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## Research Article

### COMPARATIVE STUDY OF CRYPTOSPORIDIOSIS BY MODIFIED ACID FAST STAIN AND ELISA AMONG HIV/AIDS SERO-POSITIVE PATIENTS

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HIV, Cryptosporidium parvum, CD4count, Diarrhoea, Modified Ziehl-Neelsen Staining and ELISA.

#### ABSTRACT

**Introduction:** Cryptosporidium parvum be the main frequent species in human being and most important severe persistent diarrhoea among Immuno-compromised persons. Early identification and introduction of antibiotic cure considerably decrease mortality also morbidity related along with severe diarrhea caused by this parasite .Due to higher incidence of HIV/AIDS patients in our area, i.e., Raichur district of Karnataka state, it is essential to study cryptosporidiosis and its correlation with the immune status of the patient, which is one of the predominant infection and can cause severe morbidity and mortality. Detection of this parasite will help in proper management of the patients.

##### Objectives

- To determine the Cryptosporidiosis in various age groups of HIV/AIDS sero positive patients.
- To detect cryptosporidiosis by Modified Zeihl-Neelsen staining and ELISA.
- Correlate the CD4 count with incidence of cryptosporidiosis.

##### Materials and Methods

- Stool samples of 65 patients with diarrhoea HIV/AIDS collected during 2015 to 2016 RIMS, Raichur.
- Modified Zeihl-Neelsen staining.
- ELISA: Antigen detection technique was employed for cryptosporidial antigen in the faeces for the diagnosis. (DRG Kit ELISA, Germany)

**Results:** Out of 65 samples, About 33 (51%) were males 32(49%) were females. Among 65 HIV sero-positive patients 54 patients CD4 count were below 200cells/cumm<sup>3</sup> and 11 patients CD4 count were above 200cells/cumm<sup>3</sup>. In Modified Zeihl-Neelsen acid fast staining method, Cryptosporidium parvum was observed in 63 stool samples. In ELISA, out of 65 stool samples, 64 stool samples were positive for Cryptosporidium antigen. In ELISA and MZN, maximum number of cases found between 0-200 CD4 count and minimum number of cases found between 201-500 CD4 count.

**Conclusion:** The early detection of parasite helps effectively control the cases. Knowing the prevalence of Diarrhoea associated Cryptosporidiosis in a region help to start empiric treatment, in developing countries laboratory facilities are inadequate.

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#### INTRODUCTION

Cryptosporidium parvum be the main frequent species in human being and most important severe persistent diarrhoea among immunocompromised persons<sup>1</sup>. Cryptosporidiosis

represents an immense public health problem, since numerous cases are asymptomatic, present there is no efficient cure furthermore it might exist mortal within immunocompromised patients<sup>2</sup>.

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*Cryptosporidium parvum* be a protozoan parasite to have been progressively more predictable since a cause of diarrhea equal in immunocompromised and immunocompetent cases. Studies in the developing world have frequently recognized this *Cryptosporidium* sp. as a cause in acute and persistent (> 14 days) diarrhea. Infection with this parasite cause rigorous other than self-limited diarrhea in immunocompetent individuals and repeatedly fatal diarrhea within immunosuppressed individuals, most markedly patients among AIDS. Early identification and introduction of antibiotic cure considerably decrease mortality also morbidity related along with severe diarrhea caused by this parasite.<sup>4</sup> Cryptosporidiosis, generally presents like a gastroenteritis-similar to disease, caused by means of illness through protozoan parasites of *cryptosporidium* spp. intestinal cryptosporidiosis occur within immune-compromised patients strict, chronic disease can take place moreover infection can be fatal<sup>5</sup>.

Morbidity due to Cryptosporidiosis has all the time be a significant communal health crisis within the tropics, although the prevalence and severity might differ depending on the place and time<sup>6</sup>.

