Original Article

Occurrence and clinical features of HIV and malaria in co-infected individuals in Osun State, Nigeria

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Abstract

Introduction: Human immunodeficiency virus (HIV) and malaria infections are among the major public health concerns in sub-Saharan Africa, where they are associated with high morbidity and mortality. The study was conducted to assess the occurrence and clinical features of HIV and malaria in co-infected individuals in Osun State, Nigeria. **Methods:** The study was cross-sectional, which involved 422 participants who were administered structured questionnaires for socio-demographic and clinical data. Venous blood was collected for malaria parasite detection and count from One hundred and seventy-four HIV seropositive individuals. They were re-examined clinically for HIV diagnosis, CD_4^+ T cell counts, and packed cell volume (PCV). **Results:** The mean age of the participants was 28.48 ± 15.38 while the overall predominance of malaria among the HIV-positive patients was 11.5% (20/174). The malaria prevalence was significantly higher in female patients ($P = 0.0088$) and occupational status among students ($P = 0.0001$). Malaria/HIV co-infected patients had a significantly lower mean value of PCV ($P = 0.0001$), CD_4^+ cell count (0.0001), and temperature (0.0001) compared to HIV-infected patients having no malaria. **Conclusion:** The study showed that females had relatively higher malaria infection compared to their male counterparts. To achieve better management of HIV patients against malaria infection, proper preventive measures, antiretroviral therapy (ART), and chemoprophylaxis are a useful strategy to put in place. Also, the monitoring of CD_4^+ cell count, viral load, and some hematology indices on a regular basis is crucial.

Keywords: Co-infection, HIV, malaria, Nigeria, *plasmodium falciparum*

Introduction

The human immunodeficiency virus (HIV) and *Plasmodium* species are pathogens responsible for two of the most prevalent infectious diseases in the world.[1] Both pathogens, principally *Plasmodium falciparum*, are the cause of significant stimulation and disorder of the immune system.[2] The dual infections have been reported as the life-threatening health problems of developing countries, including Nigeria, accounting for more than 2 million annual deaths globally.[3] Significant mortality has been reported as a result of opportunistic infections such as malaria and arrays of other pathogenic diseases faced by people living with HIV.[4]

The integral part of the universal anti-retroviral therapy (ART) program in developing countries, as recommended by the World Health Organization, is the administration of cotrimoxazole (CTX) prophylaxis treatment care for HIV-infected individuals.[5] This practice remains a key policy for the prevention

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of opportunistic infections among HIV-infected individuals, including *Plasmodium*. CTX has significantly decreased bacterial infections, HIV-related deaths, and hospital-acquired infections.[6] Preventive measures such as insecticide-treated bed nets[6] have been reported to reduce the risk of malaria in people living with HIV/AIDs. Remarkably, termination of CTX among HIV-infected individuals receiving ART has resulted in progressive increases in parasite intensity and malaria incidence.[7]

Reports have shown that in most clinical cases of malaria, anemia is a prognostic factor most commonly encountered hematological abnormality in HIV and malaria-infected individuals.[8-16] In most sub-Saharan African countries, where the burden of HIV and malaria co-infection is incidentally high, evaluation of clinical indices should be considered. Therefore, this study was performed to determine the occurrence and clinical features of HIV and malaria co-infected individuals in Osun State, Nigeria.

Methods

Study area

Osun State is located in the tropical zone of South Western Nigeria. The language of the majority of the people of Osun State is Yoruba, but this is however broken into scores of dialects. It is landlocked and occupies a landmass of 9,251 square kilometers and a population of 3,423,535.[17] The area experiences two seasons, the dry season (November–March) and the rainy season (April–October).[18] The mean daily temperature varies from 30°C to 34°C, and the mean annual rainfall is about 1400 mm. Malaria transmission occurs throughout the year, with the peak at the beginning and end of the rainy season.[19]

Study population

HIV-infected patients were registered, diagnosed with HIV infection, and further sought treatment at IHVN clinic, Uniosun Teaching Hospital Osogbo, General Hospital Ede, and OAUTHC Wesley Guild Hospital Ilesa. The participants included a control group of HIV seronegative individuals at the General Out-patient Department (GOPD). Only those who had given their consent for blood collection and answered the questionnaire were enrolled in the study.

