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Article

Dynamics of Liver Function among Patients with Malaria in Luanda, Angola: a Cross-Sectional Study

Running title: Liver function among malaria patients in Angola

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Abstract: Background: Malaria continues a concern in Angola. Liver dysfunction is a relevant feature of malaria. There is no published study exploring liver function dynamics during *P. falciparum* infection in Angola. **Objectives:** We investigated the dynamics of liver function among patients with malaria in Luanda, Angola. **Methods:** This was a cross-sectional study performed with 199 malaria patients, from March-August 2023. **Results:** About 71% of the patients had low parasitemia, moderate disease(54%), and used Artesunate(39.7%). Decrease in ALT(68.7 to 50.7) was observed from non-severe to severe with increases in AST(56.6 to 134, $p<0.05$), GGT(51.2 to 99.0), and Bilirubin(0.72 to 1029). We observed higher ALT values in patients using Artimeter, while higher AST and GGT in patients using Artesunate. Higher Bilirubin was observed in patients treated with Coartem. Differences in AST values depending on the antimalarial used were significant($p<0.05$). AB blood group presented high AST and GGT values, group B presented high ALT while high Bilirubin was observed in group O. Differences in GGT depending on the blood groups were significant($p<0.05$). Elevated ALT, AST, GGT, and Bilirubin were observed in Rh-positive. **Conclusions:** Several malarian patients could present high AST, ALT, GGT, and Bilirubin values indicating putative liver failure during malaria treatment in Angola.

Keywords: malaria disease; liver function; epidemiology; biological factors; angola

Introduction

Malaria remains a major public health concern in several tropical countries in Sub-Saharan African countries [1,2]. In the last twenty years, Angola has witnessed significant growth in malaria interventions, accompanied by cross-border initiatives and regional endeavours across Southern Africa [3]. According to the National Director of Public Health, in Angola, malaria continues to be a serious public health problem and is the leading cause of illness and death. In 2022, around 9,211,346 cases of malaria were reported, which constituted an increase of 0.4% compared to 2021, on the other hand, there was a 10% reduction in the number of deaths from malaria, compared to the previous year [1]. Angola has been implementing robust strategies aligned with the National Malaria Control Program with the ambitious objective of eliminating malaria by 2030, however complex factors threaten the sustainability of this progress, including environmental diversity, transmission

intensities, and mosquito vectors, as well as the internal displacement of the population and the emergence of resistance to drug and insecticide therapies are some of the challenges faced in Angola. Added to this is the emergence of new epidemiological frameworks that end up making it difficult to define priorities thus, continuous support is needed in the form of traditional malaria control methods, as well as support to implement new techniques to accelerate the decreasing trends in malaria burden [3].

Plasmodium infection is normally multiorgan and affects many organs [4]. The liver is the main organ affected during Plasmodium infection since part of the parasitic cycle takes place in this organ, and this organ plays a large role in the normal functioning of organisms among them the uptake, metabolism, phagocytosis and elimination of microorganisms and endotoxins [5–7]. Also is responsible for generating effective immunoreactivity against microorganisms and generating tolerance to avoid immunoreactivity with “proper” and harmless substances such as dietary compounds [8,9]. In malaria infection, the liver plays a protective role against blood stages of plasmodium, since it is more critical than the spleen in mediating the suppressive effects of testosterone on malaria resistance, which results in differences between men and women in terms of severity of malaria [10].

Previous studies concluded that patients with *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale* infections had elevated serum alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transferase (Gamma GT), bilirubin, and hypoalbuminemia [11]–[13]. Despite Angola's malaria-endemic status, there has been no published research exploring the dynamics of liver function in the adult population during Plasmodium falciparum infection. In this study, we investigated the dynamics of liver function among patients with malaria in Luanda, the capital city of Angola.

Methods

Study design and setting

A cross-sectional study was performed with 199 patients hospitalized due to malaria in Josina Machel, a tertiary Hospital, from March to August 2023. The patients were invited and freely consented to participate in the study. The study was approved by the scientific council of the Institute of Health Sciences of Agostinho Neto University (118/GD/ICISA/UAN/2021) and by the clinical management of Josina Machel Hospital (36/DPC/HJM/2023).

