

## Original Research Article

# Enteric parasitosis and its correlation with CD<sub>4</sub> count in human immunodeficiency virus infected patients

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**Received:** 29 April 2019

**Accepted:** 30 May 2019

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## ABSTRACT

**Background:** The gastrointestinal tract of the HIV/AIDS patient (s) is harboured by a number of luminal extracellular and intracellular parasites. These parasites are responsible for deteriorating and worsening the general health condition(s) of these immunocompromised groups of patients. Moreover, these parasitic infections are often correlated with the CD<sub>4</sub> counts of the individuals. Therefore, with a view to assess the relationship between the presence of parasitic infection and that of the CD<sub>4</sub> count, the present work is being undertaken.

**Methods:** The study was conducted in the Parasitology section of the department of Life Sciences, Manipur University and CD<sub>4</sub> count unit of Microbiology Department, RIMS hospital Imphal from 34 HIV positive Antiretroviral treatment (ART) naive patients, aged between 19 to 50 years. The samples were taken from the patients only after obtaining a signed consent form. Techniques like normal saline method, iodine wet preparation method, formol ethyl acetate concentration technique, Baermann modified funnel technique, modified Ziehl Neelsen staining technique and adhesive cellophane tape method were employed for the present investigation.

**Results:** The present study reveals that patients with CD<sub>4</sub> count <200 cells/μl were harbouring only two parasites (*Cryptosporidium sp* and *G. lamblia*) whereas individuals with CD<sub>4</sub> count within the range of 200-500 cells/μl were observed to have harboured *G. lamblia*, *Cryptosporidium sp*, *E.histolytica*, hookworm, *S. stercoralis* and *A. lumbricoides*. On the other hand, enteric parasites associated with CD<sub>4</sub> count >500 cells/μl included *G. lamblia*, hookworm, *E. vermicularis* and *S. stercoralis*.

**Conclusions:** The present study shows that occurrence of intestinal parasitism is independent of CD<sub>4</sub> count in HIV infected patients. Therefore, based on the present findings, it may be suggested that irrespective of CD<sub>4</sub> count(s) and whether the patient experiences diarrhoea or not, regular monitoring of the HIV patient(s) for the presence of enteric parasitosis by adopting standard laboratory protocols should be encouraged for maintaining a healthy life of the people living with HIV/AIDS.

**Keywords:** Parasitosis, Enteric parasites, HIV, CD<sub>4</sub> count.

## INTRODUCTION

Patients with HIV/AIDS are susceptible to a number of infectious agents including those caused by both

opportunistic and non-opportunistic parasites. It has been reported that a number of infectious or biological agents may be either associated or prevalent in the HIV/AIDS patients. In HIV/AIDS patients, opportunistic parasitic

gut infections cause various health disorders including severe diarrhoea and profoundly compromise the absorptive functions of the small intestine and cause significant morbidity and mortality.<sup>1</sup>

In Manipur, literature survey among the HIV/AIDS patients reveals the presence of enteric parasites like *Cryptosporidium parvum*, *Isospora belli*, *Entamoeba histolytica*, *Giardia lamblia*, *Cyclospora cayentanensis*, *Strongyloides stercoralis*, *Ascaris lumbricoides*, hookworm and *Enterobius vermicularis* etc without screening of their respective CD<sub>4</sub> cell counts.<sup>2-6</sup> These parasitic infections are often correlated with the CD<sub>4</sub> count of the individuals. Various workers have already done extensive work in this field.<sup>7-12</sup>

However, in Manipur, so far, no authentic work on the relationship between the enteric parasitic infection and CD<sub>4</sub> count in the HIV infected patient has been reported. Therefore, with a view to assess or establish the relationship, if any, between the presence of parasitic infection and that of the CD<sub>4</sub> count, the present work is being undertaken.

