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A Study on Cold Agglutinins in Malaria from a Tertiary Care Hospital of South India

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Method: About 150 patients diagnosed as having Malaria by Peripheral smear, QBC for malaria or by Rappid diagnostic test for Malaria were studied for occurrence of cold agglutinins by Cold agglutinin test.

Results: Out of 150 Patients, who are diagnosed as positive for malaria, 83.3% of the patients had negative for cold agglutinin and only (4%) were found to have high titers of cold agglutinins. The mean age group of the patients who were positive for the test was 45.25 ± 19.7 years. The Hemoglobin percentage and platelet count in cold agglutinin positive patients were 8.85 (± 3.72) gms% and 1, 03, 500 (± 1.16) respectively.

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A Study on Cold Agglutinins in Malaria from a Tertiary Care Hospital of South India

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Conclusion: The prevalence of cold agglutinins in patients infected with Plasmodium vivax or P falciparum was 2.67%. There was a significant change in hematological parameters like hemoglobin but not platelets.

I. Introduction

Malaria is a public health problem in Sub Saharan Africa, some parts of South East Asia and South America with considerable morbidity and mortality. This disease was almost eradicated in India during 1960's but to reemerge as a public health problem in last few decades[1]. This is mainly due to development of resistance by mosquitoes against the insecticides and to therapeutic agents by the plasmodium organism. The social and environmental changes also plays major role in this situation. Usually the disease malaria results from the parasites belonging to Plasmodium species. Human malaria is a result of four different species of plasmodium including Vivax, Falciparum, Ovale and Malariae. Nowadays, Plasmodium Knowlesii a fifth parasite which was known cause malaria in monkeys has been implicated in some parts of Karnataka.

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Simple techniques including examination of the peripheral smear is used to establish the diagnosis in Malaria. Fluorescent technique can also be employed to detect the malarial parasite. Nowadays, Malaria is often considered as grey area between the parasitology and hematology. Recent text books have considered malaria as a typical example of Hemolytic Anemia as result of acquired extra corpuscular causes. The common hematological abnormalities accompanied with the malarial infection are anemia, thrombocytopenia, spleenomegaly, mild to moderate atypical lymphocytosis and rarely DIC.[2]

The available literature had shown that, the main reason for hemolytic anemia especially in malaria is due to occurrence of cold agglutinins. The cold agglutinins are capable of agglutinating RBCs and hence result in hemolysis. But the literature available shows that the cold agglutinins occur transiently in malaria. This is mainly due to activation of polyclonal B lymphocytes which is predominantly of IgM variety. Rarely might they be either IgA or IgG variety which has the specificity for blood group antigen. The literature suggests that the hemolysis appears in 2 – 3 weeks after the malarial infection. The hemolysis is usually mild and self limiting and occasionally severe and fatal.[3, 4]

Increasing emphases is now given for these antibodies in protection against human malaria which is directed at erythrocytic stages of Plasmodium falciparum. But the protection offered by such antibodies is relatively unstable and the precise role of specificities remains unclear regarding the antigenic variability of parasite proteins. Even though parasite specific antibodies formed contribute to protection, it is not evident to what extent antibodies so formed contribute to protection[5]. Many studies have shown that these antibodies result in hemolytic anemia due to complement mediated RBC destruction in the reticuloendothelial system.

Studies regarding role of cold agglutinins are scant in India and World. This made us to take up this study in order study the profile of patients with Cold agglutinins in Malaria.

II. Materials and Methods

This cross sectional study was undertaken in the Department of Microbiology, Basaveshwara Medical College and Hospital, Chitradurga. About 150 patients who were diagnosed as having Malaria by peripheral

smear examination (both thick and thin), MPQBC or by malarial antigen assay before starting the antimalarial treatment were included in the study. An informed consent was obtained from each patient before the study was started and clearance from Intuitional ethical committee was obtained. This study was carried out between January, 2012 to March, 2013.

A detailed history was taken followed by detailed clinical examination for all the patients included in the study. These patients were also investigated for Hemoglobin estimation by cyanmethemoglobin method and Total platelet count by modified Dacie Leurs method.

