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STUDY OF HEMATOLOGICAL DYSFUNCTION IN MALARIA

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ABSTRACT

Malaria is one of oldest recorded disease known to mankind. Optimum conditions for transmission are high humidity and an ambient temperature between 20OC and 30OC. Since erythrocytes are the principle targets of the parasites, many changes occur in the infected RBC's. Anaemia results from accelerated RBC destruction and removal by the spleen in conjunction with ineffective erythropoiesis.1,2,3 Thrombocytopenia is a common feature of acute malaria and occurs in both P. falciparum and P. vivax infections regardless of the severity of infection.4,5,6, Slight coagulation abnormalities are common in falciparum malaria and mild thrombocytopenia is usual. However <5% of patients with severe malaria have significant bleeding with evidence of DIC. Total leukocyte count is usually normal; however leukocytosis can occur especially when associated with pernicious malaria and superadded bacterial infections7.8.9. Increased red cell population dispersions or red cell distribution width has been observed in malaria and has been attributed to the red cell response to malarial parasite and correlated with the degree of macrocytosis.10 In our study of 50 cases Significant haematological dysfunction occurs in malaria across all cell lines. The presence of high RDW, thrombocytopenia, leukopenia and anaemia are significantly associated with malaria.

KEYWORDS Splenomegaly, Leukopenia, Leukocytosis

INTRODUCTION -

Malaria is an acute febrile illness transmitted by mosquitoes. It is caused by Plasmodium species found all over the world from 40°s to 60°N and is endemic in many tropical countries. 300-500 million cases of malaria occur annually all over the world with an estimated 1.1-2.7 million deaths each year.[11]

Malaria is a major health problem in India. Malaria was nearly eradicated in the 1600's with less than 50,000 cases occurring annually. However massive resurgence occurred and 6.47 million cases annually were reported in the 1970's. With the launch of modified plan of operation the incidence has decreased to 2 million annual cases since 1984.[12]

A prompt and early diagnosis is key to effective management in malaria. Many acute febrile illnesses like viral fever, arboviral infections, enteric fever and leptospirosis occur in the tropics and it is difficult to distinguish malaria from these illnesses on clinical grounds alone. Microscopic diagnosis by peripheral smear examination is used for diagnosis. However it needs expertise and needs repeated examination to rule out malaria. It's a valuable technique when performed correctly but wasteful when poorly executed and when in less expert hands.

A variety of hematological alterations like progressively increasing anemia, progressively decreasing RBC counts, thrombocytopenia and leukopenia or leukocytosis occur in malaria.[13,14] These alterations if present would increase the probability of malaria in febrile patients. Such indicators may heighten the suspicion of malaria prompting a more diligent search for the parasite and prompt institution of specific therapy. Malaria is highly endemic in and around Kurnool region and hence the need to study the indirect indicators of this disease.

MATERIAL AND METHODS

Source of data:

All patients admitted in Department of Medicine, Kurnool Medical College, Kurnool during the study period of Aug 2011 to July 2012 were taken into the study after considering the inclusion and exclusion criteria.

Inclusion Criteria:

Patients with fever of less than 7 days admitted to medical word at Kurnool Medical College, Kurnool.

Exclusion criteria:

Patients who had no fever during hospital stay.

Patients in whom a localizing cause such as pneumonia, skin and subcutaneous infections, meningitis, etc. where found on clinical examination.

Sample size:

50 cases of malaria as diagnosed by peripheral smear examination. 50 cases of acute febrile illness of non-malarial etiology, who were negative for malarial parasites on peripheral smear examination.

Method of collection of data:

Information was collected through prepared proforma for each patient.

A complete clinical examination was done with special reference to the presence of fever, jaundice, bleeding spots, hepatosplenomegaly and to exclude fever with localizing signs such as meningitis, pneumonia, upper respiratory tract infection, skin and subcutaneous tissue infection, etc.

All patients were investigated with complete blood counts, peripheral smear for malarial parasite, chest film, serum biochemistry and urine microscopy. Peripheral smear positivity was taken as the gold standard for diagnosis of malaria. Other investigations like blood culture, serology for typhoid, urine culture were done where indicated.

Complete blood counts were done by an automated analyzer at the Department of Pathology, Kurnool Medical College, Kurnool.

were examined by experienced epidemiology department personnel after staining them with JSB (Singh and Bhattacharji stain).

