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Short Communication

Pseudomembranous Candidiasis Indicates High Level Drug Resistance among Patients on Antiretroviral Treatment in Nairobi East District, Kenya

Abstract

Objectives: The aim of this study was to determine antiretroviral drug resistance patterns in patients on long-term antiretroviral therapy presenting with OPC.

Methods: An exploratory survey was performed among HIV-infected patients on ART for minimum of 24 months presenting with OPC in Nairobi, Kenya. Type (pseudomembraneous or erythematous candidiasis, angular cheilitis) and previous episodes of OPC, CD4-cell counts, duration, regimen and adherence on ART were compared between patients with high (>1000copies/ml) and low HIV-RNA levels. Genotypic resistance testing was performed on those with high viral loads.

Results: Out of (n=45) patients with OPC, (n=28; 62%) had high HIV-RNA levels. The (n=28) patients who mostly presented with pseudomembraneuos candidiasis (n=26; p<.0001), had significantly more previous episodes of OPC (55% versus 18%; P<0.0373) lower median CD4 cell counts (74 versus 521; P<.0001) and higher HIV-RNA median plasma levels (111,191 copies/ml versus <20; P<.0001). The sensitivity (0.96) and specificity (0.87) of pseudomebraneous candidiasis to predict virological failure was high.

HIV genotyping performed in 22 of the 28 patients showed that most (18/22) had drug resistance mutations of which 12/18 had Lamivudine-associated M184V mutation, 14/18 had TAMS and 16/18 had NNRTI mutations. One patient had major PI mutations.

Conclusion: Virological failure and drug resistance mutations including TAMs should be suspected in patients on long-term ART that present with pseudomembraneous candidiasis. We propose to include recurrent OPC in the WHO clinical criteria for ART failure as well as to establish clinical training sessions to build competences among health care providers.

Abbreviations

ART: Antiretroviral Therapy; HIV: Human Immunodeficiency Virus; NRTIs: Non-nucleoside Reverse Transcriptase Inhibitor; NNRTI: Non-nucleoside Reverse Transcriptase Inhibitor; PI: Protease Inhibitor; OPC: Oropharyngeal Candidiasis; TAMs: Thymidine Associated analogue Mutations; CCCs: Comprehensive Care Clinics; WHO: World Health Organization; PHC: Primary Health Care; PMTCT: Prevention of Mother to Child Transmission of HIV infection Treatment

Introduction

The primary goal of antiretroviral therapy (ART) is the suppression of human immunodeficiency virus (HIV) RNA to undetectable levels and the restoration of immunity including the normalization of CD4 cell numbers and a decrease or disappearance of HIV-associated opportunistic infections [1-4]. The standard triple-drug regimen is a combination of three classes of ART drugs comprising of two nucleoside reverse transcriptase inhibitors (NRTIs) and either a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor (PI) [5].

Recurrence of opportunistic infections in ART treated patients; particularly oropharyngeal candidiasis (OPC) may indicate non response to ART and virological failure [4,6,7]. This may be as a result of drug resistance mutations to the ART regimens [8]. Drug resistance mutations include thymidine associated analogue mutations (TAMs) which induce high level resistance to most NRTIs. Continuation of ART in the presence of ongoing viral replication leads to increasing ART resistance formation whereby options for second-line treatment will decrease over time, especially if TAMs are accumulating [8,9]. Furthermore, resistant viruses may be transmitted to others whose response to first-line treatment may thereby be compromised. Patients on ART therefore need to be routinely monitored for virological failure.

In resource limited settings, viral load assays are recommended

when immunological failure (CD4 criteria) is suspected [5]. However, analyses of CD4 cell counts do not necessarily predict virological failure [10]. Alternatively, WHO clinical failure criteria may be used, consisting of occurrence of new or recurrent WHO-defined stage 3 and 4 events including OPC [11]. This criterion however does not include routine oral examination for OPC in ART treated patients.

It is unknown is whether OPC indicates early virological failure or mostly occurs in patients with resistant virus with limited effective treatment options. The aim of this study was to determine antiretroviral drug resistance patterns in patients on long-term ART presenting with OPC. The study was carried out in Nairobi East district in Kenya, where over 20,000 ART treated patients seek regular consultations [12].

Methods

This study received approval from the Kenyatta National Hospital/University of Nairobi Ethics and Research Committee (approval number KNH-ERC/A/474) and from the Ministry of Public Health and Sanitation (Ref. No. MPHS/IB/1/14 Vol. III. This trial was registered in the Netherlands Trial Register (http://www.trialregister.nl, NTR2627).

Study design

An explorative survey performed on a convenience sample of (n= 45) HIV-infected patients on ART for minimum of 24 months, who routinely attended comprehensive care clinics (CCCs) in Nairobi East district in Kenya between December 2011 and March 2013. This study was part of an ongoing project [13-15].

Selection of participants

Oral examination for OPC (pseudomembraneous candidiasis, erythematous candidiasis and angular cheilitis) was done and confirmed by a team of a trained examiner and experienced dentist respectively [14,15] using the guidelines for presumptive diagnosis [16].

