# DETECTION OF CRYPTOSPORIDIUM IN IMMUNOSUPPRESSED PATIENTS

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**Background:** Cryptosporidium is an important cause of diarrhoeal disease in children and immunosuppressed persons. It is an opportunistic pathogen. A study was done to determine the frequency of Cryptosporidium in immunosuppressed persons. **Methods:** Fecal samples were collected from Jinnah Postgraduate Medical Centre Karachi during July to December 2003 and they consisted of three categories of patients which included cancer patients (10) diabetics (20) and patients on dialysis (20). Direct microscopy in saline and iodine was done to detect parasites. Detection of Cryptosporidium from fecal samples was done by Kinyoun method. Bacteriological study was also done to determine any bacterial pathogens. **Results:** Out of 50 fecal samples Cryptosporidium was found in 20 (40%) of cases. Among the different groups Cryptosporidium was found in 80% of cancer patients, 25% diabetics and 35% dialysis patients. Distribution of Cryptosporidium according to gender indicated more cases in males (87%) among cancer patients and 40% among dialyzed patients while diabetic patients showed similar distribution. Among the different age groups male patients were found to be between 40-49 years group for all categories of patients while more positive cases were detected in females in 30-39 years age group. Direct microscopy did not indicate any ova or parasite and bacteriological study indicated Salmonella and Shigella spp. in stool samples. **Conclusion:** This study provides indication of the possibility of Cryptosporodisis in immunosuppressed patient and further comprehensive study is required to clearly establish the relationship of opportunistic infections in immunosuppressed patients.

**Keywords:** Immunosuppressed patients, Cryptosporidium, Cryptosporodosis

## INTRODUCTION

Cryptosporidium is recognized as a significant and widespread cause of diarrhoeal illness in both immunocompetent and immunosuppressed hosts.[1] Cryptosporidium may cause severe symptoms in person with weak immune system.[2] In immunocompetent individuals infection is usually self limiting but in immunocompromised individual receiving immunosuppressive drug or in AIDS patients persistent infection can be life threatening.[3] The organism may cause severe gastrointestinal infection and eventual death in immunocompromised individuals.[4] As many as 71 stools/day, and losses of up to 25 l/day of water have been observed in immunocompromised patients and the illness may persist indefinitely.[5] Potential sources of Cryptosporidium infection include pets and farm animals, infected persons, contaminated foods and water.[6] Cryptosporidium transmission occurs at a high frequency in day care centers where children are clustered within classrooms, share toilets and common play areas, or necessitate frequent diaper changing.[7] The parasite elicits a strong immunologic response in infected immunocompetent individuals as antibodies against glycoproteins and other cell determinants are detected.[8] Presence of antibodies in the intestinal lumen provides a protective effect against initial Cryptosporidium infection.[9] In the immuno-compromised cases use of immunosuppressive agents like the corticosteroids promote experimental infections.[10] No safe and effective therapy for cryptosporodiosis has been successfully developed and as it is a self limiting illness in immuno-competent individual, supportive care is the only treatment. Oral or intravenous rehydration may be necessary for particularly voluminous, watery diarrhea. Spiramycin and Dicalzuril Sodium have produced partial responses.[11] This study was done to determine the frequency of Cryptosporodosis in immunopsppressed patients of different categories using immunosuppressive drugs like cancer, diabetic and dialyzed patients.

## MATERIAL AND METHODS

Fifty stool samples were collected from Jinnah Postgraduate Medical Centre and Kidney Centre Karachi. The different categories of patients included 10 cancer patients, 20 diabetics and 20 dialyzed patients. Cancer patients were taking immuno-suppressive drugs and were admitted in cancer ward while the diabetic and dialyzed patients were also under treatment. The study was carried from July to December 2003.

Cryptosporidium was detected by Kinyoun method of staining. 40 ml of homogenized fecal sample was prepared so that even amount o fecal sample was spread on slide. Fecal smear was fixed in Methanol for 3 minutes and air dried. Basic Fuschin (1%) was applied for 3 minutes and the slide was

washed with tap water and decolorized with 1% Sulphuric acid for 1 minute. Counter stain Malachite green (0.5%) was applied for 30 seconds and the slide was washed with water and air dried. The slide was observed under low and high power objective and the number of oocysts was observed in different fields of slide.

