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Research Article

Evaluation of a Systematic Screening for Parasitic Diseases in HIV Positive Immigrant Population in Spain Five Years after its Introduction

Luis Casado-González[1], Candela Menendez[2], María Martinez-Sela[2], Noelia Moran[2], Alicia Garcia-Pérez[2], José A. Boga[3], Jonathan Fernandez[3], Mercedes Rodriguez[3], and Azucena Rodriguez-Guardado[4]*

[1]*Red Cross Hospital, Spain*
[2]*Internal Medicine Unit, The Central University Hospital of Asturias, Spain*
[3]*Microbiology Unit, The Central University Hospital of Asturias, Spain*
[4]*Internal Medicine Unit, Hospital Universitario de Cabueñes, Spain*

***Corresponding author**
Azucena Rodríguez-Guardado, Internal Medicine Unit, Hospital Universitario de Cabueñes, Spain, Tel: +34-985-158-000; Email: azucenarguardado@gmail.com

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Abstract

Objective: Human immunodeficiency virus (HIV) co-infection with parasitic diseases is an issue not only in parasite-endemic countries but also for persons who have migrated to more developed countries where the infections may go unrecognized due to lack of experience by the practitioners especially that the patients are often asymptomatic when parasite burdens are low. For this reason, we performed a systematic screening program for parasitic diseases in immigrant patients with HIV infection. The results of this program are described.

Design: A cross-sectional study was conducted to evaluate the results of this screening program.

Methods: Screening for all patients comprised blood count, biochemistry, basic urinalysis, CD4+ cell counts, HIV viral load and HIV-subtypes, Hepatitis B virus, hepatitis C virus, syphilis antibodies, and PPD test. Parasitological test included three concentrated stool samples, detection techniques for *Plasmodium* and serological detection of *Schistosoma* spp., *Strongyloides* and Chagas' disease.

Results: 63 patients were analyzed. Thirty-four patients had a parasitic disease. The geographical distribution of the patients was as follows: Central Africa (52.4%), South America (31.7%), West Africa (9.5%); North-Africa and Centro America (3.2% each). Thirty-four (54%) patients had a parasitic disease. The most frequent disease was intestinal parasites followed by filariasis and Chagas's disease. Parasitic diseases were more frequent in patients from Central Africa (11 vs 22, p=0.033, OR 3.00 [0.95-9.62])

Twenty-eight patients (44.4%) were infected with intestinal parasites and in this case viral load was significantly higher in infected patients 287,970 ± 982,009 RNA viral copies/mm3 vs 83,616 ± 196,220, (p=0.041). In the rest there are no statistical differences in age, sex, and time in Spain and the average CD4+ cell count between infected and not infected patients.

Conclusions: This study supports the use of screening for parasitic diseases in immigrant patients with HIV infection.

INTRODUCTION

Parasitic diseases are endemic in many regions of the world where Human Immuno Deficiency Virus/Acquired Immunodeficiency syndrome (HIV/AIDS) is also prevalent. Sub-Saharan Africa is among the regions where intestinal parasitic infections are entrenched and the largest burden of AIDS cases exist [1]. In Spain, there are 5,736,258 foreign-born people, with the majority arising from parasite endemic areas [2]. Many of these individuals also originate from HIV-endemic regions, suggesting that HIV and parasitic co-infection is not uncommon [2,3]. HIV co-infection with parasitic diseases is an issue not only in parasite-endemic countries but also for persons who have migrated to more developed countries where the infections may go unrecognized due to lack of experience by the practitioners especially that the patients are often asymptomatic when parasite burdens are low [4-6].

Despite the potentially severe complications from parasite co infection with HIV and the detrimental consequences because of the delays in diagnosis and in treatment, there are few data on the prevalence of parasitic disease in foreign-born persons with

HIV residing in developed countries [5-7]. Furthermore, although guidelines for screening new refugees have been developed, there are no standards for how or when to look for parasitic infections among foreign-born persons with HIV. Recently HIV British Association guidelines [8], recommended performing a *Schistosomas* spp., serology in patients with exposure longer than 1 month in sub-Saharan Africa and the Spanish Group for Aids Study [9], 2013Guidelines recommended performing *Strongyloides* serology in patients from endemic areas and those with eosinophilia.

Due to the potential interactions between HIV and parasitic diseases and the deleterious consequences of them, the Tropical Medicine Unit of Hospital Universitario Central de Asturias (HUCA, Spain) has been screening for parasitic diseases in all immigrant patients with HIV infection since 2006. A prospective, descriptive study was conducted to evaluate the results of this screening program.

