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RATE OF EXPRESSION OF ANTI-DOUBLE STRANDED DNA ANTIBODIES AMONG PATIENTS WITH MALARIA INFECTION, PRESENTED TO SELECTED PRIVATE CLINICS IN KHARTOUM STATE

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ABSTRACT

Background: According to the world wide malaria report in 2011, malaria is prevalent in 106 countries (mostly in tropical and sub-tropical areas). In most of these areas malaria is perennial problem. Annually malaria is reported to infect 250 – 660 million and kills more than a million cases worldwide (mostly among African children). Anti-double stranded DNA antibody (anti-dsDNA) is one of the group of antibodies produced against extractable nuclear antigens called anti-nuclear antibodies. The mechanism of generation of these antibodies is not well known however anti-dsDNA antibodies are incredibly specific for SLE and are therefore used for diagnosis of SLE. Auto-antibodies are detected in patients with acute malaria and in sera of healthy subjects, living in endemic areas. A correlation was found between the presence of auto-antibodies and high titer of anti-malaria antibodies (antibodies suggest that acute malaria trigger generation of auto-antibodies). This study aimed to find relation between anti-dsDNA and infection with malaria. **Methods:** Anti-dsDNA was assayed in blood of 30 patients presenting with malaria and healthy controls using *Crithidia luccilie* florescent test (Euroimmun), staining of the kinetoplast considered as positive. **Results:** Anti-dsDNA antibodies were detected in 25 of the30 malaria patients giving a percentage of 83.3% compared to only one (3.3%) of the control group. These results revealed a statistically significant difference in the percentage of

expression of the anti dsDNA between patients with malaria and the healthy control group. **Conclusion:** Malaria can be considered as possible cause of auto-immune disease.

KEY WORDS: Malaria, anti-double strand DNA antibodies.

INTRODUCTION

Malaria is an infectious disease caused by the parasite called plasmodium. Five identified species of this parasite cause human malaria (namely *P. falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi*). It is transmitted by the female anopheles mosquito. It can be treated within 48 hours, yet it can cause fatal complications if the diagnosis and treatment is delayed. It is re-emerging as number one infectious killer and it is number one priority tropical disease of the WHO.[1]

All manifestations of malaria are caused by infection of red blood cells by the asexual forms of the parasite and involvement of red blood cells makes malaria a potentially multisystem dieses.[2,3]

The development of malaria depends on the parasite biomass.[2,3] cytokines (especially pro-inflammatory cytokines), sequestration, cyto-adherance, rosetting and red cell deformity.[4,5,6,7,8,9,10]

Antibodies are detected in patients with acute malaria, chronic malaria, and in sera of healthy subjects living in endemic malarious areas. A correlation was found between the presence of auto-antibodies and high titer of anti-malrial antibodies, suggesting that acute malaria triggers generation of ANA.

However, the persistence of these antibodies didn't correlate with the level, indicating that auto antibodies are associated with chronic infection. IgG anti cardiolipin (ACL) were detected in high titers in asymptomatic *P. falciparum* carriers and IgM ACL titer correlated with cerebral malaria.[11] Globally some studies were conducted to detect presence of anti-double stranded DNA antibodies during malaria infection and other infection and other infectious diseases. A study conducted by Adu Dand and his co-workers in 1982 on Anti-ssDNA and antinuclear antibodies in human malaria, reported the absence of anti-dsDNA in the sera of studied patients.[12] Another study conducted by Koonpucknavig S. and Ekanyakul G. in 1984 on auto antibodies in sera of Thai patients with *Plasmodium falciparum* infection, reported that there is no detectable anti-dsDNA antibodies.[13] A most recent study conducted

by Virginia S. Baker and his co-workers in 2008 on Cytokines-associated neutrophil extracellular traps and antinuclear antibodies in *Plasmodium falciparum* infected children under six years of age, reported that level of ANA specific for dsDNA were significant in 81% of children both pre-treatment and post-treatment.[14]

MATERIALS AND METHODS

A total of 30 patients with malaria infection (16 *P. falciparum*, 11 *P. vivax* and 3 with both) were included in the study in addition of 30 controls. 5ml of venous whole blood were collected from each subject and used to prepare thick and thin blood films for detection of malaria parasite, parasite count and parasite differentiation techniques.[15-19] Clotted samples were used to obtain serum samples for detection of Rheumatoid factor (RF), C-reactive proteins and indirect immunoflurence technique for anti-double stranded DNA antibodies. Anti-dsDNA antibodies were assayed by crthidia luciliae-immunoflurecence test (Euroimmun®).[20]

The *crithidia* test was performed according to the manufacturer instruction. Briefly, sera diluted 1:10 in PBS were incubated on wells containing *C. luciliae*. After 30 min excess serum was washed off and bound immunoglubin detected with goat anti-human immunoglubin conjugated to Flurescence isothiocyanate. Staining of kinetoplast was regarded as positive result for anti-dsDNA antibodies. Rheumatoid factor test was used to determine presence of susceptible auto-antibodies in the studied serum, standardized semi – quantitative latex agglutination technique was used to screen the samples for rheumatoid factor and C-reactive protein according to manufacture instructions. Briefly, sera serially diluted 1/4, 1/8, 1/16, 1/32, 1/64 and test preformed, agglutination in dilution 1/16 or more was regarded as positive.

RESULTS

Among the thirty cases of malaria 16 patients (52%) were found to have *P. falciparum* infection, 11 (37%) *P. vivax* and three (10%) were with both (*P. falciparum and P. vivax*). *P. ovale* and *P. malariae* were both not detected. The degree of parasitaemia ranged between mild to moderate in most of the studied population. Fourteen patients (46%) were found to have moderate parasitaemia (++), eleven (36.6%) mild parasitaemia (+), four (13.4%) high parasitaemia (+++) and only one patient (3.4%) very high parasitaemia (++++).

