

**Study of Cryptosporidium in HIV Infected Patients and to Evaluate Their Immune Status by Absolute Lymphocyte Count****Preeti Ranjana<sup>1</sup>, Ram Shanker Prasad<sup>2</sup>**<sup>1</sup>Tutor, Department of Microbiology, Anugrah Narayan Magadh Medical College, Gaya Ji, Bihar, India.<sup>2</sup>Professor and HOD, Department of Microbiology, Radha Devi Jageshwari Memorial Medical College & Hospital, Purnea, Bihar, India.

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Conflict of interest: Nil

**Abstract**

**Background:** Cryptosporidium is one of the major causes of diarrhea in HIV-positive patients. These patients suffer from more severe and prolonged gastrointestinal disease that can be fatal; in addition, body systems other than the gastrointestinal tract may be affected. The widespread use of antiretroviral therapy does appear to be having a beneficial effect on recovery from cryptosporidiosis and on the frequency of infection in human immunodeficiency virus-positive patients.

**Methods:** A comparative study consisting of 100 HIV patients with 50 non HIV patients, who were taken as control was done during the period of January 2021 to June 2022. The study was conducted to investigate the occurrence of Cryptosporidium in HIV patients as well as to evaluate the methods of detection of Cryptosporidium in stool samples and to identify the correlation with absolute lymphocyte counts of the patients.

**Results:** Due to the emergence of Cryptosporidium infection as an opportunistic illness in HIV patients with low immunity state, we first assessed these patients' immunological status using ALC and then CD4 counts. Outside of the hospital, in a private lab, CD4 counts were performed. 12 cases of cryptosporidiosis were detected ALC < 1000 cells/ $\mu$ L, 4 cases with ALC 1001-1500 cells/ $\mu$ L, 1 case with ALC 1501- 2000 cells/ $\mu$ L was observed. Thus, in the present study patients with ALC < 1000 cells/ $\mu$ L were 6.24 times more likely to have cryptosporidium infection

**Conclusion:** Our analysis revealed a significant relationship between cryptosporidiosis and the risk of chronic diarrhea in people living with HIV. Our results suggested that cryptosporidium infection increases the risk of chronic diarrhea, and low CD4<sup>+</sup> lymphocyte cell counts aggravate the degree of diarrhea.

**Keywords:** Cryptosporidium, Human Immunodeficiency Virus (HIV), Diarrhea, CD4<sup>+</sup> T-cell count, Total Lymphocyte Counts.

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**Introduction**

Cryptosporidiosis is a worldwide ailment caused by the parasite Cryptosporidium, a protozoan that infects many vertebrate species, including humans, producing acute gastroenteritis, stomach discomfort, and diarrhoea [1]. Dillingham et al., 2002 identified Cryptosporidiosis is primarily transmitted by the fecal-oral route, which involves eating live oocysts of animal and/or human origin released with faeces that contaminated food or water [2]. Although aquatic pathogen transmission is well documented, neither the natural reservoir nor the actual infection route of cryptosporidia is well understood [3]. Tyzzer et al., identified Cryptosporidium in 1907 [4]. Meisel et al., 1976 recognized as an opportunistic pathogenic parasite, when two human instances of cryptosporidiosis

were linked to diarrhea [5]. Cryptosporidium, on the other hand, was unknown as a significant human pathogen of acute diarrhea disease until 1982, when it was identified as a causative agent of self-limiting diarrhea in the general population and a life-threatening disease in immunocompromised people such as those receiving immunosuppressive agents and AIDS patients [4]. Although Ryan et al., 2019 found the genus Cryptosporidium contains more than 30 species, only two of them, Cryptosporidium parvum and Cryptosporidium hominis, usually infect humans [6]. C. parvum is responsible for the majority of cattle illnesses and, as a result, is thought to be responsible for the majority of zoonotic infections in humans [6]. Cryptosporidium spp. show limited host specificity,

and many representatives of this genus have been documented to infect mammals, marsupials, birds, reptiles, and fish [7]. *Cryptosporidium* is an intracellular protozoan parasite that is a human and veterinary pathogen. It is a member of the phylum Apicomplexa, which includes other pathogens like as *Plasmodium* spp., *Eimeria* spp., and *Neospora* [8]. Numerous studies have found that AIDS patients with cryptosporidiosis have a lower survival rate than AIDS patients who do not have cryptosporidiosis. Despite the prevalence and bleak prognosis of cryptosporidiosis in AIDS patients, the pathophysiology is unknown, and there is presently no effective treatment. Although diarrhoea is common in AIDS patients with cryptosporidiosis, the clinical spectrum is broad. *Cryptosporidium* infection causes prolonged, profuse, cholera-like diarrhoea in some AIDS patients, whereas in others, the diarrhoea is less voluminous, sporadic, or even resolves [9].

Cryptosporidiosis pathophysiology is as variable as its clinical symptoms. In general, the parasite infects the digestive tract's epithelial cells, causing blunting of the small intestinal villi, crypt cell proliferation, and inflammatory cell infiltration of the underlying tissue. The immune response mounted against *C. parvum* infected intestinal tissue is heterogeneous, ranging from acute inflammation with many polymorphonuclear neutrophils (PMN) entering the mucosa to chronic inflammation composed of lymphocytes, histiocytes, and eosinophils [10]. The identification of cryptosporidiosis as one of the opportunistic infections affecting individuals with AIDS also suggests that immunity to this parasite requires CD4 T cells [11]. Individuals with CD4 +T cells >200 cells /  $\mu$ L display only transient disease. The fulminant and persistent disease only occurs in individuals with CD4 + counts < 50 cells / ml. Diagnosis of *cryptosporidium* is made by identification of the organisms in duodenal aspirates, stool or tissue samples [12].

Fresh or preserved stool specimens can be examined by several concentration and staining procedures which aid in visualization of *cryptosporidium* oocysts [13]. The Formalin ethyl acetate procedure to concentrate stool specimens, modified cold Kinyoun acid fast and the fluorescein tagged monoclonal antibody (Immuno fluorescent) techniques to stain *cryptosporidium* oocysts are among the most popular methods currently in use in diagnostic laboratories [14].

