



COMPARISON OF CONVENTIONAL MICROSCOPY WITH ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR DETECTION OF CRYPTOSPORIDIUM INFECTION IN PEOPLE LIVING WITH HIV.

Microbiology

Ankita Mohanty*	Senior Resident, Department of Microbiology, Lokmanya Tilak Municipal Medical College and General Hospital, Sion, Mumbai, Maharashtra, India, Pin code- 400022 *Corresponding Author
Kanchan V Joshi	Additional Professor, Department of Microbiology, Lokmanya Tilak Municipal Medical College and General Hospital, Sion, Mumbai, Maharashtra, India, Pin code- 400022
Smruti Mohanty	Assistant Professor, Department of Microbiology, Shri Shankaracharya Institute of Medical Science, Bhilai, Chattisgarh, India, Pin code- 490020
Sujata Baveja	Ex-Professor and Head, Department of Microbiology, Lokmanya Tilak Municipal Medical College and General Hospital, Sion, Mumbai, Maharashtra, India, Pin code- 400022

ABSTRACT

Background: *Cryptosporidium* gastroenteritis in humans can be severe, and life-threatening in immunocompromised. This study was conducted to assess the diagnostic accuracy of conventional microscopy and ELISA in determining *Cryptosporidium* infections in People Living with HIV. **Materials and methods:** Stool samples for three consecutive days were collected from 80 patients who had HIV infection. Each sample was subjected to Microscopy (Concentration by Sheather's Sugar Floatation technique, Wet mount, Iodine mount, Modified Trichrome stain, Modified Acid-fast stain, and Kinyoun stain) and ELISA. **Results:** Out of 80 patients, 6 (7.5%) were detected with *Cryptosporidium* infection (4 patients were detected by Microscopy and ELISA, 2 only by ELISA). **Conclusion:** All 6 positive cases (7.5%) were detected by ELISA while the maximum number of cases which was detected by microscopy was 4(5%). ELISA had a sensitivity and specificity of 100%, proving it to be an effective method for diagnosing *Cryptosporidium* infection.

KEYWORDS

Cryptosporidium, immunocompromised, gastroenteritis, microscopy

1. INTRODUCTION:

Intestinal parasites are among the most common causes of morbidity and mortality worldwide, especially among immunocompromised people(15). Several species of protozoa are associated with diarrhea in HIV (Human Immunodeficiency Virus) disease. Examples include *Cryptosporidium* species, *Cystoisospora belli*, *Microsporidium* species, *Giardia intestinalis*, *Entamoeba histolytica*, and *Cyclospora cayatanensis*. *Strongyloides stercoralis* can cause diarrhea and hyper infection syndrome in patients with immunosuppression (5).

Cryptosporidiosis is caused by protozoan parasites of the genus *Cryptosporidium* (1). Immunosuppressed individuals are at higher risk for *Cryptosporidium* diarrheal infections which is much more severe and prolonged in them (6). These infections are a leading cause of severe, life-threatening acute gastroenteritis in People Living with HIV (PLHIV), who have not taken antiretroviral therapy (12).

For early diagnosis and prompt treatment, rapid tests such as antigen detection by immunochromatographic test (ICT), ELISA (Enzyme-linked Immunosorbent assay), and microscopy are important. The sensitivity and specificity vary with different methods and kits used (9).

This study was conducted to determine and assess the diagnostic accuracy of conventional microscopy and ELISA for *Cryptosporidium* infections in PLHIV.

2. MATERIALS AND METHODS:

2.1 Study design and population: a prospective, observational study conducted for a one-year duration at a tertiary care hospital on 80 patients who were immunocompromised due to HIV infection (proven by serology).

2.2 Stool specimens and processing: Stool samples for three consecutive days were collected from 80 patients who were immunocompromised due to HIV infection (proven by serology). All samples were divided equally into two parts for Microscopy and ELISA.