## METHODS

### *Copro-parasitological analysis*

Three stool sample(s) (one fresh and the other two preserved either in 4% K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>/PBS/4% for mol saline/PVA/Schaudin's fixative) from 34 HIV positive Antiretroviral treatment (ART) naive patients, aged between 19 to 50 years visiting CD<sub>4</sub> Count unit of the Microbiology Department, Regional Institute of Medical Sciences (RIMS) hospital and admitted in different drug de-addiction centres located in the erstwhile Imphal district of Manipur were collected for the present investigation during the period of June to September 2015. There were 13 males and 21 females. All male patients were injecting drug users (IDUs) and 7 females were commercial sex workers (CSW). 30 non- HIV healthy individuals were taken as control group. These persons were staff of drug de-addiction centres working as counsellors and helpers. A brief clinical, health/hygienic and socioeconomic background/history (of the patients) were also evaluated at the time of sample collection. The samples were taken from the patients only after they have agreed to participate the said research programme by their own free will and only after getting a signed consent form from the participating patient(s)/client(s).

The detection, recovery and identification of the life cycle stage(s) of the parasite(s) were done by employing the following techniques:

#### *Normal saline method*

It was used for the detection of trophozoite/cyst/oocyst of protozoan parasite(s) and egg/ova/larvae of nematode,

trematode and cestode parasite(s) in the fresh/unpreserved stool sample(s).<sup>13,14</sup>

#### *Iodine wet preparation method*

It was used for the detection of protozoan cyst only, either in fresh or preserved faecal specimen (s).<sup>13,14</sup>

#### *Formol ethyl acetate concentration technique*

It was used for concentrating the cyst/egg/ova and larvae present in both the fresh and PVA fixed sample(s).<sup>13</sup>

#### *Baermann Modified funnel technique*

It was used for the recovery of rhabditiform larvae of *S. stercoralis*.<sup>13</sup>

#### *Modified Ziehl Neelsen staining technique*

It was used for the detection of *Cryptosporidium* oocyst.<sup>13</sup>

#### *Adhesive cellophane tape method*

It was used for the recovery and detection of egg/ova and adult (♀) of *E. vermicularis*.<sup>13</sup>

#### *CD<sub>4</sub> count*

In RIMS, the enumeration of the CD<sub>4</sub> count(s) were done in the FACS/CD<sub>4</sub> count Lab, Department of Microbiology, RIMS, Imphal by employing the Fluorescence activated cell sorting (FACS) count system (Becton Dickinson Immunocytometry system, San Jose, CA 95131-1807).

During the present study, patients were grouped into three categories, i.e. patients with CD<sub>4</sub> counts <200 cells/μl of blood, within the range of 200 - 500cells/μl and >500cells/μl respectively.

#### *Statistical analysis*

χ<sup>2</sup> test revealed the presence of intestinal parasitic infection was independent of CD<sub>4</sub> count. Statistically significant (p< 0.05).

Prior permission to conduct the present research study involving human subjects was obtained from the competent authority of Manipur University (M.U./D.Sc./ETHICS-7/09). Consents were also obtained from the clients/participants through a signed consent form towards their willingness to participate in this study.

## RESULTS

Of the 34 patients examined, 19 patients (55.88%) were found positive for parasitic infection (Table-I). Out of the 19 patients, there were 17 diarrhoeal and 2 non-diarrhoeal

patients. During the present study, among the diarrhoeal patients, *Cryptosporidium sp* and *G. lamblia* were observed in 6 and 5 patients respectively while *A. lumbricoides*, hookworm and *S. stercoralis* were observed in 2 patients each. *E. histolytica* and *E. vermicularis* were observed in 1 patient each. Other than

these parasites, commensals like *Entamoeba coli*, *Endolimax nana* and *Chilomastix mesnili* were also observed among the patients. The non-diarrhoeal patients were found to be infected with *A. lumbricoides* and hookworm. None of the patients were found to be suffering from mixed infections.