The mainly common presentation of cryptosporidiosis in patients is chronic or watery diarrhea, resulting in severe dehydration, abdominal pain, vomiting, nausea, low-grade fever, malnutrition and significant weight loss. Prolonged infections contribute to severe morbidity and mortality in HIV/AIDS patients<sup>7</sup>. Due to higher incidence of HIV/AIDS patients in our area, i.e., Raichur district of Karnataka state, it is important to study the opportunistic parasitic diseases like cryptosporidiosis and its correlation with the immune status of the patient, which is one of the predominant infection and can cause severe morbidity and mortality. Detection of this parasite will help in proper management of the patients.

## MATERIALS AND METHODS

**Type of Study:** The present work conducted is a type of cross sectional descriptive study.

**Ethical consideration:** Approval of institutional Ethical committee was taken prior to beginning of the study and written informed consent was obtained from the each participant

**Source of Data:** The study group consisted of inpatients and out patients of Medical, Pediatrics, Skin and STD, ART Centre and other departments of Raichur Institute of medical sciences, Raichur.

**Inclusion criteria:** All HIV/AIDS sero positive patients with diarrhoea were included in the study.

**Exclusion criteria:** All HIV/AIDS sero positive patients without diarrhoea and HIV/AIDS negative patients.

**Statistical Analysis:** P < 0.005 is considered to be significant, Since all the tests are < 0.001. Hence it is considered to be highly significant. Overall tests P values are highly significant. Test of significance used is Chi square test. Software used is statistical IBM SPSS 22.

The study was conducted in RIMS, Raichur, Karnataka. Stool samples of 65 patients with diarrhoea among HIV/AIDS collected during the period of January 2015 to December

2016 RIMS Hospital. The patients attending out-patients and admitted in wards, who presented with diarrhoea were collected with detailed clinical history were processed for microscopy and antigen detection. For microscopy, processing was done directly from fresh stool samples.

### Modified Ziehl-Neelsen staining

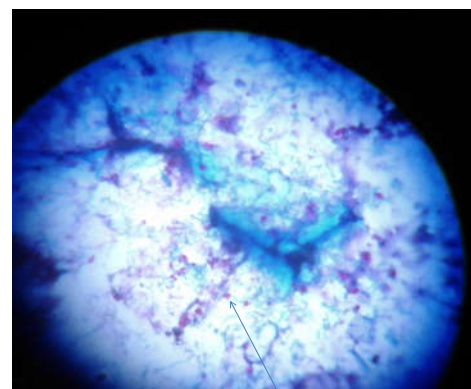
#### Procedure

After the heat fixation of the prepared fecal smear, following staining procedure will be employed.

1. Cold carbolfuchsin for 10-15 minutes.
2. Rinse with tap water.
3. Decolorize with 3%-6% sulphuric acid i.e. H<sub>2</sub>SO<sub>4</sub> rocking the slide until color stops leaking from material.
4. Rinse with tap water.
5. Counter stain with 1% Methylene blue for 30 seconds-1 minute.
6. Dry the slide, mount the smear and examine under microscope with oil immersion objective.

## RESULT

Total 65 samples examined for Microscopy, 63 samples (65.5%) were found positive by Modified Ziehl-Neelsen staining (MZN).



Oocysts of *Cryptosporidium parvum*

## ELISA

#### Procedure

- Break required wells needed from the microtitre plate and two control wells (positive and negative) place in the holder.
- Using the Micropipette add 100ul of positive control and negative control into wells.
- Add 50ul of Dilution buffer to each sample well (Don't add dilution buffer to control wells).
- Add 50ul of stool sample to each sample well and incubate for 60 minutes at room temperature (15-25<sup>0</sup>). Then wash and slap the wells to remove excess of buffer.
- Add 2 drops of Enzyme conjugate to each well at room temperature (15-25<sup>0</sup>)
- Again incubate for 30 minutes
- Then wash and slap the wells to remove excess of buffer.

- Add two drops of chromogen to each well and incubate for 10 minutes.
- Add two drops of stop solution to each well and mix the wells by tapping to side to side.
- Read the reaction after adding stop solution and read the results within 5 minutes visually or ELISA plate reader.

