

Original Research Article

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## Evaluation of CD4 Count and Its' Correlation with Opportunistic Intestinal Parasitic Infections in HIV Seropositive

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### ABSTRACT

Human Immunodeficiency Virus (HIV) infection and its end stage, Acquired Immunodeficiency Syndrome (AIDS), is an extremely serious problem worldwide. HIV seropositive patients with CD4 T cell count < 200 cells/ $\mu$ l have reported an excess risk of diarrhoea. The present study was undertaken in the at Dr. S.C.G.M.C, Nanded between November 2015 and October 2017 to identify Opportunistic Intestinal parasites in HIV positive patients presenting with or without diarrhoea and their association with CD4 T cell count. Newly diagnosed 300 patients with HIV infection confirmed by serology were included and divided into two groups Diarrhoeal and non-diarrhoeal. CD4 T lymphocyte cell count was estimated using Flow-Cytometry. Stool specimens were collected from all the patients and examined macroscopically and microscopically. We found that intestinal parasites in subjects with diarrhoea were significantly higher than without diarrhoea. *Cryptosporidium sp.* was the most common parasite detected. Intestinal parasites were found to be more common in HIV seropositive patients with CD4 cell count < 200 cells/ $\mu$ l. Thus, routine screening of the stool samples of HIV seropositive patients with diarrhoea should be done for prompt patient care and prevent the emergence of fulminant form of the disease.

#### Keywords

HIV, Opportunistic Parasitic Infections, CD4 count, *Cryptosporidium*

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### Introduction

Human Immunodeficiency Virus (HIV) infection and its end stage, Acquired Immunodeficiency Syndrome (AIDS), is an extremely serious problem worldwide.<sup>1</sup> Clinical course of HIV infection is characterized by a decrease in number of CD4 T cells and persistent viral replication, resulting in immunological decline. HIV is a window for Opportunistic Infections, by

making the conditions favourable for the survival and expansion of opportunistic infections causing disease.<sup>2</sup> HIV seropositive patients with CD4 T cell count < 200 cells/ $\mu$ l have reported an excess risk of diarrhoea compared with those having 500 cells/ $\mu$ l and above.<sup>3,4</sup> The infectious etiological agents include both opportunistic agents and non-opportunistic agents.<sup>5</sup> Studies on correlation of HIV/AIDS with enteric parasitic infection in South Asian region have reported

association of several species of pathogenic protozoa such as *Cryptosporidium spp.*, *Cystoisospora belli*, *Cyclospora cayetanensis*, *Giardia lamblia*, *Entamoeba histolytica*, *Blastocystishominis*, and *Microsporidia species*.<sup>4,6</sup> Since diarrhoeal illness due to Opportunistic Intestinal Parasitic Infections is increasing among HIV infected subjects, and only a few studies regarding enteric parasites and their association with CD4 T cell count are available from our region. Against this background the present study was carried out to identify Opportunistic Intestinal parasites in HIV positive patients presenting with or without diarrhoea and their association with CD4 T cell count.

To identify the various Opportunistic Intestinal Parasitic infections and correlate it with CD 4+ T cell count in HIV Seropositive individuals.

### **Materials and Methods**

The present study was undertaken in the Department of Microbiology and Anti-Retroviral Therapy (ART) center at Dr. S.C.G.M.C, Nanded between November 2015 and October 2017. Newly diagnosed 300 patients with HIV infection confirmed by serology were included in this study. According to their diarrhoea status the study samples were divided into two groups Diarrhoeal and non-diarrhoeal. Blood was collected aseptically to detect antibodies to HIV by using HIV Comb Aids-RS, AIDSCAN, and SD HIV1/2 3.0 Kits. Testing was carried out as per manufacturer's instructions as per HIV testing strategy II B NACO guidelines.<sup>7,8</sup>

CD4 T lymphocyte cell count was estimated using partec Flow-Cytometry Instrument (cyflow) using Partec CD4 Easy count kit.<sup>9</sup> Stool specimens were collected from all the patients irrespective of diarrhoea status. The stool specimens were examined

macroscopically and microscopically. The saline wet mount was examined for the motile trophozoites, larvae, cysts, ova, pus cells, and RBCs. The iodine wet mount was examined particularly for the study of nuclear characteristics and glycogen mass of protozoan cysts.

Modified Ziehl-Neelson (Kinyoun's) stain<sup>10</sup> was used for identification of oocysts of *Cryptosporidium*, *Cyclospora*, and *Isospora*. Modified Trichrome (Weber) stain was used for identification of *Microsporidia* spores.