Sample collection and laboratory testing

Malaria diagnoses were performed by Josina Machel Hospital professionals using rapid malaria antigen tests (SD-Bioline Malaria AG Pf/PAN) and confirmed with the microscopy technique of direct visualization of the parasite by Giemsa-stained peripheral blood thickening. Patients who presented parasitemia less than 50p/mm³ were classified as low parasitemia level, between 50 - 1000 p/mm³ as moderate level and patients who presented parasitemia above 1000 p/mm³ were classified as high parasitemia. An estimated volume of 3 mL of whole blood was collected in a tube containing EDTA. Biochemical screening was performed with the automatic biochemical analyzer Cobas C111 (Roche), using the AST (Roche, Germany), ALT (Roche, Germany), Gama GT (Roche, Germany), and Bilirubin (Roche, Germany) detection kits, for evaluation of possible liver damage resulting from malaria disease. All laboratory processing as well as the interpretation of the results were carried out according to the manufacturer's instructions. ABO/RH blood group phenotypes were characterized (Lorne Laboratories Limited, UK), following the manufacturer's instructions.

Statistical analysis

The data obtained in this study were analyzed using SPSS v29 (IBM SPSS Statistics, USA). Absolute and relative frequencies were presented as descriptive analysis. The chi-square (X²) test was used to assess the relationship between categorical variables. In addition, independent samples t-test

and One-Way ANOVA were calculated for samples with normal data distribution. All reported p-values are two-tailed and deemed significant when $p < 0.05$.

Results

Demographic profile and liver function

The demographic profile and liver function among patients with malaria are presented in Table 1. The predominant demographic characteristics of the 199 participants enrolled in the study were age group between 20–30 years old (45.2%, 90/199), female gender (61.8%, 123/199), patients from urbanized areas (53.8%, 107/199), average education level (43.2%, 86/199) and employed (75.4%, 150/199). Overall, mean liver function indices were as follows, ALT (47.0 ± 120), AST (83.1 ± 115), Gamma GT (72.6 ± 124), and Bilirubin (233 ± 3230). Although no statistical significance was observed, higher mean levels of ALT (62.3 ± 170) and AST (94.2 ± 113) were observed in patients aged over 40 years, while higher Gamma GT and Bilirubin levels were observed in patients aged between 20 and 30 years. Likewise, all mean liver function values, such as ALT (47.0 ± 120), AST (85.8 ± 95.7), Gama GT (75.6 ± 133), and Bilirubin (440 ± 4444), were elevated in residents from urbanized regions. The distribution of ALT means according to education level was statistically significant ($p = 0.038$). Unliterate patients had higher AST (87.9 ± 92.3) and lower bilirubin (0.32 ± 0.33) mean values, while patients with high educational levels had higher values for AST (132 ± 318) or gamma GT (89.2 ± 52.5) and lower values for AST (64.3 ± 49.3). Patients who reported some type of work categorized as employees, were those with the highest average values for ALT (52.0 ± 136), AST (85.7 ± 124), and Bilirubin (309 ± 3720), while the unemployed presented higher average values for gamma GT Range (104 ± 187).

Biological factors related to liver function

The predominant clinical data in the studied population were as follows, about 54% (108/199) of the patients had moderate disease, and 39% used Artesunato (39.7%, 79/199) or Coarten (38.7%, 77/199), respectively. Blood group O (46%, 91/199) and positive Rh (96%, 190/199) factors were predominant. About 71% (141/199) of the studied population had low parasitemia. Biological factors related to liver function profile among patients with malaria are presented in Table 2. A decrease in mean ALT (68.7 to 50.7) values was observed from non-severe to severe patients while we observed increases in AST (56.6 to 134), gamma GT (51.2 to 99.0), and Bilirubin (0.72 to 1029) from non-severe to severe patients. The increase in mean AST values from non-severe to severe patients was statistically significant ($p = 0.002$). Regarding the effects of antimalarials on liver function, we observed higher mean ALT values in patients who used Artimeter (85.2 ± 240), while higher mean values of AST (111 ± 145) and Gamma GT (96.3 ± 165) were observed in patients treated with Artesunate. Higher mean Bilirubin (603 ± 5209) values have been observed in patients treated with Coarten. Differences in mean AST values depending on the antimalarial used were statistically significant ($p = 0.022$). Despite the low frequency, group AB presented high mean AST and gamma GT values. High ALT values were observed in group B, while high Bilirubin values were observed in group O. The differences in the mean values of gamma GT depending on the blood groups were statistically significant ($p = 0.006$). Although no statistical significance was observed ($p > 0.05$), all elevated values of ALT (48.5 ± 122), AST (85.9 ± 117), gamma GT (74.9 ± 126) and Bilirubin (245 ± 3307) were observed in patients with positive RH factor.

