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 obtained from 57 cancer patients during chemotherapy at the Oncology Center of Cumhuriyet University Hospital and also from 65 healthy person as control groups. Personal characteristics and clinical data of patients were written in a questionnaire such as how long they have received chemotherapy. All samples were examined for Cryptosporidium spp. by Direct Fluorescent Antibody (DFA). The Cryptosporidium antigen was determined with DFA in 57 cancer patients. Also, antigen was determined with DFA in control group (P=3.87 p<0.05). The relationship between the length of the chemotherapy of cancer patients and incidence 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, immunosuppressed individuals against easily 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 haematological malignancies, primary T-cell deficiencies and HIV patients with CD4+ lymphocyte counts of <500/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 resembles secretory. 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 immunocompromised cancer patients was aimed.

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 healthy people. Demographic characteristics of the people in the control group were also recorded. All stool samples were studied by DFA test (MERIFLUOR® C/G, Cincinnati, 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%) healthy people were found positive by using DFA method (χ²=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)

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>DFA +n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung (N=10)</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>Labium (N=1)</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Column (N=12)</td>
<td>1</td>
<td>8.3</td>
</tr>
<tr>
<td>Breast (N=14)</td>
<td>4</td>
<td>28.6</td>
</tr>
<tr>
<td>Prostate (N=1)</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Rectum (N=4)</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>Larynx+Lung (N=2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cervix (N=1)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mesothelioma (N=1)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Endometrium (N=1)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Stomach (N=2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nasopharynx (N=2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ovary (N=2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Peripapillary (N=2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Esophagus (N=1)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pancreas (N=1)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total (N=57)</td>
<td>10</td>
<td>17.9</td>
</tr>
</tbody>
</table>

As seen in the table, there is no significant correlation 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.

<table>
<thead>
<tr>
<th>DFA</th>
<th>Cryptosporidium</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Chemotherapy process (week)</td>
<td>19.4</td>
</tr>
</tbody>
</table>

p<0.05 Mann-Whitney U=81.0

Depending on the length of time of the immune suppression increased the prevalence of Cryptosporidium. Chemotherapy in patients with longer duration of 20 weeks, increased incidence of Cryptosporidium 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. The first human cases of cryptosporidiosis were reported in 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 gastrenteritis 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, MacKenzie, 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%, Arik and others(1996) 38.8%, Tamer et al. (2008) 12.35% in the ratio have determined cryptosporidiosis.

In studies of the prevalence of cryptosporidiosis in the world; In Europe and America,1-3%, while in developing countries from 5-10% has been reported (Hunter, et al., 2002). Various tests have been developed for the diagnosis of Cryptosporidium. Microscopidagnostic methodisdiesethefirst. 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 technique that make use of specific monoclonal or polyclonal antibodies are commonly used in Cryptosporidiosis diagnosis. DFA compared with modified acid fast method (MAF), DFA’s sensitivity 100%, specificity was found to be 97%. In another study, Enzyme immunoassay (ELA) 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 that a relationship between colon cancer and chronic inflammation. C. parvum aprotozoan that causes pathological changes in the gastrointestinal epithelium. 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 the immunosuppressed mice, histopathological changes have been investigated. Cyclin D1 have been investigated with immunohistochemical 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 (Rafei, 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(Rafei, 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 more careful against these infectious agents. Some of these 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. Domenech 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.

REFERENCES


