“STUDY OF HEMATOLOGICAL PROFILE IN MALARIA AND DENGUE PATIENTS”

Pathology

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ABSTRACT

Background: Malaria and dengue are the two most common vector borne diseases in India. They are transmitted to human by the bite of mosquito and causes different hematological manifestations.

Aim: To investigate the alterations in hematological parameters in malaria and dengue patients and to differentiate hematological parameters in co-infection of dengue with malaria from infections of dengue and malaria alone.

Material and method: This cross sectional observational study was done in 483 malaria and/or dengue patients whose blood samples were collected in the department of Pathology and department of Microbiology at Pt. J.N.M. Medical College and associated Dr. B.R.A.M. Hospital, Raipur. The study duration was 1 year from 1st January 2018 to 31st December 2018.

Result: Out of 483 cases, 384 were dengue positive, 96 were malaria positive and 3 cases were co-infection. We observed that hematological parameters like WBC count, neutrophil %, RDW and PDW were significantly higher in malaria groups while lymphocyte %, monocyte %, RBC count, hemoglobin, hematocrit, MCH, MCHC, platelet count and plateletcrit were significantly higher in dengue group.

Conclusion: This study concluded that all the patients presented with the febrile illness should be tested for both malaria and dengue. These two diseases presented with similar clinical manifestations that is why specific diagnostic testing along with complete blood count is necessary to confirm the diagnosis and to know the severity of the illness.

KEYWORDS

Dengue, malaria, hematological parameters.

INTRODUCTION

Vector borne infections such as malaria and dengue are of major public health concern worldwide¹⁻⁶.

Malaria is a parasitic disease transmitted by Anopheles mosquito. Malaria is caused by protozoan parasite of genus plasmodium. Five species of the plasmodium such as Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium Malariae, and Plasmodium knowlesi cause malaria in humans⁷. The hematological abnormalities that have been reported to consistently companion comprise anemia, thrombocytopenia, atypical lymphocytosis, and infrequently disseminated intravascular coagulation⁸. Leucopenia, leucocytosis, neutropenia, neutrophilia, eosinophilia and monocytosis also have been reported^{9,10}. Hematological changes play a significant role in the serious complications. If hematological changes are noticed in time, the course of the disease and the outcome can surely be modified is the purpose of this study¹¹.

Dengue is a viral disease transmitted by Aedes aegypti mosquito. Dengue is caused by one of the four serotypes of the dengue virus (DEN-1, DEN-2, DEN-3, DEN-4) also referred to as an arbovirus (arthropod-borne viruses) that belongs to the genus flavivirus of the family flaviviridae^{12,13}. It is transmitted by mosquitoes of the genus Aedes aegypti. The observed hematological effects of dengue infection are changes in blood counts, hemoconcentration due to plasma leakage, leucopenia because of decreased neutrophils near the end of the febrile phase, presence of atypical lymphocytes and relative lymphocytosis before shock, thrombocytopenia and changes in blood hemostasis with frequent presence of hemorrhagic manifestations¹⁴. It is the most important merging tropical viral disease in the world today. In a geographic area where both the vectors co-exist, simultaneous occurrence of malaria and dengue in an individual cannot be ruled out¹⁵. Despite a wide overlap between vector borne infections, published reports of co-infections are very scarce till date¹⁶. Also there is insufficient data on the severity of co-infections worldwide and none from our region, therefore the aim of our study is to differentiate hematological parameters of co-infections from infections alone and determine whether patients infected by both malaria and dengue are more severe than either infection alone⁵.

MATERIAL AND METHODS

This cross sectional observational study was conducted with the permission from ethical committee in the department of Pathology and

Microbiology at Pt. J.N.M. Medical College and associated Dr. B.R.A.M. Hospital, Raipur (C.G.) for the duration of 1 year i.e. from 1st January 2018 to 31st December 2018. Patients with confirmed dengue or malaria or both positivity in the age group of 15-60 years were included in the study, fulfilling the inclusion criteria. Patient on treatment or patient having other illness like aplastic anemia, idiopathic thrombocytopenic purpura and hematological malignancies etc which can alter hematological parameters were excluded. Total 483 cases were studied and their hematological findings were analyzed. Cases were categorized into 3 groups; **Group A** includes malaria positive cases (n=96); **Group B** includes dengue positive cases (n=384) and **Group C** includes co infection cases (n=3).

Collection of sample - 4-6 ml of blood was collected in EDTA and plain vial from each febrile patient by nursing personnel, technician, male orderlies or physicians with strict aseptic precautions. All the samples were collected after obtaining the informed consent from the patients.

