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Occurrence of Hookworm–Whipworm co-infection among the HIV/AIDS patients of Noney district of Manipur, India

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Abstract: *As a part of the on-going research work and in continuation of an existing programme pertaining to the copro-parasitological survey work on the prevalence and epidemiology of enteric parasitosis associated with HIV/AIDS patients, field visits were made in a hilly district (rural) of Manipur (India), i.e. Noney district, during the period of March to June 2018, with a view to assess the occurrence/prevalence of opportunist and other parasites in these immuno-compromised group of patients. Of the 35 patients screened for the presence of enteric parasitosis, 15 (42.85%) patients were found positive for various intestinal parasites like Ascaris lumbricoides, Trichuris trichiura, hookworm and commensals like Entamoeba coli, Endolimax nana and Iodoamoeba butschli respectively. In this study, while mixed infection was observed in 6 (40%) patients, single infection was recorded in 9 (60%) patients. Of the 6 patients with mixed infections, a 47 year old HIV-HCV co-infected female patient was found to have dual infection of hookworm and whipworm (Trichuris trichiura). The present epidemiological survey work is most probably the first of its kind in this hilly district of Manipur.*

Key Words*: Hookworm, Whipworm (Trichuris trichiura), HIV-HCV co- infection, On ART, HCV treatment naive, parasitosis*

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I. Introduction

HIV/AIDS patients are prone to a number of infections caused by various biological agents, by virtue of their immuno-compromised status [(1)]. Previous workers have already done extensive investigation(s)/work(s) on the occurrence of intestinal parasitism amongst these immuno-compromised groups of patients and have already reported a number of opportunist and non-opportunist parasites to be associated with the HIV/AIDS patients of different districts of Manipur [(2-6, 15)]. They also reported the existence of regional/geographical variation with regard to the prevalence/occurrence and endemicity of certain parasite species in the different district(s) of Manipur [(4)]. In this regard, it may be mentioned that so far, no such study has been done on the occurrence/prevalence of intestinal parasitism among the HIV/AIDS patients of Noney district, a newly/recently created hill district of Manipur. Therefore, the present study was undertaken with a view to assess the occurrence/endemicity of enteric parasite(s), if any, among the HIV seropositive patient(s) of this newly created district of Manipur because of the fact that, so far, no literature/published report is available or traceable with regard to the occurrence of intestinal parasitism amongst the HIV/AIDS patients of this district.

II. Materials and Methods

For the present study, two consecutive fresh and three preserved faecal specimens (preserved in 10% buffered formalin and 2.5% potassium dichromate) were collected during the period of March to June 2018 from the HIV seropositive patient(s) of Noney district of Manipur. Altogether, 35 patients, which consisted of 20 males and 15 females were enrolled for this investigative study. Faecal /stool samples were collected as per the standard guidelines [(7)]. Detection, recovery and identification of the life cycle stages of the parasite(s) were done by adopting the following standard guidelines/ techniques [(8)]:

1. Normal saline method
2. Iodine wet preparation method
3. Formol ethyl acetate concentration technique
4. Baermann modified funnel technique

A high powered binocular compound microscope (Olympus) and a stereoscopic dissecting binocular microscope were used for the detection and observation of the parasites; the former for the detection and observation of egg/ova, cyst(s) and oocyst(s) of protozoan parasites, while the latter for the observation of larvae of helminth parasites. A calibrated compound microscope (Olympus- 71692) was used for studying the morpho-anatomical structures and for measuring the dimension(s) of the life cycle stage(s) of the parasite(s) being observed. A high resolution compound microscope (Nikon, Eclipse E-200) was used for microphotography.

III. Results

Of the 35 patients, 15 (42.85%) patients were found positive for various intestinal parasites like *Ascaris lumbricoides, Trichuris trichiura*, hookworm and commensals like *Entamoeba coli*, *Endolimax nana* and *Iodoamoeba butschli*. In this study, while single infection was observed in 9 (60%) patients, mixed infection was recorded in 6 (40%) patients only. Of the 6 patients with mixed infections, interestingly one HIV-HCV co-infected female patient was found to have dual infection of hookworm and whipworm (*Trichuris trichiura*). The HIV-HCV co-infected *On ART* but HCV treatment naive patient was apparently healthy and had a CD_4 cells count of 365 cells/µl of blood (CD_4 testing/ CD_4 cells count was done in the FACS count centre of the Dept. of Microbiology, RIMS, Imphal, Manipur) at the time of sample collection. On taking personal/clinical history of the patient, it was revealed that she was a divorce with three children and had been working as a farmer/vegetable grower in a local banana plantation farm for earning a livelihood for supporting her family since 5 years back.. Her husband was an injecting drug user. She also admitted that before joining the banana plantation farm, she also worked as a CSW (Commercial Sex Worker) for a short duration (7-8 months). Currently, she had enrolled herself in a local drug de-addiction centre for receiving relevant treatment(s) including anti-retroviral therapy (ART). There was no history of pain in the abdomen and no record of other major health problems in the last two years or so. However, she complained of experiencing abdominal discomfort, loose motion/intermittent diarrhoea, on and off for the last 3-4 months. Although she relied on anti-diarrhoeals whenever she experienced diarrhoea; the moment diarrhoea subsided/resolved, she stopped taking the drugs ignoring the prescribed period of treatment.