Discussion

Malaria continues to be a major health problem in tropical countries [1,2]. Liver dysfunction has long been known as a major clinical feature of patients with severe malaria [6,13,14]. To the best of our knowledge, this is one of the first studies that conduct a laboratory evaluation of changes in liver biochemical indices among patients infected with *P. falciparum* and treated with artemisinin derivatives in patients with different levels of malaria disease severity in Luanda, the capital city of Angola. Abnormalities in liver function values have been observed very frequently, especially in

regions endemic to *P. falciparum* [5,13]. Also, a previous study concluded that patients with *P. vivax*, *P. malariae* and *P. ovale* infections had slightly elevated serum bilirubin, aminotransferase and alkaline phosphatase levels, and hypoalbuminemia, however, these abnormalities returned to normal within a few weeks after treatment with therapies based on artemisinin derivatives [11]. When we examine indices of liver function of the present studied population, we observed certain variations in the overall mean levels of ALT, AST, Gamma GT and bilirubin that might represent clinical importance for the management of patients with malaria in Angola. Specifically, elevated levels of ALT and AST were observed among older patients, while higher levels of gamma GT and bilirubin manifest among individuals aged 20 to 30 years. Furthermore, residents of urbanized regions have elevated levels of all studied markers of liver function, accentuating the potential influence of demographic factors on the liver health of patients with malaria. It is also worth mentioning that the influence of educational level and professional status on liver function parameters reveals interesting associations. Patients with lower levels of education have elevated levels of AST, while those with higher levels of education have elevated levels of AST and Gamma GT. Similarly, employed individuals have elevated levels of ALT, AST and bilirubin, highlighting the potential impact of occupational factors on liver function (Table 1).

Regarding biological factors, clinical data reveal significant relationships between AST and Gamma GT levels with disease severity/use of antimalarial agents and different blood groups ($p < 0.05$). The increase in parasite load was not related to abnormalities in liver function values, which is similar to what was observed in previous studies that revealed that liver function abnormalities were not related to the grade of parasitaemia, fever, duration of the illness, nutritional status of the patient or associated medical problems [15]. Similar to other studies, we also identified a relatively large proportion of cases with elevated transaminases after treatment with Artimeter, Artesunate or Coartem in patients with naturally acquired malaria [5]. High levels of liver function indices were observed in the studied population, even during treatment, which could represent the hepatic effects associated with malaria after hemolysis (Table 2). Blood group O and Rh-positive are dominant and are in line with previous studies carried out in Angola, which show that these are prevalent in the Angolan population [16,17]. The influence of blood groups on liver function disorders highlights the complex interplay between genetic predispositions and liver health, with statistical significance observed in GT gamma levels across ABO blood groups. Furthermore, our findings reveal elevated liver function markers among Rh-positive individuals, although without statistical significance.

Table 1. Demographic profile and liver function among patients with malaria in Luanda, Angola.

Independent variables	N (%)	ALT (Mean ± SD)	P-value	AST (Mean ± SD)	P-value	Gama GT (Mean ± SD)	P-value	Bilirubin (Mean ± SD)	P-value
Overall	199 (100)	47.0 ± 120		83.1 ± 115		72.6 ± 124		233 ± 3230	
Age group									
<20 yo	46 (23.1)	40.8 ± 62.8	0.837	90.7 ± 94.6	0.839	48.3 ± 52.3	0.444	2.46 ± 5.14	0.752
20-30 yo	90 (45.2)	47.4 ± 135		76.6 ± 138		83.7 ± 167		515 ± 4808	
31-40 yo	29 (14.6)	37.6 ± 37.7		78.1 ± 61.1		82.1 ± 95.6		2.22 ± 4.34	
>40 yo	34 (17.19)	62.3 ± 170		94.2 ± 113		67.4 ± 62.2		0.89 ± 1.83	
Gender									
Female	123 (61.8)	43.1 ± 121	0.556	71.2 ± 84.2	0.105	70.0 ± 136	0.706	1.66 ± 3.85	0.320
Male	76 (38.2)	53.5 ± 119		103 ± 152		76.9 ± 100		612 ± 5243	
Residence area									
Non-urbanized	19 (9.50)	38.2 ± 41.0	0.540	66.4 ± 53.5	0.797	50.3 ± 41.9	0.711	3.24 ± 5.47	0.642
Periurban	73 (36.7)	36.5 ± 53.1		83.5 ± 149		74.1 ± 125		1.66 ± 4.17	
Urban	107 (53.8)	47.0 ± 120		85.8 ± 95.7		75.6 ± 133		440 ± 4444	
Educational level									
Unliterate	26 (13.1)	35.8 ± 37.7	0.038	87.9 ± 92.3	0.929	81.5 ± 176	0.916	0.32 ± 0.33	0.630
Basic	73 (36.7)	49.7 ± 126		85.7 ± 101		67.5 ± 82.1		637 ± 5353	
Mediun	86 (43.2)	34.1 ± 49.4		82.5 ± 140		71.5 ± 143		2.52 ± 5.08	
High	14 (7.00)	132 ± 318		64.3 ± 49.3		89.2 ± 52.5		2.21 ± 4.16	
Occupation									
Unemployed	49 (24.6)	32.0 ± 32.5	0.310	75.3 ± 85.7	0.585	104 ± 187	0.135	1.49 ± 3.69	0.568
Employed	150 (75.4)	52.0 ± 136		85.7 ± 124		62.2 ± 92.8		309 ± 3720	

Note: Bold number means that results were statistically significant for independent samples t-test and/or One-Way ANOVA (p<0.05).

