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HAEMATOLOGICAL CHANGES IN MALARIA POSITIVE CASES –A CROSS SECTIONAL STUDY

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ABSTRACT:

Background & Objective: Malarial anaemia is a mutifactorial disease, usually manifested in large proportion of malarial infections. Early diagnosis of malarial infection, regular haematological assessment and appropriate management may be helpful in reducing mortality and morbidity due to malaria. The objective of this study was to assess the haematological changes which occur in different type of anaemia to help clinicians diagnose the case early, assess haematological changes and institute appropriate treatment to reduce mortality and morbidity

MATERIAL & METHODS: The cross sectional study was conducted in Central Pathology Lab of a tertiary care teaching hospital in Rohilkhand region of Uttar Pradesh in India between Sept.2018 to Jan. 2020. Haemogram was done by fully automated haematology blood cell analyser (five part) model XS-1000i. Blood smears were stained with Leishman stain, and examined microscopically. Malaria was confirmed through rapid diagnostic test (MAL CARD).

Statistical analysis: Chi Square test was used

RESULTS: Out of 1256 cases of malaria, 91.40% infections were due to P. vivax, Maximum cases (29.30%) were in age group 21-30 yrs and males affected more (56.69%), Anaemia was most prevalent in 0-10 yrs age group, had sex preponderance towards female (90.26%). Anaemia was proportionately more in P. falciparum infection (94.44%), maximum (51.11%) were Normocytic Normochromic anaemia, leukopenia (14.49%), Leukocytosis (15.13%) and Neutrophilia (21.58%), Lymphocytosis (00.32%), Monocytosis (20.06%) and Eosinophilia (01.91%), thrombocytopenia (87.52%) was observed.

CONCLUSION: Malaria parasite can cause significant haematological changes with high incidence of anaemia, thrombocytopenia and monocytosis. Early diagnosis, haematological assessment and appropriate treatment is key to effective and adequate management.

KEY WORLDS: Malaria, Anaemia, Haematological, Thrombocytopenia

INTRODUCTION:

Reduction in concentration of haemoglobin, packed cell volume or red cell count below normal for age and sex of an individual in a population is anaemia. According to Scientific Group of World Health Organization, the level of haemoglobin below which anaemia is likely to occur for population living at sea level are: 11 g/dl for children aged 6 month to 6 years, 12 g/dl for children aged between 6-14 years, 13 g/dl for adult males, 12 g/dl for non-pregnant adult females and 11 g/dl for adult pregnant females. [1]

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Malarial anaemia (MA) is a mutifactorial disease for which the complex etiological basis is only partially defined. In severe malaria due to P. falciparum severe MA is main clinical presentation.[2] In malaria endemic area, aetiology include a number of discrete and overlapping features such as lysis of infected and uninfected RBCs in severe MA, [3] splenic sequestration of RBCs,[4] Dyserythropoisis and bone marrow suppression.[5] Haematological insults is an established feature in moderate and severe anaemia in infection with P. falciparum. [6]

MA is usually normocytic and normochromic without spherocytes or schistocytes. However in endemic countries the anaemia associated with malaria can also be microcytic and hypochromic due to high frequencies of haemoglobinopathies and iron deficiency. [7] Presence of inadequate reticulocytosis despite varied degree of anaemia is a feature of MA. [8] Anaemia, thrombocytopenia, atypical lymphocytosis, leukopenia, Leukocytosis, neutrophilia, eosinophilia and monocytosis are haematological changes usually observed in MA. [9,10] The objective of this study was to assess the haematological changes which occur in different type of anaemia.

MATERIALAND METHODS

It was a hospital based cross sectional study done in Central Pathological Lab of Department of Pathology in one of the tertiary care teaching institute in Rohilkhand region of Uttar Pradesh from September 2018 to January 2020. The study included 1256 malaria positive samples of patient from outpatient and inpatient department of the institute. Informed consent was taken from the patients and ethical clearance was obtained from institutional ethical committee.

All blood sample were collected in ethylenediaminetetra acetic acid (EDTA) vials by venepuncture and thin blood film was made by method described by Dacie and Lewis. [9] Blood smears were stained with Leishman stain, and examined microscopically under oil immersion lens for the presence of malaria parasite (P. falciparum or P. vivax) within RBCs , Malaria rapid diagnostic test (MAL CARD by J. Mitra and company Pvt. Ltd.) was used as diagnostic tool to diagnose and determine the type of malaria.