MATERIAL AND METHODS

We performed A cross-sectional study that includes all the immigrant patients from a developing country, greater than 18 years old and diagnosed with HIV infection and attending Tropical Medicine Unit of HUCA for the first time between June 2006- June 2011.

An epidemiological questionnaire that included demographic variables such as sex, age, country of origin, classical risk factors for parasitic infections (contact with soil, unsafe water, etc), and time from arrival in Spain to first consultation at Tropical Medicine Unit was performed. For the purposes of the study patients were classified into seven geographical areas according to their origin: Central Africa, East Africa, West Africa, North Africa, Mexico and Central America, South America, and South Asia. The geographical areas were defined according to the criteria of the Centers for Disease Control and Prevention [10].

A complete physical examination and a purified protein derivative (PPD) - test were performed at first consultation. In patients with PPD-test positive, chests X-ray were realized. No patients were treated with antiretroviral therapy. Immigrant patients lost to follow-up and patients with incomplete tests by June 2011 were excluded

Laboratory analysis

Screening for all patients comprised blood count, biochemistry included liver enzyme levels, basic urinalysis, CD4+ cell counts, HIV viral load and HIV-subtypes. Eosinophilia was defined as >500 eosinophils/mm3. Hematuria was defined as >2 red blood cells per high power field.

Hepatitis B virus (HBV), hepatitis C virus (HCV), HIV and syphilis were investigated in all patients by chemiluminescence (Architect, Abbott Laboratories, Abbott Park, IL). All of them had undergone testing for anti-HBc. Patients who were positive for HBV were tested for HBs Ag, Anti-HBc IgM HbeAg, and anti-HBe. Isolated anti-HBc was defined as positive anti- HBc with all the rest of the markers negative. HIV detection was performed to detect antigen and antibody of HIV type 1 and 2. All positive results were confirmed by Western Blot (Bio Blot HIV-1 plus®, BioKit, Barcelona Spain).

Parasitological test

Three stool samples per patient were concentrated by using Copropack Extraction Kit C100 (Cromakit, Spain) according to manufacturer's instructions, stained with lugol and screened under light microscope with a low magnification to detect helminth eggs and protozoa trophozoites and cysts. Animmuno fluorescence test (MERIFLUOR® *Cryptosporidium/Giardia* kit, Meridian Bioscience, USA) to detect *Cryptosporidium* spp., and *Giardia lamblia* were performed using concentrated stool samples.

Genome detection of *Dientamoeba fragilis* and *Entamoeba histolytica* and *E. dispar* in stool samples, previously extracted using QIAmp DNA stool Mini kit (Qiagen, Netherland), were carried out by two PCRs [11,12]. An enzyme-linked immunosorbent assay for serum anti-*S. stercoralis* antibodies was used as screening. (ELISA; DRG Diagnostics®). We considered infection whether the microscopic visualization and/or the ELISA were positives.

In all patients routine detection techniques for *Plasmodium* included Giemsa staining and microscopic examination through thick and thin blood smear were performed. Genome detection of plasmodium in blood samples, previously extracted using COBAS Ampliprep Instrument (Roche, Germany), were performed by PCR [13].

Serological detection of *Schistosoma* spp., were carried out by enzyme-linked immunoassay assays (*Schistosoma* spp., IgG ELISA, DRG Diagnostics, Germany) in all Sub-Saharan patients. In patients with abnormal basic urinalysis, three urine concentrated samples were examined microscopically for *Schistosoma* eggs. We considered that infection exits if the microscopic visualization and/or the ELISA were positive.

In all Sub-Saharan patients' blood samples were concentrated by 2% formalin (Knott technique), stained with Giemsa and screened under light microscope to detect filariasis.

All patients from Central and South America were tested for Chagas disease. An immune chromatography test (Stick Chagas®, Operon, Spain) were used to detect antibodies anti-*Tripanosoma cruzi.* Positive cases were confirmed with a second ELISA (Ortho-Clinical Diagnostics©, USA). All samples testing positive by any technique were sent to the National Microbiology Centre (Instituto Carlos III, Spain) to confirm the result by determination of anti-*T. Cruzi* antibodies by indirect immune fluorescent antibody test (IFAT) and by polymerase chain reaction (PCR).

Ethics statement

This study is a part of an overall project entitled "Study of prevalence of imported diseases in an immigrant population", which was approved by the Ethical Committee of Clinical Investigation of Asturias. The obtaining of a written consent was hindered because of the inability to read in Spanish by the most of participants and so, informed oral consent was obtained from all participants. The oral consent was registered in the patient's medical chart for the team investigator. The Ethical Committee approved the use of the oral consent procedures used in this study.

