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Study on haematological parameters and non-structural protein (NS1) profile of dengue patients

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Abstract

This study evaluated the haematological parameters and Non-structural 1 (NS1) protein profile of dengue patients with the help of ELISA method. The blood sample of the patients (N=60) was collected from Kauvery Hospital, Hosur, Tamil Nadu. Haematological tests like haemoglobin, total WBC count, differential count and total platelet count were performed. High level of hemoglobin was recorded in the age group between 26 to 35 years and lower level of hemoglobin was found in the age group between 5 to 15 years. The high value of WBC was found in the age group between 5 to 15 and the low level of total count was found in the age group between 26 to 35 years. Male patients in the age group 5 to 15 years Neutrophil-75%, Lymphocyte-25% and Eosinophil-7% whereas female patients recorded N-61%, L-35% and E-5% respectively. In the age group 16 to 25 years, male patients recorded N-65%, L-35% and E-4% whereas female patients recorded N-57%, L-36% and E-8%. The high value of platelet count was found in the age group between 16 to 25 years in the male patients. High level of IgM was recorded in the age group between 5 to 15 years and low level of IgM was found in the age group between 26 to 35 years. In the age group between 16 to 25 years and 26 to 35 years, only male patients recorded high levels of NS1 and in the same age, female patients recorded as low level of NS1. The present study showed that dengue fever most commonly affect male population compared to females especially in the age group of 16 to 35 years.

Keywords: Dengue fever, Non-structural 1 (NS1) protein, blood cells, IgM level, Platelet count

1. Introduction

Dengue Fever is a mosquito transmitted viral diseases caused by the dengue virus or flavivirus. It is mainly transmitted to humans through the *Aedes aegypti* and other species of mosquitoes [1, 2]. Many people, especially children and in teens, no signs or symptoms were occur during initial stages of dengue fever. Occurrence of symptoms typically begins after three to fourteen days, when they get bitten from dengue virus causing mosquito [3, 4]. The affected person has 104°F degree fever with sudden increase in body temperature. The severe form of dengue fever leads to hemorrhagic fever, causing severe bleeding, low levels of platelets and sudden drop in blood pressure and may also cause sudden death [5, 6]. It increases if the person is infected by second, third or fourth time respectively. DENV (Dengue virus) is an enveloped, single-stranded positive-sense RNA virus [7, 8]. The RNA genome consists of approximately 10,700 nucleotides and encodes with 3,411 amino acids long precursor polyprotein containing three structural proteins (capsid [C], precursor membrane [prM], and envelope [E]) and seven non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). The structural proteins are components of the mature virus particle whereas the NS proteins are expressed only in the infected cell and are not packaged to detectable levels into mature particles [9, 10]. Throughout the world, millions of people are affected by dengue fever. Most commonly this fever affects in the tropical and sub-tropical regions like America, South America, Asia, Australia and Africa. Causes of dengue fever include changes in climate factors, temperature and humidity. As per the Data released by Directorate of the National Vector Borne Disease Control Programme (NVBDCP) 67,000 cases of Dengue fever have been caused as of 13th October 2019 in India. World Health Organization data shows that 2, 02, 795 cases were found in Asia. India has reported more than 67,000 cases of dengue fever cases till October 2019 according to Health ministry report. Of these, In Karnataka 12,756 of these, In Karnataka 12,756 cases were recorded due to monsoon season in which the increase of Stagnant or waste water conditions allows the vectors to breed. Dengue Fever affected cases

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were recorded in different states of India includes Maharashtra (7,863 cases), Uttarakhand (7,513 cases), Gujarat (5,819 cases), Kerala (3,075 cases), Bihar (1,588 cases) and Delhi (1,431 cases). The News Minute published a report of 3000 cases of Dengue affected in Tamil Nadu. From these 2,951 persons were treated for dengue fever. In Kauvery Hospital, Hosur in the month of September 2019 alone, 40 patients are hospitalized due to suspected dengue cases. The Dengue period is around June to August during the rainy season. It is believed that climate is an important factor for dengue transmission. Researchers are working on dengue fever vaccines. Considering the above facts in view, this study evaluated the haematological parameters and Non-structural 1 (NS1) protein profile of dengue patients with the help of ELISA method.

2. Materials and methods

2.1. Specimen Collection: The blood sample of the patients (N=60) was collected from the Kauvery Hospital, Hosur and the sample was centrifuged to get the serum for virological tests.

2.2. Diagnosis of IgM antibodies: About 80 μ L of IgM diluent in the Anti-IgM dengue coated wells was filled and 100 μ L of negative control was added in the first well. About 95 μ L of diluents was prepared and 10 μ L of serum sample in an Eppendorf tube was taken and 20 μ L of mixture was added in individual wells and 100 μ L of positive control was added in all the tubes. The mixture was incubated for a period of 1 hour at 37 °C. After incubation, the excess diluent was discarded and washed six times using buffer solution. The wells were blotted using filter paper to remove water droplets. About 100 μ L of conjugate was added in the wells to remove excess presents. The solutions were washed for six times and were blotted. About 100 μ L of IgM Tetramethylbenzidine (TMB) solution was added and kept under dark field area. Finally stop solutions was added on each wells to stop Ag-Ab reactions.

2.3. Diagnosis of Non-structural antigen protein (NS1) Level: About 50 μ L of NS1 diluent in the Anti-NS1 dengue coated wells were filled and 50 μ L of negative control in the first well was added. The serum samples were added in individual wells and 50 μ L of positive control was added later. The mixture was incubated for a period of 1 hour at 37 °C. After incubation, the excess diluent was discarded and washed six times using buffer solution. The wells were blotted using filter paper to remove water droplets. About 100 μ L of conjugate was added in the wells to remove excess presents. The solutions were washed for six times and were blotted. About 100 μ L of IgM Tetramethylbenzidine (TMB) solution was added and kept under dark field area. Finally stop solutions was added on each wells to stop Ag-Ab reactions.