Tests for Malaria - Anticoagulated blood samples were tested for malarial parasite by thick and thin blood film microscopy. Films were stained with Leishman's stain for identification of species. One thousand red blood cells (RBCs) on the thin blood film and 200 white blood cells (WBCs) on the thick smear were examined before classifying a case as negative.

Detection of malarial antigen & plasmodium species was done by rapid diagnostic kit method; kit used was "ACCUCARE ONE STEP MALARIA Pf/Pv ANTIGEN TEST" by Labcare.

Tests for Dengue - Serum was separated from plain vial and tested for "Dengue DAY 1 test" for detection of dengue NS1 antigen and differential detection of IgM antibodies.

Nonstructural protein 1 (NS1) antigen detection was performed from day 0 to day 5, and indirect diagnosis based on the detection of specific anti-dengue immunoglobulin M (IgM) or immunoglobulin G (IgG) antibodies in patients' sera after day 3. At least one positive test was taken to mean a positive dengue infection.

Evaluation of hematological parameters - Evaluation of hematological parameters was done by taking 2ml anticoagulated blood samples of positive dengue and malaria patients; which was examined for CBC which includes TLC, DLC, RBC Count,

Hemoglobin, Hematocrit, MCV, MCH, MCHC, RDW, Platelet, MPV, PCT and PDW. The analysis was done by automated analyzer (BECKMAN COULTER DxH 500 and/or LH 750 HEMATOLOGY ANALYZER)

RESULT

A total of 483 cases were studied, out of which 384 (79.5%) cases were dengue positive, 96 (19.9%) cases were malaria positive and only 3 (0.6%) cases were of co-infection. Among malaria cases, 46.8% were of *P. falciparum* species, 27.1% were of *P. vivax* and 26.1% were of mixed infection of both *P. falciparum* and *P. vivax* species. Maximum numbers of dengue cases i.e. 44.8% were NS1 positive, 29.9% were IgM positive and 25.3% of the cases were both NS1 and IgM positive.

Fig 1 – Bar diagram showing age distribution among cases

In malaria and dengue groups, maximum number of cases fell in the age group of 21-30 years i.e. 31.3% and 45.8% cases respectively. 3 co-infection cases were found; all were below 40 years of age (shown in Fig 1).

Fig 2– Bar diagram showing distribution of cases in various groups based on gender

In malaria group male to female ratio was nearly equal i.e. 1.04:1; but in dengue and co-infection group it was higher i.e. 1.52:1 and 2:1 respectively (shown in Fig 2).

Maximum numbers of cases were observed in the month of August in malaria and dengue groups i.e. 22.9% and 41.6% respectively. In co-infection group; 1 case was found in the month of July, 1 in September and 1 in October.

We observed that hematological parameters like WBC count, neutrophil %, RDW and PDW were significantly higher in malaria groups while lymphocyte %, monocyte %, RBC count, hemoglobin, hematocrit, MCH, MCHC, platelet count and plateletcrit were significantly higher in dengue group. In both the groups there were no significant difference in other parameters like eosinophil %, basophil %, MCV and MPV (shown in Table 1).

Table 1: Comparison of various hematological parameters between subjects of different study groups (group C, Co-infection was not considered for comparison as only 3 subjects were present in that group)

Hematological parameters	GROUP	Mean	S.D.	S.E.M.	t	p Value
WBC (1000/cumm)	Malaria	7.46	2.66	0.27	16.013	<0.0001