The majority (83.3%) of the patients with malaria were positive for anti-dsDNA antibodies compared to only one (3.3%) in control group (Table 1). This results showed a highly significant expression of anti-dsDNA antibodies among malaria patients (*P-value= 0.00*). Among the study group, nineteen (82%) of the 23 males were found positive for anti-double stranded DNA antibodies compared to 6 (85%) of the 7 females.

The rate of detection of anti-dsDNA antibodies was almost equal among patients with *P. falciparum* and *P. vivax* (81.3% and 81.8% respectively) (Table 2).

Table (1): Show Distribution of Anti-dsDNA Antibodies Results Among the Studied Groups.

Group of study	Total examined	Result of Anti-dsDNA antibody test	
		Frequency	Percentage %
Study group	30	25	83.3%
Control group	30	1	3.3%
Total	60	26	43.3%

Table (2): Shows the Distribution of anti-dsDNA Antibodies according to Detected Species of Plasmodium.

Species of plasmodium	Total examined	Anti-dsDNA antibodies positive	
		Frequency	Percentage %
P. *falciparum*	16	13	81.3%
P. *vivax*	11	9	81.8%
Both P. *falciparum* and P. *vivax*	3	3	100%

The twenty five patients with malaria who proved to have anti double stranded DNA antibodies against kinetoplast, were also found to have other auto-antibodies against different structures (cell wall, nucleus, cytoplasm and flagella) and in different combination (patterns) as follow: kinetoplast only in (40%), kinetoplast + nucleus + cytoplasm (16%), Kinetoplast + nucleus (12%), Kinetoplast + flagella (08%), Kinetoplast + nucleus + flagella (08%), Kinetoplast + cell wall + cytoplasm (04%), Kinetoplast + cytoplasm (04%), Kinetoplast + cytoplasm + flagella (04%), Kinetoplast + cytoplasm + nucleus + cell wall + flagella (04%). (Table 3).

Table (3): Show the Distribution of Different Fluorescence Pattern Among Positive Anti-dsDNA in Study Group.

Type of pattern	Frequency	Percentage %
Group A	10	40%
Group B	1	04%
Group C	3	12%
Group D	1	08%
Group E	4	16%
Group F	2	08%
Group G	1	04%
Group H	1	04%
Group I	1	04%
Total	25	100%

Key

Group A = only kinitoplast show positive

Group B = both kinitoplast and cytoplasm show positive

Group C = both kinitoplast and nucleus show positive

Group D = both kinitoplast and flagella show positive

Group E= kinitoplast, nucleus and cytoplasm show positive

Group F= kinitoplast, nucleus and flagella show positive

Group G= kinitoplast, cell wall and cytoplasm show positive

Group H= kinetoplast, cytoplasm and flagella show positive

Group I = all copmponent of crithidalucillia (kinitoplast, nucleus, flagella, cytoplasm and cell wall) show positive

The degree of parasitaemia showed interesting results, in which the 5 patients (45%) found negative for anti-double stranded DNA antibodies were found within the total 11 patients with mild parasitaemia (+).

Patients who were found to have high and very high parasitaemia showed high variable combinations (patterns) in positive results for anti-double stranded DNA with no expression of predominant pattern kinetoplast only. Only patient who was found tho have very high parasitaemia showed unique pattern, all component (kinitoplast, nucleus, cytoplasm, cell wall and flagella) show positive result. (Table 4).

Table (4): Distribution of the combinations (patterns) of positive indirect immunofluorescent anti-ds DNA antibodies according to degree of parasitaemia.

Degree of parasitaemia	Mild (+)	Moderate (++)	High (+++)	Very high (++++)	Total
NO.	11	14	4	1	30
Negative	5	0	0	0	5
Group A	4	7	0	0	11
Group B	1	0	0	0	1
Group C	0	3	0	0	3
Group D	1	1	0	0	2
Group E	0	1	2	0	3
Group F	0	0	2	0	2
Group G	0	1	0	0	1
Group H	0	1	0	0	1
Group I	0	0	0	1	1

Key

Group A = only kinitoplast show positive

Group B = both kinitoplast and cytoplasm show positive

Group C = both kinitoplast and nucleus show positive

Group D = both kinitoplast and flagella show positive

Group E= kinitoplast, nucleus and cytoplasm show positive

Group F= kinitoplast, nucleus and flagella show positive

Group G= kinitoplast, cell wall and cytoplasm show positive

Group H= kinetoplast, cytoplasm and flagella show positive

Group I = all copmponent of crithidalucillia (kinitoplast, nucleus, flagella, cytoplasm and cell wall) show positive.

DISCUSSION

In this study anti-double stranded DNA antibodies were detected against different parts (nucleus, cytoplasm, cell wall and flagella) of *crithidia lucilliae*, in addition to the kinetoplast. Most previous studies did not show whether the auto-antibodies were directed against kinetoplast only or directed to other structures of *crithidia luciliae* [12, 13, 14]. High degree of parasitaemia was found to be associated with antibodies against different parts rather than kinetplast (nucleus, cytoplasm, cell wall and flagella). The results in this study point to the fact that infection with malaria can predispose to autoimmune diseases.

The results of this study showed statistically significant difference in the rate of detection of anti-dsDNA antibodies between malaria and healthy control (*P=0.00*).

CONCLUSIONS

Anti-double stranded DNA antibodies were detected in 83% of study population, with different patterns of results. Anti-double stranded DNA antibodies detected in both cases of *P. falciparum* and *P. vivax* equally 81% for each. 85% of females were positive for anti-dsDNA antibodies compared to 82% of males. Anti-double stranded DNA antibodies were detected in 3.3 % of control population.

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