2.3 Microscopy: Samples were subjected to concentration by Sheather's Sugar Floatation technique. Normal saline wet mounts and iodine mounts were prepared. Modified Acid-fast Staining, Kinyoun staining, and Modified Trichrome staining were performed

2.4 Antigen detection by ELISA: The Fecal *Cryptosporidium parvum* antigen ELISA Kit, manufactured by Epitope Diagnostics, San Diego, USA, was used.

2.5 Statistics: The standard formula determined the sample size, sensitivity, and specificity of different diagnostic techniques. Sensitivity and Specificity was calculated considering ELISA as the gold standard.

2.6 Ethics Approval: The study protocol was approved by the Institutional Ethics Committee Human Research with Registration Number: ECR/266/Lokmanya/Inst/MH/2013RR-16 and Reference Number: IEC/86/18.

3. RESULTS:

3.1 Comparison of *Cryptosporidiosis* with different microscopic techniques in the study group (n=80)

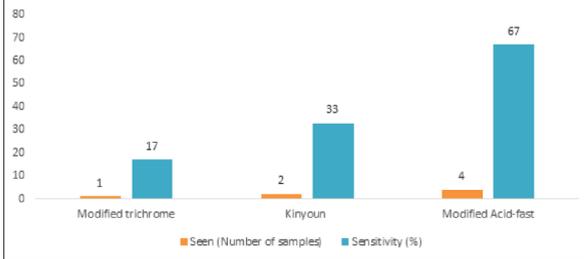
Among the various microscopic techniques, *Cryptosporidium* was detected in four patients by Modified Acid-fast staining technique (Sensitivity 67%), out of which two patients were detected by Kinyoun stain also. (Sensitivity 33%).

One patient was detected positive by all three staining techniques: Modified Trichrome (Sensitivity 17%), Kinyoun, and the Modified Acid-fast staining technique.

Specificity was 100% for all the microscopic techniques considering ELISA as the gold standard. (Table 1 and Figure 1)

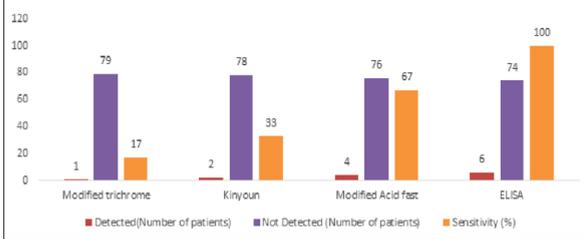
Table 1: Comparison of *Cryptosporidiosis* with different staining techniques in the study group (n=80)

Microscopy	Results		Sensitivity (%)	Specificity (%)	Percent age (%)	p-value (Fisher's exact test)
	Seen	Not seen				
Modified Trichrome Stain	1	79	17	100	1.25	0.10
Kinyoun Stain	2	78	33	100	2.5	0.004
Modified Acid-fast stain	4	76	67	100	5	<0.001

Figure 1: Comparison of Cryptosporidiosis with different staining techniques in the study group (n=80)**3.2 Detection of Cryptosporidiosis by ELISA in the study group (n=80):**

Six patients were found reactive on ELISA with 100% sensitivity and specificity. The sensitivity of ELISA was 100% whereas the highest sensitive method among the different microscopic methods was Modified Acid-fast staining (67%). The specificity of all microscopic techniques and ELISA was 100%.

Comparison between ELISA and microscopy showed that ELISA was better and this finding was statistically very very significant; p -value < 0.001 (p =Fisher's exact test). (Figure 2)

Figure 2: Comparison study of Cryptosporidiosis with different staining techniques and ELISA in the study group (n=80)**4. DISCUSSION:**

Cryptosporidium spp. are intestinal protozoan parasites of the phylum *Apicomplexa*, which cause diarrheal disease in humans worldwide. In immunocompetent individuals, infection with this parasite may be asymptomatic or cause a self-limiting diarrheal illness. However, in immunocompromised patients such as those with HIV/AIDS, *Cryptosporidium spp.* may cause severe, chronic, and possibly fatal diarrhea and muscle wasting (7)

There are limited studies in India explaining the role of ELISA in *Cryptosporidium* infection detection. Hence this study was conducted to determine *Cryptosporidium* infections in People Living with HIV and to assess the diagnostic accuracy of conventional microscopy and ELISA.