Out of total 300 patients, 189 (63%) were males and 111 (37%) were females. Majority of study subjects i.e. 139 (46.33%) belonged to age group of 31-40 years.

Out of a total 300 patients 226 (75.33%) belonged to rural areas and 74 (24.67%) people belonged to urban areas.

Diarrhoea was present in a total of 158 (52.66%) patients.

Among 158 HIV seropositive patients with diarrhoea, parasites were detected in 92 (58.22%) stool specimens and among the 142 HIV seropositive patients without diarrhoea, parasites were detected in 42 (29.57%) stool specimens.

The patients in this study were divided into three groups based on their CD4 T cell counts. 128 (42.67%) subjects in this study had CD4 T cell counts below 200 cells/ $\mu$ l while 132 (44%) had CD4 T cell counts between 200-499 cells/ $\mu$ l and 40 (13.33%) subjects had CD4 T cell counts above 500 cells/ $\mu$ l.

### **Results and Discussion**

A total of 300 newly diagnosed HIV seropositive patients were included in this study. Majority of these (46.33%) belonged to

age group 31-40 years (Fig. 1). Similar studies conducted by Uppal *et al.*,<sup>11</sup> and Munde *et al.*,<sup>12</sup> also reported that 46% HIV seropositive patients with diarrhoea belonged to age group 31-40 years. According to NACO annual report 2010-11 and UNAIDS/WHO global report 2010, majority of the HIV positive patients belonged to age group of 15-49 years (83% and 92.49% respectively).<sup>13,14</sup> Our study showed similar HIV positivity (90.67%) in 21-50years age group.

According to NACO annual report (2016-17), the HIV prevalence in males is higher than females. The prevalence in age group 15-49yrs in males was 0.30% and females 0.22%.<sup>15</sup> In present study also males (63%) predominated over females (37%).

Out of a 52.66% stool samples from patients with diarrhoea, intestinal parasites were seen in 30.66% samples whereas in the 47.33% stool samples from patients who presented without diarrhoea, intestinal parasites were seen in 42 (14%) stool samples (Table 1).

A study by Mohandas *et al.*,<sup>16</sup> showed 30% prevalence of enteric parasites in samples from HIV seropositive patients. Naik *et al.*,<sup>17</sup> reported that out of the 359 HIV positive patients, enteric parasites were seen in 195(54.31%) of HIV positive patients with diarrhoea compared to 12(12%) of HIV positive patients without diarrhoea. Mathur *et al.*,<sup>18</sup> observed that intestinal parasites in chronic diarrhoea were significantly higher than the acute diarrhoea (63.05% vs. 7.35%). Ahmed *et al.*,<sup>19</sup> found that enteric parasites were detected in 35.9% of HIV sero- positive patients having diarrhoea and 18% of HIV sero- negative patients having diarrhoea.

In the present study, intestinal parasites were seen in 58.22% of HIV seropositive patients with diarrhoea (Table 2). Various studies

from India and other countries have reported high prevalence of intestinal parasites, ranging between 30-60%.<sup>4,20,22,21,23,24</sup>

Our results are comparable with the studies conducted by Gupta *et al.*,<sup>21</sup> in 2008, Basak *et al.*,<sup>25</sup> in 2010 and Chavan *et al.*,<sup>31</sup> in 2014 who reported 55.8%, 58.8% and 58.5% intestinal parasites in HIV seropositive subjects with diarrhoea respectively.

In the present study, intestinal parasites were seen in 29.57% HIV seropositive patients without diarrhoea (Table 2). Various studies from India and other countries have reported the prevalence of intestinal parasites, ranging between 12-40%.<sup>4,5,26</sup>

In our study, out of the 83 protozoan parasites, intracellular protozoa (coccidian parasites) were found in 69 (83.13%) stool specimens while extracellular protozoa were detected in 14 (16.86%) stool specimens (Table 2) in patients with diarrhoea. In patients without diarrhoea, out of 39 protozoan parasites, intracellular protozoa (coccidian parasites) were found in 30 (76.92%) stool specimens and extracellular protozoa were detected in 9 (23.07%) stool specimens (Table 2). Basak *et al.*,<sup>25</sup> reported that, intracellular protozoa were found in (61.17%) stool specimens while extracellular protozoa were detected in (38.82%). Munde *et al.*,<sup>12</sup> found that, intracellular protozoa were found in (82.47%) while extracellular protozoa was detected in (17.43%) HIV seropositive patients with diarrhoea.