However, these methods are laborious, which requires skillful experience and have low sensitivity as compared to EIA detecting antigen which have been introduced successfully for *cryptosporidium* screening in feces [15]. Serological assays provide an alternative to

parasitologic methods for monitoring *cryptosporidium* infection [16]. *Cryptosporidium* Specific Antigens (CSA) is associated with *cryptosporidium* infection and is used as the basis for fluorescent and antigen capture immunoassay. Prospect TR, a new rapid EIA identifies CSA that is produced by *cryptosporidium* oocysts as they multiply in the intestinal tract of the host. Monitoring people with HIV/AIDS necessitates the use of expensive instruments, which are difficult to get in areas with limited resources [20].

The identification of laboratory tests that assist the doctor in predicting progression is useful for both tracking the progression of the patient's disease and determining when to start treatment [21]. When a CD4+ cell count is unavailable or unaffordable, the WHO proposed using absolute lymphocyte count as a substitute marker in April 2002: a total lymphocyte count of fewer than 1,000-1,200 lymphocytes/mm<sup>3</sup> might be employed as a threshold value to start antiretroviral medication [20]. Other viruses, such HTLV-I/II, can influence the absolute lymphocyte count as well as CD4 cell counts.

Higher CD4+ cell numbers in HIV/HTLV coinfection are not immunologically advantageous and may instead be caused by non-specific lymphocyte proliferation linked to HTLV [22, 23]. In locations where co-infection by these agents is common, this may further bring bias into the evaluation of such individuals [24]. In patients with HIV-1 infection, there is a strong link between the CD4+ T-cell count and the total lymphocyte count (TLC), according to several studies. The aim of the study was to reveal a significant relationship between cryptosporidiosis and the risk of chronic diarrhea in people living with HIV.

## Materials and Methods

**Study Design:** This study involves a total of 100 HIV positive patients confirmed as per the guide lines of WHO, included in the study & 50 non-HIV patients have been taken as controls over a period of one and half year (January 2021 to June 2022).

This study was conducted in Department of Microbiology at Darbhanga Medical College and Hospital Laheriasarai, Darbhanga (Bihar). The written informed consent has been taken from each patient. Ethical clearance has been obtained from the ethical committee of Darbhanga Medical College and Hospital.

**Source of Data:** 100 HIV positive patients confirmed as per the guide lines WHO were included in the study and 50 non HIV patients were taken as controls.

**Method of study:** 100 HIV patients were grouped into 3 groups.

- Group1 - Those with acute diarrhoea (Diarrhoea < 14days)
- Group2 - Those with chronic diarrhoea (Diarrhoea 14 days –4 months)
- Group3 - Those without history of diarrhoea as controls

**Collection of Data and Staining:** Three stool samples were collected from each patient on three consecutive days and processed as described below: The stool samples of the patient were collected in a leak-proof wide mouthed screw capped container labeled with the patient's details. Specimens were kept in separate leak proof box and transported to the laboratory and processed as soon as possible. When processing was delayed it was stored in 10% formal saline at 4°C for wet coverslip preparations and staining by Modified Kinyoun stain (cold method).

**Statistical Analysis:** A Master Chart was used to record the information collected on all the selected subjects. The Statistical Package for the Social sciences (SPSS) (v25, SPSS Inc., Chicago. IL, USA) and Microsoft Excel 2016 (Microsoft Corporation, NY, USA) were used for all statistical analysis. Frequencies, percentages, means, and

standard deviations were computed using this software. Based on the number of test group either an Unpaired t-test or one-way ANOVA were applied to calculate the  $p$ -value. A significant relationship is deemed to exist when the " $p$ " value is less than 0.05.

### Results and Analysis

**Age and sex distribution of the patients in HIV and non HIV group:** Age of the patients was grouped in different categories, which started from below 20 years of age to maximum 70 years and more. Total number of patient lies in different groups was counted and its percentage was calculated in both HIV and non HIV patients. Mean value was reported with standard deviation and  $p$ -value was also reported. Table 1 a shows the age distribution of HIV and non HIV patients. Maximum number of HIV patients was lies between 21 to 30 years of age.  $P$  value was calculated as 0.4992.

The sex distribution between HIV and non HIV group of patients showed male dominance (73% and 60% respectively) over female patients (Table 1b). These samples were sex matched with  $P=0.796$ .

**Table 1a: Age distribution of HIV and non HIV patients**

| Age of patients | HIV infected patients |       | Non-HIV infected patients |       |
|-----------------|-----------------------|-------|---------------------------|-------|
|                 | Number                | % age | Number                    | % age |
| ≤ 20            | 5                     | 5.0   | 2                         | 4.0   |
| 21-30           | 33                    | 33.0  | 21                        | 42.0  |
| 31-40           | 32                    | 32.0  | 14                        | 28.0  |
| 41-50           | 21                    | 21.0  | 9                         | 18.0  |
| 51-60           | 7                     | 7.0   | 3                         | 6.0   |
| >60             | 2                     | 2.0   | 1                         | 2.0   |
| Total           | 100                   | 100.0 | 50                        | 100.0 |
| Mean ± SD       | 35.91±11.27           |       | 34.6±10.95                |       |

**Table 1b: Sex distribution of HIV and non HIV patients**

| Sex    | HIV (n=100) |      | Non-HIV (n=50) |      |
|--------|-------------|------|----------------|------|
|        | Number      | %    | Number         | %    |
| Male   | 73          | 73.0 | 30             | 60.0 |
| Female | 27          | 27.0 | 20             | 40.0 |

### Clinical profile of HIV and Non HIV patients:

The clinical profile of the patients includes the symptoms of the infection in different patients, immune status of the patients Different symptoms associated with the infection are shown in table 2 a. Total 11 types of symptoms were observed in the patients. The number of HIV patients was 100 whereas the total count of non HIV patients was 50.

It was observed that fever was the most common symptom found in both the groups.