Smear examination was repeated twice in the next 2 days when the patient was febrile before concluding that the illness was not malaria. Malaria was diagnosed when any one of the smears was positive for malarial parasite.

Patient was labelled as having enteric fever if the blood-culture was positive for Salmonella typhi or Salmonella paratyphi.

Patients with fever, and no other localizing findings who had a chest x-ray done which revealed pneumonitis where labelled as having pneumonia.

Urine microscopy was taken as gold standard for diagnosing urinary tract infections.

When all investigations were negative and the patients responded to antipyretics and oral antibiotics the patients were labelled as having viral fever / antibiotic responsive fever.

Patients were labelled as having continuous fever, intermittent fever and remittent fever as per the following definitions.

Continuous fever - The temperature remained above normal throughout the day and did not fluctuate more than 1°C in 24 hours.

Remittent fever - The temperature remained above normal throughout the day with more than 1°C fluctuation.

Intermittent fever - The fever was present only for some hours of the day and was normal during remaining hours.

OBSERVATION AND RESULTS

This study was done at Kurnool Medical College, Kurnool during the study period of Aug 2011 to July 2012. Patients with acute febrile illness who satisfied the inclusion and exclusion criteria and who gave consent for the study were enrolled.

50 cases turned out to be smear positive for malaria. Similarly 50 cases who turned out to be smear negative were taken for comparison.

Age distribution:

Table 1: Age distribution between the two groups :

Age group (yrs)	CASES		CONTROLS	
	No.	%	No.	%
< 30	38	76%	37	74%
31-40	8	16%	9	18%
41-50	3	6%	4	8%
>50	1	2%	0	0%

The graph below shows age distribution of cases and controls which suggests that malaria is common amongst the younger population who are commonly exposed to mosquitoes by way occupation, travel, etc.

Table 2: Sex distribution of cases.

	Cases	Controls
Male	40(80%)	39(78%)
Female	10(20%)	11(22%)
Total	50(100%)	50(100%)

Of the cases 80% were males and 20% were females.78% of the non malarial cases were males and 22% were females. This sex distribution revealed no significant difference between the two groups. The Chi square value was 0.06 and p > 0.05. However there was preponderance of males in both the groups.

Symptomatology:

Table 3 : Type of fever

Type of fever	CASES		CONTROLS	
	No.	%	No.	%
Continuous	37	74	33	66
Intermittent	13	26	17	34
Total	50	100	50	100

The presence of either continuous or intermittent fever does not differentiate between non malarious and malarious fever in the study. Intermittent fever was present in 26% the cases and 34% of the controls. The Chi square value was 0.762 and the p > 0.05 which was not significant statistically.

Table 4 : Fever with chills and rigors

Fever with chills and rigors	Cases	Controls
Present	18(36%)	12(24%)
Absent	32(64%)	38(76%)
Total	50(100%)	50(100%)

Chills and rigors during the febrile episode occurred in 36% cases and 24% of the controls. The Chi square value was 1.174 and p > 0.05 which was statistically not significant.

Table 5 : Fever with splenomegaly

Fever	Cases		Controls	
Spleen +	30	60%	4	8%
Spleen -	20	40%	46	92%
Total	50	100%	50	100%

Of the 50 cases of malaria, 30 had splenomegaly. Of the 50 non malaria cases only 4 had splenomegaly. The chi-square value was 30.125 and p< 0.0001 which was statistically extremely significant. The sensitivity of splenomegaly for malaria was 60%, specificity was 92% and the positive predictive value was 88.23%.

Table 6 : Distribution of Jaundice in malaria cases

	Jaundice	No Jaundice	Total
P. falciparum	9(18%)	15(30%)	24(48%)
P. vivax	1(2%)	25(50%)	26(52%)
Total	10(20%)	40(80%)	50(100%)

The presence of jaundice was significantly associated with P. falciparum malaria. Of the 24 cases with falciparum malaria 9 had jaundice but only 1 of the 26 cases with vivax malaria had jaundice. The Chi square value with Yates correction was 6.86 and p < 0.01 was significant.

Of the 50 cases of malaria 1 died due to cerebral malaria

The following were the diagnosis amongst the patients with non malarious fever(50 cases).

