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FINDINGS OF *CRYPTOSPORIDIUM PARVUM* IN HIV/AIDS INDIVIDUALS IN RAICHUR DISTRICT BY USING MICROSCOPICTECHNIQUES AND MOLECULAR METHODS

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ABSTRACT

Background: Cryptosporidiosis due to *Cryptosporidium parvum* is an important zoonotic disease distributed world-wide and *Cryptosporidium parvum* was 1streported in 1907 in the gastric crypts of laboratory mouse by Edward Tyzzer. It is now well recognized in among immunocompromised individuals.In 1981 Michael Gottlieb and his colleagues at Los Angeles reported abroad range of opportunistic enteric parasites responsible for gastrointestinal infections like cryptosporidiosis, in patients with severe immunosuppression. *Cryptosporidium parvum* causes severe and protracted diarrhoea, is considered as one of the most important enteric opportunistic infections in AIDSHere is a need to know the higher incident rate of HIV sero-positivity /AIDS in Raichur District, Karnataka. **Aims and Objectives:**To determine the incidence of cryptosporidiosis in HIV sero-positive/AIDS patients.

- To detect and compare cryptosporidiosis in HIV sero-positive/AIDS patients with diarrhoea and without diarrhoea.
- To evaluate Modified ZiehlNeelsen staining technique (MZN) &Immunofluorescent microscopy technique (IFT) and Polymerase Chain Reaction for detection of cryptosporidiosis.
- To correlate the CD4 count with the incidence of cryptosporidiosis

Materials and methods: Stool samples were collected from 110HIV positive patients presenting with and without diarrhoea at Govt Medical College,Raichur after obtaining informed consent. Each stool sample was divided into 3 parts and subjected to modified Ziehl Neelsen staining method, immunofluorescent microscopy and PCR **Results:** Out of 110 cases studied, 65 patients presented with diarrhoea and the remaining 45 were without diarrhoea. The major group affected was 31- 40 years with mean age 34.4 years. Male preponderance was seen. Out of 110 patients, 80 (73%) patients had CD4 count less than 200 cells/cumm. Maximum positivity was in Immunofluorescent Microscopy 92.6%, Modified ZN staining 77.3%. **Conclusion:** Our study highlights the importance of routine examination of stool samples for cryptosporidium oocysts in all HIV sero-positive /AIDS patients, irrespective of gastrointestinal symptoms.

INTRODUCTION

Cryptosporidiosis due to *Cryptosporidium parvum* is an important zoonotic disease distributed world-wide and *Cryptosporidium parvum* was 1streported in 1907 in the gastric crypts of laboratory mouse by Edward Tyzzer. It is now well recognized in among immunocompromised individuals[1].

In 1981 Michael Gottlieb and his colleagues at Los Angeles reported abroad range of opportunistic enteric parasites responsible for gastrointestinal infections like cryptosporidiosis, in patients with severe immunosuppression[2]. *Cryptosporidium parvum* causes severe and protracted diarrhoea, is considered as one of the most important enteric opportunistic infections

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in AIDS[3].

Diarrhoeal disease is a recurrent problem in HIV infection. In developed countries frequency of diarrhoea is often 30-60% and around 90% in developing countries. Reports shows chronic diarrhoea in HIV sero-positive/AIDS patients due to cryptosporidiosis often considerably shorter life span as compared to the patients who do not have cryptosporidiosis [4].

If diarrhoea persists beyond one month it leads to loss of weight of around 10%, this condition is known as 'AIDS –defining condition'. Chronic diarrhoea is an independent marker of poor prognosis in patients with the AIDS[5]

The immune condition is a key factor in determining the severity of cryptosporidiosis [6]. Eighty percent of AIDS patients with cryptosporidiosis have CD4 count below 200cells/cumm[7]. Different studies have revealed various prevalence rates in varied geographical locations [8]. In India, prevalence rate of cryptosporidiosis is 80% [9].

Here is a need to know the higher incident rate of HIV sero-positivity /AIDS in Raichur District, Karnataka, it is important to know the opportunistic parasitic diseases like cryptosporidiosis and the immune status of the patient.

Primary findings of cryptosporidium will enable the clinician in effective management of the disease. There is a vital to evaluate for optimal benefit of the patients

OBJECTIVES

- To determine the incidence of cryptosporidiosis in HIV sero-positive/AIDS patients.
- To detect and compare cryptosporidiosis in HIV se-ro-positive/AIDS patients with diarrhoea and without diarrhoea.
- To evaluate Modified ZiehlNeelsen staining technique (MZN),Immunofluorescent microscopy technique (IFT) and PCR for detection of cryptosporidiosis.
- To correlate the CD4 count with the incidence of cryp-tosporidiosis

Hypothesis

Cryptosporidial infection may be linked with low CD4 counts and impaired immune system of the host and may precipitate fatal infection in HIV individuals.