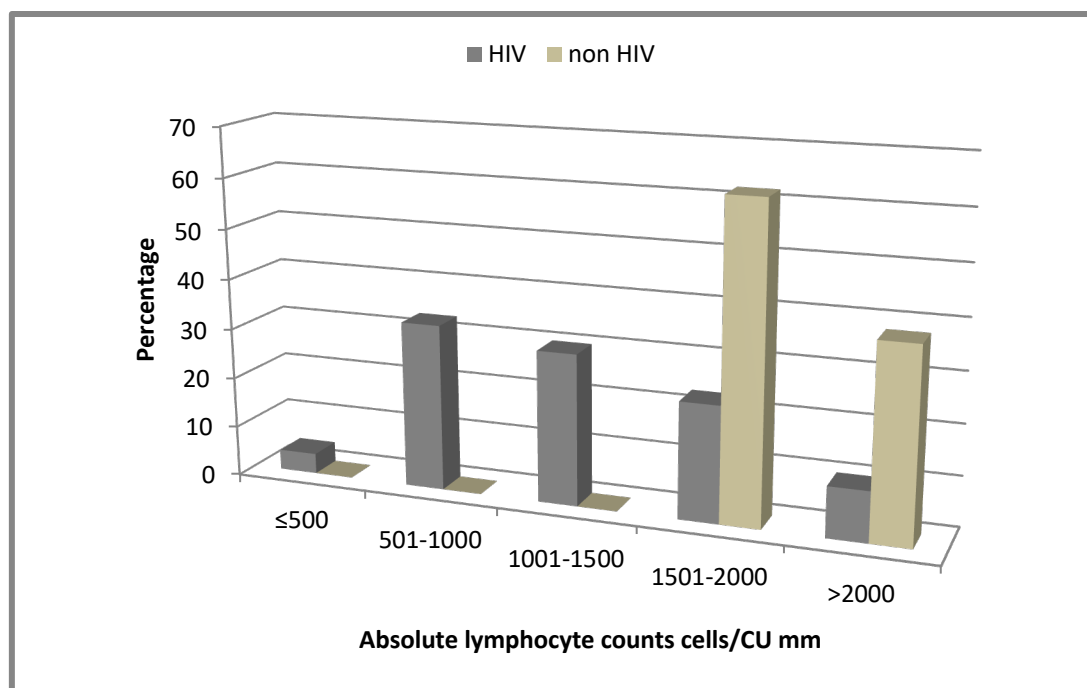
To determine and evaluate the immune status of the patients; the Absolute Lymphocyte Count (ALC) was done. Table 2 b shows the lymphocyte count of both HIV and non HIV patients, whereas Fig. 2 shows the percentage of ALC in HIV and non HIV patients.

**Table 2a: Symptoms of infection in HIV and non HIV patients**

| Symptoms                      | HIV (n=100) |      | Non-HIV (n=50) |      |
|-------------------------------|-------------|------|----------------|------|
|                               | Number      | %    | Number         | %    |
| 1.Fever                       | 58          | 58.0 | 30             | 60.0 |
| 2.Cough                       | 39          | 39.0 | 21             | 42.0 |
| 3.Weight loss                 | 42          | 42.0 | 3              | 6    |
| 4.Diarrhea                    | 56          | 56.0 | -              | -    |
| 5.Nausea                      | 15          | 15.0 | 7              | 14.0 |
| 6.Vomiting                    | 13          | 13.0 | 10             | 20.0 |
| 7.Abdominal cramps            | 12          | 12.0 | 4              | 8.0  |
| 8.Generalised Lymphadenopathy | 12          | 12.0 | -              | -    |
| 9.CNS manifestation           | 12          | 12.0 | -              | -    |
| 10.Generalized weakness       | 62          | 62.0 | 18             | 36.0 |
| 11.Oral thrush                | 24          | 24.0 | -              | -    |

**Table 2 b: Absolute Lymphocyte Count (ALC) of both HIV and non HIV patients**

| Absolute Lymphocyte counts | HIV (n=100)  | Non-HIV (n=50) | P value          | OR (HIV)     |
|----------------------------|--------------|----------------|------------------|--------------|
| cells/ $\mu$ l             |              |                |                  |              |
| $\leq 500$                 | 4 (4.0%)     | -              | 0.152            | $\leq 3.59$  |
| 501-1000                   | 33 (33.0%)   | -              | $P < 0.001^{**}$ | $\leq 47.67$ |
| 1001-1500                  | 30 (30.0%)   | -              | $P < 0.001^{**}$ | 24.50        |
| 1501-2000                  | 23 (23.0%)   | 31 (62.0%)     | 0.890            | 1.05         |
| $> 2000$                   | 10 (10.0%)   | 19 (38.0%)     | $P < 0.001^{**}$ | 0.04         |
|                            | 1295.00      | 1947.00        |                  |              |
| Mean $\pm$ SD              | $\pm 551.14$ | $\pm 214.62$   | $P < 0.001$      |              |

**Fig 1: Percentage of Absolute Lymphocyte Counts (ALC) in HIV and non HIV patients**

**Analysis of Stool samples to detect the presence of Cryptosporidium:** Stool samples from HIV and non HIV patients were collected and screening of it was done to detect the presence of Cryptosporidium in it. Maximum stool samples were found semisolid (38% in HIV and 46% in non HIV patients)

followed by formed (35% in HIV and 32% in non HIV), watery (15% in HIV and 22% in non HIV) and mucus mixed (12% in HIV). None of the sample from non HIV patients was found the mucus mixed stool. Table 3 shows the result of stool macroscopy of HIV and non HIV patients.

**Table 3: Result of stool macroscopy of HIV and non HIV patients**

| Stool macroscopy | HIV (n=100) | Non-HIV (n=50) |
|------------------|-------------|----------------|
| Formed           | 35 (35.0%)  | 16 (32.0%)     |
| Semi solid       | 38 (38.0%)  | 23 (46.0%)     |
| Watery           | 15 (15.0%)  | 11 (22.0%)     |
| Mucus mixed      | 12 (12.0%)  | 0 (0.00%)      |

**Detection of different parasites in stool sample:**

All the samples of the collected stool were screened for Cryptosporidium by (i) wet mount, (ii) modified ZN staining and (iii) Formalin Ether concentration technique followed by modified ZN staining. It was

observed that many other parasites were also present along with Cryptosporidium. Total 33 parasites were detected in HIV patients and 13 parasites were detected in non HIV patients (Table 4).

**Table 4: Various parasites detected in the stool samples of HIV and non HIV patients**

| Parasites detected          | HIV (n=100)                                                                                      | Non-HIV (n=50) |
|-----------------------------|--------------------------------------------------------------------------------------------------|----------------|
| 1. Ascaris lumbricoides.    | 4 (4.0%)                                                                                         | 6 (12.0%)      |
| 2. Ancylostoma duodenale.   | 2 (2.0%)                                                                                         | 4 (8.0%)       |
| 3. Cryptosporidium          | 17 (17.0%)                                                                                       | -              |
| 4. Cyclospora cayetanensis  | 1 (1.0%)                                                                                         | -              |
| 5. Entamoeba histolytica    | 3 (3.0%)                                                                                         | -              |
| 6. Enterobius vermicularis. | 1 (1.0%)                                                                                         | -              |
| 7. Isospora belli           | 4 (4.0%)                                                                                         | -              |
| 8. Trichuris trichiura      | 1 (1%)                                                                                           | 3 (6%)         |
| Inference                   | Cryptosporidium infection is approximately 16.9 times more likely in HIV patients with (P=0.003) |                |

**Association of age with the incidence of Cryptosporidium:** It was observed that the incidence of Cryptosporidium infection had no specific correlation with the distribution of age. Table 5 shows the association of the age of patients with the incidence of Cryptosporidium.