Cold agglutinin test was used to detect cold agglutinins in the malarial patients. The cold agglutinins if present in patient's serum in high titers may be pathologic and result in cold agglutinin disease. Serum or plasma of all the malarial patients was separated at 37^0 C from a Blood sample collected. A pool of 2 or more examples of washed group O1 adult red cells and Phosphate buffered saline (PBS) at pH 7.3 were used as reagents. A serial two fold dilutions of the patient's serum or plasma in PBS were prepared followed by Two drops of each dilution 1 drop of a 3% to 5% suspension of red cells was mixed. The solution was mixed and incubated at 4^0 C for 1 to 2 hours.

The tubes were centrifuged for 15 to 20 seconds at 900 to 1000 X g. Then it was placed in ice water bath. The tubes were examined microscopically for agglutination, starting with the tube at highest dilution. The results were graded and recorded.

All the details were collected in a pre-structured, self administered proforma. The data thus obtained was compiled and analysed in the form of frequency and proportions. Chi square test and Student T test were used as significance test by using Statistical Package for Social Sciences (SPSS vs 18). A p value of less than 0.05 was considered as statistically significant.

III. Results

Table 1 : Cold agglutinin test results of the study group

	Frequency	Percent
Negative	125	83.3
Positive at 4 deg at 1/256 dilution	3	2.0
Positive at 4 deg at 1/64 dilution	1	0.7
Positive at 4 deg at 1/32 dilution	3	2.0
Positive at 4 deg C at 1/16 dilution	5	3.3
Positive at 4 deg at 1/8 dilution	10	6.7
Positive at 4 deg at 1/4 dilution	3	2.0
Total	150	100.0

On observing the results cold agglutinin test, about 83.3% of the patients had negative cold agglutinin test. Only 4 patients who had malaria were positive for the cold agglutinin test since the cut of value for the positive test was above 1:64 dilution.

Table 2 : Distribution of the Malarial patients according to age group and result of Cold agglutinin test

Age group	Cold agglutinin test		Total n (%)
	Negative n (%)	Positive n (%)	
Less than 20 yrs	25 (17.1)	1 (25.0)	26 (17.3)
21 - 30 yrs	57 (39.0)	0	57 (38.0)
31 - 40 yrs	25 (17.1)	0	25 (16.7)
41 - 50 yrs	17 (11.6)	2 (50.0)	19 (12.7)
51 - 60 yrs	10 (6.8)	0	10 (6.7)
61 yrs and above	12 (8.2)	1 (25.0)	13 (8.7)
Total	146 (100)	4 (100)	150 (100)
Mean ± Std dev	34.09 ± 15.55	45.25 ± 19.7	34.39 ± 15.7

t (148)= 1.406 p=0.162, NS

Table no 2 shows the age group wise distribution of the study group. The mean (± SD) age of subjects in the study group was 34.39 ± 15.7 years. The mean age group of the patients who were positive for the test was 45.25 ± 19.7 years. About 50% of the subjects who had positive test belonged to 51 – 60 years age group, one patient was aged less than 20 years and another aged more than 60 years. There was no statistically significant difference between the age of the patients and result of the Cold agglutinin test.

Table 3 : Distribution of the Malarial patients according to sex and result of Cold agglutinin test.

Sex	Cold agglutinin test		Total
	Negative n (%)	Positive n (%)	n (%)
Male	104 (71.2)	3 (75.0)	107 (71.3)
Female	42 (28.8)	1 (25.0)	43 (28.7)
Total	146 (100)	4 (100)	150 (100)

χ^2 value = 0.027 df=1 p=0.869

About 75.0% of the patients who were positive for the cold agglutinin test were males and 25% were females. Of 146 patients who had shown negative results for the test, 71.2% were males and 28.8% were females. This difference was not statistically significant.

Table 4 : Distribution of the Malarial patients according to the level of hemoglobin and result of Cold agglutinin test.