On laboratory investigation, the sample was found negative for blood and mucus; however, a number of bile stained, oval/barrel shaped egg(s) having an unsegmented protoplasmic mass with bipolar mucoid plug [having a dimension of 50-55µm (in length) and 22-24 µm (in breadth)] characteristic of *Trichuris trichiura* (whipworm, Fig. I) were observed. A large number of vermiform, elongated, worm/larvae like structures were also observed in the sample. No cyst or trophzoite was detected in the sample. On further examination using Baermann modified funnel and ethyl acetate concentration technique, a number of fusiform worm like structures were observed. When the concentrate was again examined using a stereoscopic dissecting microscope, structures very much akin to that of rhabditiform larvae of nematodes were observed. These structures were then examined under a compound microscope having a high resolution power (Nikon, Eclipse E-200), so that the detailed morphological and anatomical structures can be observed and studied. Ultimately, they were identified as rhabditiform larva (L1) of hookworm based on WHO criteria [7, 8]. Using a calibrated compound microscope (Olympus-71692), the dimensions of the larva and its internal accessory structures were measured. The larvae were found to have possessed a fusiform body having a length of 255.4 – 294.6µm and a breadth of 15-17µm when measured at the body median, an anal pore at a distance of 80µm from the posterior end, a characteristic small genital primordium having a dimension of 7µm at the posterior region of the larva, besides possessing a buccal cavity having a length of 15µm. The presence of a 15 µm long buccal cavity is the most important and taxonomically valid identifying character for the differentiation of rhabditiform larva (L_1 stage) of hookworm from those of the other rhabditiform larvae (viz, *S. stercoralis*).

Therefore, based on the presence of the above mentioned systematically important & taxonomically valid diagnostic characters and identifying criteria for the rhabditiform larvae of hookworm [7], the present larvae have been identified and confirmed as the rhabditiform larva of hookworm (Fig. II).

Thus, based on the above mentioned parasitological observation(s) and finding(s), the patient was confirmed as a case of hookworm-whipworm co-infection. This laboratory finding report was submitted to the relevant authorities (Medical Officer in-charge) of the drug de-addiction centre where the patient was enrolled for treatment compliance.

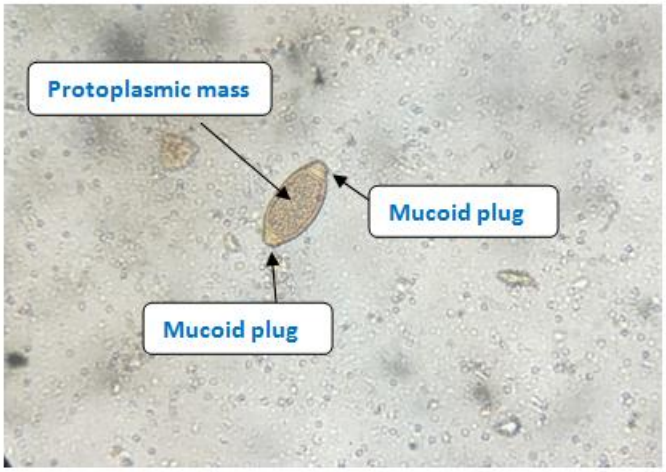

Fig. I Bile stained, oval/barrel shaped egg/ova of *Trichuris trichiura* having an unsegmented protoplasmic mass with bipolar mucoid plug [Ethyl acetate concentration technique, 40x]

