DFA positive on average 19.4 weeks, while the group of patients who were DFA negative average of 12.7 weeks (p<0,05 Mann-Whitney U=81,0 ).In Conclusion, immun-suppressed individuals against easly transmitted parasites from environment such as *Cryptosporidium* spp. must be protected. Particularly in patients receiving cancer treatment, if chemotherapy process becomes longer, may increase the risk of cryptosporidiosis

**Key words:** Cryptosporidium spp Chemotherapy, DFA

## INTRODUCTION

Cryptosporidiosis, usually presenting as a gastro-enteritis-like syndrome, caused by infection with protozoan parasites of *Cryptosporidium* spp. intestinal cryptosporidiosis occurs in immune-compromised patients severe, chronic disease may occur and infection can be fatal.The majority of human infections are with *C. parvum* and *C. hominis* and infection occurs in both immune-competent and immune-compromised populations. Disease ranges in seriousness from mild to severe and signs and symptoms depend on the immune status of the host. Diarrhoea is generally watery and voluminous; between three and six stools may be passed each day. Other symptoms are abdominal pain, nausea or vomiting, anorexia and weight loss can be occur. Blood and leukocytes are not present in the stool. Symptoms last up to three weeks. Oocysts may continue to be shed for a mean period of 7 days. At least eight of the currently identified 20 *Cryptosporidium*species have been detected in humans. Patients with T-cell immune deficiency are at most risk, including those with haematologicalmalignancies, primary T-cell deficiencies and HIV patients with CD4+ lymphocyte counts of <50/mm3. The oocysts survive in moist environments. *Cryptosporidium* spp. Oocysts are resistant to used disinfectants recommended concentrations. Infection occur by ingestion of oocysts, direct contact with human or animal faeces, contaminated water, food. The causative mechanisms of Cryptosporidiosis have not been fully elucidated. The watery nature of the diarrhoea resemblessecretory. But, *Cryptosporidium* toxin has not been isolated yet. There are four clinical syndromes of cryptosporidiosis in AIDS patients with CD4+ counts <200/mm3: transient diarrhoea, relapsing illness, chronic diarrhoea, and cholera-like illness. Chronic diarrhoea and cholera- like illness with severe weight loss predominated in these patients. In this study, based on the above information to investigate the relationship between the process of chemotherapy and the prevalence of cryptosporidiosis in immunocomprimised cancer patients was aimed.

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## MATERIALS AND METHODS

### Collection of samples

Stool samples were collected from 57 cancer patients receiving chemotherapy at the Center of Medical Oncology of Cumhuriyet University between September 2009 to September 2012. Patients who planned to collect a stool sample were informed in advance regarding study and were asked the samples of stool after approval. The samples were brought to the laboratory in plastic containers by the patients.

The patient's name, surname, age and gender was written to the label on the containers. In addition, for each patient, what type of cancer they had, when they started chemotherapy, such information was taken. Stool samples were placed in 10% formalin for DFA studies. Stool specimens could be taken once from patients. Creating working groups not only from patients with diarrhea, at that time all of the patients who agreed samples were taken. The ages of the patients in the study ranged from 2 to 65 and 30 male 26 were female. To create a control group fecal specimens were obtained from 65 healty people. Demographic characteristics of the people in the control group were also recorded. All stool samples were studied by DFA test (MERIFLUOR® C/G, Cincinati, Ohio USA). Tests were run as the manufacturer suggested and using the fluorescent microscope (Olympus BX50, Japan) were evaluated.

## RESULTS

10 of the 57(17.5%) cancer patients receiving chemotherapy, 4 of the control group consisting of 65 (6.1%) healty people were found positive by using DFA method ($\chi^2$=3.87 p<0.05). Cancer types in the oncologic patient group and *Cryptosporidium* incidence are presented in Table 1.

**Table 1.Patient group and positivity by using DFA method (N: Total patient no, n: positive no)**

| | DFA +n | % |
|---|---|---|
| Lung (N=10) | 2 | 20 |
| Labium (N=1) | 1 | 100 |
| Column (N=12) | 1 | 8.3 |
| Breast (N=14) | 4 | 28,6 |
| Prostate (N=1) | 1 | 100 |
| Rectum (N=4) | 1 | 25 |
| Larynx+Lung(N=2) | 0 | 0 |
| Cervix (N=1) | 0 | 0 |
| Mesothelioma(N=1) | 0 | 0 |
| Endometrium(N=1) | 0 | 0 |
| Stomach (N=2) | 0 | 0 |
| Nasopharynx(N=2) | 0 | 0 |
| Ovary (N=2) | 0 | 0 |
| Periampullary(N=2) | 0 | 0 |
| Esophagus(N=1) | 0 | 0 |
| Pancreas (N=1) | 0 | 0 |
| Total (N=57) | 10 | 17,9 |

As seen in the table, there is no significant corralation between the cancer types and the presence of *Cryptosporidium* spp. However, the common features are getting chemotherapy. Furthermore, false-positive and cross reaction rarely found as a result of the DFA method.

| DFA | Cryptosporidium | |
|---|---|---|
| | + | - |
| Chemotherapy process (week) | 19,4 | 12.7 |

p<0,05 Mann-Whitney U=81,0

Depending on the length of time of the immune suppression increased the prevalence of *Cryptosporidium*. Chemotherapyin patients with longerduration of20 weeks, increasedincidence ofCryptosporidium spp. and the difference between the two groups was statistically significant. Also in the direct examination of stool samples of patients in the study group; in two patients *Giardia intestinalis*, in one patient *Entamoeba histolytica* and in one patients *Blastocystis hominis* was found.