Micro wells containing the anti-cryptosporidium antibodies. Enzyme conjugate used diluted cryptosporidium antibodies conjugated to horseradish peroxidase Kit used is DRG International, USA. (EIA-3467)

**Result:** Total 65 samples were subjected to ELISA, 64 (98.4%) samples were found positive.



Negative control = A, Positive control = B and Positive sample

**Results:** Total 65 stool samples of HIV/AIDS patients have been collected with detailed clinical history of the patients, regarding duration & frequency of diarrhoea, weight loss & abdominal symptoms, past history of blood transfusion, TB & Personal history.

Fresh stool samples were collected in wide mouthed, clean, dry plastic containers with tight fitting lid (Because each specimen represents a potential source of infections) and the specimen brought to laboratory immediately without any delay. After receiving, samples are labeled properly.

The specimens were processed and later preserved for further tests. Stool specimens were examined macroscopically for Colour, Odour, Consistency, and Presence of blood, mucus, live worms & segments of worms. Microscopic examination was done Modified Kinyoun's acid-fast staining. Stool samples also subjected to ELISA.

Out of 65 samples, About 33 (51%) was males 32(49%) were females (Chart -1). Among 65 HIV sero-positive patients 54 patients CD4 count were below 200cells/cumm<sup>3</sup> and 11 patients CD4 count were above 200cells/cumm<sup>3</sup> (Table-1). In Modified Ziehl-Neelsen acid fast staining method, Cryptosporidium parvum was observed in 63 stool samples. In ELISA, out of 65 stool samples, 64 stool samples were positive for Cryptosporidium antigen (Table-2). In ELISA and MZN, maximum number of cases found between 0-200 CD4 count and minimum number of cases found between 201-500 CD4 counts. Correlation of CD4 counts, Diarrhoea & cryptosporidial positivity were positively correlated (Table-3).

**Sex wise distribution in the study group**

Out of 65 cases Of HIV/AIDS studied the female predominance was observed 33(51%) followed by female population 32 (49%).

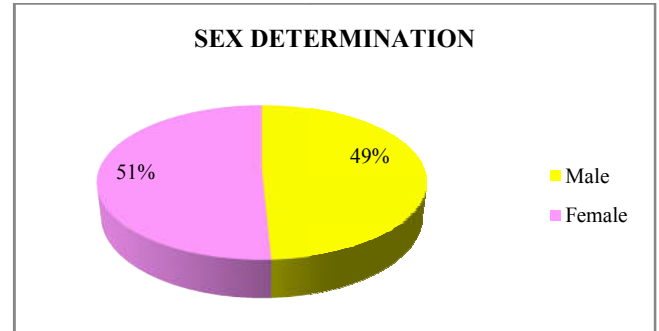


Chart 1

CD4 Count and Diarrhoea Table 1

CD4 counts(cells/ cumm <sup>3</sup> )	Diarrhoea (%)
0 – 200	54(83%)
201 – 500	11(17%)
>500	00
Total	65(100%)

In maximum number of cases found between 0-200 CD4 i.e54 (83%) count and minimum number of cases i.e11 (17%)found between 201-500 CD4 count.

Detection of Modified Zeihl-Neelsen Table 2

Method	Total -65	Sensitivity	Specificity
MZN	63(97%)	97%	100%
ELISA	64(98.4%)	98%	100%