In our study *Cryptosporidium spp.* was the most common enteric pathogen detected in 32.27% followed by *Isospora belli* in 8.86% of stool specimens of patients presenting with diarrhoea (Table 2). *Cryptosporidium spp.* was the most common 97 enteric pathogen detected in 21.12% in stool specimens of patients without diarrhoea (Table 2). Studies

conducted by Naik *et al.*,<sup>27</sup> Mathur *et al.*,<sup>18</sup>, Munde *et al.*,<sup>12</sup> and reported *Cryptosporidium spp.* (15.87%, 24.80% and 27.40% respectively) as the most common parasite detected followed by *Isospora belli* (10.02%, 7.70% and 8.66% respectively) in HIV seropositive patients.

Kaur *et al.*,<sup>5</sup> found that the prevalence of *Cryptosporidium spp.* among the diarrhoeal and non-diarrhoeal group of patients was 40.41% and 21.42% respectively and the prevalence of *Isospora belli* was 11.64% in the diarrhoeal group and no *Isospora* were detected in the non-diarrhoeal group.

This is similar to findings in our study where no *Isospora* were detected in the non-diarrhoeal group.

*C. cayetanensis* was found in 2.53% of stool specimens from patients with diarrhoea in our study (Table 2). In a study conducted by Mohandas *et al.*,<sup>16</sup> *C. cayetanensis* was reported in 3.3% subjects, Kulkarni *et al.*,<sup>23</sup> *C. cayetanensis* reported in 0.7% of the HIV-infected individuals presenting with diarrhoea. Other studies by Khalil *et al.*,<sup>4</sup> Munde *et al.*,<sup>12</sup> and Swathirajan *et al.*,<sup>2</sup> found *C. cayetanensis* in 0.8%, 2.40% and 1.3% HIV seropositive patients respectively. In our study, *Giardia lamblia* was detected in 6.32% of stool specimens from patients with

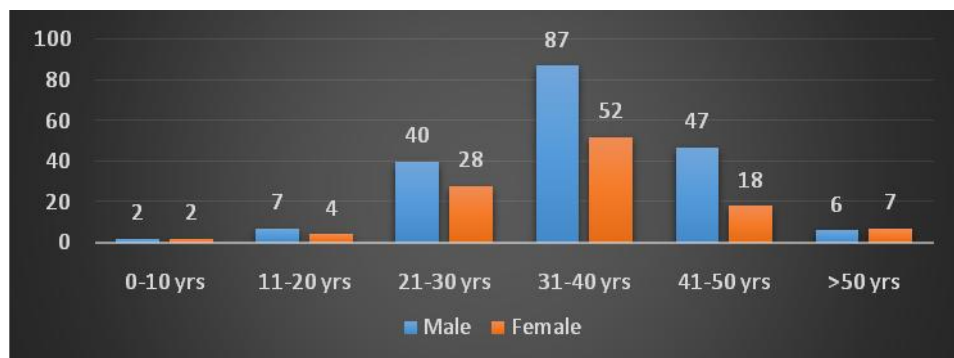
diarrhoea and 4.92% in patients without diarrhoea and *Entamoeba histolytica* in 2.53% stool specimens of patients with diarrhoea and 1.40% of patients without diarrhoea (Table 2). Basak *et al.*,<sup>25</sup> reported *Giardia lamblia* and *Entamoeba histolytica* in 8.1% and 14.1% patients respectively.

Kaur *et al.*,<sup>5</sup> detected *Giardia lamblia* in 10.27% of stool specimens from patients with diarrhoea and 5.24% in patients without diarrhoea and *Entamoeba histolytica* in 4.79% stool specimens of patients with diarrhoea. Swathirajan *et al.*,<sup>2</sup> reported *Giardia lamblia* in 1.4% and *Entamoeba histolytica* in 3.4% of HIV seropositive patients with diarrhoea.

*S. stercoralis* was found in 2.53% stool specimens of HIV seropositive patients with diarrhoea (Table 2). Kaur *et al.*,<sup>5</sup> found *S. stercoralis* in 1.36% stool specimens of HIV seropositive patients with diarrhoea.

In our study *Cryptosporidium spp.* was detected as the most common parasite in the study. Prevalence of *Cryptosporidium spp.* among the diarrhoeal and non-diarrhoeal group of patients was 32.27% and 21.12% respectively (Table 2). The difference in prevalence of *Cryptosporidium spp.* ( $p < 0.029$ ) was significant in diarrhoeal group in comparison to non-diarrhoeal group.