MATERIALS AND METHODS

Type of Study: The present study undertaken is a cross \ sectional descriptive study.

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

Inclusion criteria:All HIV Sero-positive/AIDS patients with and without diarrhoea were included in the study.

Exclusion criteria: HIV Sero-negative patients were excluded.

Data Collection Procedure

Ethical consideration"Approval is taken by Institutional Ethical committee of RIMS, Raichur was obtained. Before including in the study, informed consent was obtained from each participant.

Statistical analysis

All characteristics were summarized descriptively. For continuous variables, the summary statistics of mean± standard deviation (SD) were used. Chi-square (χ^2) test was used for association between two categorical variables.

The formula for the chi-square statistic used in the chi square test is:

$$\chi_c^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

The subscript "c" is the degrees of freedom. "O" is observed value and E is expected value.

Specimen collection and Transport

Stool samples were collected from 110 HIV positive patients presenting with and without diarrhoea at RIMS, Raichur after obtaining informed consent. Stool samples were collected in a clean dry sterile plastic container, labeled with information of the patient and sent for laboratory examination immediately along with requisition form with detailed clinical history.

Processing of samples

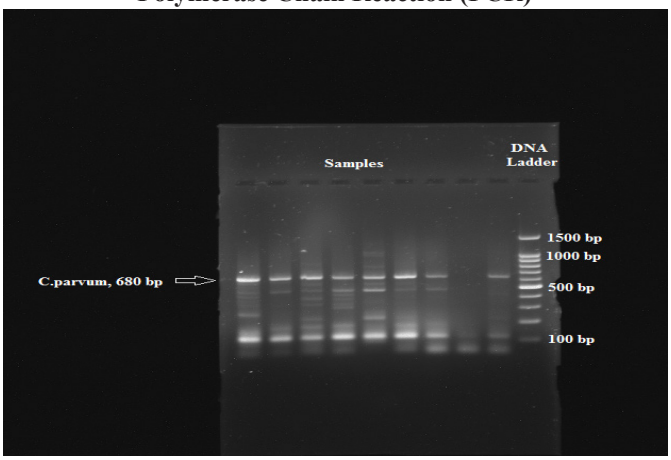
Macroscopic examination

The stool samples were observed for colour, odour, PH & consistency. The presence of blood or mucous, live worms or segments of the worms was also noted.

All sample was divided into 3 portions.

- First portion is used to prepare the smear and stained by Modified Ziehl Neelsen staining technique (MZN).
- Second portion was used to prepare the smear, fixed with ethanol and stored at room temperature for Immunofluorescent microscopy technique (IFT).
- Third portion was preserved in ethanol and stored at -4^{0}C for further analysis by Polymerase chain reaction (PCR).

Polymerase Chain Reaction (PCR)



RESULTS

The study was conducted on total of 110 stool specimens obtained from HIV infected patients admitted to RIMS government Hospital, Raichur.

Diarrhoeal Vs Nondiarrhoeal is $\chi^2 = 3$, P- value is <0.05 SIG. Out of 110 cases studied 65 patients were with diarrhoea and the remaining 45 were without diarrhoea

Age wise distribution of study individuals

The distribution of the patients in different age groups ranged

from 01-60 years. Maximum number of cases was in the age group of 31-40 years. Least number of cases was found in the age groups 0-10 years & 11-20 years. Mean age of cases in the study is 34.4 years. Amongst the 110 HIV sero-positive /AIDS cases studied, male predominance was observed i.e. 61(55%) and female population was of 49 (45%). Male to Female ratio was 10:6. The commonest age group affected amongst males was 31-40 years and lowest was 0-10years. Amongst the female maximum cases belonged to 31-40 years age group and the lowest group affected was 11-20 years (χ^2 =3.1 & P =0.692).

Description of CD4 counts among 110 cases of HIV sero-positive /AIDS

Out of 110 patients 80 patients (73%) CD4 count is less than 200cells /ul, 29 patients (26%) CD4 count is less than 500cells/ ul

Detection and comparison of cryptosporidiosis in HIV sero-positive /AIDS patients with diarrhoea and without diarrhea

Results of Modified Ziehl Neelsen staining

Out of 110 cases, cryptosporidium positive cases are 85 , 61cases were with diarrhoea and 24 cases without diarrhoea. P value is <0.001 (sig).

Results of IFT Method

Amongst of 110 cases, cryptosporidium positive cases are102 positive cases, 64 cases with diarrhoea and 38 cases without diarrhoea. P value is 0.005(sig).

Results of PCR Method: Out of 110 cases. 73 positive cases, 52 cases were with diarrhoeaand 21 cases without diarrhoea. P value is 0.002 (sig).

