INCIDENCE OF CRYPTOSPORIDIUM SPP. IN THE HUMAN POPULATION OF MALATYA IN TURKEY

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ABSTRACT

Aims: Cryptosporidium species, settled in the microvilli of digestive and respiratory systems of vertebrates, infect mammals, poultry, fish and reptiles. Cryptosporidium species are common worldwide. Symptoms vary depending on species of infected host, condition of the immune system and age. In healthy patients with strong immune system it is characterized by self-limiting diarrhea in approximately 2 weeks, whereas in people with suppressed immune systems it can cause diarrhea such as cholera and could create a life-threatening clinical condition.

The aim of this study is to determine the epidemiology of Cryptosporidium species in the human population of Malatya province and around.

Materials and methods: In Malatya province in 2006, 2,281 stool samples were taken from patients admitted to the Inonu University Faculty of Medicine Parasitology Department with the gastrointestinal complaints and analyzed. On the other hand a questionnaire has been prepared to determine the epidemiology of Cryptosporidium species in the human population of Malatya province. Each patient included in the study filled out and signed a patient information form.

Results: In the studies, it has been reported that the Cryptosporidium infection rate is 6.1% in the developing countries and it is 2.1% in the developed countries. In different studies carried out in Turkey, it has been reported that the prevalence of infection is between 0-35.5%. Stool samples were examined by direct microscopy and acid-fast staining method and 161 (7.1%) of them were positive. In this study, a significant relationship hasn’t been found in patients with loss of appetite, general body itching, allergies, immunosuppressive and cancer, dyspnea, ulcerative colitis, diarrhea, abdominal pain, salivation, constipation, nausea, vomiting, growth retardation, the rectal itching and anemia, but in the statistical analysis, the difference is statistically significant.

Conclusion: Consequently, molecular studies are needed to determine the common Cryptosporidium species and their sources in Malatya province and around. This will guide in determining strategies of public health education.

Key words: Cryptosporidium, Digestive System, Epidemiology, Acid-fast Staining, Malatya.

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Introduction

Cryptosporidium species are coccidian parasites that settled in digestive and respiratory systems of vertebrates infect mammals, poultry, fish and reptiles. Symptoms vary depending on species of infected host, the condition of the immune system and age. Cryptosporidium species are common and may cause infection not only in humans but also in animal species. Therefore, transmission to human may be in many different ways. The transmission is easier for old people or children and day care centers. Although the transition from animals to humans especially in rural areas is mentioned in many publications, this issue is debatable. The incidence of infection is high for some professions engaged in animal husbandry, veterinary, laboratory staff, nursery staff, children and the elderlies, people who travel to endemic regions, unhygienic places and when you contact with infected people. Some Cryptosporidium outbreaks originating from public swimming pools, common meals, well water and unhygienic drinking water sources are known.

While the Cryptosporidium may cause asymptomatic infection or diarrhea in patients with strong
immune system, it can cause severe and chronic diarrhea, pancreatic, biliary and respiratory tract infections and even death in people with suppressed immune systems\(^9\).

Cryptosporidium infections are more common in developing and less developed countries. In the studies of diarrheal stools of patients; the prevalence of Cryptosporidium has been reported as 1-4\% in Europe and North America and 3-20\% in Africa, Asia, Australia, South and Central America. It has been reported that the lack of clean water and sanitation facilities, crowded home environment and close relationship of the reservoir animals with the people increase the prevalence of parasites\(^9\). In various studies conducted in Turkey, prevalence of the infection has been reported between 0-35.5\%\(^6,\)\(^10\). The aim of this study is to determine the epidemiology of Cryptosporidium species in the human population of Malatya province and around.

Different parasites analyzed during the study period has been found in the samples. However, they were not assessed; because the destination of the study is to determine the Cryptosporidium species.

Materials and methods

The patients who admitted to the outpatient clinics of Inonu University School of Medicine with gastrointestinal complaints from Malatya and its surroundings between 1st. January. 2006- 31st. December. 2006 have formed the population of the study. When selecting the study sample, considering the patients coming from the Department of Parasitology, the target in order to reach individuals who suffer from digestive system was set and for this purpose 2281 stool samples have been examined.

Subjects answered a questionnaire used for the determination of the Cryptosporidium in samples collected from the study group. Each subject included in the study filled out a Patient Information Form and signed it. A questionnaire for collecting data that can be associated with the presence of cryptosporidium has been developed in order to be implemented during the receipt of stool samples.

The data collection instruments used in the study were applied to the patients who admitted to Inonu University Turgut Ozal Medical Center Department of Parasitology with digestive system complaints. The patients were given special containers for stool specimens. The stool collection containers were given to the patients and they were informed that the diarrheal patients had to put 3-4 tablespoons of their stool samples in this container and the non-diarrheal had to put as the size of a walnut and after closing its mouth tightly they had to deliver them within 1 hour to the Parasitology Laboratory. In addition, a questionnaire about the cryptosporidium were applied to the patients by the investigator. The characteristics measured by the specific informations of the examined cryptosporidium questionnaire form constitute the limitations of the study. It has also been assumed that the prepared form has adequate validity and reliability. Stool samples were examined by direct microscopy and acid-fast staining method.