Fig.1 Age and Sex distribution of the study population (n=300)



**Table.1** Total Parasites detected in the study (n=300)

Diarrhoea	Parasites		Total
	Present	Absent	
Present	92 (30.66%)	66 (22%)	158 (52.66%)
Absent	42 (14%)	100 (33.33%)	142 (47.33%)
Total	134 (44.66%)	166 (44.33%)	300 (100%)

**Table.2** Parasitic profile of diarrhoeal and non-diarrhoeal group

Parasites	Diarrhoeal group (n=158)	Non-diarrhoeal group (n=142)
<i>Cryptosporidium</i> spp.	51 (32.27%)	30 (21.12%)
<i>Isoospora</i> spp.	14 (8.86%)	-
<i>Cyclospora</i> spp.	04 (2.53%)	-
<i>Giardia lamblia</i>	10 (6.32%)	07 (4.92%)
<i>E. histolytica</i>	04 (2.53%)	02 (1.40%)
<i>S. stercoralis</i>	04 (2.53%)	-
<i>A. lumbricoides</i>	02 (1.26%)	02 (1.40%)
<i>A. duodenale</i>	02 (1.26%)	01 (0.70%)
<i>Taenia</i> spp.	01 (0.63%)	-
<b>Total</b>	<b>92 (58.22%)</b>	<b>42 (29.57%)</b>

**Table.3** Association of parasites with CD4 T cell count in patients with diarrhea

Parasites	No. of subjects with CD4 T cell count			Total (n=158)
	< 200 cells/ $\mu$ l (n=91)	200-499 cells/ $\mu$ l (n=58)	$\geq$ 500 cells/ $\mu$ l (n=9)	
<b>Protozoa</b>				
<i>Cryptosporidium</i> spp.	41 (45.05%)	09 (15.51%)	01 (11.11%)	51 (32.27%)
<i>Isoospora</i> spp.	11 (12.08%)	03 (5.17%)	-	14 (8.86%)
<i>Cyclospora</i> spp.	03 (3.26%)	01 (1.72%)	-	04 (2.53%)
<i>Giardia lamblia</i>	06 (6.59%)	04 (6.2%)	-	10 (6.32%)
<i>E. histolytica</i>	02 (2.19%)	01 (1.72%)	01 (11.11%)	04 (2.53%)
<b>Helminths</b>				
<i>S. stercoralis</i>	04 (4.39%)	-	-	04 (2.53%)
<i>A. lumbricoides</i>	01 (1.09%)	01 (1.72%)	-	02 (1.26%)
<i>A. duodenale</i>	01 (1.09%)	01 (1.72%)	-	02 (1.26%)
<i>Taenia</i> spp.	-	01 (1.72%)	-	01 (0.63%)
<b>Total</b>	<b>69 (75.82%)</b>	<b>21 (36.20%)</b>	<b>02 (22.22%)</b>	<b>92 (58.22%)</b>



**Table.4** Association of parasites with CD4 T cell count in patients without diarrhea

Parasites	No. of subjects with CD4 T cell count			Total (n=142)
	< 200 cells/ $\mu$ l (n=37)	200-499 cells/ $\mu$ l (n=74)	$\geq$ 500 cells/ $\mu$ l (n=31)	
<b>Protozoa</b>				
<i>Cryptosporidium</i> spp.	18 (48.64%)	11 (14.86%)	01 (3.22%)	30 (21.12%)
<i>Isoospora</i> spp.	-	-	-	-
<i>Cyclospora</i> spp.	-	-	-	-
<i>Giardia lamblia</i>	03 (8.10%)	03 (4.05%)	01(3.22%)	07 (4.92%)
<i>E. histolytica</i>	-	01 (1.35%)	01 (3.22%)	02 (1.40%)
<b>Helminths</b>				
<i>S. stercoralis</i>	-	-	-	-
<i>A. lumbricoides</i>	-	01 (1.35%)	01 (3.22%)	02 (1.40%)
<i>A. duodenale</i>	-	01 (1.35%)	-	01 (0.70%)
<i>Taenia</i> spp.	-	-	-	-
<b>Total</b>	<b>21 (56.75%)</b>	<b>17 (22.97%)</b>	<b>04 (12.90%)</b>	<b>42 (29.57%)</b>

Other Opportunistic parasites such as *Isoospora* spp. and *Cyclospora* spp. were seen in diarrhoeal group only. Non-opportunistic parasites such as *Giardia lamblia*, *E. histolytica*, *A. lumbricoides*, *A. duodenale* were also seen in both diarrhoeal and non-diarrhoeal group.

Similar result was seen study conducted by Kaur *et al.*,<sup>5</sup> where prevalence of *C. parvum* among the diarrhoeal and non-diarrhoeal group of patients was 40.41% and 21.42% respectively.