Detection of Cryptosporidium parvum by methods

P< 0.005 is considered to be significant Maximum detection of Cryptosporidium parvum identified in both the methods comparatively maximum in Immunofluorescent Microscopy 92.6% & follows with Modified ZN Microscopy77.5% and PCR 66.4%

DISCUSSION

The present study depicts a high incidence of cryptosporidiosis i.e. 92.6% amongst HIV sero-positive/AIDS cases in Raichur District of Karnataka. One of the studies by A. Singh from Manipal incidence of cryptosporidiosis was 42.9% quite lower than our study and another study by Anand from Manipur it was 94.4% which is equal to my study[10,11]. Globally the incidence rate of cryptosporidiosis is 60.3%[12].

In the present study, amongst 110 HIV sero-positive / AIDS cases, 61(55%) were males and 49 (45%) were females. Male predominance was observed (Male to Female ratio was 10:6). These findings are in accordance with the reports of Darji et al (2013) at Gujarat (males-61% and females-39%), Paudyal et al (2013) at Kathmandu (males-66% and females-34%) and A.Singh et al (2003) at Manipal (males-97% and females-3%)[13,14].Males are more prone to develop HIV infection as compared to females due to increased mobility, work-related issues and similar findings have been reported in other studies conducted by S.V. Kulkarin et al and S. Gupta etal[15,16].

Cryptosporidial infection and diarrhoea were common in HIV infected patients with low CD4 cell counts. Rate of diarrhoea was found to be inversely proportion in HIV patients with CD4 counts <200 cells/cumm. According to M. Darji et al and Newman RD et al *C. parvum*is an important cause of persistent diarrhoea in patients with AIDS and it can cause severe symptoms in immunocompromised patients[25] (Baqai R et a) [13,17,18].

In the present study, positivity of cryptosporidial infection by Modified Ziehl-Neelsen staining was 77.3% which is closer to the results of two studies i.e Darji's et al at Gujarat (74%), Ibrahim R Aly Shalash et al at Egypt (72.2%)[13, 18,19]. The advantage of this method is that it is inexpensive and adoptable method, for this reason all underdeveloped and developing countries still depend on this method (Connelly et al)[20].

Cryptosporidial positivity by immunofluorescent microscopy was 92.7% which varies with the findings of two studies 66.67% by Salah H Elsafi at Damman (2008) and 27% by M. Srisuphanut at Thailand, which is quite lower than our study (2005)[21,22].

In the present study PCR showed an incidence of cryptosporidial infection of 66.4%.Resultsof the studies conducted by UU Nwodo at Nigeria was 65.7% and in another study by Reza Ghafari's at Iran was 70. 8% which is quite higher than our study[23,24].

SUMMARY AND CONCLUSION

- The incidence of cryptosporidiosis amongst the HIV sero-positive/AIDS patients was 95.4%, in Raichur District of Karnataka.
- Major group affected was 31-40 years with mean age 34.4 years and male preponderance was seen.
- Seventy three percent of the cases had CD4 count of <200 cells/cumm.
- Cryptosporidial positivity rate was 98.4% amongst the patients who presented with diarrhea and 91.1% in non-diarrhoeal cases.
- Maximum positivity was detected by Immuno-fluorescent microscopy (92.6%), followed by modified Ziehl Neelsen staining (77.3%).
- This study enhanced the awareness of cryptosporidiosis among HIV sero-positive / AIDS patients in Raichur District of Karnataka.
- Our study highlights the importance of routine screening for cryptosporidiosis in all HIV sero-positive/AIDS patients, irrespective of gastrointestinal symptoms
- Immunofluorescent microscopy be considered the most reliable method for the detection of cryptosporidial oocysts in fecal specimens.

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References

1. IJMM Review article AIDS-associated parasitic diarrhoea. Dr. Arora, B. Arora 2009; vol-27:3:185-190.
2. Talib SH, Singh J.A study on opportunistic enteric parasites in 80 HIV seropositive patients. Indian J Pathol-Microbiol 1998; 41: 31-7.

