Immunological, Virological, Parasitic and Biological Profile of Malaria/HIV Co-Infection in 18 Years Old and Above Patients in Lubumbashi (DR Congo)

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Abstract

Introduction: Malaria infection and HIV infection are major public health issues in several parts of the world. Together they have caused more than a million deaths per year. Africa, and Sub-Saharan in particular are the most affected. Our study objective is to determine the prevalence of Malaria/HIV co-infection and describe its immunological, virological, parasitic and biological characteristics. Methodology: This is a descriptive, transversal and multi centric study done on 18 years old and above HIV positive patients, for a period extending from December 2008 to October 2009 in 5 different HIV treatment centres. Parameters studied were gender, age, CD4 count, viral load, parasitic density and haemoglobin level. The HIV diagnosis was made according to the AIDS National Program and malaria according to the Malaria National Program. Statistic analyses were done using Epi Info 7 software and the Yates corrected Chi Square test or the Fischer Exact test (when recommended) was used to check any link between different parameters studied. Statistical significance was fixed at <0.05. Results: 405 HIV seropositive patients were compiled. The malaria prevalence in these patients was 6.9%. Majority of co-infected patients were aged between 26 and 49 years (67.9%), and had a CD4 count <200 μl (67.9%), a parasitic density <10,000 trophozoites/μl (75%) and an Hb level <11 g/dl. With regards to correlations between co-infected patients parasitic density and the CD4 count, viral load and Hb level, none of the studied parameters showed any statistic significance difference. Conclusion: A prevalence of 6.9% among HIV/malaria co-infected patients and 67.7% of these patients had a CD4 count less than 200 cells/μl. Thus, both national programs must promote an early testing for HIV infected patients and reinforce preventive measures in the management of malaria.

Keywords
Malaria/HIV Co-Infection, CD4, Viral Load, Parasite Density, Haemoglobin

Subject Areas: HIV, Infectious Diseases

1. Introduction
Malaria and HIV infection are both public health issues in several regions worldwide. Together, they have caused more than a million deaths a year, mainly in Africa, Asia and South America [1]. Africa has the highest number of deaths [2][3] and especially Sub-Saharan Africa [3].

Malaria is caused by the protozoan parasite Plasmodium and it’s transmitted by Anopheles mosquitoes. It is endemic in most tropical and subtropical region of the world. Of the four Plasmodium species that infect human, P. falciparum is the most virulent and is responsible for the majority of morbidity and mortality due to malaria [4].

Malaria is preventable and treatable when recommended interventions are properly implemented. These preventive measures may be most-efficient when combined with systematic screening and treatment of asymptomatic individuals in high transmission settings [5].

Malaria is known for its influence on HIV infection [6]-[10]. A Zambian study has reported that patients who are co-infected have a higher viral load [8] and low CD4 count [11]. Conversely, HIV infection impacts on malaria incidence, severity and malaria drug’s efficacy [12]-[14]. The effect of HIV-1 on malaria seems to be driven mainly by the incapacity of the immune system to control parasite load, leading to a higher prevalence of infection, a higher incidence of clinical malaria, and a risk for treatment failure in immune-suppressed HIV-1 patients [8].

The Centre for Disease Control and Prevention (CDC) since 2009 believes that malaria should be considered as HIV/AIDS opportunistic infection [15].

In the Democratic Republic of Congo, few studies have reported malaria/HIV co-infection. However, in 2015, a study reported a 5.7% malaria/HIV co-infection among pregnant women [16]. And this just demonstrates a partial picture of the scourge in our midst.

So this study’s objective is to determine malaria prevalence among HIV positive patients and describe immune, virological, parasitic and biological (Haemoglobin level) characteristics of this co-infection.

2. Methods
2.1. Type of Study
This is a retrospective, transversal, multi centric study done on HIV positive patients from December 2008 to October 2009 in Lubumbashi (DR Congo) in 5 different HIV centres: Lubumbashi University Clinics, Janson-Sendwe General Hospital, Kenya General Hospital of Reference, SNCC Hospital and AMO CONGO.

2.2. Population
Were included, all HIV positive patients aged 18 years old and above, presenting with 48 hours fever or fever at the time of consultation with a positive thick film for malaria.

Patients with active tuberculosis were excluded.

Study parameters were: gender, age, CD4 count, viral load, haemoglobin level and parasitic density

2.3. Laboratory Analysis
The HIV diagnosis was made according to the HIV/AIDS National Program protocol. A sample of 4 ml of venous blood was collected from the median vein in the elbow in a tube of EDTA anticoagulation for measuring the level of CD4 T-cell and the determination of viral load. The CD4 count was determined by flow cytometry using the BD FACS Count system and the viral load was done by the AMPLICOLOR HIV-1 MONITOR Test, ver-
The malaria diagnosis was made according to the malaria National Program protocol where parasitemia was quantified. Thick and thin blood smears were made on grease-free slides and stained with Giemsa to determine species of malaria parasites and parasite density. Parasite densities were estimated by counting the number of Plasmodium malaria parasites (parasite count) per 200 leukocytes per high power field (number of parasites/µl of blood). All stained slides were examined by microscopy using 100 power fields under oil immersion.