	Dengue	3.97	1.67	0.09		
Neutrophils (%)	Malaria	68.85	9.91	1.01	17.50	<0.0001
	Dengue	41.62	14.41	0.74		
Lymphocyte (%)	Malaria	22.59	8.08	0.83	-15.12	<0.0001
	Dengue	45.25	14.11	0.72		
Monocyte (%)	Malaria	5.85	2.35	0.24	-10.14	<0.0001
	Dengue	10.10	3.93	0.20		
Eosinophils (%)	Malaria	2.45	1.74	0.18	-0.14	0.892
	Dengue	2.48	2.00	0.10		
Basophils (%)	Malaria	0.26	0.26	0.03	-1.47	0.144
	Dengue	0.31	0.32	0.02		
RBC (10 ⁵ /cumm)	Malaria	3.33	0.79	0.08	-14.11	<0.0001
	Dengue	4.49	0.71	0.04		
Hemoglobin (g/dl)	Malaria	9.53	2.27	0.23	-10.22	<0.0001
	Dengue	11.72	1.76	0.09		
Hematocrit (%)	Malaria	27.89	6.80	0.69	-13.73	<0.0001
	Dengue	38.25	6.57	0.34		
MCV (fL)	Malaria	83.73	8.86	0.90	-1.55	0.122
	Dengue	85.18	8.03	0.41		
MCH (pg)	Malaria	26.82	3.23	0.33	-4.78	<0.0001
	Dengue	28.52	3.09	0.16		
MCHC (g/dl)	Malaria	32.01	1.74	0.18	-5.74	<0.0001
	Dengue	33.43	2.25	0.11		
RDW (%)	Malaria	18.89	4.31	0.44	9.34	<0.0001
	Dengue	15.94	2.22	0.11		
PLT (1000/cumm)	Malaria	57.34	26.83	2.74	-6.60	<0.0001
	Dengue	87.99	43.49	2.22		
MPV (fL)	Malaria	10.08	1.38	0.14	-1.58	0.114
	Dengue	10.36	1.60	0.08		
PCT (%)	Malaria	0.06	0.03	0.00	-7.03	<0.0001
	Dengue	0.09	0.04	0.00		
PDW (%)	Malaria	18.64	3.03	0.31	2.98	0.003
	Dengue	17.59	3.10	0.16		

Among different malaria species mixed infection had significantly low RBC count, hematocrit and platelet count compared to subjects with *P. falciparum* and *P. vivax* infection. Hemoglobin was found to be significantly lower in *P. falciparum* and mixed infection subjects compared to *P. vivax* positive subjects. No significant difference was found in other hematological parameters.

Significantly low WBC count, neutrophil %, platelet count and plateletcrit but significantly high lymphocyte %, monocyte %, MCHC and RBC count were observed in subjects with both NS1 and IgM positivity compared to subjects with isolated NS1 and IgM positivity.

Among malaria cases mean hemoglobin was 9.53gm/dL, mean hematocrit was 27.89%, mean TLC was 7,460/cumm and mean platelet count was 57,340/cumm.

Among dengue cases mean hemoglobin was 11.72gm/dL, mean hematocrit was 38.25%, mean TLC was 3,970/cumm and mean platelet count was 87,990/cumm.

Distribution of study population on the basis of variation in the hematological parameters like hemoglobin, hematocrit, total leucocyte count and platelet count are shown in **Table 2, 3, 4 and 5**.

Table 2: Hemoglobin level among study population.

Hemoglobin gm/dL	Malaria (n=96)		Dengue (n=384)	
	N	Frequency (%)	N	Frequency (%)
3-5.9	7	7.3	4	1
6-8.9	30	31.2	11	2.9
9-11.9	48	50	204	53.1
12-15.9	10	10.5	162	42.8
>16	1	1	3	0.8

Table 3: Hematocrit among study population.

Hematocrit (%)	Malaria (n=96)		Dengue (n=384)	
	N	Frequency (%)	N	Frequency (%)
≤20	11	11.5	4	1
20.1-30	52	54.2	22	5.7
30.1-40	30	31.2	214	55.8
40.1-50	2	2.1	132	34.4
>50	1	1	12	3.1

Table 4: Total leucocyte count among study population.

TLC (per cumm)	Malaria (n=96)		Dengue (n=384)	
	N	Frequency(%)	N	Frequency(%)
≤4000	11	11.5	241	62.8
4,001-6,000	22	22.9	101	26.3
6,001-8,000	22	22.9	32	8.3
8,001-11,000	30	31.2	7	1.8
>11,000	11	11.5	3	0.8

Table 5: Platelet count among study population.

Platelet count (per cumm)	Malaria (n=96)		Dengue (n=384)	
	N	Frequency(%)	N	Frequency(%)
≤20,000	10	10.4	18	4.7
20,001-50,000	35	36.5	64	16.6
50,001-75,000	26	27.1	65	16.9
75,001-1,50,000	25	26	206	53.7
>1,50,000	0	0	31	8.1

DISCUSSION

In our study total number of cases were 483, out of which maximum number of cases were dengue positive i.e. 79.5% (384) followed by malaria positive i.e. 19.9% (96) and only 0.6% (3) co-infection cases were found. Our study had the maximum number of dengue cases due to the epidemic, which occurred during monsoon period in 2018. Study done by **M.K. Mohapatra et al¹⁶** found similar result in which out of 469 cases, 72.5% (340) were dengue positive, 21.8% (102) were malaria positive and 5.7% (27) cases were of co-infection. In **Arundhati Barua et al¹⁷** study 54.5% cases were dengue positive, 35.3% cases were malaria positive and 10.2% cases were of co-infection out of 156 total cases. Out of 340 cases in **Rajendra Kumar Verma et al¹⁸** study; dengue, malaria and co-infection cases were 45.8%, 38.8% 15.4% respectively. **Dhanya P. R. et al¹⁹** study also had highest number of dengue cases i.e. 93.9% followed by malaria 4.5% and only 1.6% cases were of co-infection cases among 332 total cases.