1.	Enteric fever	9 cases
2.	Brucellosis	1 case
3.	Viral fever/antibiotic responsive fever	21 cases
4.	Viral fever with arthralgia (Chikungunya fever)	10 cases
5.	Pneumonitis	3 cases
6.	Urinary tract infection	5 cases
7.	SLE	1 case

Table 7: hematological dysfunction in malaria

Parameter	Mean ±SD	't' Value	'p' Value	Significance
Hb - g %	8.532±2.864	21.07	<0.0001	Highly significant
TC - cell / mm^3	6022±3409.4	12.49	<0.0001	Highly significant
RDW %	17.44±4.232	29.49	<0.0001	Highly significant
Platelet count cell / mm^3	117476±97475	8.522	<0.0001	Highly significant

The table shows that significant haematological dysfunction occurs in malaria. The haematological dysfunction in malaria was studied with the help of single 't' test. All 4 index tests revealed statistically highly significant dysfunction. The p value was < 0.0001 for all 4 parameters

Table 8 : Analysis of Hb values

Hb g%	Cases		Controls	
< 10	33	66%	21	42%
> 10	17	34%	29	58%
Total	50	100%	50	100%

The presence of anaemia had a sensitivity of 66% and a specificity of 58% for malaria. The positive predictive value was 61%. On comparing the two groups it was found that anaemia was significantly associated with malaria with p<0.05.

Mean Hb : Cases : 8.532 g% with a standard deviation of ± 2.864g%
Range : 3g% - 14.9g%
Mean Hb : Controls :10.66 g% with a standard deviation of ± 2.631g%
Range 5.9 g% - 15.1g%
Sensitivity: 66%
Specificity: 58%
Positive predictive value: 61%
Chi square value : 5.797
p < 0.05- significant

Table 9: Analysis of total leukocyte count

Total Count cells / mm^3	Cases		Controls	
< 4000	14	28%	0	0%
4000-11000	33	66%	44	88%
>11000	3	6%	6	12%

Cases : Mean TC : 6022 WBC / mm^{3}with a standard deviation of ± 3409.4 cells/mm3
Range 2400-21500 WBC / mm^3
Control : Mean TC : 8392.2 WBC / mm^3 with a standard deviation of ± 2794.9 cells/mm^3
Range 4300-18800 WBC/mm^3

On comparing the total counts between the two groups, the chi square value was 16.571 and p < 0.001 which was very significant.

Table 10: Analysis of leukopenia

Total count	Cases		Controls	
Leukopenia	14	28%	0	0%
Normal count / leukocytosis	36	72%	50	100%
Total	50	100%	50	100%

The sensitivity for leucopenia in malaria was 28% and the specificity was 100%. The positive predictive value was 100%.

Table 11 : Analysis of RDW

RDW	Cases	Controls
< 15%	14(28%)	31(62%)
> 15%	36(72%)	19(38%)
Total	50(100%)	50(100%)

Mean RDW - Cases 17.43% ; standard deviation ± 4.232%
Mean RDW - Controls 15.014% ; standard deviation ± 2.298%
Range 11% - 36.8% (cases)
Range 11.8% - 23.5% (controls)
Sensitivity: 72%
Specificity: 62%
Positive predictive value: 65%

The sensitivity of high RDW for malaria was 72% and the specificity was 62%. The positive predictive value was 65%. 72 % of the cases had a RDW value of more than 15% whereas 38% of the controls had a high RDW. The Chi square value was 11.677 and the p < 0.001 which was highly significant.

Table 12 : Analysis of platelet count

	Cases	Controls
With thrombocytopenia	37(74%)	1(2%)
Without thrombocytopenia	13(26%)	49(98%)
Total	50(100%)	50(100%)

Sensitivity : 74%
Specificity : 98%
Positive predictive value :97%
Cases: Mean platelet count – 1,17,476 / mm3
Range 18,000 - 4,69,000/mm3
Controls:Mean platelet count – 2,61,800/mm3
Range 1,40,000-4,48,000/mm3

The standard deviation for cases was ± 97475/mm3 and controls was ± 75062/mm^3 The Chi square value was 55.008 and p value < 0.0001 which was extremely significant.