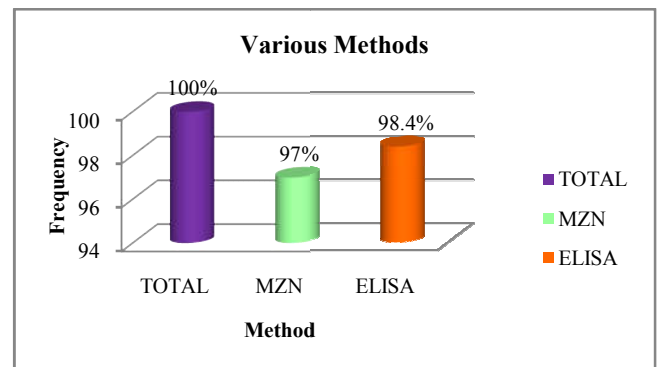


Chart 2

Correlation Cd4 Count, Diarrhoea & Cryptosporidial Positivity Table 3

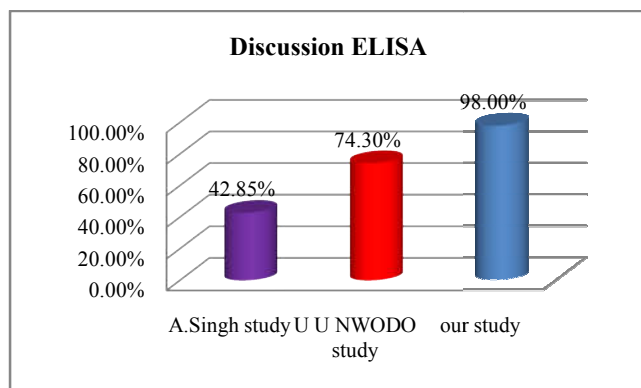
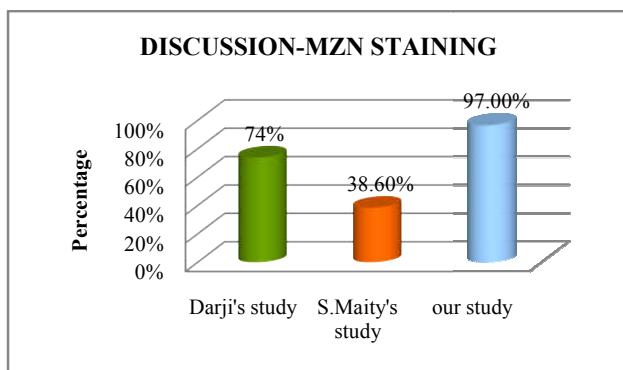
CD4 counts(cells/ cumm <sup>3</sup> )	Diarrhoea	Cryptosporidial positivity
0 – 200	54	54(84.4%)
201 – 500	11	10(15.6%)
>500	00	00
Total	65	64(100%)

CD4 Count Versus Cryptosporidium positivity is correlated positively with 0.267 correlation coefficient. CD4 Count Versus Diarrhoea is correlated positively with 0.332 correlation coefficient. Diarrhoea Versus Cryptosporidium positivity is correlated positively with 0.085 correlation coefficient.

## DISCUSSION

Prevalence of Cryptosporidiosis was 98.4% i.e. 64 out of 65 among HIV sero-positive patients in RIMS, Raichur. Parasitic infection leftover a important source of morbidity and mortality in developing countries mainly with HIV infected persons. HIV infection is a important risk factor for acquiring an Cryptosporidium infection.

In the present study, males were found more prone to develop Cryptosporidial infection as compared to females possibly due to work-related ground. The point of education among the HIV positive patients considerably affects the occurrence of Cryptosporidial infection i.e. Laborers and farmers were much affected although businessman and serviceman were least affected. In present study, prevalence of Cryptosporidial infection by Modified Zeihl-Neelsen staining was 97%.The prevalence of Cryptosporidium parvum in other two studies quite lower than my study, i.e. 74% in the Darji's study at Gujarat<sup>8</sup> and in another study by Soumendra nath Maity, Hyderabad<sup>11</sup> was quite lower than my study i.e.39%.



In present study, prevalence of Cryptosporidial infection by ELISA was 98.4%.The other two studies prevalence of Cryptosporidium parvum quite lower than my study, i.e., 74.30% by U U Nwodo at Nigeria<sup>10</sup> and another study at Manipal by A. Singh i.e. 42.85%<sup>12</sup>.on the other hand both MZN Staining and ELISA were the main ideal techniques due to its high specificity and sensitivity,

## CONCLUSION

Cryptosporidial infection plays a key role in cause of morbidity and mortality of HIV/AIDS individuals. The early detection of parasite helps effectively to control the cases. Knowing the prevalence of Diarrhoea associated Cryptosporidiosis in a region help to start empiric treatment, in developing countries

where laboratory facilities are inadequate. Routine examination for cryptosporidium oocysts in stool samples should be perform in all AIDS patients Detection of Cryptosporidium will help in proper management in patients and increase quality of life among HIV/AIDS patients

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