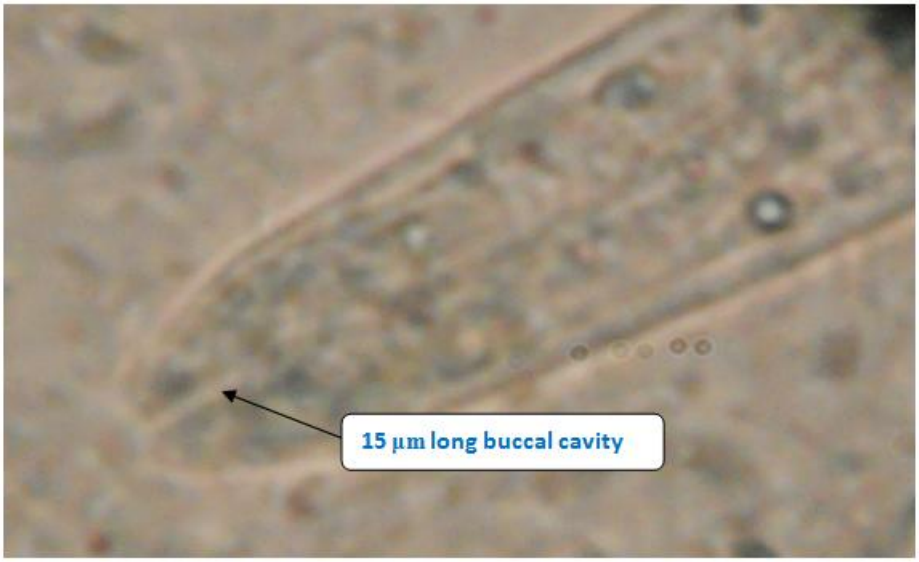

Fig. II Rhabditiform larva (L1 stage) of hookworm showing the characteristic buccal cavity having a length of 15µm [stained with Dobell's Iodine, 10x]

IV. Discussion

HIV disease progression is influenced by helminthic infection by way of regulating the host immune response both at cellular and humoral level, as the result of a strong T-helper 2 type (Th2) cytokine profile [9 - 11]. Parasites like hookworm, *Trichuris trichiura, Strongyloides stercoralis* and *Ascaris lumbricoides* which are known to be strongly associated with a Th2 cytokine shift accompanied by eosinophilia could represent a condition associated with rapid immuno-competence deterioration [11-14].

Hookworm and *Trichuris trichiura* are soil transmitted intestinal nematodes that infect human population especially those people living in the tropical and subtropical regions. The normal habitat of these parasites is the small intestine although the preferred site of feeding is the mucosal lining of the upper part of the small intestine. These parasites damage the mucosal and epithelial layers of the gastrointestinal tract either by mechanical action or through the release of parasite specific excretory or secretory antigens that are lethal/deleterious or toxic to host tissue or cell.

Human infection occurs percutaneously in case of hookworm (mediated by the infective filarifrom larvae) via contaminated soil, while ingestion (egg/ova) via contaminated food/water is the general rule in case of *T. trichiura*. In immuno-competent hosts, infection with these enteric parasitic nematode(s) cause a minor health problem with symptoms like transient diarrhoea, abdominal pain etc (when there is moderate to heavy infection) but in some individuals, there may be no signs and symptoms when the parasite density/population is low.

In Manipur, previous workers have reported a prevalence rate of 5% to 18.7% in case of hookworm infection and 9.7% to 43.7% in case of *Trichuris trichiura* infection amongst the HIV seropositive non-diarrhoeal patients of Imphal, Churachandpur and Ukhrul districts, depending upon variation in the regional endemicity (rural/urban), occupation (susceptible or high risk population includes farmers and/or vegetable growers), geographical factors, level of literacy, standard of living, knowledge of health & hygienic practices, and whether the patient experienced diarrhoea or not [4,5,15]. However, as far as literature review and our existing knowledge is concerned, the present study is probably the first epidemiological/coproparasitological work ever conducted in this newly created district of Manipur. Moreover, this is the first report on the occurrence of hookworm-whipworm co-infection in an apparently healthy HIV-HCV co-infected *On ART* but HCV treatment naive tribal patient of this district. So far, no published work/report on the association of enteric parasites with either HCV infected or HIV-HCV co-infected patient(s) is available or traceable.

The probable reason(s) for the occurrence/observation of enteric parasitosis amongst the studied population in general and this hookworm-whipworm co- infected patient in particular might be due to poor sanitation, unavailability of adequate supply of safe drinking water (lack of potable water), unhygienic practices, low standard of living, lack of health awareness, habitual practice of open defecation and easy accessibility of the host to the infective stage(s) of the parasite(s) when people consume (either drink/eat) the infective stage impregnated water/food or work in the field(s) contaminated with night soil. Although avoiding exposure to the infective stage(s) of parasite(s) can probably reduce the incidence of acquiring these infections, this is not always practicable. Therefore, with a view to cater the immediate healthcare and prompt treatment requirement of these immuno-compromised group of patients, whenever an HIV infected individual complains of diarrhoea or any other gastrointestinal problem, and if the patient is suspicious of such an infection, then there should be compulsory testing of the patient to ascertain the involvement of any parasite or pathogen. Thus, early detection of parasitic infection(s), if any, will be of immense importance and value for suggesting effective interventional, management and treatment/prophylactic measures towards improving the quality of life for the people living with HIV/AIDS (PLHA), thereby minimizing the morbidity and mortality.

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