Haemoglobin level was determined using the Crosby method with Drabkin reagent (cyanmethemoglobin method).

2.4. Encoding and Statics Analysis

Data were captured using Excel 2010 and Epi Info 7. Statistically we have used Means, standard deviation, corrected chi square test of Yates or Fischer Exact test when needed in order to link different parameters studied. A p value < 0.05 was considered statically significant.

2.5. Ethics Considerations

The study was approved by the Ethical Committees of Lubumbashi’s University.

For ethics and code of practice reasons and trying to avoid stigma on our HIV centres, we didn’t produce results per centre and data were collected in such a manner that patients remained anonymous after obtaining their consent.

3. Results

For this study we have collected data for 405 HIV positive patients and the malaria prevalence in this group was 28 out of 377 patients (6.9%) (Figure 1).

Table 1 shows that from 405 HIV seropositive patients captured 71.6% are female, it also shows that majority of co-infected patients are aged between 26 and 49 years (67.9%), the CD4 count <200 µl (67.9%), a viral load ≥100,000 species/l (46.4%) and an Hb level <11 g/dl (57.1%).

Table 2 presents correlations between HIV and malaria co-infected patient’s parasitic density with CD4 count, their viral load and the hemoglobin. None of the tested parameters has a statistics significance difference.

4. Discussion

Malaria and HIV both have a high prevalence in the Sub-Saharan Africa; and HIV seropositive patients are more
susceptible to plasmodium infection [17]. In our study, 405 HIV positive patients were captured from which 71.4% are female giving a female to male ratio of 2.52. The 25 to 45 years old age range is the most affected. This finding corroborates findings from the second DR Congo Demographic and Health Survey [18] done in 2013 where the F/M ratio was 2.7 and the 25 - 45 years old age range being the most affected. The advent of protection of mother to child transmission (PMCT) can explain the high ratio in favour of women, and they easily adhere to the voluntary testing compared to men. With regard to the age, all different age ranges are noted but our results could be explained by the fact that the most affected age range are people who are almost permanently sexually active.

In our series the prevalence of malaria is 6.9%. Our results seem to be very low compared to the Tay and Fo studies where prevalence were respectively 11.75% and 11.8% [19] [20]. However compared to Adu-Gyasi in Ghana (4.4%) our prevalence is high [21]. Difference in timing of study would explained difference in results obtained in Tay and Fo due to the fact that these studies were conducted in the period of the year where malaria endemicity is high [22]. The difference with the Adu-Gyasi study can be explained by the fact that 86.7% of HIV/malaria co-infected patients in their series were on antiretroviral treatment and cotrimoxazole prophylaxis.

### Table 1. Seropositive patients socioeconomic, immunologic, virological, parasitic and biological characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Total n = 405 (%)</th>
<th>Malaria (+) n = 28 (%)</th>
<th>Malaria (-) n = 377 (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>290 (71.6)</td>
<td>21 (75)</td>
<td>269 (71.4)</td>
<td>0.8448</td>
</tr>
<tr>
<td>M</td>
<td>115 (28.4)</td>
<td>7 (25)</td>
<td>108 (28.6)</td>
<td></td>
</tr>
<tr>
<td><strong>Age, years</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>10 (2.5)</td>
<td>0 (0)</td>
<td>10 (2.7)</td>
<td></td>
</tr>
<tr>
<td>26 - 45</td>
<td>297 (73.3)</td>
<td>19 (67.9)</td>
<td>278 (73.7)</td>
<td>0.4383</td>
</tr>
<tr>
<td>46 - 45</td>
<td>91 (22.5)</td>
<td>9 (32.1)</td>
<td>82 (21.8)</td>
<td></td>
</tr>
<tr>
<td>&gt;60</td>
<td>7 (1.7)</td>
<td>0 (0)</td>
<td>7 (1.8)</td>
<td></td>
</tr>
<tr>
<td>Means</td>
<td>40.9 ± 9.8</td>
<td>39.3 ± 9.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CD4, µl</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>242 (59.8)</td>
<td>19 (67.9)</td>
<td>223 (59.2)</td>
<td></td>
</tr>
<tr>
<td>200 - 349</td>
<td>148 (36.5)</td>
<td>9 (32.1)</td>
<td>139 (36.9)</td>
<td>0.6544</td>
</tr>
<tr>
<td>≥350</td>
<td>15 (3.7)</td>
<td>0 (0)</td>
<td>15 (3.9)</td>
<td></td>
</tr>
<tr>
<td>Means</td>
<td>166 ± 85.3</td>
<td>171.7 ± 110.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Viral load, species/ml</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 - 999</td>
<td>8 (2.0)</td>
<td>2 (7.1)</td>
<td>6 (1.6)</td>
<td></td>
</tr>
<tr>
<td>1000 - 9999</td>
<td>42 (10.4)</td>
<td>5 (17.9)</td>
<td>37 (9.8)</td>
<td>0.7152</td>
</tr>
<tr>
<td>10,000 - 99,999</td>
<td>125 (30.9)</td>
<td>8 (28.6)</td>
<td>117 (31.0)</td>
<td></td>
</tr>
<tr>
<td>≥100,000</td>
<td>230 (56.7)</td>
<td>13 (46.4)</td>
<td>217 (57.6)</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>114,883.5 (809 - 4,467,610)</td>
<td>151,232 (220 - 218,840,430)</td>
<td></td>
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</tr>
<tr>
<td><strong>Hb, g/dl</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;11</td>
<td>218 (53.8)</td>
<td>16 (57.1)</td>
<td>202 (53.6)</td>
<td>0.8663</td>
</tr>
<tr>
<td>≥11</td>
<td>187 (46.2)</td>
<td>12 (42.9)</td>
<td>175 (46.4)</td>
<td></td>
</tr>
<tr>
<td>Means</td>
<td>10.5 ± 1.7</td>
<td>10.7 ± 2.0</td>
<td></td>
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</tr>
</tbody>
</table>
Table 2. Correlation between parasitic density and CD4 count, viral load and hemoglobin in patients infected with Malaria/HIV coinfection.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Frottis &gt;10,000 Trophozoites/µL n = 21 (%)</th>
<th>Frottis ≤10,000 Trophozoites/µL n = 7 (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4, µl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>19 (67.9)</td>
<td>13 (61.9)</td>
<td>6 (85.7)</td>
<td>0.2487</td>
</tr>
<tr>
<td>≥200</td>
<td>9 (32.1)</td>
<td>8 (38.1)</td>
<td>1 (14.3)</td>
<td></td>
</tr>
<tr>
<td>Viral load, species/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10,000</td>
<td>5 (17.9)</td>
<td>4 (19.0)</td>
<td>1 (14.3)</td>
<td>0.6333</td>
</tr>
<tr>
<td>≥10,000</td>
<td>23 (82.1)</td>
<td>17 (81.0)</td>
<td>6 (85.7)</td>
<td></td>
</tr>
<tr>
<td>Hb, g/dl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;11</td>
<td>16 (57.1)</td>
<td>13 (61.9)</td>
<td>3 (42.9)</td>
<td>0.3275</td>
</tr>
<tr>
<td>≥11</td>
<td>12 (42.9)</td>
<td>8 (38.1)</td>
<td>4 (57.1)</td>
<td></td>
</tr>
</tbody>
</table>