Selection criteria

Inclusion criteria

Confirmed HIV-infected patients, duly registered into the anti-retroviral treatment (ART) program, presenting with uncomplicated *falciparum* malaria with the presence of fever (≥ 37.5°C) in accordance with the WHO criteria.

Exclusion Criteria

The exclusion criteria were the patients that have been on anti-malarial medication for two weeks prior to the period of blood collection, as well as the patients that neither showed interest nor gave consent.

Sample size determination

Sample size was determined using.[20]

$$\text{Sample size } (n) = \left[\frac{Z\left(1-\frac{\alpha}{2}\right)2 \cdot e(1-e)}{d2}\right]$$

Where n = Sample size

Z = Z score, i.e., Standard normal variate at 5% type 1 error ($P < 0.05$)

e = Standard deviation or expected proportion in population based on previous studies

d = Margin of error or absolute error (5%)

Z = 1.96

e = 0.5.

The sample size obtained was 422. A total of 174 HIV seropositive individuals who sought care at the study locations were recruited in the study.

Ethical clearance

The study was approved by the Uniosun Teaching Hospital (LTH/EC/201/11/243) and Osun State Ministry of Health Ethical Committee Osogbo (*OSHREC/PRS/569T/41*) before this research was carried out. Written informed consents were obtained from patients prior to recruitment into this study. Consent for the children was provided by the parents/guardians while some of the children participants provided the assents by nodding and thumb printing.

Structured questionnaire

A questionnaire in local (Yoruba) and official (English) languages, was included in the basic socio-demographics of the study subjects as well as their knowledge and prevention of malaria. For consistency of thought, two independent translators were engaged; one translated English to Yoruba, the other, who had no prior copy of English, was tasked to translate the local language back into English. Individuals were given unique codes on the questionnaires and their laboratory specimens.

Determination of axillary temperature and weight

The weight was determined using a weighing scale, and the axillary temperature was also determined before enrolment using a digital clinical thermometer. The tip of the thermometer was inserted under the armpit of the included subjects, and the numeric value of axillary temperature was recorded.[21]

Detection and parasite density of malaria parasite

Laboratory investigation was conducted using 2 µl of blood sample was spread at an angle 30°C with a clean spreader to

form a thin blood smear with a tail at the end of the film and 6 µl of blood for a thick film. The smear was thoroughly allowed to dry, fixed with methanol, and Giemsa stain stock diluted in 1:10 in buffered distilled water was used for both films for 10 minutes. The stained smears were washed off with buffered water (pH 7.2), allowed to air-dry, and examined microscopically under x100 (oil immersion) objective of an Olympus CX 22 light microscope (Olympus Optical Co. Ltd., China).[22]

The slide was considered positive if asexual forms/gametocytes of any *Plasmodium* species were observed in the blood films. The corresponding thin films were observed to determine the species of *Plasmodium* present. Malaria parasites were counted against 200 white blood cells (WBCs) in the thick film. The parasite density was recorded as the number of asexual parasites per microlitre of blood.[23]

HIV diagnosis

HIV test was conducted using determine Rapid Test kit (Abott Laboratories, Co., Ltd. Minato-Ku, Tokyo Japan) was used to test the supposed HIV negative subjects for HIV-1. Test results were read after 15 min from corresponding colour changes on the strip according to the manufacturer's guidelines and instructions. All HIVpositive results by the test kit were confirmed using UNI GOLD or STAT PAK concurrently according to the serial algorithm of the Federal government of Nigeria.[24] Both test kits are immuno-chromatographic rapid tests for the qualitative detection of antibodies specific to HIV in human serum, plasma, or whole blood.