**Table 5: Association of age with the incidence of Cryptosporidium**

| Age in years | Cryptosporidium in HIV patients |                |
|--------------|---------------------------------|----------------|
|              | Absent (n=83)                   | Present (n=17) |
| < 20         | 4 (4.8%)                        | 1 (5.9%)       |
| 21-30        | 28 (33.7%)                      | 5 (29.4 %)     |
| 31-40        | 27 (32.5 %)                     | 5 (29.4%)      |
| 41-50        | 18 (21.7%)                      | 3 (17.6 %)     |
| 51-60        | 5 (6.1 %)                       | 2(11.8 %)      |
| >60          | 1 (1.2 %)                       | 1 (5.9%)       |

**Association of sex with the incidence of Cryptosporidium:** Table 6 shows the association of the sex of patients with the incidence of Cryptosporidium. It was observed that the incidence of Cryptosporidium was equally distributed in both male and female HIV patients.

**Table 6: Association of sex with the incidence of Cryptosporidium**

| Sex    | Cryptosporidium in HIV patients |                |
|--------|---------------------------------|----------------|
|        | Absent (n=83)                   | Present (n=17) |
| Male   | 60 (72.3%)                      | 13 (76.5 %)    |
| Female | 23 (27.7%)                      | 4 (23.5%)      |

**Association of Symptoms with Cryptosporidium infection:** Table 7 shows the association of the different symptoms with Cryptosporidium infection. Maximum number of patients was from suffering fever and weakness. It was observed that about 52.9 % patients had lost their weight due to the infection.

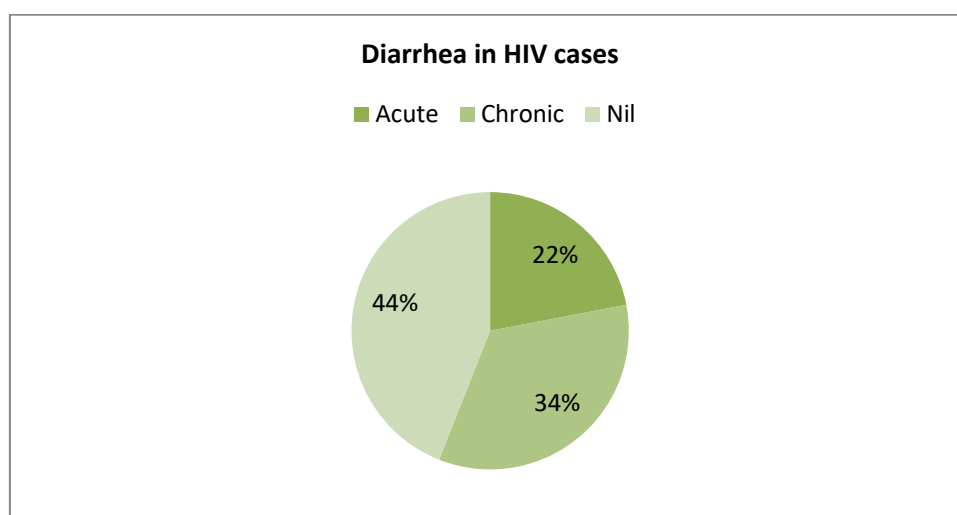
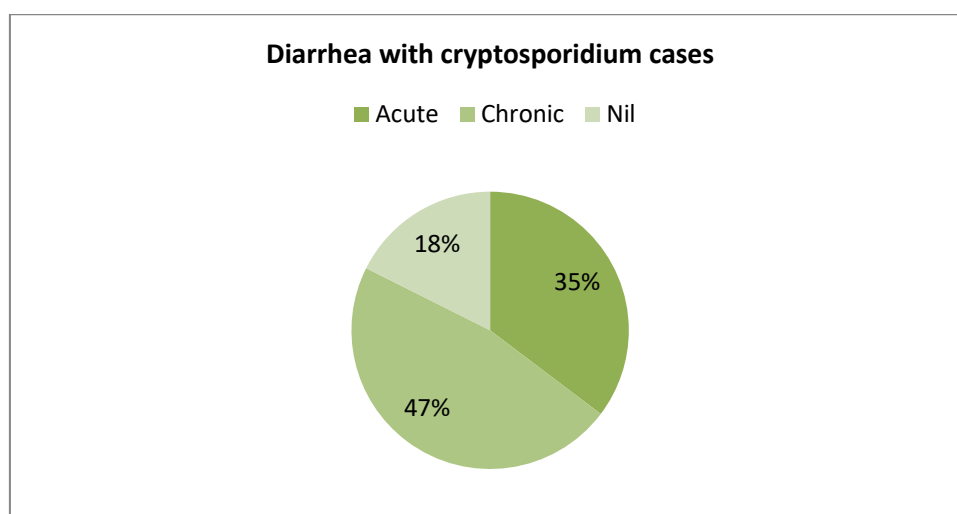
**Table 7: Association of Symptoms with Cryptosporidium infection**

| Symptoms                      | HIV patients |                               |            |
|-------------------------------|--------------|-------------------------------|------------|
|                               | Total        | Number cryptosporidium (n=17) | Percentage |
| 1.Fever                       | 58           | 2                             | 11.8       |
| 2.Cough                       | 39           | -                             | -          |
| 3.Weight loss                 | 42           | 9                             | 52.9       |
| 4.Diarrhea                    | 56           | 13                            | 76.5       |
| 5.Nausea                      | 15           | 3                             | 17.6       |
| 6.Vomiting                    | 13           | 4                             | 23.5       |
| 7.Abdominal cramps            | 12           | 5                             | 29.4       |
| 8.Generalised Lymphadenopathy | 12           | -                             | -          |
| 9.CNS manifestation           | 12           | -                             | -          |
| 10.Generalized weakness       | 62           | 13                            | 76.5       |
| 11.Oral thrush                | 10           | -                             | -          |

**Duration of Diarrhea in HIV & Cryptosporidium cases:** Out of 100 HIV patients, 44 had no diarrhea, 22 had acute diarrhea and 34 had chronic diarrhea. In non HIV patients 26 had diarrhea and 24 had no diarrhea. Cryptosporidium was found in 6 out of 22 patients with acute diarrhea, 8 out of 34 chronic diarrhea and 3 out of

44 without diarrhea patients. The incidence of Cryptosporidium was not detected in non HIV patients.