And Merminn, in his study done in Uganda, maintains that cotrimoxazole prophylaxis have a protective effect on malaria [22].

Majority of patients (67.9%) had a CD4 count below 200 cells/µl and the Means CD4 count among co-infected patients was 166 cells/µl ±85.3. Tay and Browne have reported same findings and consider the major reason of these results is simply that patients present themselves very late for consultations. Also a CD4 count below 200 cells/µl remains a high risk for opportunistic infections [19] [23]. Fear of stigma, lake of awareness and inadequate counseling on the part of our staffs are risk factors in favour of late consultations of our seropositive patients. The viral load was superior and equal to 100,000 copies/ml 46.4% of our co-infected patients. This somewhat confirms the poor immune state of most in our hospital institutions patients.

57.1% of co-infected patients had anaemia thus confirming several other literatures [19] [24]-[26]. Anaemia remains the principle malaria marker. But it should be remembered that its aetiology is multi factorial and could be malnutrition, iron deficiency, vitamins A, C and B12 deficiencies, falciparum anaemia, thalassemia, HIV or other parasites like ascaris or schistosom [27].

With regards to the parasite density, 75% had a parasite load superior to 10,000 trophozoites/µL in our series while Sanyaolu in his study in Nigeria noticed that all his co-infected patients were in this category [24] and the Means parasite density (5606.67 ± 6329.9) in our series seemed superior compared to Rutto’s results in Kenya [28]. However, in our study no significant correlation between parasite density and CD4 count in co-infected patients, viral load and haemoglobin level couldn’t be demonstrated. This would give the impression that the virus effect on malaria never exist as Bloland reported in a study done in Malawi [29]. But studies done in area where HIV and malaria are highly endemic found a very high viral load [30] and a very low CD4 count in connected patients [31].

5. Conclusion

In our series, a prevalence of 6.9% was obtained for HIV/malaria co-infection with 67.9% of patients having a CD4 count below 200 cell/µl and 46.4% having a viral load above 100,000 copies/ml. This sufficiently shows that early detection of HIV patients remains an activity to improve in our environment and preventive measures against malaria, in particular, the use of insecticide-treated nets should be encouraged in highly endemic areas, as DR Congo, not only for children below 5 years old and pregnant women but also people living with HIV.

Author’s Contributions

KC, MO, MC and LO conceived and designed the study. KC, KE and MC conducted and collected data. KC, MO, MM, MA and MP contribute to data analysis, interpretation and manuscript review. KC, MM, MA and TB wrote the manuscript.
Conflict of Interests
The authors declare they have no competing interests.

References


**Abbreviations**

HIV: Human Immunodeficiency Virus  
CD4: Cluster Differentiation Antigen 4  
AIDS: Acquired Immune Deficiency Syndrome  
Hb: Haemoglobin