Data was given as average, standard deviation or the number / percentage. The chi-squared test was used for statistical analysis. Moreover, multivariate logistic regression analysis was used to assess the relationship between the parasite and some variables including age, cancer, (RGD) retarded growth and development, and ulcerative colitis. P values <0.05 were considered as significant. Data were evaluated by using the SPSS 11.5 program.

Results

A total of 2281 stool samples were examined. Direct microscopy (figure 1) and acid-fast (figure 2, 3) methods were used for the diagnosis of Cryptosporidium, the positivity was determined as 7.1\%.

Figure 1: Cryptosporidium oocysts saline (400X).

According to the results of the study, percentage of Cryptosporidium has been given in Table 1. The distribution of Cryptosporidium positivity according to the months has been given in Graph 1. The average age of the patients are 24.3 ± 19.7.
The positivity rate has been given in Graph 2. A significant difference has not been found between age and incidence of parasite. The distribution of patients according to the provinces has been given in Table 2. The distribution of patients according to the complaints has been given in Table 3.

Stool samples were examined by direct microscopy and acid-fast staining method and the positivity was found as 161 (7.1%). In the statistical analysis a significant relationship was not found in patients who have loss of appetite, general body itching, allergies, immunosuppressive + cancer, dyspnea, ulcerative colitis, diarrhea, abdominal pain, salivation, constipation, nausea, vomiting, growth retardation, the rectal itching and anemia problems, but among the positive results the difference was statistically significant. Average age of the patients in the study was determined as 24.3 ± 19.7.

The parameter estimates of the logistic regression model, standard errors, Wald statistics, degrees of freedom, the odds ratios and information about confidence intervals have been given in Table 4.
According to the results, significant relationship has been determined between incidence of parasite and RGD (P <0.05).

A significant relationship has not been observed between cancer and ulcerative colitis. However, in ulcerative colitis and cancer cases, the incidence of parasite has increased a half times.

Discussion

Patients were examined for determining epidemiology of Cryptosporidium and the findings were discussed and interpreted.

In developed countries, while viral agents (especially rotavirus) can cause to gastroenteritis as 30-40%, in developing countries bacterial and parasitic agents are more prominent. Cryptosporidium initially has been seen in patients with primary and secondary immunodefiency (especially AIDS) Acquired Immune Deficiency Syndrome). But with the help of new laboratory techniques it has started to be seen in non-immunocompromised people during diarrhea epidemics. The incidence of Cryptosporidium shows regional differences in the world. The prevalence of Cryptosporidium has been found as 5-17% in developing countries where socio-economic status is low, 1-3% in developed countries. Ratnam et al. have reported in their international studies that the prevalence is 1.2% in Canada, 10% in Kuwait, 7.4% in Africa, 2.9% and 7% in Iran and 17% in Egypt.

In abroad studies about the epidemiology of the parasite, the rates have been reported as 6.4% in children without immune system deficiency in the United States of America and 4.7% in asymptomatic pre-school children under five years old in Saudi Arabia. In the various studies conducted in Turkey, Dogan et al. reported that they have detected 2.2% of interference in Eskişehir, Ozdemir et al. reported that they have detected 3% of interference in Ankara and Akyön et al. reported that they have detected 3.5% of interference. Otag et al. have detected 5.5% of Cryptosporidium parasite in 8-12 age group students studying at four elementary schools in Mersin and its neighborhood, and Cicek and yilmaz have detected 2.2% of Cryptosporidium parasite in Van. Similarly Börekci et al. have reported 3% of cryptosporidium oocysts in the stool samples of a total of 361 normal healthy patients who have not any diarrhea complaint. Again Alvin et al. have met (1.5) Cryptosporidium parasite in 8 of 519 samples in Bursa. Sönmez Tamer and Gülenc examined 65 samples in Kocaeli and they found a rate of 6.25% of parasite. In the Aegean Region In the studies of Koturoğlu et al. 13.5% of Cryptosporidium have been found in Aegean region and Aksoy et al. have detected 7.8% of Cryptosporidium parasite from the same region. 3.5% of Cryptosporidium oocysts were detected in the study of Akyön et al. in Ankara. In the study, 2281 stool samples were examined by direct microscopy and acid-fast staining method and 161 (7.1%) of them were found positive.