In this study, a total of 80 patients (immunocompromised due to HIV) clinically suspected of having *Cryptosporidium* infection were tested. Among the various microscopic techniques, *Cryptosporidium* was detected in four patients by Modified Acid-fast staining technique (Sensitivity 67%), out of which two patients were detected by Kinyoun stain also. (Sensitivity 33%).

One patient was detected positive by Modified Trichrome (Sensitivity 17%). *Cryptosporidium* oocysts were also seen in the stool sample of the same patient by Kinyoun and the Modified Acid-fast staining technique. Specificity was 100% for all the microscopic techniques. This finding is comparable with a study done by Tamomh *et al*, which also shows the Modified Acid-fast staining technique as a better method (10).

All six patients were found reactive on the ELISA with a sensitivity and specificity of 100%. The sensitivity of ELISA is higher in comparison to microscopic techniques. The sensitivity of ELISA was 100% whereas the highest sensitivity among the different microscopic methods was of Modified Acid-fast staining i.e. 67%.

The specificity of all microscopic techniques and ELISA was 100%.

The above findings match with the findings in the study by Uppal *et al* which showed a specificity of the staining technique (Modified Acid-fast) and ELISA as 100% (13).

A study by Garcia *et al* also showed Modified Acid-fast staining method superior to other techniques for *Cryptosporidium* diagnosis (4).

In this study, all six positive cases (7.5%) were detected by ELISA out of which only four patients (5%) were positive by microscopic techniques also [two patients (2.5%) by both Modified Acid-fast staining and Kinyoun and two patients (2.5%) by only Modified Acid-fast staining] which means almost one-third of positive cases (33%) were missed by microscopy. There was one positive patient detected by all the staining techniques i.e. Modified Trichrome stain, Kinyoun stain, Modified Acid-fast staining, and ELISA.

The above findings match the study done by El-Shazly *et al* where *C. parvum* is diagnosed in stool samples as 5.3% and 8.3% by Modified Acid-fast staining and ELISA respectively (3). In past studies, it has been stated that the Acid-fast staining method is reliable, specific, and has a high diagnostic value among staining methods used for the identification of *Cryptosporidium spp.* oocysts. ELISA is preferred in resource-limited laboratories due to its high specificity and sensitivity, easy usage, fast application and scoring, and easy standardization for the determination of *Cryptosporidium spp.* antigens in stool samples. It can be used as a stand-alone method wherever possible to avoid missing the positive cases.

This study showed the overall prevalence of Cryptosporidiosis as 7.5% which matches with the prevalence of the various studies done by Patel *et al* (8), Tian *et al* (11), Iqbal *et al* (2), and Utami *et al* (14).

REFERENCES:

- Ahmadpour, E., Safarpour, H., Xiao, L., Zarean, M., Hatam-Nahavandi, K., Barac, A., Picot, S., Rahimi, M. T., Rubino, S., Mahami-Oskoue, M., Spotin, A., Nami, S., & Baghi, H. B. (2020). Cryptosporidiosis in HIV-positive patients and related risk factors: A systematic review and meta-analysis. In *Parasite* (Vol. 27). EDP Sciences. <https://doi.org/10.1051/parasite/2020025>
- A.L. Lim, Y. (2012). Cryptosporidiosis in HIV-Infected Individuals: A Global Perspective. *Journal of AIDS & Clinical Research*, 01(11). <https://doi.org/10.4172/scientificreports.431>
- El-Shazly AM, Gabra A, Mahmoud MS, & Aziz SS SW. (2002). The use of Ziehl-Neelsen stain, enzyme-linked immunosorbent assay and nested polymerase chain reaction in diagnosis of cryptosporidiosis in immuno-competent, -compromised patients. *Journal of the Egyptian Society of Parasitology*, 155-166.
- Garcia, L. S., Bruckner, D. A., Brewer, T. C., & Shimizu, R. Y. (1983). Techniques for the Recovery and Identification of *Cryptosporidium* Oocysts from Stool Specimens. In *JOURNAL OF CLINICAL MICROBIOLOGY* (Vol. 18, Issue 1).
- Gupta, P., Bhatia, M., Gupta, P., & Omar, B. (2018). Emerging biocide resistance among multidrug-resistant bacteria: Myth or reality? A pilot study. *Journal of Pharmacy and Bioallied Sciences*, 10(2), 96-101. https://doi.org/10.4103/JPBS.JPBS_24_18
- Hossein Nahrevanian, & Mehdi Assmar. (2008). Cryptosporidiosis in immunocompromised patients in the Islamic Republic of Iran - PubMed. *Journal of Microbiology Immunology and Infection*.
- O'Connor, R. M., Shaffie, R., Kang, G., & Ward, H. D. (2011). Cryptosporidiosis in patients with HIV/AIDS. In *AIDS* (Vol. 25, Issue 5, pp. 549-560). <https://doi.org/10.1097/QAD.0b013e3283437e88>
- Patel, P., Patel, S., Sood, N., Rao, P., Radadiya, D., & Professor, A. (2015). A Study Of The Prevalence Of *Cryptosporidium Parvum* In Stool Samples Of Patients Of Tertiary Care Hospital, Ahmedabad. In *NJIRM* (Vol. 6, Issue 4).
- Sponseller, J. K., Griffiths, J. K., & Tzipori, S. (2014). The evolution of respiratory cryptosporidiosis: Evidence for transmission by inhalation. *Clinical Microbiology Reviews*, 27(3), 575-586. <https://doi.org/10.1128/CMR.00115-13>
- Tamomh, A. G., Agena, A. E. M., Elamin, E., Suliman, M. A., Elmadani, M., Omara, A. B., & Musa, S. A. (2021). Prevalence of cryptosporidiosis among children with diarrhoea under five years admitted to Kosti teaching hospital, Kosti City, Sudan. *BMC Infectious Diseases*, 21(1). <https://doi.org/10.1186/s12879-021-06047-1>
- Tian, L. G., Chen, J. X., Wang, T. P., Cheng, G. J., Steinmann, P., Wang, F. F., Cai, Y. C., Yin, X. M., Guo, J., Zhou, L., & Zhou, X. N. (2012). Co-infection of HIV and intestinal parasites in rural area of China. *Parasites and Vectors*, 5(1). <https://doi.org/10.1186/1756-3305-5-36>
- Tzipori, S., & Widmer, G. (2008). A hundred-year retrospective on cryptosporidiosis. In *Trends in Parasitology* (Vol. 24, Issue 4, pp. 184-189). <https://doi.org/10.1016/j.pt.2008.01.002>
- Uppal, B., Singh, O., Chadha, S., & Jha, A. K. (2014). A comparison of nested PCR assay with conventional techniques for diagnosis of intestinal cryptosporidiosis in AIDS cases from Northern India. *Journal of Parasitology Research*, 2014. <https://doi.org/10.1155/2014/706105>
- Utami, W. S., Murhandarwati, E. H., Artama, W. T., & Kusnanto, H. (2020). Cryptosporidium Infection Increases the Risk for Chronic Diarrhea Among People Living With HIV in Southeast Asia: A Systematic Review and Meta-Analysis. In *Asia-Pacific Journal of Public Health* (Vol. 32, Issue 1, pp. 8-18). SAGE Publications Inc. <https://doi.org/10.1177/1010539519895422>
- Vanathy, K., Parija, S. C., Mandal, J., Hamide, A., & Krishnamurthy, S. (2017). Detection of *Cryptosporidium* in stool samples of immunocompromised patients. *Tropical Parasitology*, 7(1), 41-46. https://doi.org/10.4103/tp.TP_66_16