Direct microscopy for ova and parasite was done in saline and iodine and slide was observed under low and high power objective.

Bacteriological study was done by inoculating fecal samples on MacConkey's agar and Salmonella Shigella agar. Plates were incubated at 37$^{o}$ C for 24 hours and colonies were observed the next day. Identification was done by inoculating on triple sugar iron agar and other biochemical tests.

## RESULTS

Out of 50 patients Cryptosporidium was detected in 20 (40%) of cases. Among the different groups, Cryptosporidium in cancer patients was found in 8 (80%) of cases. In 20 diabetic patients it was found in 5 (25%) and in 20 dialyzed patients it was found in 7 (35%) of cases.

Among cancer patients Cryptosporidium was found to be more in males (85.7%) as compared to females (66.6%). In diabetics the percentage of patients affected was similar (25%). Among dialyzed patients 40% males were infected as compared to females (30%).

Patients were mainly from two age groups 30-39 and 40-49 years. More positive cases for all categories pf patients were found in 40-49 years age group.

Among female cancer patients the percentage was similar in both groups while diabetic patients positive for Cryptosporidium were found to be more in 40-49 years group while dialyzed patients were more in 30-39 years group.

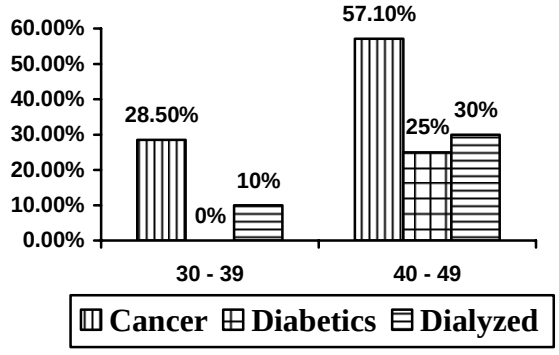


**Figure-1: Distribution of Cryptosporidium according to Age group in Males**

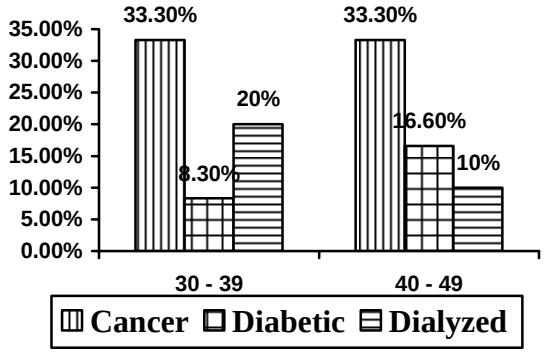


**Figure-2: Distribution of Cryptosporidium according to Age group in Females**

Direct microscopic examination of fecal samples did not reveal any parasites Bacteriological study of fecal samples indicated the presence of E.coli, Salmonella and Shigella in stool samples of cancer patients. E. coli was only detected from diabetic patients while dialyzed patients showed E.coli and Shigella in stool samples.

## DISCUSSION

Cryptosporidium, Isospora, Cyclospora and Microsporidia are increasingly becoming prevalent in AIDS patients.[12,13] In India prevalence rates of human cryptosporidiosis was reported as 23% in AIDS patients.[14] Major mortality and morbidity occur in patients with multiple parasites and CD4 count below 116 /cmm.[15] Cryptosporidiosis can be acquired at any time during the course of HIV infection. Cancer is the abnormal growth of cells and cancer patients are highly susceptible to opportunistic infections. Among cancer patients the disease condition and treatment of disease makes the immune system weak.[16] Diabetes is a disorder that leads to hyperglycemia. It is a metabolic disorder that affects many cells of our body and maker our immune system weak.[17] Out of our diabetic patients 25% were positive for Cryptosporidium and infection was more in males above 40 years. Dialysis is done for those patients who have defective kidney or kidney failure.[18] Blood and tissues of these individuals get exposed to machines so there is greater chance of infection. Some drugs used by these patients suppress the immune system. In our study 35% of patients were positive for Cryptosporidium and more cases were in males above 40 years of age. From our study it can be stated that Cancer patients are more immuno-compromized and they are more likely to be infected with Cryptosporidium but a more comprehensive study is required as the number of cases in our study was limited.