First-line ART treatment consisted of Lamivudine and AZT or d4T or Tenofovir disoproxil fumarate (Tenofovir) in combination with either Nevirapine or Efavirenz. Patients on first line treatment included those who had received prior antiretroviral treatment such as prevention of mother to child transmission of HIV infection treatment (PMTCT) and intra-class ART switch for instance from AZT to TDF. In case of second-line treatment, PIs were part of the regimen that includes two NRTIs as well. Patients were included if all criteria a) confirmed HIV infection b) presently on ART and for a minimum of 24 months c) confirmed OPC d) not currently on anti-mycobacterium drugs, were fulfilled and informed consent was signed for. All patients were taking cotrimoxazole.

Data collection

A clinical recording form (CRF) was adapted from the WHO clinical recording form for oral mucosal diseases [11] and the Kenya Ministry of Health patient's routine clinical cards.

To assess patients' adherence to ART pill counts were done. Satisfactory adherence of >95% was defined as patients having missed two or less ART doses per month [17]. Additional information was obtained from clinical records and patient interviews regarding previous pill counts, keeping of appointments, previous treatment interruptions, knowledge of ART dosage and social support networks such as partner disclosure, HIV support groups and treatment supporters.

CD4, viral load and resistancy testing

Records for CD4 counts and viral loads at baseline were absent or scanty as these two tests were not prerequisite laboratory investigations for initiation and monitoring of ART treatment [5,17].

Patients with OPC were immediately referred to the testing laboratory where blood for CD4 cell counts and viral load testing was drawn for assays using standard methods [18]. As per the World Health Organization and the Kenya clinical guidelines [5, 17] samples with HIV-RNA plasma levels exceeding 1,000 copies/ml were considered as having virological failure and were submitted for genotypic (population based sequencing of protease and partial reverse transcriptase regions) resistance assays.

NRTI resistance mutations considered were Lamivudine associated M184V mutation, TAMs (defined as various combinations of mutations at positions 41,67,70,210,215 and 219) and K65R mutation (which results in a four-fold decrease of Tenofovir susceptibility).

NNRTI resistance mutations considered were K103N (which confers resistance to Nevirapine and Efavirenz), G190A/S, K101E, Y181C and Y188L.

Resistant mutations for the PI gene included I54V, M46V and V82A [19].

Statistical analysis

Data were manually entered into an Excel file and checked for accuracy and completeness.

Independent variables were the patients' response to ART which was categorized into those with high (>1000 copies /ml) and those with low HIV-RNA plasma levels.

Socio-demographic characteristics were analyzed to identify possible potential confounders.

Statistical differences between dependent variables under low and high HIV-RNA plasma viral loads categories were analysed using SAS version 9.2 (SAS Institute, Cary, NC, USA) for frequencies using Fisher's exact test and for medians using Wilcoxon tests. The level of significance was set at 0.05.

Patients were categorized as having pseudomebraneous candidiasis if this lesion appeared alone or in combination with another type of OPC.

Results

A total 45 patients were evaluated, predominantly female (n=33).

Except for age, patients with low and high HIV-RNA levels were similar in sociodemographic characteristics, indicating that these variables did not influence HIV-RNA plasma levels (Table 1).

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Analysis of clinical characteristics and OPC risk factors is presented in Table 2.

Patients (n=28) with high HIV-RNA plasma levels, mostly presented with pseudomembraneous candidiasis(n=26; p<.0001), had significantly higher episodes of OPC in the past one year (16 episodes versus 3; P=0.0132), lower CD4 counts (median 74 copies/ml versus 521; P<.0001) and higher HIV-RNA median plasma levels (111,191 copies/ml versus <20; P<.0001) compared to those with low HIV-RNA plasma levels. Those (n=17) with low HIV-RNA plasma levels mainly presented with erythematous candidiasis (n=13). There was no statistical difference regarding the other variables. The sensitivity (0.96) and specificity (0.87) of pseudomebraneous candidiasis to predict virological failure was high.

After excluding (n=7) inadequate samples, genotypic resistance testing was performed on 22 samples of patients with high HIV-RNA plasma levels.

The 22 patients had HIV-1 subtypes A1 (n=13), D (n=6) and C (n=1) while (n=2) were unassigned. All were using ART regimens which included Lamivudine (3TC).

Most (18/22) patients had resistant mutations as further described below:

Regarding NRTI resistance mutations most patients had: a) Lamivudine-associated M184V (n=12/18) b) least one TAM (14/18), and mostly (12/18) presenting as multiple TAMs. Distribution of TAMs was: T215F/Y (12/18), M41L (12/18), L210W (5/18), D67N

Table 1: Socio-demographic characteristics of 45 patients on long-term ART Presenting with oropharyngeal candidiasis in Nairobi East district.