Antigen Histadine release protein II test was used for detection of P. falciparum pLDH (parasite lactate dehydrogenase) for any plasmodium species (P. vivax). It is an immunoassay based on sandwich principle. The conjugate contains colloidal gold conjugated monoclonal anti-pan specific pLDH antibody. The test uses monoclonal anti Pf (Plasmodium falciparum) pLDH antibody.

Haemogram was done by a fully automated haematology blood cell analyser (five part) model XS-1000i supplied by Transasia (SYSMEX MACHINE).

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Hb level below 12g/dl. was defined as Anaemia. Severity of Anaemia was graded as Severe, moderate and mild anaemia on the basis of Hb level below 7gm/dl, 7-9.9gm/dl and 10-11.9gm/dl respectively. [10]

Thrombocytopenia was graded into following 5 grades according to National Cancer Institute common terminology criteria for adverse events version 3.0 grading of thrombocytopenia. [11]

Grade 0: within normal limit, platelet count 1, 50,000 or above
Grade I: platelet count between 75,000- 1, 50,000
Grade II: platelet count between 50,000-75,000
Grade III: platelet count between25, 000-50,000
Grade IV: platelet count <25,000

The data were compiled and statistically analysed using SPSS version 18.0 All data were fed in excel spreadsheet. For statistical analysis Chi Square test was used

RESULTS:

More than 90% cases were of P. vivax, having maximum cases in 21-30 yrs age group, while P. falciparum mostly (22.22%) occured in 11-20 year age group. Overall also, age group 21-30 yrs was most affected having 29.30% cases. Malaria was more prevalent in males (57%) (**Table 1)**

Age Group	Type of Malaria			Total	Chi-sqare P-value
	P. vivax	P. falciparum	Mixed infection		
0-10 yrs age group	111 09.67% 90.98%	01 05.56% 00.82%	10 11.11% 08.82%	122 09.71% 100.00%	29.886 .008
11-20 yrs age group	232 20.21% 90.98%	04 22.22% 01.57%	19 21.11% 07.45%	255 20.30% 100.00%	
21-30 yrs age group	356 31.01% 96.74%	03 16.67% 00.81%	09 10.00% 02.45%	368 29.30% 100.00%	
31-40 yrs age group	194 16.90% 88.18%	04 22.22% 03.48%	22 24.44% 10.00%	220 17.52% 100.00%	
41-50 yrs age group	101 08.80% 87.83%	04 22.22% 03.48%	10 11.11% 08.69%	115 09.16% 100.00%	
51-60 yrs age group	100 08.71% 89.28%	01 05.56% 01.78%	11 12.22% 09.83%	112 08.92% 100.00	
61-70 yrs age group	46 04.00% 82.14%	01 05.56% 01.78%	09 10.00% 16.08%	56 04.45% 100.00%	
71-80 yrs age group	08 00.70% 100.00%	00 00.00% 00.00%	00 00.00% 00.00%	08 00.64% 100.00%	
Total	1148 100,00%	18 100.00%	90 100.00%	1256 100.00%	

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	91.40%	01.43%	07.17%	100.00%	
Statistically significant					
Correlation of sex with type of malaria					
Sex	Type of malaria			Total	Chi-square P-value
	P. vivax	P. falciparum	Mixed infection		
Male	649 56.53% 91.15%	09 50.00% 01.26%	54 60.00% 07.59%	712 56.69% 100.00%	.741 .690 #
Female	499 43.47% 91.73%	09 50.00% 01.65%	36 40.00% 06.62%	544 43.31%	
Total	1148 100.00% 91.40%	18 100.00% 01.43%	90 100.00% 07 .17%	1256 100.00% 100.00%	
Statistically insignificant #					

Table 1:- Demographic profile of malaria patients

Its evident from Table 2 shows that 0-10 years is the worst affected group (98.36%) with anaemia was preponderant (90%) among females.

Table 2:- Demographic profile with anaemia in malarial positive cases and type of malaria

Age group	Anaemia			Chi-square P-value
	Present	Absent	Total	
0-10 yrs age group	120 11.65% 98.36%	02 .88% 01.64%	122 09.71% 100.00%	51.505 .000*
11-20 yrs age group	230 22.33% 90.20%	25 11.06% 09.80%	255 20.30% 100.00%	
21-30 yrs age group	280 27.18% 76.90%	88 3894% 23.91%	368 29.30% 100.00%	
31-40 yrs age group	165 16.02% 75.00%	55 24.34% 25.00%	220 17.52% 100.00%	
41-50 yrs age group	90 08.74% 78.26%	25 11.06% 21.74%	115 09.16% 100.00%	
51-60 yrs age group	94 09.13% 83.93%	18 07.96% 16.07%	112 08.92% 100.005	
61-70 yrs age	45 04.37%	11 04.87%	56	

