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RETROSPECTIVE CASE SERIES STUDY OF HEMATOLOGICAL PARAMETERS OF DENGUE FEVER PATIENTS FROM THE BANGLADESHI POPULATION

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ABSTRACT
Background: The objective of the present study was to evaluate the haematological parameters as screening markers for differentiating patients with dengue fever. **Methods:** This study was a retrospective case series study in Dhaka, Bangladesh. All data of patients of dengue infection from January 2019 to August 2019 was retrieved anonymously from computerized medical records including laboratory results. For statistical analysis, all analysis was performed by software GNU PSPP stable release 1.4.1/September 5, 2020. **Result:** The results showed that among 250 confirmed patients with dengue infection, MCV, MCH, HCT and PCT were significantly lower in patients with dengue fever (DF). The RBC count was found to be a little bit lower in patients with dengue infection. Hb was found to be much lower in female patients than in males as per standard limit. The platelet count was significantly lower in patients with dengue infection compared to patients with non-dengue infection. **Conclusion:** Alteration of haematological parameters can be combined with other clinical and laboratory markers to help physicians make an early diagnosis of dengue fever on the first day of admission to help closely monitor patients with dengue and prevent developing dengue haemorrhagic fever (DHF).

KEYWORDS: Dengue, Hematological parameters, Markers.

INTRODUCTION
Dengue fever (DF) is caused by one of the four serotypes of the dengue virus, including DEN-1, DEN-2, DEN-3, and DEN-4. This virus is an arbovirus or arthropod-borne virus that belongs to the genus Flavivirus of the family Flaviviridae.[1,2] Dengue has a variety of presentations, ranging from asymptomatic to an undifferentiated fever (dengue fever, DF) to the more severe forms, such as dengue haemorrhagic fever (DHF).[3] According to estimates by the World Health Organization (WHO), about 50 million cases of DF occur annually worldwide and 2.5 billion people live in risk areas.[4]

Nowadays, laboratory techniques to confirm dengue infection are detection of viral nucleic acid and viral antigens/antibodies. The detection depends on the phase of illness. At the beginning of the illness, the virus can be detected in serum, plasma, circulating blood cells and other tissues for 4-5 days. At the onset of illness, virus isolation, and nucleic acid testing or antigen detection can be performed to diagnose the dengue infection.[5,6] In routine screening tests for dengue, a rapid dipstick test of Dengue non-structural protein 1 antigen (NS1 Ag) Strip will serve as a useful diagnostic tool.[7] NS1 Ag, a highly conserved non-structural glycoprotein secreted by virus infected cells, was found to increase during the acute phase of DF infection.[8] Other than antigen detection, at the end of the acute phase of infection, antibody detection is the method of choice for diagnosis.[6] However, antibody detection may take several days.[9]

A previous study has shown that leukopenia is the most prominent haematological change with counts of less than $2x10^3$/L.[10] Moreover, thrombocytopenia can occur in 88% of cases.[10] The decreasing platelet counts have been found to predict the severity of the disease and are associated with increased haematocrit, increased liver enzymes, and altered coagulation profile.[11] Early distinction between dengue and non-dengue could help clinicians to identify patients who should be closely monitored for signs of DHF because only patients with severe DF and DHF cases should be hospitalized. The objective of the present study was to evaluate the hematological parameters as screening markers for patients who come to the hospital for dengue diagnosis. This will help identify patients with dengue fever from

non-dengue fever in order to help the physician with better management of those patients.

MATERIALS AND METHODS

Study design

The study was cross-sectional case series study to monitor the effects of Dengue Virus infection on different blood parameters attending for medical treatment at the Labaid Diagnostic Limited, Gulshan 2, Dhaka, Bangladesh.

Study Location

The study was conducted among the Dengue Virus infected patients at Labaid Diagnostic Limited, Gulshan 2, Dhaka, Bangladesh during January 2019 to August 2019.

Study Subjects

250 Dengue Virus infected patients.

Study Time

January 2019 to August 2019.

