**Strongyloides stercoralis** infection among HIV infected individuals in Osogbo, Osun State, Nigeria

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**Abstract**

**Introduction:** *Strongyloides stercoralis* is endemic in tropical and subtropical regions and hyper-infection and dissemination could occur in immunocompromised patients. Regular parasitic assessment of HIV patients is needed to prevent disseminated *S. stercoralis* infection. This study therefore determined the prevalence of *S. stercoralis* among HIV individuals in Osogbo, Nigeria using stool microscopy and Enzyme-Linked Immunosorbent Assays (ELISA).

**Methodology:** Stool and serum samples were collected from HIV individuals who were receiving antiretroviral treatment at Ladoke Akintola University of Technology (LAUTECH) Teaching Hospital Osogbo, Nigeria. Stool samples were examined for *S. stercoralis* by microscopy. Serology was performed using commercially available ELISA kit to detect parasite-specific immunoglobulin G (IgG).

**Results:** Out of 188 (55 males and 133 females) HIV-infected individuals recruited for the study, 8.5% were positive for *S. stercoralis* by microscopy while 16.0% were positive by ELISA and 39 (20.7%) HIV individuals were positive with any of the two methods. The highest detection rate (73.1%) was observed with ELISA while microscopy recorded a detection rate of 39%. HIV individuals with CD4 ≤200 had a significantly higher prevalence (29.5%) compared with those with CD4 ≥200 (22.4%) (p = 0.0064).

**Conclusion:** The findings of this study show that the combination of both ELISA and microscopy may improve the problem associated with the diagnosis of *S. stercoralis* in Nigeria.

**Keywords:** *Strongyloides stercoralis*, HIV, Microscopy, Serology, Nigeria
INTRODUCTION

Strongyloides stercoralis, the parasite responsible for strongyloidiasis is a cosmopolitan neglected disease with high prevalence in Latin America, Southeast Asia, and sub-Saharan Africa. Although the precise data on its prevalence is unknown, it is estimated that 30–100 million people are infected worldwide [1,2]. Strongyloidiasis is found most commonly in rural areas, institutional settings, and lower socio-economic groups [3,4]. In immunocompromised persons, strongyloidiasis accounts for about 60–85% mortality rate [5,6] and this fatality is enhanced by the ability of the parasite to develop chronic condition which is due to its unique replicative ability in the human host [1,7]. Clinical manifestation of strongyloidiasis ranges from asymptomatic chronic infection to acute hyper-infection and fatal disseminated syndrome [8,9]. However, adult worms can chronically survive in hosts for decades while causing merely intermittent gastrointestinal symptoms and weight loss [10].

Routinely, diagnosis of strongyloidiasis is usually performed by simple and inexpensive microscopic examination of stool samples, although this method is reported to have low sensitivity [11] and leads to frequent misdiagnosis of the disease since many infections remain asymptomatic [12]. The need for improved diagnostic tests with good sensitivity and specificity has led to the use of different faecal concentration, culture, serological diagnosis with good sensitivity and specificity has led to the use of different faecal concentration, culture, serological diagnosis and molecular techniques. Serological diagnosis using serum has been reported to provide useful indirect evidence for laboratory diagnosis of strongyloides infection in both immunosuppressed and immunocompetent patients [13]. Several immunodiagnostic assays have been established and evaluated, and these have shown variable sensitivities and specificities based on the antigen preparation, immunoglobulin isotypes and population tested. Indirect ELISA is the most convenient and widely used procedure which detects specific serum IgG, the IgG subclasses (IgG1, IgG2 and IgG4) and IgE antibodies using crude extracts of S. stercoralis larvae [12]. The availability of locally relevant data is important in order to determine whether screening for Strongyloides should be considered during the management of HIV individuals. As a step toward addressing this issue, the present study was designed to assess the prevalence of S. stercoralis in HIV individuals in Nigeria.

METHODOLOGY

Study Area and Population

The study was conducted at the Anti-Retroviral (ART) clinic of Ladoke Akintola University of Technology (LAUTECH) Teaching Hospital, Osogbo, Nigeria. This clinic routinely delivers HIV counselling, testing for HIV status and treatment for HIV individuals in and around Osogbo. Osogbo is the capital city of Osun State with approximately 845,000 people based on 2006 census provisional results and it is located in the heart of southwest Nigeria.

Ethical Consideration

Ethical approval was obtained from the ethical committee of the LAUTECH Teaching Hospital, Osogbo. Before the commencement of the study the purpose of the study was explained to the patients. The benefits of the study and some discomfort that could be associated with sample collection were explained. Informed consent was obtained from all willing participants and only those that gave their consent were recruited into the study.

Microscopic Detection of S. stercoralis

Labelled sterile containers with a collecting spoon were provided to all the participants. They were told to pass the stool specimen directly into the sterile container, or to pass the stool on a piece of paper and use the provided spoon to transfer it to the sterile labelled container immediately. The stool samples were transported to the microbiology laboratory on the same day avoiding any unnecessary delay. The formol-ether concentration technique was used for quantitative determination of helminths ova. Stool samples were processed within 12 hours of collection and examined microscopically within 1 hour of preparation to avoid missing hookworm ova. The intensity of infection was expressed as the number of eggs per gram of faeces (epg). Water- or urine-contaminated stools were rejected.