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group	80.36%	19.64%	04.45%	
71-80 age group	06 .58% 75.00%	02 .88% 25.00%	56 04.45% 100.00%	
Total	1030 100.00% 82.01%	226 100.00% 17.99%	1256 100.00% 100.00%	
Statistically significant*				
Sex	Anaemia			Chi-square p-value
	Present	Absent	Total	
Male	539 52.33% 75.70%	173 76.55% 24.30%	712 56.69% 100.00%	44.274 .000*
Female	491 47.67% 090.26%	53 23.45% 09.74%	544 43.31% 100.00%	
Total	1030 100.00% 82.01%	226 100.00% 17.99%	1226 100.00% 100.00%	
Statistically significant*				

Correlation of anaemia with type of malaria positive cases

Anaemia	Type of malaria				Chi-square P-value
	P. vivax	P. falciparum	Mixed infection	Total	
Present	925 80.57% 89.81%	17 94.44% 01.65%	88 97.78% 08.54%	1030 82.01% 100.00%	18.652 .000*
Absent	223 19.43% 98.68%	01 05.56% 00.44%	02 02.22% 00.88%	226 17.99% 100.00%	
Total	1148 100.00% 91.40%	18 100.00% 01.43%	90 100.00% 07.17%	1256 100.00% 100.00%	
Statistically significant*					

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Table No. 3 shows that maximum anaemia was of Normocytic Normochromic anaemia (51.11%) and it was in P. vivax (50.70%), P. falciparum (50.00%) and in mixed infection (56.67%).

Table 3: Type of anaemia with type of malaria positive cases

Type of anaemia	Type of malaria parasite				Chi-square Pvalue
	P. vivax	P. falciparum	Mixed infection	Total	
Normocytic Normochromic blood picture	296 25.78% 98.01%	02 11.11% 00.66%	04 04.44% 01.33%	302 24.04% 100.00%	688.933 .000*
Normocytic Normochromic anaemia	582 50.70% 90.65%	09 50.00% 01.40%	51 56.67% 07.95%	642 51.11% 100.00%	
Microcytic Hypochromic anaemia	101 08.80 % 92.66%	01 05.56% 00.92%	07 07.78% 06.42%	109 08.68% 100.00%	
Microcytic anaemia	19 01.66% 79.17%	02 11.11% 08.33%	03 03.33% 12.50%	24 01.91% 100.00%	
Dimorphic anaemia	27 02.35% 58.70%	01 05.56% 02.17%	18 20.00% 39.13%	46 03.67% 100.00%	
Normocytic Hypochromic anaemia	123 10.71% 92.485	03 16.66% 02.26%	07 07.78% 05.26%	133 10.59% 100.00%	
Total	1148 100.00% 91.40%	18 100.00% 01.43%	90 100.00% 07.17%	1256 100.00% 100.00%	
Statistically significant*					

Leukopenia was present in 14.5% malarial positive cases and leukocytosis in 15.53% cases. Leukocytosis was more prevalent (55.56%) in P. falciparum. (Table 4)

Table 4: Type of Leukocyte with type of malaria positive cases

Total leukocyte count	Type of Malaria Parasite				Chi-square P value
	P. vivax	P. falciparum	Mixed infection	Total	
Leukopenia	176 15.33% 96.70%	00 00.00% 00.00%	06 06.67% 03.30%	182 14.49% 100.00%	44.778 .000*

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Normal count	815 70.99% 92.72%	08 44.44% 00.91%	56 62.22% 06.37%	879 69.98% 100.00%	
Leukocytosis	157 13.68% 80.51%	10 55.56% 05.13%	28 31.11% 14.36%	195 15.53% 100.00%	
Total	1148 100.00% 91.40%	18 100.00% 01.43%	90 100.00% 07.17%	1256 100.00% 100.00%	
Statistically significant*					

There was no statistical difference between different types of leucocytosis in malarial positive cases

Thrombocytopenia was present in 87.52%. However there was no statistical difference among different grades of Thrombocytopenia (Table 5)