Haemoglobin %	Cold agglutinin test		Total
	Negative n (%)	Positive n (%)	
More than normal	35 (24.0)	2 (50.0)	37 (24.7)
Less than normal	111 (76.0)	2 (50.0)	113 (75.3)
Total	146 (100)	4 (100)	150 (100)
Mean ± Std dev	11.40 ± 2.67	8.85 ± 3.72	11.33 ± 2.72

t (148)= 1.862 p=0.065, NS

The mean hemoglobin level of the study subjects was 11.33 (± 2.72) gm%. Patients negative cold agglutinin test results had a mean hemoglobin level of 11.4 (± 2.67) gm% and those with positive test had a mean hemoglobin level of 8.85 (± 3.72) gm%. About 50% of the patients had normal level of hemoglobin and 50% had less than normal level of hemoglobin. There was statistically significant difference in the hemoglobin levels between the negative and positive cold agglutinin test patients.

Table 5 : Distribution of the malarial patients according to the level of Platelets and result of Cold agglutinin test

Platelet Count	Cold agglutinin test		Total n (%)
	Negative n (%)	Positive n (%)	
Less than 1,65,000	132 (90.4)	3 (75.0)	135 (90.0)
Normal	13 (8.9)	1 (25.0)	14 (9.3)
More than 4,15,000	1 (0.7)	0	1 (0.7)
Total	146 (100)	4 (100)	150 (100)
Mean ± Std dev	97,832.2 ± 63,824	1,03,000 ± 1.16	97,970.0 ± 65,099.4

t (148)= 0.156 p= 0.876, NS

The mean platelet count in test negative patients was 97,832.2 (± 63,824) and in test positive patients was 1, 03,500 (± 1.16). This difference was not statistically significant. The platelet count was low in 91.4% of test negative and 75% of the test positive patients.

Table 6 : Distribution of the Malarial patients according to the type of malarial parasite and result of Cold agglutinin test

Type of Malarial Parasite	Cold agglutinin test		Total n (%)
	Negative n (%)	Positive n (%)	
Pl. Vivax	98 (67.1)	0	98 (65.3)
Pl. Falciparum	32 (21.9)	2 (50.0)	34 (22.7)
Mixed	16 (11.0)	2 (50.0)	18 (12.0)
Total	146 (100)	4 (100)	150 (100)

$\chi^2 = 8.985$ df=2 p=0.011, Sig

Table no 6 shows the type of malarial parasitic infection and results of the Cold agglutinin test. About 67.1% of the test negative patients were infected with Plasmodium Vivax. About 50% of the malarial patients with positive cold agglutinin test were infected with Plasmodium vivax and 50% had mixed Vivax and Falciparum infection. The difference in type of parasite and results of the Cold agglutinin test was statistically significant.

IV. Discussion

This study was mainly undertaken to study the prevalence of cold agglutinins in malaria patients. The results from this study had shown that about 2.67% of the malarial patients were positive for cold agglutinins with titres above 1:64 dilutions. In a study in 1980 the authors have failed to demonstrate any cold agglutinins in 1980.[6] A study of monoclonal gammopathies of cold agglutinin disease in mayo clinic had shown that the prevalence was 1.1%. A study by Torres et al had shown prevalence similar to that observed in this study.[7]

The mean age group of the patients who were positive for the test was 45.2 (± 19.7) years. About 50% of the patients who were positive for the test belonged to 41 – 50 years and 61 years and above age group. However there was a statistically significant difference between age group and result of the Cold agglutinin test. The finding from our study shows that the prevalence of cold agglutinins was common in the patients above 40 years. In contrary to these findings, Sharon[4] states that the secondary cold agglutinin disease is common in children and young adults. About 75% of the patients who had cold agglutinins were males in this study.

The mean haemoglobin levels in the positive patients for cold agglutinins was 8.85 (± 3.72) gm% in this study. About 50% of the patients had hemoglobin more than normal and 50% had hemoglobin below normal. This study is able to demonstrate haemolysis similar to the study by Gertz et al[18]. There was no drop in platelet count in patients who were positive for cold agglutinins in this study. Reduced levels of Haemo-globin and Platelet count in malaria patients were recorded by other author [8].

About 67.1% of the test negative patients were infected with Plasmodium Vivax. About 50% of the patients' positive for Cold agglutinin test had mixed malarial parasitic infections. The difference in type of parasite and results of the Cold agglutinin test was significant. A study from Ouagadougou also reported the similar results.

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