Study Samples

Whole blood from participants was drawn by venipuncture into dipotassium ethylenediamine-tetraacetate (K2-EDTA)-containing evacuated Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) after informed consent was obtained. Samples were packaged according to CDC guidelines and transported by courier in insulated containers to this CLIA-certified laboratory. Most samples are delivered within 4 hours of collection. Samples held overnight before delivery are kept at room temperature (65-76°F).[12]

RBC (indices), WBC, Platelet (indices) test method

The Sysmex XT-2000i used the electric resistance detecting method (impedance technology) with hydro dynamic focusing to measure RBC, PLT, MPV, MCV write out on first reference, and HCT. Fluorescence flow cytometry was used to measure WBC, Diff, the optical PLT count, and the reticulocyte count. The system employed a 633nm semi-conductor laser for flow cytometry analysis. For the measurement by flow cytometry of the proportional count, expressed as percent of the total WBC, of neutrophils (NEUT), lymphocytes (LYMPH), monocytes (MONO), and eosinophils (EOS), white cells were stained with fluorescent dyes that bind to both DNA and RNA. Side Scatter (SSC) is employed to determine the internal complexity of the cell-the size, shape, and density of the nucleus and granules of the cell. Fluorescence and scatter measurements were combined to characterize white cell populations. Basophils (BASO) were measured separately using cell size and SSC properties. Hb was measured photocolorimetrically using SLS-HGB, a cyanide-free method.[12]

The reagents required for the operation of the Sysmex XT-2000i are supplied by Sysmex America (Mundelein, IL) and are listed as follows.[12]

Reagent[12]	Function[12]
Cellpack	RBC/PLT and Hb Diluent; rinsing of instrument; hydrodynamic focusing
Stromatolyser-4DL	Diff lysing reagent
Stromatolyser-4DS	Diff stain
Stromatolyser-FB	Diluent for WBC count and lyses all cells except BASO
Sulfolyser	Non-cyanide Hb lyse (sodium lauryl sulphate)
Ret-Search (II)	Dilutes sample for reticulocyte analysis
Ret-Search (II) Dye	Stains reticulocytes and platelets for analysis

HCT and MCV were direct measurements on the Sysmex XT-2000i. The MCV is an average of all RBC size measurements collected in the impedance counter. The HCT is the sum of all the RBC size measurements and reported in proportion to the total volume of the analysis sample. Calculated red cell indices are MCH write out first reference, MCHC write out first reference, and RDW. RDW is reported on the Sysmex XT as both standard deviation from the mean red cell size (RDW-SD) and as coefficient of variation from the mean (RDW-CV).[12]

The Sysmex XT-2000i provided 2 PLT counts. One was an impedance count that both enumerates the platelets (I-PLT) and estimates MPV. The other was an optional optical count obtained in 1 of the flow analysis channels (O-PLT). The instrument also performed an optional reticulocyte count in 1 of the flow analysis channels. RBCs were stained, counted, and measured for size and fluorescence. Counts were expressed as percent of RBC (RET%).[12]

This laboratory operated under Clinical Laboratory Improvement Act (CLIA) certification. Calibration of the instrument was confirmed each day using 2 levels of controls (Sysmex e-Check Hematology Control for Sysmex X-Series Analyzers, Sysmex America), according to the manufacturer's recommendations. These recommendations were consistent with CLIA Interpretive Guidelines 493.1256(a)-(c) and 493.1256(d), Standard: Control procedures. Repeated analysis of a sample obtained from a healthy donor was used daily to confirm instrument precision, as per CLIA Interpretive Guideline 493.1253(b)(1)(i)(B), Precision (Reproducibility).[12]

Statistical Analysis

Data were assessed using the free software GNU PSPP stable release 1.4.1/ September 5, 2020. It has a graphical user interface and conventional command-line interface. It is written in C and uses GNU Scientific Library for its mathematical routines. The name has "no official acronymic expansion".[13]

RESULTS

In this study 250 infected patients blood parameters data were collected, and among them 120 participants were male and 130 patients were female.

Table-1: Distribution of Dengue Virus infected patients among different age and sex groups (n=250).