**Table 1: Association of enteric parasites with CD<sub>4</sub> count and presence or absence of diarrhoea in ART naive patients (n=19).**

Age	Sex	Diarrhoea	CD <sub>4</sub> Count (cells / $\mu$ l)	Parasite(s) detected
48	M	+	343	<i>Giardia lamblia</i>
35	F	+	139	<i>Giardia lamblia</i>
26	M	+	301	<i>Ascaris lumbricoides</i>
50	F	+	170	<i>Cryptosporidium sp</i>
45	F	+	197	<i>Cryptosporidium sp</i>
40	F	+	200	<i>Strongyloides stercoralis</i>
36	F	+	666	Hookworm
30	F	+	54	<i>Cryptosporidium sp</i>
23	M	+	776	<i>Giardia lamblia</i>
23	F	+	610	<i>Enterobius vermicularis</i>
23	F	+	447	<i>Entamoeba histolytica</i>
42	M	+	219	<i>Cryptosporidium sp</i>
26	M	+	109	<i>Cryptosporidium sp</i>
45	F	+	713	<i>Strongyloides stercoralis</i>
40	F	+	52	<i>Cryptosporidium sp</i>
26	F	+	34	<i>Giardia lamblia</i>
50	F	+	52	<i>Giardia lamblia</i>
45	M	-	266	Hookworm
42	M	-	317	<i>Ascaris lumbricoides</i>

**Table 2: Distribution of HIV-associated parasites in ART naive patients having different categories of CD<sub>4</sub> count (n=19)**

Name of the Parasite(s)	Number of patient(s) in each category of CD <sub>4</sub> count(s)			Total no. of patient(s) positive for each parasite
	(<200 cells/ $\mu$ l)	(200-500 cells/ $\mu$ l)	(>500 cells/ $\mu$ l)	
<i>Giardia lamblia</i>	3	1	1	5
<i>Cryptosporidium sp</i>	5	1	0	6
<i>Entamoeba histolytica</i>	0	1	0	1
<i>Ascaris lumbricoides</i>	0	2	0	2
Hookworm	0	1	1	2
<i>Enterobius vermicularis</i>	0	0	1	1
<i>Strongyloides stercoralis</i>	0	1	1	2
	08	07	04	19

Table-2 shows the distribution of parasites among the different categories of CD<sub>4</sub> counts.

The study revealed that cryptosporidiosis was observed in 6 patients. Out of which 5 patients had CD<sub>4</sub> count <200 cells/ $\mu$ l while 1 patient had CD<sub>4</sub> count within the range of 200-500 cells/ $\mu$ l. Amoebiasis and giardiasis were observed in 1 and 5 patients respectively. The lone patient with amoebiasis had a CD<sub>4</sub> count within the range of 200-500 cells/ $\mu$ l. In case of giardiasis, a CD<sub>4</sub> count of

<200 cells/ $\mu$ l was observed in 3 patients and while a CD<sub>4</sub> count of >500 cells/ $\mu$ l was observed in 1 patient, a CD<sub>4</sub> count within the range of 200-500 cells/ $\mu$ l was observed in the other patient.

Ascariasis was observed in 2 patients whose CD<sub>4</sub> count was within the range of 200-500 cells/ $\mu$ l. Hookworm infection was observed in 2 patients, out of which 1 patient had a CD<sub>4</sub> count within the range of 200-500 cells/ $\mu$ l while the other patient had a CD<sub>4</sub> count >500

cells/ $\mu$ l. In the present study, while enterobiasis was observed in a lone patient who had a CD<sub>4</sub> count >500 cells/ $\mu$ l, strongyloidiasis was observed in 2 patients, out of which while 1 patient had a CD<sub>4</sub> count within the range of 200-500 cells/ $\mu$ l the other had a CD<sub>4</sub> count >500 cells/ $\mu$ l. In case of cryptosporidiosis, as 5 patients out of 6 had a CD<sub>4</sub> count <200 cells/ $\mu$ l, it can be presumed that occurrence of this disease is higher in patients with low CD<sub>4</sub> count as far as the present study is concerned. However, the occurrence of other enteric helminthic infections seems to be more associated with patients having a CD<sub>4</sub> count  $\geq$ 200 cells/ $\mu$ l.

Based on the findings of the present study, it may also be inferred that while the occurrence(s) of parasite(s), especially of *G. lamblia* and *Cryptosporidium sp* were higher in patients whose CD<sub>4</sub> count was < 200 cells/ $\mu$ l, isolation/occurrence rate of other parasite species was more in patients whose CD<sub>4</sub> count value lies within the range of 200-500 cells/ $\mu$ l.