Serological Detection of S. stercoralis

About 5 ml of blood samples were aseptically collected from all HIV individuals whose stool samples were collected. The blood samples were collected using a sterile plain bottle. Within 2 hours of collection, the samples were centrifuged at 1,500 × g for 20 min at room temperature. After centrifugation, serum aliquots were prepared, which were frozen at −20°C until testing. ELISA was used to detect the IgG to S. stercoralis in the serum using a commercially available kit (Diagnostic Automation, Woodland Hills, USA). The procedure was carried out according to the manufacturer’s instruction. The reaction was read with the aid of ELISA plate reader within 5 minutes after adding stop solution. Absorbance reading...
of 0.15 OD (optical density) units and above indicated the sample contains *S. stercoralis* antibody and taken to be positive.

**CD4 Count Determination**

Blood samples were analyzed to determine the CD4+ count using a two-color single platform flow cytometer (Cyflow Partec) (Sysmex Corporation, Chuo-ku Kobe, Japan). 20µl of well gently mixed whole blood was incubated in the dark for 15 minutes at room temperature. 800 µl of CD4 buffer was added and mixed gently and the result was read in the flow cytometer.

**Statistical Analysis**

Data obtained were analyzed using SPSS software 16.0 (SPSS, Chicago, IL). Significant differences between categorical variables were determined using chi square, and p<0.05 was taken as significant value.

**RESULTS**

A total of 188 HIV positive individuals (55 were males and 133 females) were recruited into the study. The general characteristics and the overall prevalence of intestinal parasitic infection of the HIV individuals studied are shown in table 1. The mean age and the Mean CD4 count of the studied population is 42.46 ± 10.4 years (age range 25 – 74 years) and 365.25 ± 245.4 mm3 respectively. Out of the 188 HIV patients investigated for *S. stercoralis*, 8.5% were positive by microscopy while 16.0% were positive by ELISA. The difference in the number detected between the two methods were statistically significant (p=0.005). Other intestinal parasites recovered by microscopy include *Entamoeba histolytica* (11.2%) and *Ascaris lumbricoides* (4.3%).

Table 2 shows the distribution of *S. stercoralis* by sex and age using the combine prevalence of ELISA and Microscopic methods. Out of the 54 males examined, 11 (20.4%) were positive while, 28 (21.0%) were positive out of the 134 females examined and the difference was not statistically significant. The highest positivity rate was overserved in the older age groups 66-75 years (37.5%) and 55-64 years (35.3 years) but the difference was not statistically significant.

The association between the prevalence of *S. stercoralis* and CD4 count is shown in Table 3. HIV patients with CD4 ≤200 had a significantly higher prevalence of *S. stercoralis* (29.5%) compared with those with CD4 ≥200 (22.4%) (p = 0.0064)

**Table 1: Characteristics of study subjects and prevalence of *S. stercoralis* based on diagnostic methods**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of individuals</td>
<td>188</td>
</tr>
<tr>
<td>Mean age (yyears) ± SD</td>
<td>42.46 ± 10.4</td>
</tr>
<tr>
<td>Sex: Male/Female</td>
<td>54 / 134</td>
</tr>
<tr>
<td>Mean CD4 count (cells/mm³)</td>
<td>365.25 ± 245.4</td>
</tr>
<tr>
<td><em>S. stercoralis</em> Positive by Microscopy (%)</td>
<td>16 (8.5)</td>
</tr>
<tr>
<td><em>S. stercoralis</em> Positive by ELISA (%)</td>
<td>30 (16.0)</td>
</tr>
<tr>
<td><em>Ascaris lumbricoides</em></td>
<td>8 (4.3)</td>
</tr>
<tr>
<td><em>Entamoeba histolytica</em></td>
<td>21 (11.2)</td>
</tr>
<tr>
<td><em>S. stercoralis</em> and <em>E. histolytica</em></td>
<td>2 (1.1)</td>
</tr>
<tr>
<td><em>E. histolytica</em> and <em>A. lumbricoides</em></td>
<td>4 (2.1)</td>
</tr>
</tbody>
</table>

**Table 2. Distribution of *S. stercoralis* by Sex and Age**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Frequency</th>
<th>No. Positive (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>54</td>
<td>11 (20.4)</td>
<td>1.00</td>
</tr>
<tr>
<td>Female</td>
<td>134</td>
<td>28 (21.0)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25-34</td>
<td>49</td>
<td>11 (22.4)</td>
<td></td>
</tr>
<tr>
<td>34-44</td>
<td>61</td>
<td>11 (18.0)</td>
<td></td>
</tr>
<tr>
<td>45-54</td>
<td>51</td>
<td>8 (15.7)</td>
<td>0.36</td>
</tr>
<tr>
<td>55-64</td>
<td>17</td>
<td>6 (35.3)</td>
<td></td>
</tr>
<tr>
<td>65-75</td>
<td>8</td>
<td>3 (37.5)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>188</td>
<td>39 (20.7)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3. Association between the prevalence of *S. stercoralis* and CD4 count in HIV individuals**