Age (Years)	Sex				Total	%
	Male	%	Female	%		
00-10	05	4.16	00	0.00	05	4.20
10-20	14	11.66	13	10.00	27	10.80
20-30	20	16.66	34	26.15	54	21.60
30-40	24	20.00	36	27.69	60	24.00
40-50	22	18.33	20	15.38	42	16.80
50-60	18	15.00	12	9.23	30	12.00
60-70	11	9.16	10	7.69	21	8.40
70-80	6	5.00	3	2.30	9	3.60
80-90	0	0.00	2	1.53	2	0.80
Total	120	100.00	130	100.00	250	100

In the male subjects, 4.16% (5) were in the 0-10 year age group. 11.66% (14), 16.66% (20), 20.00% (24), 18.33% (22), 15.00% (18), 9.16% (11), 5.00% (6), and 0.00% (0) male participants were in the 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, and 80-90 age groups consecutively. In the female subjects, 8.11% (6) were in the 0-10years age group. 0.00% (0), 10.00% (13), 26.15% (34), 27.69% (36), 15.38% (20), 9.23% (12), 7.69% (10), 2.30% (3), and 1.53% (2) female participants were in the 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, and 80-90 age groups consecutively. For males, the highest number of patients was found in the 30-40 year age group and then in the 20-30, 40-50 year age group. For females, the highest number of patients was found in the 30-40 year age group and then in the 20-30, 40-50 year age groups (Table-1).

Table-2: RBC count between different sex of the dengue virus infected patients (Male=120, Female=130).

RBC[10^6/uL]		Male	%	Female	%	Average
Bellow	3.90	15	12.50	41	31.53	22.40
Standard	3.90-4.80	45	37.50	70	53.84	46.00
Above	4.80	60	50.00	19	14.61	31.60

Among the male subjects, 12.50% (15) were with less than standard RBC count, 37.50% (45) with standard and 50.00% (60) with above the standard count. For the female subjects, 31.53% (41) with less than the standard count, 53.84% (70) with standard and 14.61% (19) with above the standard count (Table-2).

Table-3: Hb content between different sex of the dengue virus infected patients (Male=120, Female=130).

HB [g/dl]		Male	%	Female	%	Average
Bellow	12	36	30	104	80	56
Standard	12-15	84	70	26	20	44
Above	15	0	0	0	0	0

In respect of the male subjects, 70% (84) were with standard Hb content, 30% (36) with less than the standard and 0% (0) with above the standard content. The scenario was similar for female subjects. 20% (26) with standard content; and 80% (104) with less than the standard and 0% (0) with above the standard content.

Table-4: MCV of RBC between different sex group of the dengue virus infected patients (Male=120, Female=130).

MCV (femtoliters/cell)		Male	%	Female	%	Average
Bellow	81	56	46.66	64	49.23	48
Standard	81-101	64	53.33	64	49.23	51.20
Above	101	00	00	02	1.53	0.80

MCV was measured for both types of sex group. For male 53.33% (64) and female 49.23% (64) subjects were in standard range; 46.66 (56) male and 49.23% (64) female were in the below the standard range. 00% (0) male and 1.53% (2) female subjects were found with above the standard MCV value (Table-4).

Table-5: MCH content of RBC between different sex group of the Dengue Virus infected patients (Male=120, Female=130).

MCH (picograms/cell)		Male	%	Female	%	Average
Bellow	27	41	34.16	52	40	37.20
Standard	27-32	75	62.50	70	53.84	58
Above	32	04	3.33	08	6.15	4.80

MCH was measured for both types of sex group. For male 62.50% (75) and female 53.84% (70) subjects were in standard range of MCV value; 34.16% (41) male and 40.00% (52) female were in the below the standard range. 3.33% (4) male and 6.15% (8) female subjects were found with above the standard MCH content (Table-5).

Table-6: HCT/PCV Percentage between different sex of the Dengue Virus infected patients (Male=120, Female=130).

HCT (%)		Male	%	Female	%	Average
Bellow	39.60	77	64.17	122	93.48	79.60
Standard	36.90-54.50	43	35.83	08	6.15	20.40
Above	54.50	00	00	00	00	00

HCT for 35.83% (83) male and 6.15% (8) female subjects were within the standard range; 64.16%(77) male and 93.84% (122) female were below the standard range (Table-6).