## REFERENCES


1. Eckman Laurent F Kagnoff MNF Intestinal Epithelial cells Apoptosis following Cryptosporidium parvum infection Infection and Immunity 2000;68:1710-3
2. Langer R, Riggs MW. Cryptosporidium parvum apical complex glycoprotein CSL contains a sporozoite ligand for intestinal epithelial cells. Infect Immune 1999;67:5282-52
3. Riggs MW, Stone PA, Yount RC, Langer MJ, Arrowood DL. Bentle**y** Protective monoclonal antibody defines a circumsporozoite-like glycoprotein exoantigen of cryptosporidium, parvum sporozoites and merozoites. J Immunol 1997;158:1787-95
4. Schroeder A, Lawrence ACE, Abrahamsen MS**.**. Differential mRNA display cloning and characterization of a Cryptosporidium parvum gene expressed during intracellular development. J Parasitol 1999;85:213-20
5. LeChevallier MW, Di Giovanni GD, Clancy JL, Bukhari Z, Bukhari S. Comparison of Method 1623 and Cell Culture-PCR for Detection of cryptosporidium, spp. in Source Waters. Appl Environ Microbiol 2003;69:971-9
6. Ward HD, Cevallos M. Cryptosporidium: molecular basis of host-parasite interaction. Adv Parasitol 40:151-185
7. Xiao L, Fayer R, Ryan U, Upton SJ. Cryptosporidium Taxonomy: Recent Advances and Implications for Public Health. Clin Microbiol Rev 2004;17:72-97
8. Schaefer DA, Auerbach-Dixon BA, Riggs MW. Characterization and formulation of multiple epitope-specific neutralizing monoclonal antibodies for passive immunization against cryptosporidiosis. Infect Immun 2000;68**:**2608-16
9. Guyot K, Follet-Dumoulin A, Lelievre E, Sarfati C, Rabodonirina M. Molecular Characterization of cryptosporidium, Isolates Obtained from Humans in France. J Clin Microbiol 2001;9:3472-80
10. Valdez LM, Dang H, Okhuysen PC, Chappell CL. Flow cytometric detection of cryptosporidium oocysts in human stool samples. J Clin Microbiol 1997;35:2013-17
11. MManabe YC, Clark DP, Moore RD. Cryptosporidiosis in patients with AIDS: correlates of disease and survival. Clin Infect Dis 1998;27:536.
12. Prasad KN, Nag VL, Dhole TN, Ayyagari A. Identification of enteric pathogens in HIV positive patients with diarrhoea in Northern India. J Health Popul Nutr 2000;8(1):23-26.
13. Ballal M, Prabhu T, Chandran A, Shivananda PG. Cryptosporidium and Isospora belli diarrhoea in immunocompromised host. Indian J Cancer 1999;36(1):38-42.
14. Lanjewar DN. Immunopathology of HIV: Proceedings of International symposium on AIDS; 1995 Maay; Pune, India.
15. Shah UV, Purohit BC, Chandralekha D, Mapara MH. Coinfection with cryptosporidium, isospora and s.stercoralis in a patient with AIDS - a case report. Indian J Med Microbiol 2003;21(2):137-8.
16. Malgrange VB, Escande MC, Theobald S. Validity of earlier positivity of central venous blood cultures for diagnosing cancer related bactermia in cancer patients. J Clin Micro 2001;39:274-8
17. Carol JC, Bougneres PE. Treatment of diabetic patients with insulin. Experience and Future Hoem Res 1996;45: 4-47
18. Schleifer CR, Benz RL, McAlack J, Poupard J, Calman J. Lactobacillus acidophilus peritonitis in CAPD. Peritoneal Dialysis Int 1990;9:22.



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