Figure 8a and b shows the duration of Diarrhea in HIV & Cryptosporidium cases.

**Fig 2a: Percentage of the types of diarrhea in HIV patients****Fig 2b: Percentage of the types of diarrhea with cryptosporidium cases in HIV patients**

**Association of Stool macroscopy with Cryptosporidium infection:** Table 9 shows the association of stool macroscopy with Cryptosporidium infection. 35% stool sample was found formed whereas 38% was semi solid kind of stool. 15% from the total sample was watery.

**Table 8: Association of stool macroscopy with Cryptosporidium infection**

| Stool macroscopy | HIV (n=100) | Number of Cryptosporidium infection (n=17) |
|------------------|-------------|--------------------------------------------|
| Formed           | 35 (35.0%)  | -                                          |
| Semi solid       | 38 (38.0%)  | 2 (11.7 %)                                 |
| Watery           | 15 (15.0%)  | 6 (35.3%)                                  |
| Mucus mixed      | 12 (12.0%)  | 9 (53.0%)                                  |

**Comparison of methods of detecting cryptosporidium in HIV cases:** Among various methods used to detect Cryptosporidium, wet mount detected only one case, modified ZN staining detected 8 cases. In formol ether concentration technique, there was further increase in number of oocysts per oil immersion field in 14 cases. Result has been presented in table 10.

**Table 9: Comparison of methods of detecting cryptosporidium in HIV cases**

| Methods                                               | Number (n=100) | %    |
|-------------------------------------------------------|----------------|------|
| Wet mount                                             | 1              | 1.0  |
| Modified ZN staining                                  | 8              | 8.0  |
| Modified ZN staining after Formol Ether sedimentation | 14             | 14.0 |

**Comparison of various methods of detecting Cryptosporidium:** Comparison of various methods of detecting Cryptosporidium has been presented in table 10. It was observed that there were some improvement after the Modified ZN staining after formol ether sedimentation.

**Table 10: Comparison of various methods of detecting Cryptosporidium**

| Cryptosporidium Cases | Wet mount | Modified ZN staining | Modified ZN staining after formol ether sedimentation |
|-----------------------|-----------|----------------------|-------------------------------------------------------|
| 1                     | ✓         | ✓                    | Improved                                              |
| 2                     | x         | X                    | x                                                     |
| 3                     | x         | ✓                    | same                                                  |
| 4                     | x         | X                    | x                                                     |
| 5                     | x         | ✓                    | Improved                                              |
| 6                     | x         | ✓                    | Same                                                  |
| 7                     | x         | ✓                    | Improved                                              |
| 8                     | x         | ✓                    | Improved                                              |
| 9                     | x         | ✓                    | Improved                                              |
| 10                    | x         | ✓                    | Same                                                  |
| 11                    | x         | ✓                    | Improved                                              |
| 12                    | x         | x                    | Same                                                  |
| 13                    | x         | x                    | Same                                                  |
| 14                    | x         | x                    | Improved                                              |
| 15                    | x         | x                    | Improved                                              |
| 16                    | x         | x                    | Improved                                              |
| 17                    | x         | x                    | x                                                     |

**Association of ALC with Cryptosporidium infection:** When presence of Cryptosporidium infection was correlated with ALC, significant number of Cryptosporidium infection was found in patients with ALC<1000 cells/μl (Table 12).

**Table 11: Association of ALC with Cryptosporidium infection**

| Absolute Lymphocyte counts cells/μl | HIV (n=100)    | Number of Cryptosporidium infection (n=17) |
|-------------------------------------|----------------|--------------------------------------------|
| □500                                | 4 (4.0%)       | 2 (11.8%)                                  |
| 501-1000                            | 33 (33.0%)     | 10 (58.8%)                                 |
| 1001-1500                           | 30 (30.0%)     | 4 (23.5%)                                  |
| 1501-2000                           | 23 (23.0%)     | 1 (5.9%)                                   |
| >2000                               | 10 (10.0%)     | -                                          |
| Mean ±SD                            | 1295.00±551.14 | 1022.87±598.92                             |

**Association of CD4 counts with Cryptosporidium infection:** The CD4 count of the patients with

Cryptosporidium infection was done to know the actual immune status of these patients. It was found that Cryptosporidium infection was common in patients with CD4 counts < 100 cells/ $\mu$ l (Table 13).

**Table 12: Association of CD4 counts with Cryptosporidium infection**

| CD4 counts/ $\mu$ l | HIV (n=100)                                                                                                | Number of Cryptosporidium infection (n=17) |
|---------------------|------------------------------------------------------------------------------------------------------------|--------------------------------------------|
| 0-50                | 9 (9.0%)                                                                                                   | 6 (35.3%)                                  |
| 51-100              | 22 (22.0%)                                                                                                 | 6 (35.3%)                                  |
| 101-150             | 22 (22.0%)                                                                                                 | 2 (11.8%)                                  |
| 150-200             | 15 (15.0%)                                                                                                 | -                                          |
| 201-250             | 15 (15.0%)                                                                                                 | 1 (5.8%)                                   |
| >250                | 17 (17.0%)                                                                                                 | 2 (11.8%)                                  |
| Inference           | Patients with CD4<100/ $\mu$ l are 6.09 times more likely to have Cryptosporidium infection with P=0.002** |                                            |

## Discussion

A serious issue for HIV patients is intestinal parasite infections. Sadraei et al. (2005) [25] conducted a study on diarrhea, CD4+ cell counts and opportunistic protozoa in Indian HIV-infected patients. Results showed that, Low CD4 counts decrease immunity, and intestinal parasite infections frequently because diarrhoea that causes considerable weight loss and cachexia. 90% of AIDS patients in developing nations experience diarrhoea. Similar study done by Kumar et al. (2002) [26] on Intestinal parasitic infection in HIV infected patients with diarrhoea in Chennai and Wiwanitkit V (2001) [27] on Intestinal parasitic infections in Thai HIV-infected patients with different immunity status revealed that diarrhea and Cryptosporidium species are frequently linked in HIV patients.