The symptoms in the patients with diarrhea are similar. It is difficult to separate the clinical findings such as abdominal pain, vomiting, fever from the Cryptosporidium diarrhea which is one of the other diarrhea factors. In the study of Koturoğlu et al., a correlation was not observed between the symptoms and Cryptosporidium diarrhea. Karlı et al. has detected dehydration in 37.5% of the patients with Cryptosporidium. They also have found mild dehydration in 25% of cases. In different studies dehydration frequency has been reported as 13-81%. It has been reported that Cryptosporidium frequency can increase in children with malnutrition. Karlı et al. has detected malnutrition in 75% of patients with Cryptosporidium. In the study conducted in Iran, it has been detected that 47% of patients with Cryptosporidium are slim. But, in the studies conducted in urban areas of Bangladesh, the malnutrition frequency was not high and there was no correlation between malnutrition and Cryptosporidium. It has been reported that the Cryptosporidium enteritis often cause watery diarrhea.

Moreover, in the study, a significant relationship has not been found in the general evaluation of the patients who has loss of appetite, general body itching, allergies, immunosuppressive + cancer, shortness of breath, ulcerative colitis, diarrhea,
abdominal pain, saliva, constipation, nausea, vomiting, growth retardation, rectal itching and anemia problems but the difference is significant in the statistical evaluation made between the positives. The percentage of occurrence of interference in patients suffering from abdominal pain and diarrhea has been determined at a higher rate than the others.

Also, in the logistic regression analysis, a significant relationship has been found between the occurrence percentage of the interference and RGD. The percentage of occurrence of interference has increased 1.79 times in children who has growth retardation problem. Aynı şeyi anlatıyordu sildim

In this study, in Malatya 7.3% of Cryptosporidium parasites, in Adıyaman 4.7% of Cryptosporidium parasites, in Kahramanmaraş 5.7% of Cryptosporidium parasites have been detected. The percentage of incidence of parasite has been high for the patients coming from Adıyaman and Kahramanmaraş. It has been thought that with various studies to be conducted in those provinces, clear data can be obtained about the epidemiology of the parasite.

The Cryptosporidium frequency increases in immunocompromised individuals. High prevalence has been reported in adults particularly in HIV-positives. The frequency of Cryptosporidium increases in cases other than primary immunodeficiency suppressed by the immune system and HIV infection for example; in leukemia, solid tumors, lymphoproliferative disease, chronic renal failure. It has been emphasized that in children with leukemia whose bone marrow and solid organ transplants were performed and in children who received immunosuppressive therapy after transplantation, the prevalence increases due to decreasing of immune resistance and in the event of gastrointestinal symptoms, the Cryptosporidium oocysts should be investigated in these patients.[35,36]

For the patients with primary and secondary immunocompromised, the clinical situation may be very severe. It may lead to symptoms such as diarrhea and cholera. Daily dehydration up to 20 liters and systemic involvement is possible. In a four-year-old patient who has been followed with the diagnosis of acute lymphoblastic leukemia and reported by Russell et al two months of ongoing severe diarrhea has been found and also in the case of weight loss Cryptosporidium has been found[36].

Atambay et al.[37] have examined 500 stool samples of the patients suffering from diarrhea in Malatya province by Kinyoun’s acid fast staining method and they have reported 1.6% of Cryptosporidium in the stool samples. In this study, 7.1% of Cryptosporidium parasite were detected. This situation may be caused by the wide universe of the study and the methods. Also in logistic regression analysis a significant relationship was not observed between cancer and incidence of parasite but the rate of Cryptosporidium in cancer cases has been determined a half times more.

In the laboratory diagnosis of Cryptosporidium, Kinyoun’s modified acid-fast method has become the main method in many laboratories, because the results can be taken in a short time and easily. The diagnosis can be supported by (IFA) immunofluorescence and (ELISA) Enzyme-Linked ImmunoSorbent Assay methods. With these methods it is possible to investigate organism itself or its soluble antigen. The IFA is important for the diagnosis of the disease in the early stages but it is an expensive technique. Specific anti-Cryptosporidium IgG, IgM may be detected by ELISA method. Recently, (PCR) Polymerase Chain Reaction techniques have been developed for diagnosis, however they have been limited for use in diagnosis because they are expensive and also they cause false positive. Using multiple methods in combination with the microscopic method for the diagnosis increase the reliability of the results[31, 38]. In the study, stool samples were stained with modified acid-fast method and examined by the parasitolog.

In conclusion, studies are needed to determine the common Cryptosporidium species and their sources in Malatya province and around. This will guide in determining strategies of public health education.

According to the research findings the suggestions are presented below;

- As the study for the epidemiology of Cryptosporidium species is confined to the region of Malatya, studies on the epidemiology of interference should be carried out also in other regions of the country.

- Experimental studies about the transmission of Cryptosporidium species should be done and information efforts about the protection methods should be made.

- Considering the clinical signs in the diagnosis of Cryptosporidium species, the patient should be directed to the laboratory to investigate the parasite for the prolonged gastrointestinal complaints.
The relationship between the positivity of the Cryptosporidium species and RGD complaint should be investigated.

The search of Cryptosporidium species is necessary for the BGG complaints and in the presence of the parasite.

In the immunosuppressive and cancer diseases, considering the complaints of patients during and after treatment, sample taking should be performed for the diagnosis of Cryptosporidium species.

References