Table 5: Platelets with malaria positive cases

Platelet count	Type of malaria parasite				Chi-square p-value
	P. vivax	P. falciparum	Mixed infection	Total	
Normal	146 12.72% 93.59%	02 11.11% 01.28%	08 08.89%	156 12.48% 100.00%	13.256 0.103#
Thrombocytopenia Grade I	300 26.14% 90.63%	07 38.89% 02.12%	24 26.67% 07.25%	331 26.35% 100.00%	
Thrombocytopenia Grade II	195 16.99% 90.28%	01 05.55% 00.46%	20 22.22% 09.26%	216 17.20% 100.00%	
Thrombocytopenia Grade III	250 21.78% 88.34%	06 33.33% 02.12%	27 30.00% 09.54%	283 22.53% 100.00%	
Thrombocytopenia Grade IV	257 22.39% 95.19%	02 11.11% 00.74%	11 12.22% 04.07%	270 21.50% 100.00%	
Total	1148 10.00% 91.40%	18 100.00% 01.43%	90 100.00% 07.17%	1256 100.00% 100.00%	
Statistically insignificant#					

DISCUSSION

In our study out of total 1256 malarial positive cases the percentage of P. vivax, P. Falciparum, and Mixed malarial infection was 91.40%, 01.43% and 07.17% respectively. In various studies, Jairaj Puri et al,[12] Gill MK et al, [13] Reddy et al, [14] reported P. vivax (87.74%, 76.67%, 61.20% respectively) P. falciparum (03.77%. 15.00%, 36.80% respectively) and Mixed malarial infection (08.49%, 08.33%, 02.00% respectively). In contrast Akhtar S et al, [15] Kashinunti M et al,[16] reported maximum cases of P. falciparum

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(52,71%, 50.00% respectively) followed by P. vivax (36.48%, 40.00% respectively) and Mixed malarial infection (10.81%, 10.00% respectively). In our study we found maximum malarial positive cases (29.30%) were reported in 21-30 yrs age group, and higher numbers were reported among males (56.69%). Similar findings were observed by Jairaj pui et al, [12] Khuraiya et al, [17] (38.20, 34.61% respectively) in 21-30 yrs age group. Ahmad et al, [18], Mandokhel S et al, [19] Gill MK et al, [13] have reported 52.00%, 58.00%, 63.33% cases respectivelyamong males.

In this study anaemia was reported in 82.01% malarial positive cases and maximum incidence (98.36%) was reported in 0-10 yrs age group. Anaemia was reported in 90.26% malarial positive females. Similar findings of anaemia in malarial positive cases have been reported by Akhtar S et al, [15] Sharma SK et al, [20], Kashinkunti M et al, [16] Awoke N et al, [21] (86.48%, 86.70%, 69.00%, 60.00% respectively.

Anaemia was reported in 97.78%, 94.44% and 80.57% malarial positive cases in mixed infection, P. falciparum and P. vivax respectively. Very high incidence of malarial anaemia

in P. falciparum infection was observed by Agravat AH et al, [22] Akhthar S et al, [15] Shah et al, [23] Jain et al, [24] (93.00%, 89.70%, 87.50% and 56.06% respectively.

In present study we found maximum Normocytic Normochromic anaemia (50.70%) in maximum anaemic malarial positive cases. Akhthar S et al [15] has also reported comparable findings (47.30%). However, Mandokhel S et al [19] found Normocytic Normochromic anaemia in 93.00% of cases which is quite high.

We reported Leukopenia and Leukocytosis in 14.49% & 15.53% cases respectively while Akhthar S et al [15] found Leukopenia(10.80%) as most remarkable findings in leucocyte. Leukocytosis (13.30%, 1.00% and 08.10%) has been reported by Sharma SK et al, [20] Kasinkunti M et al [16] and Akhthar S et al [15] respectively.

We found Neutrophilia, Lymphocytosis, Monocytosis, and Eosinophilia in 21.58%, 00.32%, 20.06% and 01.91% respectively.. While Kasknkunti M et al [16] (11.00%), Akthar S et al[15] has predominantly found Neutrophilia (10.80%), Lymphocytosis by Akthar et al[15] in 06.76%, Monocytosis by Akthar S et al [15] in 18.90%, Kasinkunti M et al [16] in 15.00% and Eosinophilia reported by Akhthar S et al, [15] (12.16%), and Kasinkunti M et al, [16] (05.00%).

Thrombocytopenia was reported in 87.54% malaria positive cases in our study. Comparable finding (91.54%) reported by Agarwal Ak et al, [25]

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Jairajpuri et al [12] (92.00%), Agravat et al, [22] (81.90), Gupta et al, [26] (77.83%) and Akthar et al [15] (71.61%).

CONCLUSION:

Anaemia is quite common in malaria parasite infections. Common haematological parameters alterations observed in malarial infections are thrombocytopenia, anaemia, leukopenia and monocytosis. Timely diagnosis of infection and haematological investigations may be quite helpful for clinicians in early diagnosis and timely and appropriate treatment of malaria.

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