Statistical analysis

Qualitative variables were compared using the χ^2 test, the Fisher exact test, when necessary. For quantitative variables, the Student t test for non- paired variables or the Mann-Whitney U test were used. Significance was designated at $p<0.05$. All tests were performed with the SPSS 15 software for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Patient characteristics

During the period of the study 63 patients were analyzed. The median age was 36 years (range 18–59years) and forty were female (63.5%). The average time in Spain was 679 ± 760 days. Twenty-eight patients were in Spain for less than one year. The median CD4+ cell count was 356 cells/µl (range 15–1002), and thirteen patients had CD4+ cell counts less than 200 cells/µl. The most frequent HIV genotypes were: CRF02_AG (50.8% patients), B (44.1%), K/C (3.4%), CRF01-AE (1.7%).Average viral load was 166,017[642,145] RNA viral copies/mm^3. The characteristics of patients are described in Table 1.

Table 1: Clinical and epidemiological Characteristics of patients.

Parameters	n (% or SD) n=63
Demographic characteristics	
Sex (Female)	40(63.5%)
Age (Years)	36±10
Median time in Spain (Days)	679±760
Areas of origin	
Central Africa	33(52.4%)
South America	20(31.7%)
West Africa	6(9.5%)
North Africa	2(3.2%)
Centro America	2(3.2%)
Clinical Characteristics (Yes)	
Median CD4+cell count (cells/µl)	356 ±200
Median viral load (RNA copies/mm^3)	166,017±642,145
Most frequent Diseases	
Chronic hepatitis B	6 (9.5%)
Hepatitis C virus	4(6.3%)
Syphilis	21(33.3%)
Latent Tuberculosis	9(14%)
Tuberculosis	6(9.5%)
Parasitic Diseases	34(54%)
No disease	22(35%)

Cosmopolitan diseases

Twenty (31.7%) patients were immune to hepatitis B and 6 (9.5%) had a chronic hepatitis B. All hepatitis B chronic patients were from Africa Central (p=0.0254, OR 2.11 [1.61-2.78]. Four (6.3%) patients had hepatitis C virus infection, most of them (3 cases) from Central Africa.

Twenty-one patients (33.3%) were diagnosed by syphilis. Seventeen of them were from Equatorial Guinea (p=0.0031, OR 2.13 [1.37-3.29]) and 4 from South America. Nine patients (14%) had a latent tuberculosis (TB) infection and six had an active TB (9.5%). Although both types were more frequent in Central Africa only the risk of latent TB disease were significantly higher (p=0.0145, OR 1.95 [1.36-2.79]).

Parasitic diseases

Thirty-four (54%) patients had a parasitic disease. The characteristics of both groups are showed in Table 2. No statistically differences in sex, age, time in Spain, viral load or CD4+ cell count were observed. Parasitic diseases were more frequent in patients from Central Africa (11 vs 22, p=0.0339, OR 3.00 [0.95-9.62])

Twenty-eight patients (44.4%) were infected with intestinal parasites including ten (35.7%) with *Entamoeba histolytica*, eight (28.5%) with *Trichuris trichuria*, two (7%) with *Giardia intestinalis*, (3.5%) with *Blastocystis hominis*. Serologic evidence of *S. stercoralis* infection was detected in 13(46%) patients; no larvae were detected by stool examination. Three patients (11%) had a positive serology for *Schistosoma* spp., ten patients had a mixed infection

Most of patients (60%) with intestinal parasites came from Central Africa, especially from Equatorial Guinea (56%) although without statistically significance. There is no statistically differences in age (36.6 vs 35.93 years), sex (27female vs 13), time in Spain (627 [555] vs 708 [857] days) and the average CD4+ cell count (350 [209] vs 360 [192] cells/µl) between infected and not infected patients. The viral load was significantly higher in infected patients 287,970 [982,009] RNA viral copies/mm3 vs 83,616[196,220], (p=0.0412).

Regarding to *S. stercoralis*, 13(46%) patients were positive, seven (53.8%) come from Central Africa and six (46.2%) from South America. The infection was significantly more frequent in women (12 vs 28 p=0.022, OR= 9.429 [1.137-78.162]). Were observed no statistically differences in age, time in Spain, and countries of origin. Seven patients were asymptomatic (53.8%). None of the 13strongyloidiasis-infected individuals had evidence of infection by HTLV. Although patients with strongyloidiasis had lower CD4+ cell counts (mean 294 [160] cells/µl) than those not infected (median 373 [203] cells/µl), the difference was not significant (p = 0.084).