The present study shows that while maximum number of Cryptosporidium infected patient(s) were encountered in patients having low CD<sub>4</sub> count (< 200 cells/ $\mu$ l), patients suffering from giardiasis were observed to be independent of CD<sub>4</sub> count. However, diseases like amoebiasis and other enteric helminthic infections were observed to have been associated with patients having a CD<sub>4</sub> count  $\geq$  200 cells/ $\mu$ l.

## DISCUSSION

In the present study, out of the 5 patients with giardiasis, three patients had a CD<sub>4</sub> count of < 200 cells/ $\mu$ l (the patients were having CD<sub>4</sub> counts of 34, 52 and 139 cells/ $\mu$ l), one patient had a CD<sub>4</sub> count within the range of 200-500 cells/ $\mu$ l (the patient was having a CD<sub>4</sub> count of 343 cells/ $\mu$ l) and the other patient had a CD<sub>4</sub> count > 500 cells/ $\mu$ l (the patient had a CD<sub>4</sub> count of 776 cells/ $\mu$ l) respectively. The present finding of giardiasis being observed in three patients having CD<sub>4</sub> counts of 34, 52 and 139 cells/ $\mu$ l respectively is more or less in close agreement with the finding of Dalvi et al who reported the occurrence of the disease in patients having a CD<sub>4</sub> count within the range 50-200 cells/ $\mu$ l.<sup>8</sup> Moreover, the present observation of one patient having a CD<sub>4</sub> count of 343 cells/ $\mu$ l is in tally with the findings of Suryawanshi et al and Surekha et al who reported the observation of this disease in patients having CD<sub>4</sub> counts > 200 cells/ $\mu$ l and within the range of 200-500 cells/ $\mu$ l respectively.<sup>15,16</sup>

Although in the present study, a patient with giardiasis was observed to have a CD<sub>4</sub> count of 776 cells/ $\mu$ l of blood, none of the above mentioned investigators reported a similar observation.

During this study, out of 6 patients with cryptosporidiosis, 5 patients had a CD<sub>4</sub> count <200 cells/ $\mu$ l (the patients were having CD<sub>4</sub> counts of 52, 54, 109, 170, and 197 cells/ $\mu$ l) and 1 patient had a CD<sub>4</sub> count

of >200 cells/ $\mu$ l (i.e. 219 cells/ $\mu$ l). The present observation of two patients having a CD<sub>4</sub> cell counts of 52 and 54 cells/ $\mu$ l falls within the range the of 32-106 cells/ $\mu$ l as reported by Attili et al.<sup>7</sup>

Three cryptosporidiosis patients of the present investigation having CD<sub>4</sub> counts of 109, 170 and 197 cells/ $\mu$ l are also in agreement with the findings of Assefa et al, Venkatesh et al, Agarwal et al and Surekha et al who also reported the association of the disease with patients having CD<sub>4</sub> count <200 cells/ $\mu$ l.<sup>16-19</sup> The revelation of 1 patient of the present study being recorded with a CD<sub>4</sub> count of 219 cells/ $\mu$ l corroborates the observations of Surekha et al and Assefa et al who reported the occurrence of the disease in patients with CD<sub>4</sub> counts >200 cells/ $\mu$ l i.e. 200-500 cells/ $\mu$ l and within the range of 200-349 cells/ $\mu$ l.<sup>16,17</sup>

The only patient diagnosed as suffering from amoebiasis was observed to have a CD<sub>4</sub> count of 447 cells/ $\mu$ l of blood. This finding is consistent with the findings of Surekha et al and Venkatesh et al, who reported the occurrence of this disease in the HIV patients having CD<sub>4</sub> counts within the range of 200-500 cells/ $\mu$ l.<sup>16,18</sup> However the present observation is not in tally with the findings of Attili et al and Agarwal et al who reported the occurrence of amoebiasis in patients having CD<sub>4</sub> counts within the range of 28-186 cells/ $\mu$ l and < 200 cells/ $\mu$ l.<sup>7,19</sup>