Characteristic	Number (%)		High HIV-RNA plasma levels (>1000 cp/ml) n=28	Low HIV-RNA plasma levels (<1000 cp/ml) n=17	P value
Gender					
Male	12	(27%)	10	2	0.096
Female	33	(73%)	18	15	
Age (yrs)					
0 - 39	21	(47%)	18	3	0.005
> 39	24	(53%)	10	14	
Education level					
Primary	29	(64%)	25	4	1.000
Higher (secondary, diploma, graduate)	16	(36%)	2	14	
Socio-economic class					
Lower	39	(87%)	24	15	1.000
Middle / Upper	6	(13%)	4	2	
Occupation					
Employed (profession, skilled)	29	(64%)	19	10	0.212
Unemployed/unskilled	16	(33%)	9	7	

Table 2: Analysis of clinical characteristics and OPC risk factors of 45 patients with high and low HIV-RNA plasma levels on chronic ART presenting with oropharyngeal candidiasis in Nairobi East district.

Characteristics	High HIV-RNA plasma levels (>1000 cp/ml) n=28	Low HIV-RNA plasma levels (<1000 cp/ml) n=17	P value
Type of OPC			
pseudomembraneous	26	2	<.0001
erythematous	1	13	
angular cheilitis	1	2	
Had OPC episodes in the past one year	16	3	0.0132
CD4 at OPC diagnosis (median)	74	521	<.0001
Viral loads at OPC diagnosis (median)	111,191	<20	<.0001
Years since HIV diagnosis (median)	6.5	6	0.15
Years on ART (median)	6	5	0.15
History of TB	19	8	0.2162
On current co-medications	9	3	0.69
Adherence to ART:			
adheres to ART>95% (pill count)1	25	16	1.000
ART treatment interruptions2	12	3	0.1097
adheres to ART(knows ART dosage)	24	10	0.0721

[1]Patient having missed two or less ART doses per month.
[2]Patient having skipped more than one month between regimens.

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(4/18), K219Q (3/18) and K70R (2/18) No K65R mutation was not present.

Nearly all, patients (16/18), had NNRTI resistance mutations comprising of all (n=13) those on first- line ART.

All the (12/18) patients with Lamivudine-associated M184V resistance mutation also had NNRTIs mutations.

Only one patient had major PI mutations.

Discussion

In this study we report three important findings.

First finding is that most (62%) patients on long-term ART presenting with OPC had high HIV-RNA plasma levels. Our study, in line with previous studies [1-4,6,7] underlines the potential of OPC, as a clinical marker of virological failure in this setting.

Second finding is that of the three types of OPC, pseudomebraneous candidiasis presented in nearly all (93%) of the patients with high HIV-RNA plasma levels. The high sensitivity and specificity demonstrated the validity of this lesion to predict virological failure in this setting. This lesion should be recognized by all health workers in this high HIV prevalence setting. Our earlier study [15] demonstrated however that primary health care (PHC) workers, the main HIV service providers, do not perform routine oral examination and are not competent to identify the lesion. PHC providers may also fail to acknowledge the clinical importance of OPC because neither oral examination nor OPC are emphasized in the WHO criteria for treatment failure. In congruence with this study, 55% of the patients with high HIV-RNA plasma levels had previously experienced OPC, which was not recognized as a possibility of virological failure since they all continued first- line treatment.

Third finding is that most (82%) of patients on long-term ART who presented with OPC had resistant mutations including TAMs thus indicating the potential OPC as marker of a resistant virus. This finding is particularly important in this setting high ART resistance setting [20] where viral loads are not routinely monitored. Clinical and immunological criteria may delay identification of virologic failure and may further contribute to development of drug-resistance mutations [10]. Our study confirms these statements, as significant numbers of NRTIs and NNRTIs drug-resistance mutations, including TAMs, were found among patients with virologic failure. Previous episodes of OPC, long median durations on ART and the presence of TAMs in most (74%) patients indicates that drug resistance mutations had been accumulating over a long period. TAMs induce high level resistance in both AZT and d4T and in most other NRTIs especially when in combination with M184V [18]. Also, the response to Tenofovir may be limited by K65R mutation in certain patterns of TAMs [21]. The high prevalence of TAMS combined with the M184V, may therefore seriously limit the effectiveness of the present available second-line treatment in Kenya consisting of a boosted protease inhibitor and two NRTIs [17]. Our concern involves also patients who were already on second-line regimens as one or more TAMs were found in 4 out of the 9 patients with high HIV-RNA plasma levels.

The small size of our study population however limits the interpretation of the data. Comparison with data from the previous studies [1-4,6,7] was also limited due to wide variations in duration of patients on ART and in the 'high HIV-RNA plasma level' cut offs which ranged from 2,000 – 100,000 copies/ml. It was remarkable to observe that although a selected group of patients participated in our study, the high prevalence of mutations including TAMs was consistent with that of a larger study [9].

Conclusions

Virological failure and resistance mutations including TAMs should be suspected in patients on long-term ART that present with pseudomembranous candidiasis. We propose to include recurrent OPC in the WHO clinical criteria for ART failure as well as to establish clinical training sessions to build competences among health care providers.

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The authors declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere. There are no other competing interests.

Authors' contributions

LNK, WJMVDS, TMAWM, NHJC and AVDV created the study design and participated in the discussions about the study design. LNK, AVDV and EOD participated in the field work. JM performed the statistical analysis. LNK, AVDV, WJMVDS, JM and FFS drafted the manuscript. All authors have read and approved the final manuscript.

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