**Conclusion**

Cryptosporidiosis in humans and animals of any age, but they appear to be more prevalent and symptomatic especially at a young age. Thefirsthumancasesof cryptosporidiosiswere reportedin 1976 (Griffiths, 1998). Cryptosporidium is now recognized as a significant cause of diarrhea in humans and as a zoonosis may be acquired from animals (Griffiths, 1998). *Cryptosporidium* can create gastroenteritis in these individuals. However, individuals with inadequate immune system, can be fatal.Cryptosporidiosis are heavily in AIDS patients and can be life threatening, also parasites can be detected in tissue and organs such as respiratory system, liver, biliary tract (Griffiths, 1998.,Tzipori,*et al.*,2008.,Mac Kenzie, *et al.*,1994., Mosier,*et al.,*2000). Serological methods are also used in the diagnosis of the disease. Microscopic diagnosis is usually wants to experience. Different methods in the studies of the prevalence of cryptosporidiosis have been used in our country.In these studies in immunocompromised hosts;Tanyüksel *et al.*(1995) 17% (16), Ok et al 18.8-35.5% (Ok, *et al.*,1996., Ok, *et al.*,1997), Dökmetaş *et al.*(1998) 19.1%, Arikan and others(1996) 38.8%, Tamer *et al.* (2008) 12.35% in the ratio have determined cryptosporidiosis.

In studiesof the prevalenceofcryptosporidiosisin the world; InEurope and America,1-3%, while in developing countries tovary from5-10% has been reported (Hunter, *et al*., 2002). Various tests have been developed for the diagnosis of *Cryptosporidium*. Microscopicdiagnostic methodsistheirfirst. Oocysts can be detected using distinctive staining methods from feces and tissue. In tests using polyclonal and monoclonal antibodies are very high sensitivity and specificity such as DFA, ELISA, but the cost is higher (Graczyk, *et al*., 1996). Fluorescent techniquesthat make use ofspecificmonoclonal or polyclonal antibodiesare commonly used in Cryptosporidiosisdiagnosis.DFA compared with modified acid fast method (MAF), DFA's sensitivity 100%, specificity was found to be 97%.In another study, Enzyme immunoassay (EIA) sensitivity 93% and specificity was found as 99% (Graczyk, *et al*., 1996).

In this study, the presence of Cryptosporidium oocysts were investigated using the DFA method in stool samples of cancer patients, In 10 of 57(17.5%) cancer patients were obtained *Cryptosporidium*spp. oocysts. It is thought thata relationship betweencolon cancerandchronic inflammation.*C.parvum*is aprotozoanthat causespathologicalchanges in the gastrointestinalepithelium.*Cryptosporidium* spp. are accused of causing colorectal cancer in immunocompromised persons. In colorectal cancer, cyclin D1, which is the basis of cell growth cycle oscillation, were found to have above normal. Creating infection with *Cryptosporidium*in immun system suppressed mice, histopatlogical changes have been investigated. Cyclin D1 have been investigated with immunohisto chemical staining method. In these infected mice were found to have intestinal dysplastic changes. Cyclin D1, the detection of intestinal dysplasia has also been reported to be a good marker (Abdou *et al.*, 2013). PCR tests are also used in the diagnosis of *Cryptosporidium* spp. in recent years (Rafiei, *et al.,*2014., Melrose, *et al.,*2007). In one of these studies, *Cryptosporidium* spp. were detected in 16 (4.1%) patient in the patient group consisting of 390 individuals, by PCR. 11 of these 16 cases is C.parvum, 4 cases is C.hominis, one is designated as C.meleagridis(Rafiei, *et al.,* 2014).

Tandon et al, in a case with haematolymphoid malignancies, reported that they have identified cryptosporidiosis. This patient diagnosed with multiple myeloma was reported to be watery diarrhea since 15 days (Tandon, *et al.,* 2014). Tanyüksel *et al.*(1995) in a similar study in cancer patients, 17% percent were detected positive. However, they did use different diagnostic methods. Sönmez*et al.* (2008), in child leukemia and lymphoma patients the prevalence of cryptosporidiosis have been found 12.5%.This rate is lower than our findings. In our study, long-term chemotherapy has been found to be higher than the incidence of parasites in patients compared with patients receiving chemotherapy shorter time difference between them was statistically significant ($p < 0.05$).A comparison in this way not found in similar studies. Uppal *et al*.(2014), have been investigated *Cryptosporidium* spp. in 58 AIDS patients by Ziehl-Nielsen (ZN), ELISA and PCR methods. *Cryptosporidium*spp. were observed in 17 patients (29.4%) by ZN, in 39 patients (67.3% ) by ELISA, in 45 patients (77.5%) by nested PCR (Uppal *et al*.,2014). These rates are higher than the rate in patients with cancer detected earlier.

In cancer patients who we studied, *Cryptosporidium* spp. was detected in 17.5% by DFA. However Arikan *et al*. (1996) all of five HIV-positive patients in the study were also observed *Cryptosporidium* spp. According to these results, *Cryptosporidium* spp were observed higher in HIV patients. Izadi *et al*.(2012) have been found *Cryptosporidium* spp 6%, in 13 immunodeficiency patients by using 18SrRNA gene amplification and sequencing and by ZN. Then genotyping was performed. While *C.parvum* was detected in 8 patients, *C.hominis* have been obtained in 5 patients. In the another study, *Cryptosporidium* spp have been found in two lymphoblastic leukemia patients. Domeneck reported the development of cholangitis in one of these patients due to *Cryptosporidium* (Domenech, *et al.,* 2011).

It is known that the immun system of insufficient or immunocompromised individuals is more easily affected by the from infectious agents. These individuals must be protected, against parasites such as *Cryptosporidium* spp. can be transmitted easily from the environment.In particular, the longer the process of chemotherapy in cancer patients, it is necessary to be more careful against these infectious agents.

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