Table-7: T-WBC count between different sex group of the Dengue Virus infected patients (Male=120, Female=130).

WBC[10^3/uL]		Male	%	Female	%	Average
Bellow	4	7	5.83	5	3.85	4.80
Standard	4-11	101	84.17	105	80.78	82.40
Above	11	12	10	20	15.37	12.80

Among the male participants 84.17% (101) were counted with standard range and 10% (12) were with above the standard range of the WBC count. 80.78% (105) female subjects' WBC count found within the standard range, 15.37% (20) were with above the standard count and 4.80% (12) found with less than 4000cells/µL (Table-7).

Table-8: Neutrophil count between different sex groups of the Dengue Virus infected patients (Male=120, Female=130).

Neutrophil (%)		Male	%	Female	%	Average
Bellow	2	11	9.16	6	4.61	6.80
Standard	2-7	98	81.70	100	76.92	79.20
Above	7	11	9.16	24	18.46	14.00

Both male and female (81.70% and 76.92%) Dengue Virus infected subjects showed standard neutrophils count those were within 2.00-7.00%. 9.16% (11) male and 4.61% (6) female subjects neutrophils count were below the 2-7% range (Table-8).

Table-9: Eosinophil count between different sex groups of the Dengue virus infected patients (Male=120, Female=130).

Eosinophil (%)		Male	%	Female	%	Average
Bellow	0.02	10	8.33	13	10	9.20
Standard	0.02-0.50	103	85.83	110	84.61	85.20
Above	0.50	7	5.83	7	5.38	5.60

In this study most of the male and female (85.83% and 84.61%) subjects eosinophils count were within the standard range 8.33% (10) male & 10% (13) female respondents showed count below the standard limit (Table-9).

Table-10: Lymphocytes count between different sex group of the Dengue Virus infected patients (Male=120, Female=130).

Lymphocyte (%)		Male	%	Female	%	Average
Bellow	1	6	5	9	6.93	6
Standard	1-3	92	76.67	91	70.00	73.20
Above	3	22	18.33	30	23.70	20.80

Lymphocytes count showed that most of the male and female (76.67% and 70.00%) subjects were within standard count. In case of more than standard count 18.33% were male and 23.70% were female subjects (Table-10).

Table-11: Monocytes count between different sex group of the Dengue virus infected patients (Male=120, Female=130).

Monocytes (%)		Male	%	Female	%	Average
Bellow	0.20	10	8.33	10	7.69	8
Standard	0.20-1	109	90.83	120	92.30	91.60
Above	1	1	0.83	0	0	0.40

In our study for male 0.20% (10) subject was found with low number of monocyte and for female 7.69% (10) was found with low monocytes. 90.83% (109) male and 92.30% (120) female subjects monocytes counts were within the standard range (0.20-1%) (Table-11).

Table-12: Platelets count between different sex of the Dengue Virus infected patients (Male=120, Female=130).

PLT ([10^3/uL]		Male	%	Female	%	Average
Bellow	150	98	81.67	100	77	79.20
Standard	150-400	21	17.50	30	23	20.40
Above	400	1	0.83	0	0	0.40

Number of 81.67% (98) male and 77% (100) female respondents were found with thrombocytopenia. 17.50% (21) male and 23% (30) female respondents were fit with standard platelet count. 0.83% (1) male and none of female precipitants platelets count found above the standard limit (Table-12).

Table-13: MPV of platelet cell between different sex of the dengue virus infected patients (Male=120, Female=130).

MPV (femtoliters)		Male	%	Female	%	Average
Bellow	6.50	00	0	0	0	0
Standard	6.50-12	73	60.83	90	69.23	65.20
Above	12	47	39.16	40	30.76	34.80

Regarding average size or MPV of platelet 60.83% (73) male and 69.23% (90) female subjects showed standard phenomena. None of male & female showed bellow phenomena. 39.16% (47) male and 30.76% (40) female subjects were found with above the standard MPV limit (Table-13).