Amongst the 158 patients who had diarrhoea, majority i.e 91 (57.59%) had CD4 T cell counts below 200 cells/ $\mu$ l, while 58 (36.70%) had CD4 T cell counts between 200-499 cells/ $\mu$ l and 9 (5.69%) had CD4 T cell counts above 500 cells/ $\mu$ l.

Also out of the 142 patients without diarrhoea, 37 (26.56%) had CD4 T cell counts below 200 cells/ $\mu$ l while majority, 74 (52.11%) had CD4 T cell counts between 200-499 cells/ $\mu$ l and 31 (21.83%) patients had CD4 T cell counts above 500 cells/ $\mu$ l.

Our results are comparable to the study conducted by Munde *et al.*,<sup>12</sup> where out of total 208 HIV seropositive patients with chronic diarrhoea majority (68.75%) had CD4 T lymphocyte counts below 200 cells/ $\mu$ l, 26.92% had CD4 T lymphocyte counts between 200-499 cells/ $\mu$ l and 4.32% had CD4 T lymphocyte counts above 500 cells/ $\mu$ l. Kaur *et al.*,<sup>5</sup> found that out of 300 study subjects, 146 had diarrhoea and 154 did not have diarrhoea. Amongst the diarrhoeal group 69 (47.26%) had CD4 T lymphocyte counts between 0-200, 44 (30.13%) had CD4 T lymphocyte counts between 201-400, 27 (18.49%) had CD4 T lymphocyte counts between 401-800 and 6 (4.10%) had CD4 T lymphocyte counts above 800. In the non-diarrhoeal group 47 (30.51%) had CD4 T lymphocyte counts between 0-200, 51 (33.11%) had CD4 T lymphocyte counts between 201-400, 47 (30.51%) had CD4 T lymphocyte counts between 401-800 and 9 (5.84%) had CD4 T lymphocyte counts above 800.

In various studies, parasites associated with HIV were more likely encountered as the

CD4 cell count fell below 200 cells/ $\mu$ .<sup>28,29</sup> This may be because immunodeficient patient are either more susceptible to acquire particular parasites and/ or unable to clear once infection is established.<sup>30</sup>

In the present study amongst the patients with diarrhoea, opportunistic parasites such as *Cryptosporidium spp.*, *C. cayetanensis*, and *I. belli* were detected in 60.43% samples with CD4 count < 200 cell/ $\mu$ l and 33.51% in samples with CD4 count > 200 cell/ $\mu$ l (Table 3). Opportunistic parasites in patients with CD4 count < 200 cell/ $\mu$ l were significantly higher than CD4 count > 200 cell/ $\mu$ l (p value is less than 0.0001).

In our study amongst the patients without diarrhoea, *Cryptosporidium spp.*, was detected in 48.64% samples with CD4 count < 200 cell/ $\mu$ l and 18.08% in samples with CD4 count > 200 cell/ $\mu$ l (Table 4). Opportunistic parasites in patients with CD4 count < 200 cell/ $\mu$ l were significantly higher than CD4 count > 200 cell/ $\mu$ l (p value is less than 0.0001).

In our study, opportunistic intestinal parasitic infections were high in patients with CD4 count < 200 cells/ $\mu$ l. No such significant predominance was observed in case of non-opportunistic parasites.

This result shows the importance of the association of opportunistic intestinal parasites with or without diarrhoea in the HIV infected patients particularly with CD4 count < 200 cells/ $\mu$ l. This association is especially significant in patients with diarrhoea as prevalence of opportunistic parasites were significantly higher in subjects with diarrhoea than without diarrhoea.

While non-opportunistic intestinal parasite infections are still a problem in HIV-infected patients at any level of CD4 count.

Conclusions of the study are as follows:

Diarrhoea is an important clinical problem among HIV seropositive individuals and is associated with significant impairments in health and quality of life.

There is male preponderance with maximum patients (69%) from sexually active group 21-40 years. Hence one should focus on this age group for prevention of transmission of HIV.

Intestinal parasites were present in both group of patients, who presented with or without diarrhoea but intestinal parasites in subjects with diarrhoea were significantly higher than without diarrhoea.

Intestinal parasites were found to be more common in HIV seropositive patients with CD4 cell count < 200 cells/ $\mu$ l.

With decline in CD4 cell counts < 200/ $\mu$ l in HIV infected patients, the risk of acquiring opportunistic intestinal parasitic infection increases.

Thus, routine screening of the stool samples of HIV seropositive patients with diarrhoea should be done for prompt patient care so as to administer early and specific treatment and prevent the emergence of fulminant form of the disease.

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