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Coombs' Direct Antiglobulin Test Among Individuals Co-Infected with Malaria and HIV in Iwo Community, Southwestern Nigeria

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Abstract: In the present study, direct antiglobulin test (DAT) was performed using poly-antiglobulin serum on a total of 138 individuals; 20 of whom had HIV infection only, 50 malaria only, 18 both malaria and HIV and 50 non-infected with either malaria or HIV in order to determine the effect of co-infection with malaria and HIV on DAT status. Malaria parasite test was done by microscopic examination of thick and thin blood films stained with 3% Giemsa. Antibodies to HIV were determined using Capillus rapid HIV 1/HIV 2 test kit, enzyme linked immunosorbent assay (ELISA) and then confirmed with Western blot (WB). Three (15%) of the 20 HIV infected individuals, 9 (18%) of the 50 malaria infected individuals, 10 (55.6%) of the 18 individuals co-infected with malaria and HIV and 1 (2%) of the 50 non-infected individuals were positive to DAT. Direct antiglobulin test positivity rate was significantly higher in individuals co-infected with malaria and HIV than in those infected with malaria only ($\chi^2 = 7.62$; $p<0.001$) or HIV only ($\chi^2 = 5.76$; $p<0.001$). Direct antiglobulin test positivity rates in subjects with HIV only (15%) and malaria only (18%) were not statistically different ($p = 0.8$). The high prevalence of positive DAT observed in individuals co-infected with malaria and HIV which suggested aggravated effect resulted from positive interactions between malaria and HIV.

Key words: Direct Antiglobulin Test · Malaria, HIV · Co-Infection

INTRODUCTION

The previous studies revealed that the microbial interactions can be result in neutral [1, 2], ameliorative [3,4] or aggravated [5,6] effect. Studies on Direct antiglobulin test (DAT) reactivity and malaria have reported high prevalence of positive DAT among malaria patients [7, 8]. Like malaria, high prevalence of positive DAT has been reported in patients with HIV/AIDS [9, 10]. Co-infection with m alaria and HIV is believed to be common in sub-Saharan African but reports on concurrent infection with malaria and HIV are inconclusive [11]. Information on the DAT status of subjects co-infected with HIV and malaria could help resolve some of these conflicting reports. The aim of this study was to compare the prevalence of positive DAT in subjects co-infected with malaria and HIV interaction, to those with single infection of malaria or HIV.

MATERIALS AND METHODS

The study was carried out in Iwo, a semi-urban community in Southwestern Nigeria. It is located between Latitudes 7°37′30″ and 7°38′30″N, Longitudes 4°10′30″ and 4°12′00″S. A total of 138 subjects were screened in the study. They included 20 with HIV infection only, 50 with malaria only, 18 with both malaria and HIV and 50 non-infected with either malaria or HIV. Ethical approval for this study was obtained from the Joint Ethical Committee of Ladoke Akintola University Teaching Hospital, Osogbo and Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.

Malaria parasite test was done by microscopic examination of thick and thin blood films stained with 3% Giemsa. Antibodies to HIV were determined using Capillus rapid HIV 1/HIV 2 test kit (Trinity Biotech Plc, Ireland), enzyme linked immunosorbent assay (ELISA) (GenScreen plus HIV Ag-Ab test kit, Pasteur, Paris) and

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then confirmed with Western blot (WB) (New-Lav Blot 1, Bio-Rad, France). Haematocrit, haemoglobin concentration, leucocyte count and platelet count were done using an automated Coulter counter STKS model.

For direct antiglobulin test, 1ml red blood cell suspension was washed 4 times in comparatively large volume (4 ml per wash) of 0.9% saline by centrifugation and reconstituted with saline to 5% Packed Cell Volume. One drop of this suspension was mixed with two drops of poly-antiglobulin sera (DIAGAST laboratories, Cedex, France), followed by centrifugation at 1,200 xg for 1 minute. The sample was immediately inspected macroscopically for agglutination and negative or doubtful result was re-examined by light microscopy.

Statistical Analysis: Chi-Square test and Fisher's exact test were used to compare differences between percentages and proportions. Student's t test was used to compare sample means. A p-value of < 0.05 was considered significant.