Because the condition is self-limiting, failing to detect cryptosporidiosis in immunocompetent patients with diarrhoea seldom has a negative impact. Eisenberg et al. (2001) reported that despite the fact that there is no widely accepted treatment for cryptosporidium, the detection of the condition is crucial in immunocompromised individuals since it may interfere with therapeutic treatments [28].

Keeping this view, we studied 100 HIV positive patients and 50 non HIV patients, which were taken as control. Out of 100 HIV patients 56 had diarrhea and 44 did not have diarrhea. Out of 56 patients, 22 had acute diarrhea and 34 had chronic diarrhea. Out of 50 controls no one had diarrhea. In the present study both HIV and non HIV (70%) patients recruited were in the age group of  $\leq 20$  years to maximum 75 years with a male preponderance (HIV 73 % & non HIV 60 %). The samples were age matched with P=0.485 & sex matched with P=0.796. Similar result was observed by Aggarwal et al. (2005) [29].

The clinical profile of HIV patients included fever (58%), diarrhea (56%), generalised weakness (62%), weight loss (42%), cough (39%), oral thrush (24%), nausea (15%), vomiting (13%), abdominal cramps (12%), and CNS manifestations (12%),

generalised lymphadenopathy (12%) Out of 56 HIV patients with diarrhea, 22 had acute diarrhea, (<14 days duration) and 34 had chronic diarrhea (>14 days duration). The results of the clinical profile of our patients were matched with the observations of various other studies too [30,31]. Among non HIV patients diarrhea was not found. These patients were mostly suffering from fever (60%), vomiting (20%), abdominal cramps (8%), generalised weakness (36%), cough (42%) and nausea (14%).

The main cause of diarrhea is the intestinal parasitic infestations in patients who have low immune status, so, the immune status of these patients were evaluated by their Absolute Lymphocyte Count (ALC). A study published by Kimani et al. (2003) states that according to the antiretroviral guidelines of WHO- 2002, Total Lymphocyte Count (TLC) of 1200 cells/ cumm and below can be used to initiate antiretroviral therapy but it is not validated in resource poor settings [32]. Mahajan et al. (2004) also states that, as the cost of CD4 count test is highly expensive, it has been the major obstacle in administration of HAART [33].

According to two different studies by Jacobson et al. (2003) and Mwamburi et al. (2005) within the first two years of HAART, a change in the direction of TLC appears to be a strong marker for concurrent change in CD4 count, and ALC or TLC could have clinical utility as a basis for figuring out when HIV patients should start HAART in places where CD4 count assays are unavailable. [34, 35] Numerous other research have revealed that CD4 counts and ALC may not correlate well, Akanmu et al. 2001 suggested that ALC may not be a perfect substitute for CD4 as an immune reaction to therapy monitor [36]. However the main barrier to administering HAART is now the high expense of CD4 count testing according to Mahajan et al (2004) [33].

Because to the high cost of CD4 counts, we used ALC in our study to assess the patients' immunological state. Only patients with positive cryptosporidium tests had CD4 counts performed.

Some patients' medical records were consulted to determine their CD4 numbers. In the present study 67/100(67%) HIV patients had ALC <1500 cells/ $\mu$ L and 38% non HIV patients had ALC > 2000 cells/ $\mu$ L which showed that ALC of < 1500 cells/ $\mu$ L is significantly associated with HIV infection than non HIV patients with  $P < 0.001$ .

During the study, three stool samples from each patients were collected on consecutive days and these were screened for the presence of *Cryptosporidium* by wet mount, modified ZN staining and formalin ether concentration methods. Intestinal parasites were encountered in 33 (33%) HIV patients of whom 13 (13%) had *Cryptosporidium* infection followed by *Isospora belli* 4% *Ascaris lumbricoides* 4%, *Entamoeba histolytica* 3%, *Ancylostoma duodenale* 2%, *Trichuris trichiura* 1%, *Enterobius vermicularis* 1%, *Cyclospora cayetanensis* 1%.

In non HIV group 13 out of 50 patients i.e. 26 % patients had intestinal parasites mainly comprising of *Ascaris lumbricoides* 12%, *Ancylostoma duodenale* 8% *Trichuris trichiura* 6%. *Cryptosporidium*, *Cyclospora cayetanensis* *Entamoeba histolytica*, *Enterobius vermicularis* and *Isospora belli* were not detected in the patients who were not infected with HIV. These results might be because of the small sample size or it may be, because these parasites cause self-limiting diarrhea in immune-competent patients, they must would not have pursued medical advice.

Our study showed significant number of cryptosporidium infection in HIV patients than in non HIV patients with  $P < 0.003$ . Different studies have reported 10.8 to 51.42% prevalence of cryptosporidium in various parts of our country [26,29, 37, 38, 39].

Present study correlates with the findings of Aggarwal et al. (2005) [29] and the other similar study done by Mohandas et al (2002) [37]. In the present study, there was no significant difference in the correlation of *Cryptosporidium* infection with age and sex. Similar findings were observed in other studies [40].

The clinical profile of patients with *Cryptosporidium* infection included diarrhea (76.5%) generalized weakness (76.5%), weight loss (52.9%), abdominal cramps (29.4%), vomiting (23.5%), nausea (17.6%) and fever (11.8%). Out of 13 patients with diarrhea 6 had acute diarrhea and 7 had chronic diarrhea. A study done by Awole et al. (2003) [41] showed that among the 192 HIV infected patients 54 (28.1%) and 45(23.4%) of them had chronic and acute diarrhea respectively. The prevalence of intestinal parasites were 28(51.9%) in patients with chronic and 17(37.8%) in those acute with diarrhea. *C. parvum*, *I. belli*,

and *C. catenensis* oocyst were detected only in HIV infected patients with chronic diarrhea. Our clinical profile of the patients also matched with the various other studies, [26 ,42, 43].

It was found that *Cryptosporidium* oocysts were maximum in stool samples mixed with mucus (53.0%) than in watery (35.3%) and semisolid (11.7%) kind of stools. Similar correlations were also observed in the studies done by Eisenberg et al. (2001) [28] and Chen et al. (1992) [43].

In this study, different methods of detection of *Cryptosporidium* in stool samples was also evaluated by microscopy - wet mount, modified ZN staining, formalin ether concentration technique methods.