Regarding other parasites, seven of Sub-Saharan patients (17.9%) were infected by *Mansonella perstans,* all from Equatorial Guinea. Two patients (9%) had a positive antibody test for Chagas' disease. Four patients (6.3%), all from Equatorial Guinea had malaria caused by *Plasmodium falciparum.*

Only 14 patients showed eosinophilia in blood. Eosinophilia was significantly higher in patients infected with *T. trichuria* (709 [1,019] vs 306 [345]. p=0.0001) and *M. perstans* (835[1,056] vs 314 [304], p=0.002) but not in *Strongyloides* group (497 [512] vs 325 [485]. p=0.258).The eosinophilia was also higher in mixed infections (935[1,079] vs 368 [422], p=0.033)

Fourteen per cent of patients had two or more parasites. Thirteen patients had CD4+ cell counts below 200 cells/µl without differences in sex, age or risk of parasitic diseases Table 3.

In twenty-two (35%) patients the screening didn't show any disease included cosmopolitan diseases, (p=0, 0132; OR=5.64; [1.57-21.17]). The risk of parasite diseases was significantly higher in patients from Central Africa (p=0.0002 OR=2.12[1.34-

Table 2: Characteristics of infected and not infected patients.

	Infected with any parasite n=34 (54%)	Non Infected n= 29 (46%)	p-value*	OR
Age (years ± SD)	35.93 ± 8.69	36.38 ± 9.33	0.4360	-
Sex (Female/Male)	20/14	20/9	0.4046	0.64[0.20-0.2.06]
Regions of origin				
Central Africa	**22**	**11**	**0.0339**	**3.00[0.95-9.62]**
West Africa	2	4	0.2863	0.39[0.05-2.80]
North Africa	1	1	0.9089	0.85[0.02-32.84]
Central America	0	2	0.2078	0.00[0.00-3.51]
South America	9	11	0.3300	0.59[0.18-1.95]
Time in Spain (days ± SD)	662.82 ± 693.104	700.82 ± 846.83	0.9760	-
CD4+ cells count (cells/µl ± SD**)**	365 ± 198	346 ± 198	0.1060	-
Viral load (RNA viral copies/mm^3)	228,495 ± 857,291	94,920 ± 220,358	0.6840	-

*χ2 test or Fisher exact test, when necessary

Table 3: Comparison between patients with CD4+ cells count below and higher 200 cells/ µl.

	> 200 CD4+ cells/µl n=50 (79%)	< 200 CD4+ cells/µl n=13 (21%)	p-value*
Age	36.20 [9.36]	36[7.30]	0.7290
Sex (Female/Male)	31/19	9/4	0.4439
Regions of origin			
Central Africa	26 (52%)	7 (54%)	0.9054
West Africa	4 (8%)	2 (15,4 %)	0.4190
North Africa	16 (32 %)	4(30,6%)	0.9323
Central Americav	2 (4 %)	0 (0%)	0.4636
South America	2(4%)	0 (0%)	0.4636
Time in Spain (days ± SD)	626.62 ± 702.87	902.33 ± 967.39	0.822
Patients infected with any parasite	29 (58%)	5 (38.5%)	0.2079
M. perstans	6 (12%)	1 (7.5%)	0.5539
S. stercoralis	9(18%)	4 (31%)	0.2563
Intestinal parasites	20 (40%)	5(38.5%)	0.9195
Malaria	4(%)	0 (0%)	0.3866
Asymptomatic	27 (54%)	10 (77%)	0.1347

*χ2 test or Fisher exact test, when necessary

3.36]) and lower in South America (p=0.07; OR 1.60 [0.95-2.69]. Regarding two countries the risk of parasitic diseases was significantly higher in Equatorial Guinea (p=0.0003; OR= 9.87 [2.45-4.45]).

DISCUSSION

Over 34 million people are living with HIV/AIDS, the majority (more than 23.5 million) of whom live in sub-Saharan Africa and 1.4 million in South America [1], where parasitic diseases are endemics. It has long been recognized that parasite infections and HIV interact bidirectional and synergistically with each other [3]. Previous studies have shown that HIV sero-positive patients were more likely to have intestinal parasitic infections, malaria or schistosomiasis [14]. On the other hand, HIV infection increases the risk of the severity of parasite infection, which has been associated to higher HIV viral loads [15,16].

In spite of this there is a scarce number of studies about the necessity of perform a screening for parasitic diseases [5-7], although recently several guidelines recommend performing a screening of *Strongyloides* or *Schistosoma* infection in these patients [8,9].