Summary of statistical analysis
Table 13: Haematological dysfunction in malaria.

Parameter	Mean ±SD	't' Value	'13' Value	Significance
Hb - g%	8.532±2.864	21.07	<0.0001	Highly significant
TC — cell / mm^3	6022±3409.4	12.49	<0.0001	Highly significant
RDW %	17.44±4.232	29.49	<0.0001	Highly significant
Platelet count cell / mm^3	117476±97475	8.522	<0.0001	Highly significant

Table 14: Comparison of haematological parameters between the two groups (unpaired t test and chi square test)

Parameter	Mean ± SD		't' value	'p' value	SF	z2	'p' value	SF
	Cases	Controls						
Hb - g%	8.531+2.864	10.66±2.631	3.870	<0.001	VS	5.797	<0.05	S
TC — cell / MM^3	6022±3409.4	8392.1+2394.9	3.802	<0.001	VS	16.571	<0.001	VS
RDW %	17.44±4.232	15.014±2.298	3.56	<0.001	VS	11.677	<0.001	VS
Platelet count cell /mm	117476±97475	260240±75062	8.205	<0.0001	HS	55.008	<0.0001	HS

x^2 - Chi Square, SF — Significance, S — Significant, VS — Very significant, HS — Highly Significant.

DISCUSSION

The present study demonstrates that significant haematological dysfunction occurs in malaria across all cell lines. The study also demonstrates that haematological parameters can be used as predictors of malaria. The presence of leucopenia and thrombocytopenia emerged as the strongest indicators of malaria followed by high RDW and the presence of anaemia.

In our study there was significant haematological dysfunction in malaria as demonstrated by the highly significant p values (less than 0.0001) as studied by the single 't' test.

Low platelet count emerged as one of the strongest predictors of malaria. Amongst the various studies conducted to study the haematological profile in malaria, Ekhart LM et al[15] reported that platelet counts of less than 1,50,000 increase the likelihood of malaria by 12 - 15 times.

Lathia TB and Joshi R[16] in a study at Sevagram, Maharashtra concluded that thrombocytopenia was 60% sensitive and 88% specific for the diagnosis of malaria. Combination of anaemia and thrombocytopenia had higher sensitivity (69%), a specificity of 74% and a positive likelihood ratio of 2.77.

When the presence of the index parameters in malaria was compared with that in non malarious acute fever, thrombocytopenia emerged as extremely significant predictor of malaria. The sensitivity of thrombocytopenia in malaria was 74% and the specificity was 98%. The positive predictive value was 97%. Of the 50 patients with malaria 37(74%) had thrombocytopenia whereas only 1 of the patients with non-malarious fever had thrombocytopenia. The Chi square value was 55.008 and the $p < 0.0001$ which was extremely significant.

Anaemia was detected in 66% of the cases and 42% of the non-malarious patients. It was found to be a less significant indicator than the other parameters. The sensitivity was 66% and the specificity was 58%. The positive predictive value was 61%. The Chi square value was 5.797 and $p < 0.05$ which was significant.

In our study 28% of the patients with malaria had leukopenia as compared to non malarious patients in whom none had leukopenia . The sensitivity for leukopenia in malaria was 28% and the specificity was 100%. The positive predictive value was 100%. However leukocytosis was more common in the non malarious group. The Chi square value for the changes in leukocyte count was 16.571 and the $p < 0.001$ which was very significant.

RDW was found not to be predictive of malaria in a study done by Lathia TB and Joshi R.[49] Our study in contrast showed that it was also very significant predictor of malaria with a $p < 0.001$. The sensitivity of high RDW for malaria in our study was 72% and the specificity was 62%. The positive predictive value was 65%.

Leaving aside all these laboratory parameters, the presence of splenomegaly was seen to be significantly associated with malaria. Splenomegaly was present in 60% of our cases as compared to in only 8% of the other group. The $p < 0.0001$ which was extremely significant. The sensitivity was 60%, specificity was 92% and the positive predictive value was 88.23%.

CONCLUSION

Significant haematological dysfunction occurs in malaria across all cell lines. The presence of thrombocytopenia and leukopenia increases the probability of malaria. The presence of high RDW, and anaemia are also significantly associated with malaria. These findings along with a clinical suspicion should prompt a more diligent search for the malarial parasite.

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