Our observations were similar of various other studies. Claudio Viera Silva et. al, 2003 – 3/52 cases were detected positive by modified ZN staining and ELISA, 4 out of 52 only by ELISA and 45 out of 542 were negative by both methods [28]. Wet mount, Modified ZN staining and ELISA method was used for staining by Tejashree et al. 2017 [44] whereas Direct wet mount, modified Z-N and Giemsa stain techniques was used by other different researchers [45-48].

Due to the emergence of *Cryptosporidium* infection as an opportunistic illness in HIV patients with low immunity state, we first assessed these patients' immunological status using ALC and then CD4 counts. Outside of the hospital, in a private lab, CD4 counts were performed. 12 cases of cryptosporidiosis were detected ALC< 1000 cells/ $\mu$ L, 4 cases with ALC 1001-1500 cells/ $\mu$ L, 1 case with ALC 1501- 2000 cells/ $\mu$ L was observed. Thus, in the present study patients with ALC< 1000 cells/ $\mu$ L were 6.24 times more likely to have cryptosporidium infection with  $P=0.002$ .

## Conclusions

Due to their reduced immunity, people with HIV/AIDS are more susceptible to infections, particularly opportunistic intracellular parasites that target the small intestine and have dire prognoses. In AIDS patients, parasites can cause protracted, potentially fatal diarrhoea. Early detection of these opportunistic parasites will allow the practitioner to treat the patient effectively and prevent the patient's mortality from rising. *Coccidia* made up the majority of the protozoan parasites discovered in HIV patients, which were more prevalent than helminths. The most prevalent parasite found was *Cryptosporidium parvum*. Diarrhea, especially chronic diarrhea, was more severe in HIV-infected people with decreased CD4 counts and immunity. Lower immune status individuals frequently had parasites. *C. parvum* was found in people with a variety of immunological conditions, including those who weren't diarrhoeic, but the infection

incidence was highest in people with CD4 counts between 100 - 200 cells/mm<sup>3</sup>. In this work, a number of intestinal parasites were successfully identified in HIV patients using straightforward techniques, including wet mounts and modified acid-fast staining. The formalin ether concentration process improved the sensitivity of detection.

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### References

1. Mary T. Parisi and Philip M. Tierno, JR. Evaluation of New Rapid Commercial Enzyme Immunoassay for Detection of Cryptosporidium Oocysts in Untreated Stool Specimens. J Clin Microbiol. 1995; 33(7):1963-1965.
2. Michael J Arrowood. In Vitro Cultivation of Cryptosporidium Species. Clin Microbiol Rev. 2002;15(3):390-400.
3. Miao YM, Gazzard BG. Management of protozoal diarrhoea in HIV disease. HIV Med. 2000;1:194-9.
4. Logan C, Beadsworth MB, Beeching NJ. HIV and diarrhoea: what is new? Curr Opin Infect Dis. 2016;29:486-94.
5. Shimelis T, Tassachew Y, Lambiyio T. Cryptosporidium and other intestinal parasitic infections among HIV patients in southern Ethiopia: significance of improved HIV-related care. Parasit Vectors. 2016;9:270.
6. Iribarren JA, Rubio R, Aguirrebengoa K, Arribas JR, Baraia-Etxaburu J, Gutierrez F, et al. Prevention and treatment of opportunistic infections and other coinfections in HIV-infected patients: may 2015, Enferm Infecc Microbiol Clin. 2016;34:516.e1-516.e18.
7. Goodgame RW. Understanding intestinal spore-forming protozoa: Cryptosporidia, microsporidia, isospora, and cyclospora. Ann Intern Med. 2016;124:429-41.
8. Mbae C, Mulinge E, Waruru A, Ngugi B, Wainaina J, Kariuki S. Genetic diversity of Cryptosporidium in children in an urban informal settlement of Nairobi, Kenya. PLoS One, 2015;10:e0142055.
9. Marcos LA, Gotuzzo E. Intestinal protozoan infections in the immunocompromised host. Curr Opin Infect Dis. 2013;26:295-301.
10. Michael A Poles ,Edmund J Bini and Jonathan Cohen. Gastrointestinal infections in patients infected with HIV-1 . Curr Opin Infect Dis. 2017;5:219-235.
11. Dioniso D. Cryptosporidiosis in HIV-infected patients. J postgrad Med 2002;48(3):215-216.
12. W L Current . Cryptosporidiosis. Chapter18. Topley and Wilson's Microbiology and Microbial Infections. Volume 5 .9th Edition. Volume editors: Libero Ajello and Roderik J Hay. Arnoldpublishers.
13. Rainer Weber , Ralph t. Bryan, and Dennis D. Juranek. Improved Stool Concentration Procedure for Detection of Cryptosporidium Oocysts in Fecal Specimens. J Clin Microbiol 2012;30(11):2869-2873.
14. Beth L.P.Ungar. Cryptosporidium. Chapter 262. Mandell, Douglas and Bennet's Principles and Practice of Infectious Diseases. Fourth Edition. New York Churchill Livingstone 2015.
15. DP Casemore, RL Sands, A Curry. Cryptosporidium species a "new" Human pathogen .J clin Pathol 1985;38:1321-1336.
16. Meamar AR, Rezaian M, Mohraz M, Zahabiun F, Hadighi R, Kia EB, A Comparative Analysis of Intestinal Parasitic Infections between HIV+/AIDS Patients and Non-HIV Infected Individuals. Iranian J Parasitol. 2017; 2(1):1-6.
17. Sauda FC, Zamarioli LA. Prevalence of Cryptosporidium sp. and Isospora belli among AIDS patients. J Parasitol. 2016; 79(3):454-56.
18. Brandoniso O, Maggi P. Prevalence of Cryptosporidiosis in HIV-infected patients with diarrhoea illness. Eur J Epidemiol. 2013; 9 (2):190-94.
19. Hazrati-Tappeh KH, Gharavi MJ. Makhdoumi K. Rahbar M, Taghizadeh A. Prevalence of Cryptosporidium spp. Infection in Renal Transplant and Hemodialysis Patients. Iranian J Publ Health. 2016; 35 (3):54-7.
20. Akinola O.O., Adediran I.A., Onayemi O., et al. The search for a predictor of CD4 cell count continues: Total lymphocyte count is not a substitute for CD4 cell count in the management of HIVinfected individuals in a resource-limited setting. Clin Infect Dis 2004;39: 579-81.
21. Pascale J.M.I.M., Contreras P., Gomez B., et al. Immunological markers of disease progression in patients infected with the Human Immunodeficiency Virus. Clin Diagn Lab Immunol 1997;4(4): 474-7.
22. Guimarães M.L.B.F., Telles P.R., Galvão-Castro B., et al. Retrovirus infections in a sample of injecting drug users in Rio de Janeiro City. Brazil: prevalence of HIV-1 subtypes. and co-infection with HTLV-I/II. J Clin Virol 2001;21:143-51.
23. Willy R.J.S.C., Macalino G.E., Rich J.D. Long-term non-progression of HIV-1 in a patient coinfecting with HTLV-II. Diagn Microbiol Infect Dis 1999;35: 269-70.
24. Schechter M.Z.R., Machado L.L., Pinto M.E., et al. Predicting CD4 counts in HIV-infected Brazilian individuals: a model based on the World Health Organization Staging System.