CD_4 count estimation (Partec cyflow technique)

The CD_4^+ T- lymphocyte count of the study population was evaluated using flow cytometry. (Partec, GmBH, Germany).[25] 20 µl of CD_4^+ - PE monoclonal antibody was put in labelled Partec (Rohren) tubes, and 20 µl of well-mixed ethylenediamine tetraacetic acid (EDTA) blood was added. This content was mixed together several times for 2 min, and incubated in the dark for 15 min at room temperature with intermittent mixing every 5 min. After incubation, 800 µl of CD_4 diluting buffer was added to each preparation, mixed properly before being analysed on the cyflow counter as described by the equipment manufacturer.[25] CD4 cell counts were categorized as very low or advanced stage (<250 cell/µl), 250–350 cell//µl (low), lower normal (350–500 cell/µl) and higher normal or asymptomatic stage (≥500 cell/µl).[26]

Packed cell volume (PCV) and Haemoglobin concentration (Hb)

The packed cell volume (PCV) and haemoglobin concentration (Hb) were estimated with Sysmex XT- 21N Haematology Analyser Automated machine (Sysmex Corporation, Japan 2012 Model) with strict adherence to the manufacturer instruction.[27] Anaemia was defined as Hb <11.0 g/dl and further classified as described by Cheesbrough (2010) as severe (Hb <7 g/dl), moderate (Hb between 7.0 g/dl and 10.0 g/dl), and mild (>10.0 g/dl and <11 g/dl).[28,29]

Statistical analysis

After validation, data analysis was done using GraphPad Prism 5 (GraphPad Software Inc. USA) to generate means, standard deviation, median, and frequency distributions. The significant difference between groups was determined using a T-test for continuous variables such as age and parasitaemia that were normally distributed. Analysis of variance was used to investigate the relationship between the variables. The association between categorical variables was tested using the Chi-square test (<2). Statistical significance was defined as a P value < 0.05.

Results

A total of 174 participants with a mean age of 28.48 ± 5.38 were enrolled in the study. The study population comprised nearly two-third females and one-third males, distributed across six different age groups. Almost half of the study population were married (48.9%), and 40.8% were single, while the remaining (10.4%) were considered divorced/widowed. The greater proportion of the studied population had completed some level of education from primary to tertiary levels. A major proportion of the studied participants was self-employed, while about 13.3% were civil servants, students were found to be 27.6%, and 8.6% were unemployed, as presented in Table 1. The place of residence showed that more participants were residing in rural than in urban settings [Table 1].

The level of anaemia among the malaria-infected participants was 38.5% anaemic while 61.5% non-anaemic for malaria non-infected individuals. Thirty-one out of 174 (17.8%) of the study participants were malaria positive. The proportion of participants who used insecticides treated nets (ITNs) was higher (114 (81.0%)) compared to their counterparts that utilized insecticide sprays (105 (60.3%)). One hundred and thirty-two (75.9%) were on daily cotrimoxazole prophylaxis. The number of participants with a CD4 cell count less than 200 was 31.0% higher than the CD4 cell count of greater than or equal to 500 of 12.6%, as shown in Table 2.

Comparison of Socio-demographic characteristics of HIV positive individuals with malaria

The age group of 26–45 years was recorded with the highest percentage of malaria infection (10 (32.2%)). The age group, place of residence, and level of education showed no significant difference in the prevalence of HIV and malaria co-infection. The female participants (20 (64.5%)) had a higher malaria positive than male counterpart (11 (35.5%)) with a significant difference among the gender ($\chi^2 = 6.864$; df = 1; $P < 0.0088$) as shown in Table 2. The occurrence of malaria infection was higher among the students while the unemployed had the least malaria positive with a significant difference between occupation and malaria ($\chi^2 = 29.29$; df = 4; $P < 0.0001$). A total of 125 (71.8%) were observed to be under ART treatment while the remaining were not on ART treatment. Nineteen (61.3%) had anaemia, while twelve (38.7%) were non-anaemic. However,

the difference was not statistically significant ($\chi^2 = 1.636$; df = 2; $P < 0.4413$) [Table 3].

Table 1: Socio-demographic characteristics of the respondents

Variable	Frequency	Percentage (%)
Age group (years)		
1-5	40	23.0
6-12	31	17.8
13-18	20	11.5
19-25	24	13.8
26-45	40	23.0
>45	19	10.9
Sex		
Male	67	38.5
Female	107	61.5
Marital Status		
Single	71	40.8
Married	85	48.9
Divorced/Widowed	18	10.4
Place of residence		
Rural	99	56.9
Urban	75	43.1
Level of Education		
No formal Education	22	12.6
Primary Education	56	32.2
Secondary education	54	31.0
Tertiary	42	24.1
Occupation		
Civil Servant	23	13.3
Business	29	16.7
Artisan	59	33.9
Unemployed	15	8.6
Student	48	27.6