The two patients with ascariasis were recorded to have CD<sub>4</sub> counts of 301 and 317 cells/ $\mu$ l of blood respectively. These values are well within the range of 200-500 cells/ $\mu$ l and this observation is in conformity with the finding of Venkatesh et al who reported the association of this disease with patients having CD<sub>4</sub> counts within the range of 200-500 cells/ $\mu$ l.<sup>18</sup> However, the present finding is contradictory with the finding of Attili et al who reported the occurrence of helminth infection in those patients having CD<sub>4</sub> counts within the range of 125-155 cells/ $\mu$ l.<sup>7</sup>

Hookworm infection was detected in two patients whose CD<sub>4</sub> cells counts were 266 and 666 cells/ $\mu$ l of blood respectively. This observation is in close agreement with the findings/observations made by Suryawanshi et al. (2012) and Vankatesh et al. (2012) as these CD<sub>4</sub> count values are consistent with their observations.<sup>15,18</sup> While the former reported the occurrence of this disease in patients having a CD<sub>4</sub> count of >200 cells/ $\mu$ l, the latter reported the observation of this infection in patients with CD<sub>4</sub> count of >500 cells/ $\mu$ l.

Strongyloidiasis was observed in two patients whose CD<sub>4</sub> cells counts were found to be 200 and 713 cells/ $\mu$ l of blood respectively. The present finding is contradictory with the observations of Suryawanshi et al and Agarwal et al who reported the occurrence of this disease in HIV patients having CD<sub>4</sub> count < 200 cells/ $\mu$ l.<sup>15,19</sup> However, the present observation of a patient having a CD<sub>4</sub> count of 200 cells/ $\mu$ l fits well and is consistent with the finding of Assefa et al and Venkatesh et al who reported the

presence of this disease in patients having CD<sub>4</sub> count within the range of 200-349 cells/μl and 200-500 cells/μl.<sup>17,18</sup> Moreover, the present observation of strongyloidiasis in a patient with a CD<sub>4</sub> count value of 713 cells/μl is in agreement with the finding of Assefa et al, who also reported the occurrence of this parasitic disease in HIV patients having CD<sub>4</sub> counts ≥500 cells/μl of blood.<sup>17</sup>

In the present study, enterobiasis was observed in a patient whose CD<sub>4</sub> count value was found to be 610 cells/μl. None of the currently referred/cited workers reported the observation of this disease among their studied population.

During the present study, higher occurrence of cryptosporidiosis and giardiasis were observed in patients having a CD<sub>4</sub> count of <200 cells/μl. The present finding of higher occurrence of enteric parasitosis in patients whose CD<sub>4</sub> count lies within the range of 200-500 cells/μl is contradictory with the finding of Sherpa et al who reported intestinal parasitic infection to be more common in patients whose CD<sub>4</sub> count lies within the range of 100-200 cells/μl.<sup>4</sup>

## CONCLUSION

The present work is the first investigative study of its kind as far as the relationship between the occurrence of enteric parasitosis and CD<sub>4</sub> count in the HIV infected ART naive patients of the Manipur state is concerned. Based on the present finding, it may be suggested that irrespective of CD<sub>4</sub> count(s) and whether the patient experiences diarrhoea or not, regular monitoring of the HIV patient(s) for the presence of enteric parasitosis by adopting standard guidelines for coprological analysis should be encouraged in the best interest of the HIV patients towards maintaining a quality life for the people living with HIV (PLHIV).

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge Head(s), Department of Life Sciences, Manipur University and Department of Microbiology, RIMS and staff and patient(s) working/visited/admitted in the Department of Microbiology, RIMS and RIMS hospital and different drug de-addiction centres (NGOs) located in the erstwhile Imphal district of Manipur for their help, assistance and co-operation. The first author gratefully acknowledges Manipur University, Canchipur for supporting this research programme by giving financial assistance in the form of University Fellowship (PhD programme), vide Manipur University order No. 437, dated 15th June 2015.

*Funding: No funding sources*

*Conflict of interest: None declared*

*Ethical approval: The study was approved by the Institutional Ethics Committee (M.U./D.Sc./ETHICS-7/09).*

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**Cite this article as:** Devi RH, Th NS, Singh AL, Singh RKG. Enteric parasitosis and its correlation with CD4 count in human immunodeficiency virus infected patients. *Int J Res Med Sci* 2019;7:2690-5.