Table-14: PCT Percentage between different sex of the dengue virus infected patients (Male=120, Female=130).

PCT (%)		Male	%	Female	%	Average
Bellow	0.19	99	82.50	103	79.20	80.80
Standard	0.19-0.39	19	15.80	22	17	16.40
Above	0.39	02	1.70	05	3.80	2.80

Regarding average size or MPV of platelets, 60.83% (73) male and 69.23% (90) female subjects showed standard phenomena. None of the males or females showed the below phenomena. 39.16% (47) male and 30.76% (40) female subjects were found to be above the standard MPV limit (Table-13).

DISCUSSION

In the present study DF was more common in females (52%). This is in accordance with a study in Delhi, India indicated that Dengue seropositive was found to be significantly associated with the female gender.[14,15,16] However, general studies both sexes are equally affected although a male to female ratio of 0.65:1.[17] Some studies found twice the number of male patients infected with dengue compared to females.[18,19]

DF was found in different age groups, the largest proportion was seen in the age group of 30-40 years. This was not in accordance with previous reports in

Thailand indicated that the age group with the highest incidence changed from those aged 5-9 years to those over 15 years of age.[20,21,22] This finding was consistent with the idea that the observed age shift might be a consequence of the demographic transition in Thailand.

This study revealed haematological changes in study population. WBC count was significantly okay in dengue infected patients. This is not in accordance with previous studies indicated that patients with dengue had significantly lower white blood cell (WBC) counts.[23,24] This study, neutrophils and lymphocytes counts were significantly good in DF. This was not in accordance with results of previous studies.[23,24,25,26,27] Monocytes and eosinophil count were found significantly good in DF compared to standard value. This is not in accordance with a previous study indicated leukopenia was the most prominent haematological changed during dengue infection.[24,28] Ration of low number of white blood cell and their absolute counts may be due to the reduction in WBC due to bone marrow suppression by dengue virus.[29] Our study did not match with these observations.

This study, lower RBC count (12.50% male, 31.53% female), MCV (46.66% male, 49.23% female), and MCH (34% male, 40% female) in patients with dengue were observed. This never been reported in any previous studies. This could be explained by occurring of haemolyticanaemia in dengue infected patients. However, haemolyticanaemia in dengue fever is considered rare and has been described in case reports in Malaysia, Sri Lanka, India and in a British traveler.[30,31,32,33]

This study, lower platelet count was observed in patients with dengue compared to standard count. This is in accordance with a previous study indicated significantly lower platelet.[23] According to the results, low platelet count is one of the criteria for diagnosis of DHF.[34] The ration behind thrombocytopenia among patients with dengue is currently unknown. However, previous studies indicated decreased production of platelets in DF and increased destruction of platelets in DHF.[24,35]

The previous studies indicated that routine laboratory markers help to reduce the cost for laboratory diagnostic screening.[25,36] In routinely diagnosis of dengue infection, dengue non-structural protein 1 antigen (NS1) is an antigen presenting in sera of dengue patients during acute phase of infection and responsible for pathogenesis of dengue.[37] NS1 Ag strip has a rapid, sensitive, and easy to use for the early diagnosis of DF at the presentation.[38] Moreover, several commercial kits available on the market for detecting anti-dengue antibodies such as Immunochromatographic test (IgM/IgG detection).[39,40] This study found that routine laboratory markers including MCV, MCH, MPV, PCT are useful in detecting laboratory-confirmed dengue infection. This predictive technique could be used to decide the priority of NS1 Ag strip.

There are several limitations in this study. Firstly, this study was the retrospective and single based-hospital might not reflect the large groups of dengue patients. However, these results may provide useful routine laboratory markers for diagnosing dengue in endemic area, which may help alertness of medical technologist or medical sciences technician performing dengue diagnosis as no single laboratory marker available for predicting DF infection.[41]

CONCLUSION

Alteration of hematological parameters can be combined with other clinical and laboratory markers to help physicians make an early diagnosis of dengue fever on the first day of admission to help closely monitor patients with dengue and prevent developing dengue haemorrhagic fever (DHF).

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