Table 2: Frequency of all variables among the respondents

Variable	Frequency	Percentage (%)
Anemic Status		
Anaemic	67	38.5
Non-anaemic	107	61.5
Malaria		
Positive	31	17.8
Negative	143	85.1
Use of Insecticides treated Nets		
Yes	141	81.0
No	33	19.0
Use of Insecticides Spray		
Yes	105	60.3
No	69	39.7
CD4 T cell count (cells/μl)		
<200	54	31.0
200-300	48	27.6
301-499	50	28.7
≥500	22	12.6
On prophylaxis Cotrimoxazole		
Yes	132	75.9
No	42	24.1

In this study, the percentage of HIV and malaria co-infection was 31 (17.8%) in a total of 174 detected by blood smear microscopy, a gold-standard method [Figure 1].

Effect of usage of preventive measures on the spread of malaria parasite

The use of insecticide-treated nets was significant with malaria among HIV-infected participants ($\chi^2 = 4.054$, $P < 0.044$) as shown in Table 4. Participants that adhere to good environmental sanitation were further observed to be significant as a preventive measure against malaria parasite among the HIV-infected individuals ($\chi^2 = 6.881$, $P < 0.046$).

Clinical features, CD4 and PCV of respondents with malaria status

The body temperature was significantly higher in HIV and malaria co-infected participants compared to HIV mono-infected counterparts. The CD4 T-cell count was observed to be significantly lower in the HIV individuals diagnosed to be positive for malaria. Likewise, packed cell volume was significantly lower in HIV-positive participants with malaria infection as shown in [Tables 4 and 5].

Discussion

HIV and malaria co-infection may occur simultaneously in individuals and constitute two of the most devastating global health problems in sub-Sahara Africa, including Nigeria. This study was designed as a cross-sectional study to determine the occurrence and clinical features of HIV and malaria co-infected patients attending clinics in the study population. Of the species of *Plasmodium* that were tested, only *P. falciparum* was found to be present in this study.

The burden of malaria in HIV-positive participants in this study was 17.8% of the total study cohort, conforming to a similar study conducted in Osogbo by Ojurongbe *et al.*, 2014[9] where a prevalence of 18.5% was recorded. Although the 17.8% is lower,

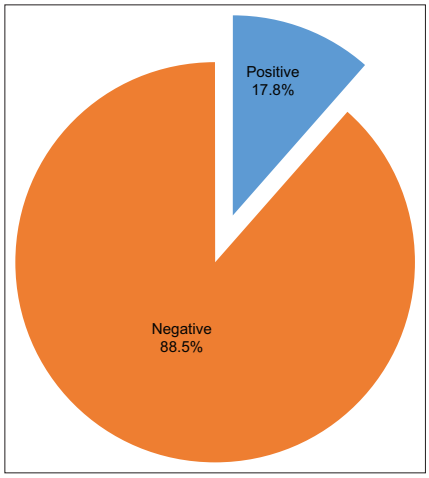

Figure 1: Prevalence of malaria among HIV positive participants

Table 3: Comparison of Socio-demographic characteristics of HIV positive individuals with Malaria

Variables	Malaria Negative	Malaria Positive	Df	χ^2	*P*
Age					
1-5	15 (10.5)	7 (22.6)	5	6.976	0.2224
6-12	12 (8.4)	5 (16.1)			
13-18	10 (6.9)	4 (12.9)			
19-25	17 (11.9)	3 (9.7)			
26-45	39 (27.2)	10 (32.2)			
>45	50 (35.0)	2 (6.5)			
Sex					
Male	52 (36.4)	11 (35.5)	1	6.864	0.0088*
Female	91 (63.6)	20 (64.5)			
Place of Residence					
Rural	72 (50.4)	19 (61.3)	1	3.155	0.0757
Urban	71 (49.7)	12 (38.7)			
Level of Education					
No Education	27 (18.9)	6 (19.4)	3	6.748	0.0804
Primary	51 (35.7)	7 (22.6)			
Secondary	45 (31.5)	7 (22.0)			
Tertiary	20 (14.0)	11 (35.5)			
Occupation					
Civil Servant	23 (16.1)	4 (12.9)	4	29.29	0.0001*
Business	20 (14.0)	6 (19.3)	1		
Artisans	34 (23.8)	7 (22.6)	2		
Unemployed	19 (13.3)	3 (9.7)			
Students	47 (32.9)	11 (35.8)			
Use of ART					
Yes	116 (66.7)	9 (29.0)		0.4176	0.5181
No	27 (15.5)	22 (71.0)			
Anemic Status					
Anemic	48 (33.6)	19 (61.3)		2.116	0.3413
Non-anemic	95 (66.4)	12 (38.7)			