RESULTS

Out of the 138 individuals tested, 23 (16.7%) were positive for DAT. The prevalence of DAT reactivity among infected and non-infected subjects is given in Table 1. Three (15%) of the 20 individuals positive for HIV infection only, 9 (18%) of the 50 individuals infected with malaria only, 10 (55.6%) of the 18 individuals co-infected with malaria and HIV and one (2%) of the 50 non-infected individuals had positive DAT. Direct antiglobulin test positivity rate was significantly higher in: (i) HIV positive infected subjects than in HIV negative subjects ($\chi^2 = 11.8$; df = 1; $p<0.001$) (ii) malaria positive subjects than in malaria negative subjects ($\chi^2 = 14.7$; df = 1; $p<0.001$) and (iii) individuals co-infected with malaria and HIV than in those infected with malaria only ($\chi^2 = 7.62$; df = 1; $p<0.001$) or HIV only ($\chi^2 = 5.76$; df = 1; $p<0.001$). DAT positivity rates in subjects with HIV only (15%) and those with malaria only (18%) were not statistically different ($p = 0.8$). DAT positivity rate was significantly higher in subjects with malaria only (18%) than in the non-infected subjects (2%) ($p = 0.01$) and in subjects with HIV only (15%) than in the non-infected subjects (2%) ($p = 0.03$).

The mean haematological values of DAT positive and DAT negative infected subjects are given in Table 2. Infected subjects who were DAT positive had significantly lower mean values of haematocrit, total leucocyte count and haemoglobin concentration compared to those who were DAT negative.

Table 1: Prevalence of Direct Antiglobulin Test (DAT) Reactivity among Infected and Non-infected subjects

Infection Status	No. Examined	Number Positive for DAT (%)
HIV only	20	3 (15.0)
Malaria + HIV	18	10 (55.6)
Malaria	50	9 (18.0)
No infection	50	1 (2.0)
Total	138	23 (16.7)

Table 2: Comparison between Mean Haematological Values of DAT-Positive and DAT- Negative Infected Subjects

Parameter	DAT negative subjects n=54 Mean±S.D	DAT positive subjects n=23 Mean±S.D	p value
Haematocrit (%)	35.1±4.7	30.6±5.1	$p < 0.001$
Total leucocyte Count ($10^3/\mu l$)	3.7±1.3	3.4±1.1	$p = 0.14$
Platelet Count ($10^3/\mu l$)	135.0±31.2	131.4±30.3	$p = 0.27$
Haemoglobin Conc. (g/dl)	12.2±1.6	10.7±1.7	$p < 0.001$

DISCUSSION

Malaria infection has been shown to increase DAT reactivity [7, 8] and a similar effect has been associated with HIV infection [12, 13]. While DAT positivity rates in subjects with only HIV and those with only malaria were not statistically different in this study, co-infection with malaria and HIV resulted in significant increase in DAT positivity rate. Co-infection with malaria and HIV therefore produced a synergistic effect resulting in increased prevalence of direct antiglobulin test.

Direct antiglobulin test positive subjects had lower haemoglobin concentration compared to DAT negative subjects. This is in line with the study of Lai *et al*. [10] and implies that anaemia in these cases is partly immune mediated. It has been suggested that complement or immunoglobulin-mediated immune complex formation contributes to haemolysis in malaria or HIV infection [10, 14]. Immunoglobulin M (IgM) and complement components have been found on the red cells of patients with malaria or HIV. Although the subjects investigated in this study were not separately tested with anti-IgM and anti-complement reagents to ascertain whether the DAT positivity was due to IgM, complement or both, previous studies had shown that infections with these microbes stimulated complement activation [8,15]. The complement activation has been associated with reduced haemoglobin levels [16] by direct lysis of infected red blood cell [17]. A central molecule in this association appears to be C3d that can opsonise the red blood cell for erythrophagocytosis which is mediated by macrophages,

as indicated by increased levels of neopterin [18,19] which is a marker of macrophage activation [20]. The combination of activated macrophages and red blood cell opsonised by complement after activation will lead to increased erythrophagocytosis and decreased haemoglobin as demonstrated in other studies [8, 21].

CONCLUSION

Positive interactions between malaria and HIV resulted in high prevalence of positive DAT observed in individuals co-infected with malaria and HIV.

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