Table 2. Biological factors related to liver function profile among patients with malaria in Luanda, Angola.

Clinical characteristics	N (%)	ALT (Mean ± SD)	P-value	AST (Mean ± SD)	P-value	Gamma GT (Mean ± SD)	P-value	Bilirubin (Mean ± SD)	P-value
Overall	199 (100)	47.0 ± 120		83.1 ± 115		72.6 ± 124		233 ± 3230	
Parasitaemia level, p/mm ³									
Low (<50)	141 (70.9)	53.8 ± 141	0.449	86.4 ± 132	0.793	79.5 ± 142	0.463	1.86 ± 4.29	0.085
Moderate (50 – 1000)	22 (11.1)	26.9 ± 18.2		69.8 ± 55.1		58.9 ± 59.5		1369 ± 7852	
High (>1000)	36 (18.1)	32.3 ± 21.8		78.2 ± 57.0		53.4 ± 48.6		233 ± 3230	
Clinal status									
Non-severe	46 (23.1)	68.7 ± 189	0.309	56.6 ± 56.6	0.002	51.2 ± 49.6	0.180	0.72 ± 2.00	0.179

Moderate	108 (54.3)	36.5 ± 98.1		72.9 ± 89.7		70.5 ± 134		1.78 ± 3.66	
Severe	45 (22.6)	50.7 ± 64.6		134 ± 182		99.0 ± 146		1029 ± 6800	
Antimalarial therapy									
Artimeter	43 (21.6)	85.2 ± 240	0.060	71.1 ± 110	0.022	63.4 ± 113	0.087	1.91 ± 4.59	0.453
Artesunate	79 (39.7)	36.4 ± 35.6		111 ± 145		96.3 ± 165		3.04 ± 5.48	
Coarten	77 (38.7)	36.6 ± 53.5		61.7 ± 71.5		53.8 ± 63.1		603 ± 5209	
ABO blood groups									
A	53 (26.6)	41.3 ± 62.5	0.918	71.5 ± 72.7	0.176	67.3 ± 75.3	0.006	1.60 ± 3.74	0.752
B	42 (21.1)	58.1 ± 186		72.9 ± 50.2		59.0 ± 68.9		2.80 ± 5.24	
AB	13 (6.50)	42.7 ± 31.6		147 ± 148		188 ± 329		1.89 ± 3.85	
O	91 (45.7)	45.8 ± 114		85.3 ± 146		65.5 ± 108		515 ± 4808	
RH blood group									
RH-	9 (4.50)	15.4 ± 5.87	0.418	25.1 ± 6.20	0.061	25.2 ± 6.48	0.241	0.21 ± 0.06	0.825
RH+	190 (95.5)	48.5 ± 122		85.9 ± 117		74.9 ± 126		245 ± 3307	

Note: Bold number means that results were statistically significant for independent samples t-test and/or One-Way ANOVA ($p < 0.05$).

This study has potential limitations that should be considered. Studies have documented that abnormal liver function profiles in malaria patients return to normal within a few weeks of treatment [7], however, this was not observed in this study due to the cross-sectional nature of the study. However, future prospective studies should be carried out in Angola, to evaluate the liver function of individuals two or more weeks after infection with *P. falciparum*, or another species treated with artemisinin derivatives. Even so, our findings present the patterns of variations in liver function indices in patients with *P. falciparum* treated with different antimalarials, whether Artimeter, Artesunate or Coarten in Angola. Further investigation may clarify other contributing either demographic or biological factors to abnormalities in liver profiles among patients with malaria in Angola.

Conclusion

In summary, this study elucidates the role of demographic and biological determinants on liver function in patients with malaria, highlighting the multifactorial determinants that shape liver health in this vulnerable population. Our findings not only improve our understanding of malaria-associated histopathology but also highlight the need for personalized interventions that address demographic and biological determinants to mitigate liver morbidity in malaria-endemic regions. Further studies may clarify other contributing demographic or biological factors to abnormal liver profiles among patients with malaria in Angola.

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