3. Current WL. Garcia I Cryptosporidiosis microbiol Rev.1991:4:325-58.
4. CT Costiniuk and JB Angel. Human immunodeficiency virus and the gastrointestinal immune system: does highly active antiretroviral therapy restore gut immunity: Mucosal Immunology. Nov 2012;5: 596-604.
5. Masarat, F Ahmad, M Chisti, S. Hamid and B.Ahmad-Sofi .Prevalance of Cryptosporidiumspecies among HIV positive asymptomatic and asymptomatic immigrant population in Kashmir, India Iran J microbial. 2012; 4(1):35-39.
6. Sax PE, Rich JD, Pieciak WS, Trnka YM. Instestinalmicrosporidiosis occurring in a liver transplant recipient, Transplantation 1995; 60; 617-8.
7. Clayton F, Clayton CH. Gastroinstestinal pathology in HIV-infected patients.GastrenterolClin North Am 1997; Gastrointestinal Clin North Am 1997; 26 (2): 191-240.
8. Lew EA, Polei MA, Dieterich DT. Diarrhoeal disease associated with HIV infection. GastroenterolClin-NorhAM 1997; 26(2): 259 -90.
9. Ramakrishna BS.Prevalence of instestinal pathogens in HIV patients with diarrhoea : Implications for treatment .Implications for treatment . Indian J Pediatr 1999; 66:85-91.
10. A. Singh, I. Bairy. Cryptosporidiosis in HIV seropositive patients in Kasturba Hospital. Manipal, Karnataka, South India.Journal of Institute of Medicine,2005; 27(2),18-19.
11. Anand L, Dhanachand C and Brajachand N. Prevalence and Epidemilogical characteristics of opportunistic and non-opportunistic intestinal parasitic infections in HIV positive patients in Manipur. J. Commun. Dis. 1998; 30 (1): 19-22.
12. Selma Baiderson, Panagiotis, Karanis. Water borne transmission of Protozoan parasites: Review of Worldwide outbreaks –An update 2004-2010. 2011, vol, 45, issue 20.
13. S.M. Darji, J.D Pethani, P.D Shah, M.T Kada. Study of Intestinal Parasitic Infection in HIV Infected Patients. Gujarat, GCSMC J Med Sc., July-December 2013; Vol (II) No (II).
14. Paudyal S, Karna SR, Khatiwada S, Joshi LR, Tiwari A, Shrestha SP. Study on the prevalence of Cryptosporidium in calves and HIV infected humans in the periphery of river basins of Kathmandu valley. Int J Infect Microbial 2013; 1(2); 7-11.
15. Kulkarin S.V, Kairon R, Sane S.S, Padmavan P.S, Kale V.A, Thakar M.R, Risbad A.R; Opportunistic parasitic infection in HIV/AIDS patients presenting with diarrhoea by the level of immunosuppression. Indian J Med Res130, July 2009; pp63-66.
16. S. Gupta, S. Narang, S. Nunavath, V.Singh. 'Chronic Diarrhoea in HIV Patients Prevalence of Coccidian Parasites'. Indian Journal of Medical Microbiology, 2008; Vol. 26, No.2, pp. 172-175 (doi:10.4103/0255-0857.40536)
17. Newman RD, Zu S-X, Wuhib T, Lima AAM, Guerrant RL, Sears CL. Household epidemiology of Cryptosporidium parvum infection in an urban community in Northeast Brazil. Annal Inter Med 1994; 120:500–5.
18. Baqai R, Anwar S, Kazmi SU. Detection of cryptosporidium in immune-suppressed patients. J Ayub Med Coll Abbottabad 2005; 17:3.
19. Ibrahim R AlyShalashRababZalat,gehan El-Enain,Mohamed EL-Mohandes Mohamed EL-Faramawy and EmadAly. Comparision between Modified Acid fast-staining and Antigen Detection Assay as Diagnostic Techniques for Cryptosporodiumparvum. Worid Journal of Medical sciences 13 (1):72-78, 2016.
20. Connelly J.T, Nugen S.R, Borejsza-Wysocki W, Durst R.A, Montagna A.J. Human pathogenic Cryptosporidium species bioanalytical detection method with single oocyst detection capability. Anal. Bioanal. Chem. 2008; 36, 450–457.
21. Salah H. Elsafi,,Somaya S. Al-Sheba, Khalid M. Al-Jubran, Mohamed M. Abu Hassan, M.Sc. andEidan M. Al Zahrani, a Clinical Laboratory Journal Taibah University Medical Sciences, 2015;9(4),263-267.23.
22. Srisuphanunt,Saksirisampant and Karanis . Prevalence and genotyping of Cryptosporidium isolatedfrom HIV/AIDS patients in urban areas of Thailand. Annals of tropical Medicine & Parasitology,2011;Vol. 105, No. 6, 463–468.
23. Beauty E, Omoruyi, Uchechukwu, U. Nwodo, Chukwuneke S., Udem, Francis O Okonkwo. Comparative Diagnostic Techniques for Cryptosporidium Infection. Nigeria, Mol, 2014; Vol (II) No (II).
24. Reza Ghafaria, AbdollahRafieia, Mehdi Tavallaa, ParastooMoradiChoghakabodib, RohangezNashibic, Reza Rafiei. Prevalence of Cryptosporidium species isolated from HIV/AIDS patients in southwest of Iran. Comparative Immunology, Microbiology and Infectious Diseases 56 2018; 39–44.

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