In our study all groups had higher male to female ratio i.e. 1.04:1, 1.52:1 and 2:1 in malaria, dengue and co-infection respectively. Study done by **M.K. Mohapatra et al¹⁶**, **Rajendra Kumar Verma et al¹⁸**, **Sarita Shrivastava et al²⁰** and **Dhanya P.R. et al's¹⁹** are in concordance with our study, they all found higher male to female ratio in all groups. The incidence is more common in males than females, is because males are more frequently exposed to the risk of acquiring infection than females because of their outdoor life which they lead. Further, female in India are usually better clothed than males, hence they are less exposed²¹.

In our study mean WBC count, neutrophils % and RDW are higher in malaria than dengue cases; while lymphocyte %, monocyte %, RBC count, hemoglobin, hematocrit and platelet are higher in dengue cases than malaria. Among rest of the parameters, there is no significant difference in malaria and dengue. Anemia in malaria can be due to hemolysis of parasitized RBC's, ineffective erythropoiesis, decreased RBC deformability which causes splenic pooling and phagocytosis. Increased RDW was also observed in malaria, which could be due to macrocytosis and red cell response to malarial parasite²². Unlike most of the study previously done, our study had less significant thrombocytopenia among dengue cases. This might be due to the reason that during our study period there was a dengue epidemic and due to awareness and early treatment seeking, patients presented early to the hospital after the onset of symptoms. In **Neha Chaudhary et al²³** study there is no significant difference among malaria and dengue in parameters like mean WBC count, DLC, RBC count, MPV, PCV and PDW. Hemoglobin, HCT, MCV, MCH and MCHC are higher in dengue cases while RDW and platelet were higher in malaria cases. Like in our study, **Manas Kotepui et al²⁴** study had mean WBC count and neutrophil % higher in malaria than dengue while lymphocyte % RBC count, hemoglobin and hematocrit were higher in dengue than malaria. But unlike our study mean eosinophil % was higher in dengue and mean platelet count was slightly higher in malaria cases. Rest of the parameters had no significant difference.

In our study, among malaria positive cases maximum number of cases seen in the month August followed by July, June and September. In dengue positive cases maximum number of cases also seen in the month August followed by September and October. 3 co-infection cases were found in 1 in the month of July, 1 in September and 1 in October. This is in concordance with the study done by **Anita Tahlan et al²⁵** who did study on dengue positive cases in 2017 and observed

maximum number of cases in August followed by July and September. In **Sarita Shrivastava et al²⁰** study September month had more number of cases, which was followed by October month. According to the above mentioned study; during monsoon months (June July, August) cases were lesser than the post monsoon/early winter months (September/October). Water stagnation and mosquito breeding could be the important reason behind this rise in number of cases in later months. Collection of water in various sites like coolers, pots, tyres etc favours the mosquito breeding. India being a tropical country provides suitable weather for mosquito to grow and increase the disease burden.

CONCLUSION

Vector borne diseases like malaria and dengue are not uncommon in our geographical area. Although the percentage of malaria and dengue co-infection is very less in our study, the chances of getting co-infection cannot be neglected and proper laboratory diagnosis is required to differentiate between malaria and dengue mono-infection with malaria and dengue co-infection.

Dengue infection formed the largest group in the present study. We recommend that all the patients presented with the febrile illness should be tested for both malaria and dengue. The two infections are clinically indistinguishable and specific diagnostic testing along with complete blood count is needed to confirm the diagnosis and to know the severity of the illness. So that the treatment would start early.