<table>
<thead>
<tr>
<th>CD4 count values</th>
<th>Number Examined</th>
<th>No. Positive (%)</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 ≥200</td>
<td>116</td>
<td>26 (22.4)</td>
<td></td>
</tr>
<tr>
<td>CD4 ≤200</td>
<td>44</td>
<td>13 (29.5)</td>
<td>0.006</td>
</tr>
<tr>
<td>Total</td>
<td>160</td>
<td>39 (24.4)</td>
<td></td>
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</table>

**Discussion**

There is an underestimation in the prevalence of *S. stercoralis* which is partly due to inadequate diagnostic techniques and issues relating to the sensitivity and specificity of the diagnostic methods [14,15]. This study evaluates the prevalence of *S. stercoralis* among HIV
individuals using microscopy and ELISA methods. Microscopy detected *S. stercoralis* in 8.5% of the faecal samples compared to 16.0% detected by ELISA. Overall ELISA techniques seem to perform better with a detection rate of 77% compared to 41% in microscopy. A number of factors have been associated with the low *S. stercoralis* detection by microscopic examination. These include the use of single stool sample which may lack sensitivity thereby failing to detect *S. stercoralis* larvae in up to 70% of cases coupled with low worm load in asymptomatic individuals [5]. Nevertheless, the microscopic techniques have always been preferred due to its low cost and ease of performance, especially when involving large surveys [16]. ELISA demonstrated higher sensitivity in comparison to microscopy in this study. However, the possibility of cross-reactivity with antibodies of other helminthic infections such as *A. lumbricoides*, detected in this study using ELISA could not be ruled out [17,18]. Also the positive ELISA reactions which detect the IgG would not only be detecting current active infection but would also be detecting persisting level of antibody from a past infection that cleared naturally or following antihelminthic treatment [19]. ELISA therefore does not distinguish between past and current infections and it is difficult in many cases to know whether low-level autoinfection is continuing [19]. It is widely believed that while stool examination generally underestimates the prevalence, serological examination may overestimates it in population-based studies [7]. The use of both serology and microscopy together is therefore recommended in other to obtain a fairly true estimate of the prevalence of *S. stercoralis* infection. In settings where it is available, Polymerase Chain Reaction provides highly specific and sensitive molecular method for diagnosis of *S. stercoralis* genome in human faeces [20]. However, Polymerase Chain Reaction entails higher overall costs and requires high expertise, which makes serology and microscopy together a more cost-effective methodology in a resource poor setting.

In this study, *S. stercoralis* infection was not associated with gender among the HIV individuals. Previous studies from Thailand and India have reported an increased prevalence of strongyloidiasis in males and HIV-infected patients with CD4 count less than 100 mm$^3$ [21]. In our study, the number of males sampled was low compared to females which could have influenced the results. Male generally hide their HIV status in this environment because of male ego and stigmatization. It is therefore not surprising that the number of females sampled doubled their male’s counterpart. The prevalence of *S. stercoralis* was higher among the older age groups 55-64 year (35.3%) and 65-75 years (37.5%) but the difference was not statistically significant. It has been previously suggested that *S. stercoralis* infection may first occur in household where young children play, usually without shoes [22]. After contracting the parasite, the infection may remain for decades in the host if the infection is not treated, which would account for the increased prevalence that is associated with age [22,23].

Similar to other studies [24,25], the prevalence of *S. stercoralis* was higher among HIV individuals with CD4 count<200 cells/mm$^3$ compared to those with higher CD4 count and the difference was statistically significant. In most studies *Strongyloides* are now mostly detected at or around the time of detection of HIV suggesting that it is an opportunistic infection in HIV. Helminths including *Strongyloides* spp. are known to promote Th2 response that leads to the suppression of Th1 function and decrease in CD4 cell counts [24,26]. This may be an alternative cause for the low CD4 cell counts in HIV patients with strongyloidiasis in this study. This observation also shows that *S. stercoralis* infection can be a marker of a severe immune deficiency in HIV as the infection is associated with a reduction in CD4 T-lymphocytes.

In conclusion, although many study have reported the prevalence of *S. stercoralis* in Nigeria [27,28], this is the first study that has shown the usefulness of using both ELISA and microscopy for the detection of *S. stercoralis* among HIV individuals in Nigeria. The study concludes that the combination of both method ELISA and microscopy will improve the problem associated with diagnosis of *S. stercoralis* in Nigeria. The study also indicates that, strongyloidiasis is common in HIV-positive patients with low CD4. Although the clinical impact of *S. stercoralis* was not investigated in this study, it could be assumed that since higher prevalence of infection correlated with low CD4 count, development of fatal complicated infection may occur.

**Acknowledgements**

We thank all the consenting participants for their cooperation.

**Conflict of interest**

The authors declare that they have no conflicts of interest.

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