In this paper, the most frequent parasites found in HIV-infected immigrants were intestinal parasites, including chronic strongylodiasis infection. Previous studies have shown that intestinal parasites appear between 6-19% of HIV-infected immigrants [5,17], which is a lower rate than that found in the present study. A possible explanation is the origin of our patients with the majority arising from Sub-Saharan Africa, where several studies have described intestinal parasites in 24-34% of HIV positive patients, similar rate to that found by us [15,18], and significantly higher (81%) in studies performed in Equatorial Guinea, the most frequent country of origin in our group of patients [19].

Strongyloides stercoralis was the most frequent intestinal parasite (59% of intestinal parasites, 20% of global parasites) such as has been reported in other papers [5]. Several studies have documented increased rates of *S. stercoralis* infection among HIV-infected individuals [20]. Assefa et al. [21], found a

21-fold increased prevalence of *S. stercoralis* infection among HIV-positive compared to HIV-negative patients in southern Ethiopia. Other studies report similar results in Brazil [22]. However, this increased predilection for *S. stercoralis* infection among HIV-infected individuals does not seem to be predictive of an increased incidence of hyper infection and dissemination. Although *S. stercoralis* infection was more frequent in women, such as has been reported in other papers [6], no differences in age, viral load, or time of stay in Spain have been found.CD4 + T cell counts are used as a measure of immunity and HIV disease progression [8,9] and counts less than 200 cells/μl increase the risk of opportunistic infections. In this study, HIV patients with CD4+ T cell counts less than 200 cells/μl were not at risk of acquiring either single or combined parasitic infections, except in strongyloidiaisis group where CD4+ T cell count was lower, although without significant differences probably due to few cases number. This finding is consistent with previous reports [23-27]. Thus, Assefa et al. [21], found that the rate of parasitic infection was increased with decreasing CD4 T-cell count among HIV infected individuals. Similarly, an increased rate of mixed parasitic infection was observed at the same lower counts of CD4 T-cells. Although fourteen per cent of patients had mixed infections, no difference by HIV status were observed. Increased rate of mixed infections among HIV positive individuals, particularly in those with CD4 counts below 200 cells/μL, maybe because of higher prevalence of certain parasites among the risk group, which favors the presence of mixed infections.

Seven patients were infected by *Mansonella perstans,* which is a little known but wide spread human filarial parasite in many parts of Sub-Saharan Africa. Infections have been reported in 33 countries of this region, and infection prevalence is often very high in endemic areas, especially among children. Thus, *M. perstans* can be considered one of the most prevalent parasites of man in tropical Africa [28]. *M. perstans* might potentially interfere with the host's regulatory mechanisms and influence the outcome of other infections such as malaria, tuberculosis and HIV, which often thrive in the same environment.

Previous studies [5,7] use serological techniques as diagnostic method of filariasis, but filarias serology is not useful in asymptomatic patients, showing cross reactions with other parasites like *Ascaris lumbricoides*. Our study supports the useful of microscopic techniques as diagnostic method in patients from endemic areas, especially in those with eosinophilia and absence of skin symptoms. Many clinicians rely on eosinophilia as an indicator of parasitic infection. However, only 14 patients (22%) of our cohort had a peripheral eosinophillia. Screening based on an eosinophilia alone would have missed patients with positive results. For this reason, in our evaluation, eosinophilia was not useful to identify the patients to be screened, which is consistent with other studies [4,6,7]. In our results, lack of eosinophilia should not preclude consideration of parasitic infection in the differential diagnosis in the context of HIV [5,7].

There are several limitations to our study, such us the relatively small sample size and the use of serological techniques as an indication of previous parasitic infection for *Strongyloides* and *Schistosoma*. Limitations of serological testing, such as cross-reactivity between parasites or the fact that patients with an advanced HIV infection may have an impaired antibody formation (although the major of our patients had high levels of CD4+ T cell counts), may limit the utility of these tests.

Despite these limitations, this study showed the results of a prospective screening program in an immigrant population from several geographical regions concluding that HIV-infected patients from countries endemic for parasitic disease, which are not routinely screened for these infections because they are often asymptomatic and lack eosinophilia, are at high risk for chronic parasitic diseases. The high prevalence of parasitic diseases in our patients suggests that a screening program, which includes intestinal parasites, *Strongyloides* and *Schistosoma*, filarias and Chagas disease according to the patient's geographic origin, would be very useful.

CONCLUSION

In conclusion, both parasitic and cosmopolitan distribution diseases are frequent in immigrant population infected with HIV. Systematic screening programs are a useful tool for early detection of these pathologies. Our study supports the use of screening for parasitic diseases in immigrant patients with HIV infection.

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