- JAIDS 1994;7:163-8.
25. Sadraei J, Rizvi MA, Baveja UK. Diarrhea, CD4+ cell counts and opportunistic protozoa in Indian HIV-infected patients. *Parasitol res.* 2005;97:270-3.
  26. Kumar SS, Ananthan S, Lakshmi P. Intestinal parasitic infection in HIV infected patients with diarrhoea in Chennai. *Indian J Med Microbiol.* 2002; 20(2):88-91.
  27. Wiwanitkit V. Intestinal parasitic infections in Thai HIV-infected patients with different immunity status. *BMC gastroenterology.* 2001;1(1):1-3.
  28. Eisenberg JN, Priest JW, Lammie PJ, Colford Jr JM. The Serologic response to Cryptosporidium in HIV-infected persons: implications for epidemiologic research. *Emerging infectious dis.* 2001;7(6):1004.
  29. Aggarwal A, Arora U, Bajaj R, Kumari K. Clinicomicrobiological study in HIV seropositive patients. *J Indian Acad Clin Med.* 2005;6(2):142-5.
  30. Ayyagiri A, Sharma AK, Prasad. Spectrum of opportunistic infections in Human Immunodeficiency virus [HIV] infected cases in a tertiary care hospital. *Indian J Med Microbiol* 1999;17:78-80
  31. Talib SH, Singh J. A study on opportunistic enteric parasites in HIV Seropositive patients. *Indian J Path* 1998;41:31-37.
  32. Kimani J, Irungu E, Thottingal P, Njeri J, Kariri A, Wachihi C. Is a total lymphocyte count a surrogate marker for absolute CD4+ cells count among HIV-1 infected patients in Nairobi Kenya. *Antivir Ther* 2003;8 (sSuppl. 1).
  33. Mahajan AP, Hogan JW, Snyder B, Kumarasamy N, Mehta K, Solomon S, Carpenter CC, Mayer KH, Flanigan TP. Changes in total lymphocyte count as a surrogate for changes in CD4 count following initiation of HAART: implications for monitoring in resource-limited settings. *JAIDS.* 2004;36(1):567-75.
  34. Jacobson MA, Liu L, Khayam-Bashi H, Deeks SG, Hecht FM, Kahn J. Absolute or total lymphocyte count as a marker for the CD4 T lymphocyte criterion for initiating antiretroviral therapy. *AIDS.* 2003;17(6):917-9.
  35. Mwamburi DM, Ghosh M, Fauntleroy J, Gorbach SL, Wanke CA. Predicting CD4 count using total lymphocyte count: a sustainable tool for clinical decisions during HAART use. *American J trop med hygiene.* 2005;73(1):58-62.
  36. Akanmu AS, Akinsete I, Eshofonie AO, Davies AO, Okany CC. Absolute Lymphocyte Count as Surrogate for CD4+ Cell Count in monitoring Response to Antiretroviral. *Nigerian Postgraduate Med J.* 2001;8(3):105.
  37. Mohandas K, Sehgal R, Sud A, Malla N. Prevalence of intestinal parasitic pathogens in HIV-seropositive individuals in Northern India. *Japanese J infec dis.* 2002;55(3):83-4.
  38. Singh A, Bairy I, Shivananda PG. Spectrum of opportunistic infections in AIDS cases. *Indian J med sci.* 2003;57(1):16-21.
  39. 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.
  40. Casemore DP, Sands RL, Curry A. Cryptosporidium species a "new" human pathogen. *J Clin Pathol.* 1985;38(12):1321-36.
  41. Awole M, Gebre-Selassie S, Kassa T, Kibru G. Prevalence of intestinal parasites in HIV-infected adult patients in southwestern Ethiopia. *Ethiopian J Health Dev.* 2003;17(1):71-8.
  42. Nimri LF, Batchoun R. Prevalence of Cryptosporidium species in elementary school children. *J clin microbiol.* 1994 ;32(4):1040-2.
  43. Chen YG, Yao FB, Li HS, Shi WS, Dai MX, Lu MI. Cryptosporidium infection and diarrhea in rural and urban areas of Jiangsu, People's Republic of China. *J clin microbiol.* 1992;30(2):492-4.
  44. Tejashree A, Pooja PB, Babu KS. Comparative Evaluation of Microscopy and ELISA in Diagnosis of Cryptosporidiosis in HIV and Non-HIV Patients. *Int. J. Curr. Microbiol. App. Sci.* 2017;6(1):232-9.
  45. Udeh EO, Obiezue RN, Okafor FC, Ikele CB, Okoye IC, Otuu CA. Gastrointestinal parasitic infections and immunological status of HIV/AIDS coinfecting individuals in Nigeria. *Ann Global Health.* 2019; 85(1): 99, 1–7.
  46. Shimelis T, Tassachew Y, Lambiyo T. Cryptosporidium and other intestinal parasitic infections among HIV patients in southern Ethiopia: significance of improved HIV-related care. *Parasit Vectors.* 2016;9:1-7.
  47. Sangaré I, Bamba S, Cissé M, Zida A, Bamogo R, Sirima C, Yaméogo BK, Sanou R, Drabo F, Dabiré RK, Guiguemdé RT. Prevalence of intestinal opportunistic parasites infections in the University hospital of Bobo-Dioulasso, Burkina Faso. *Infect Dis Poverty.* 2015;4:1-6.
  48. Singh DD, Singh V. Prevalence of Intestinal parasitic infections in HIV infected patients presenting with diarrhea and their association with CD4+ Counts. *Int J Appl Sci Biotechnol.* 2015;3(1):96-100.