*=Significant value

Table 4: Association between preventive measures and malaria among HIV positive participants

Preventive measures	Malaria +ve	Malaria -ve	df	χ^2	*P*
Insecticide Treated Nets (ITNs)					
Yes	8 (25.8)	97 (67.8)	2	4.054	0.044*
No	23 (74.2)	46 (32.2)			
Insecticide Spray					
Yes	10 (32.3)	131 (91.6)			
No	21 (67.7)	41 (8.4)	2	5.240	0.014
Window/door nets					
Yes	27 (87.1)	123 (86.0)			
No	4 (12.9)	20 (14.0)	2	1.240	0.265
Mosquito repellent Creams.					
Yes	26 (83.9)	103 (72.0)			
No	5 (16.1)	40 (28.0)	2	1.283	0.319
Environmental Sanitation					
Yes	6 (19.4)	85 (59.4)			
No	25 (80.7)	58 (40.5)	2	6.881	0.046*

it is indicative that the transmission rate of malaria is gradually declining, which could be due to religious preventive adherence by the population and government policy programmes initiated in the control and elimination of malaria, such as campaign on a prompt diagnosis of malaria with the use of rapid diagnostic test (RDTs), the use of Artemisinin- combination therapy according to WHO recommendation,[30,31] alertness on the regular use of long-lasting insecticides nets and free supply of ART and CTX recommended for HIV-infected patients in Africa which have proffered protection as well as improving health management of HIV/AIDs infected individuals.[30-32]

In comparison with studies conducted in other region of the country, 17.8% from this study is higher than 14.2% and 14% reported among HIV patients in Uyo[33]; 16.2% in North Central Nigeria[34]; and lower than 22.9% malaria co-infection prevalence by Gumel *et al.*[35]; 24% in Jos[36]; 56.8% in Keffi[37]; 59.2% in Kaduna.[38] Other studies around the world showed as 14% among HIV seropositive patients in Cameroon[30]; 36% in South Africa[39] and 61.7% in Mozambique.[40]

Among the HIV-positive people with malaria, it was observed that sex and occupation influenced the prevalence of malaria in this study. HIV-positive females with malaria co-infection were higher than their male counterparts. This is similar to the report of Tay *et al.*,[16] who also described a higher level of parasitemia in females in contrast to their male counterparts; Bello and Ishaleku,[41] from Keffi; and Amadi *et al.*[33] in Uyo, Nigeria. This marked difference may be due to the female engaging in household chores while staying outside during the mosquitoes' active biting hours. On the contrary, Akinbo *et al.*[42] reported a statistically significant higher prevalence in males than in females. A study in Nasarawa showed that male participants showed a higher prevalence than females, reported by Dikwa *et al.*[43] and Okokon *et al.*[44] A study conducted in Kano reported an undifferentiated pattern and frequency between both sexes.[8] The highest type of occupation in which co-infection of malaria and HIV occurred was observed among students. The parasite caused by the occupation observed among the students could be due to poor environmental sanitation and their careless attitude in following preventive measures against *Plasmodium* infection.

The participants using anti-retroviral drugs (ART) displayed low parasitemia (29.0%) than non-ART observers (71.0%). This is in line with a study conducted by Gennano *et al.*,[45] and Sandie *et al.*,[30] which reported a higher prevalence of malaria in HIV-positive individuals who are not on ART than those on ART. This could be as a result of the reformation of their immune system connected with the drugs given to them, signifying the effectiveness of ART as well as the administration of cotrimoxazole as the component of their chemotherapy, which is known to have certain anti-malarial elements, thus lowering the occurrence of malaria in HIV-infected individuals. This is coherent with findings report on the protective capacity of CTX with other preventive measures among people living with HIV.[36]