REFERENCES

- Charrel RN, Brouqui P, Foucault C, de Lamballerie X. Concurrent dengue and malaria. *Emerg Infect Dis* 2005;11:1153-4.
- Deresinski S. Concurrent Plasmodium vivax malaria and dengue. *Emerg Infect Dis* 2006;12:1802.
- Thangarathnam PS, Jeevan MK, Rajendran R, Samuel PP, Tyagi BK. Dual infection by Dengue virus and Plasmodium vivax in Alappuzha District, Kerala, India. *Jpn J Infect Dis* 2006;59:211-2.
- Bhalla A, Sharma N, Sharma A, Suri V. Concurrent infection with dengue and malaria. *Indian J Med Sci* 2006;60:330-1.
- Ward DI. A case of fatal Plasmodium falciparum malaria complicated by acute dengue fever in East Timor. *Am J Trop Med Hyg* 2006;75:182-5.
- Kaushik RM, Varma A, Kaushik R, Gaur KJ. Concurrent dengue and malaria due to Plasmodium falciparum and P. vivax. *Trans R Soc Trop Med Hyg* 2007;101:1048-50.
- Swati Aundhakar, Piyush Prajapati, Shakuntala Prajapati, Aditya Aundhakar, Divyen Kothia, Dany John, Akshay Shrisath, Mohit Khatri. Study of Clinical and Hematological Profile of Plasmodium vivax Malaria in a Tertiary Care Hospital in Western Maharashtra. *International Journal of Scientific Study* 2017;5(3):257-60
- Facer CA. Hematological aspect of malaria In: *Infection and Hematology*. Oxford Butterworth Heinemann Ltd;1994:259-94.
- Murphy GS, Oldfield EC. Falciparum malaria. *Inf Dis Clin North Am* 1996;10:747-75.
- Jandle JH. Hemolytic anemias caused by infection of red blood cells. In: *Blood*. 2nd edition. New York: Little brown and company, 1996:473-501.
- Rakesh Kumar Yadav, Sujit Kumar et al. To study hematological profile in malaria patients. *Int J Adv Med*. 2017 Jun;4(3):707-712
- World Health Organisation, 2009. Dengue : Guidelines for Diagnosis, Treatment, Prevention and Control. New Edition, World Health Organisation and TDR for research on diseases of poverty.
- Francisca Raimunda F.Guerreiro Azin et al. Dengue: profile of hematological & biochemical dynamics. *Rev Bras Hematol Hemoter*.2012;34(1):36-41.
- DENGUE. Guidelines for diagnosis, treatment, prevention and control, 2nd Edition, World Health Organization, Geneva. 2009;1-144.
- Richa Giri, Nirmala Singh, Chirag Gupta, Arisht Jain, Rajendra Kumar Verma et al. A study on Clinical Presentation and Outcome of Concurrent Malaria and Dengue Infection from a Malaria Endemic Zone of North India.
- M.K. Mohapatra et al. Manifestation and outcome of concurrent malaria and dengue infection. *J Vector Borne Dis* 49. 2012 December:262-265
- Arundhati Barua, Niharika Gill. A Comparative Study of Concurrent Dengue and Malaria Infection with their Mono-infection in a Teaching Hospital in Mumbai. *Journal of The Association of Physicians of India*. 2016 August;64:49-52
- Rajendra Kumar Verma et al. A Study on Clinical Presentation and Outcome of Concurrent Malaria and Dengue Infection from a Malaria Endemic Zone of North India. *JMSCR*. 2016 December;4(12):15116-27
- Dhanya P. R. et al. A comparative study on dengue and malaria infections and analysis on the prevalence of co-infection from a rural tertiary care hospital. *Int J Res Med Sci*. 2018 Jun;6(6):2111-211
- Sarita Shrivastava et al. Clinical, Hematological and Biochemical Profile of Malaria and Dengue in Kharghar, Navi Mumbai - A Hot Spot of Mosquito Breeding. *JMSCR*. 2017 July;05(07):24356-24362
- Kailash C. Meena et al. A study of hematological profile in dengue fever at tertiary care center, Kota Rajasthan, India. *International Journal of Advances in Medicine*. *Int J Adv Med*. 2016 Aug;3(3):621-624
- Lathia TB et al. Can hematological parameters discriminate malaria from nonmalarious acute febrile illness in the tropics? *Indian J Med Sci*. 2004 Jun;58(6):239-44.
- Neha Chaudhary et al. Comparison of Hematological Parameters in Various Acute Febrile Illnesses. *National Journal of Laboratory Medicine*. 2016 Jul;5(3):49-53
- Kotepui M et al. Differentiating between dengue fever and malaria using hematological parameters in endemic areas of Thailand. *Infectious Diseases of Poverty* (2017) 6:27 DOI 10.1186/s40249-017-0238-x
- Anita Tahlan et al. Haematological profile of dengue fever. *Int J Res Med Sci*. 2017 Dec;5(12):5367-5371