Finding from the study revealed that a greater number of participants using insecticide-treated nets are over and above other preventive measures practiced. This could be attributed to a religious adherence to the use of preventive measures, which

Table 5: Physiological feature, CD4, and PCV of respondents with malaria status

Variables	Malaria -ve/ HIV pos. Mean±SD	Malaria +ve/ HIV pos. Mean±SD	*t*	*P*
Weight (kg)	53.14±1.87	52.42±0.05	1.006	0.315
Temp. (0°C)	37.14±0.06	39.21±0.11	15.14	<0.0001*
CD_4 Count	325.98±9.24	203.09±6.30	7.512	<0.0001*
PCV (%)	34.32±0.21	29.26±0.30	14.92	<0.0001*

serves as an obstacle to mosquitoes and so prevents the spread of the parasites. This result is in line with the finding in Kenya[46] that reported that the use of ITNs greatly reduces the chance of mosquito contact and malaria transmission. Among HIV-infected participants, those who practice more than one preventive measure against malaria stand a chance of absolute protection, which helps in combating malaria transmission and infection.

Good environmental sanitation was statistically significant ($P = 0.009$) among study participants. Among those who adopted good sanitation practices, the malaria positivity was significantly lower in the HIV-infected participants (19.4%) than those who did not practise it. This result is consistent with a study in Cameroon where a high prevalence of malaria was reported among school children who had bushes around their residential homes.[47] The low parasitemia recorded in this study may be a result of the effort of the government enforcing periodic environmental sanitation, most importantly in urban areas due to heavy overcrowding and dirty surroundings.

The HIV and malaria co-infected participants displayed high body temperature compared to uninfected HIV counterparts. This finding confirms the previous studies done in Jos Nigeria,[44] there was a strong correlation between body temperature and malaria and HIV co-infection. The rise in temperature could have resulted from the tumult in the schizogonic cycles due to heterogeneity of the malaria parasite; hence the immune system is stimulated to secrete TNF-α, IL-1, and other body cytokines, which causes resistance that leads to up-regulation of endothelial cells that generate febrile paroxysm.[48,49]

The low mean value of PCV in malaria-infected HIV individuals was observed in this study. Although there is no scientific evidence that malaria is associated with anaemia, malaria stimulated anaemia could be a result of lysis of red blood cells (RBCs), which accelerates the clearance of parasitized and non-parasitized RBCs,[50,51] as well as complications attached to some anti-retrovirals taken by HIV-infected individuals, which have been documented to cause anaemia in conjunction with the destruction of red blood cells by *Plasmodium* malaria, thus decreasing PCV and hemoglobin concentrations. This is in line with the findings of Bawah *et al.*,[52] who reported higher rates of malaria-inducing anemia among children in Ho municipality. Also, studies conducted in Ghana by Sakzabre *et al.*,[53] and in Nigeria by Osaro *et al.*[54] linked anaemia to malaria parasitism. This contrasts with the findings in Oshogbo, Nigeria, which stated that there is no association between malaria parasites and anaemia.[9]

The mean CD4 of malaria-infected HIV individual was low compared to the uninfected HIV counterpart, which showed a significant difference as similarly observed by Ojurongbe *et al.* and Tay *et al.*[9,16] This could be due to the associated decrease in CD4 among those infected with HIV. However, several studies have separated malaria from a low CD4 count among HIV-infected individuals.[9,55] Although, it is not quite the same as Njunda *et al.*,[56] where malaria was higher in CD4 below 200 cells/μL.

Conclusion

In conclusion, malaria parasites prevalence was found to be high in HIV-positive individuals among the study population. Immune recovery of HIV-positive patients being monitored by the change in CD4 count may be affected by malaria co-infection, especially in a recurrent malaria attack and compliance with ART regimen. Therefore, to achieve better management of HIV-positive patients against malaria infection, primary health care physicians and healthcare providers should harness the use of preventive measures and chemotherapy as a useful strategy to mitigate this public health challenge. Also, early malaria and HIV diagnosis, monitoring of CD4 count, viral load, and haematological indices on a regular basis is crucial. In addition, concerted efforts by primary care physicians should be channeled to completely eradicate HIV and malaria co-infection through public enlightenment and sensitization.

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Conflicts of interest

There are no conflicts of interest.

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