2.4. Haematological parameters

2.4.1. Estimation of Hb: The calibrated tube of the hemoglobinometer was filled up to the mark (0.3 g/100 mL) with 0.1N hydrochloric acid. The blood specimen was drawn into the Sahli pipette (20 μ L) from anticoagulated venous blood. The blood was blow into the acid solution inside graduated tube. The specimen mixture was allowed to stand for 10 minutes at 37 °C.

The color of the diluted blood in the tube was compared with

the reference tube. The hemoglobin concentration was calculated in g/dL.

2.4.2. Estimation of total WBC count: Pipetted out 0.4 mL of the WBC diluting fluid into the test tube and 20 μ L of capillary blood was sucked in it. The blood sample was filled in the counting chamber. "W" marked areas were counted in all four squares. WBC (cells/mm³) was counted using the following formula: Number of WBC X $\frac{1}{4}$ X depth factor (0.1 mm) X dilution factor (20).

2.4.3. Estimation of differential count: A blood smear was made using glass slide. Leishman's stain was added to the blood smear and kept for 2 minutes and washed with distilled water. The slide was dried and a particular portion in the slide was examined under microscope and the blood cells were counted and expressed in percentage.

2.4.4. Estimation of total platelet count: The blood was drawn in the RBC pipette up to 0.5 mark and diluted with platelet diluting fluid up to 10 mark and mixed thoroughly. The counting chamber was cleaned and a glass cover was placed over it and a drop of this mixture was added and counting was done in the centrally located rolling as in terms of lakhs.

3. Results and discussion

3.1. IgM level of dengue patients: Laboratory diagnosis of dengue virus infection can be made by the detection of specific virus, viral antigen, genomic sequence, and/or antibodies. Serological detection of antibodies based on capture immunoglobulin M (IgM) enzyme-linked immunosorbent assay (ELISA) has become the new standard for the detection and differentiation of primary and secondary dengue virus infections. In this study, the male patients in the age group between 5 to 15 years recorded 1.50 index values whereas female patients recorded 2.46 index values respectively. The high level of IgM was recorded in the age group between 5 to 15 years and low level of IgM was found in the age group between 26 to 35 years (Table 1). In a study by Vaughn *et al.* [11] showed that primary infection is characterized by the presence of significant or rising levels of IgM antibodies in the period 3–5 days after onset of infection, and can persist for 3–5 months.

Table 1: IgM (Index values) of the dengue patients.

Age group (Years)	Male	Female
5 to 15	1.50	2.46
16 to 25	1.33	2.00
26 to 35	1.10	1.45

3.2. NS1 protein level of dengue patients: A dengue (NS1) test was diagnosed using ELISA kit method. The average NS1 level in the male patients between the age group of 5 to 15 years recorded 145.80 RU/mL, whereas female patients recorded under this age group recorded 74.71 RU/mL respectively. In the age group between 16 to 25 years and 26 to 35 years, both male and female patients recorded high levels of NS1 (Table 2). It was found that NS1 can be used as an early diagnostic marker during dengue infection because of the large amounts circulating in patient serum during the acute phase of the disease. The structural proteins are components of the mature virus particle whereas the NS proteins are

expressed only in the infected cell and are not packaged to detectable levels into mature particles [12].

Table 2: NS1 (RU/mL) of the dengue patients.

Age group (Years)	Male	Female
5 to 15	145.80	74.71
16 to 25	596.38	123.4
26 to 35	570.13	122.10

3.3. Haemoglobin level of the dengue patients: Male patients in the age group 5 to 15 years recorded 9.4 gms% whereas, female patients recorded 9.1 respectively. High level of hemoglobin was recorded in the age group between 26 to 35 years and lower level of hemoglobin was found in the age group between 5 to 15 years (Table 3). In this study, the dengue patients showed normal haemoglobin levels. A study revealed [13] that cross-reaction of proinflammatory mediators such as tumor necrosis factor (TNF)-alpha and anti-NS1 antibodies with surface proteins on endothelial cells causing apoptosis of these cells subsequently causes plasma leakage and increased haemoglobin levels.

Table 3: Average HB (GMS %) of the dengue patients.

Age group (Years)	Male	Female
5 to 15	12.4	11.6
16 to 25	14.5	13.3
26 to 35	15.5	14.6

3.4. Total WBC Count of the dengue patients: The white blood cell (WBC) count in enteric or dengue fever is often low. Leukocytosis is common in the first 10 days in children and may also result from bacteremia, localized infection, bowel perforation, or other extraintestinal complications. Male patients in the age group 5 to 15 years recorded 13,500 WBC cells whereas; female patients recorded 11,600 cells respectively. In the age group 16 to 25 years, male patients recorded 12,600 WBC cells whereas female patients recorded 5,300 cells respectively. The high value of WBC was found in the age group between 5 to 15 and the low level of total count was found in the age group between 26 to 35 years (Table 4). The levels of leucocytes can be used as an early marker of dengue infection as claimed by various studies [14, 15].

Table 4: Average WBC (cells/cu.mm) of the dengue patients.

Age group (Years)	Male	Female
5 to 15	13,500	11,600
16 to 25	12,600	5,300
26 to 35	7,600	12,400

3.5. Differential count of the dengue patients: Leukocytes are the cells that make up the majority of the immune system. It is the part of the body that protects itself against foreign substances and various types of infections and exists in all parts of the body, including the connective tissue, lymph system, and the blood stream. Male patients in the age group 5 to 15 years Neutrophils-75%, Lymphocyte-25% and Eosinophil-7% whereas female patients recorded N-61%, L-35% and E-5% respectively. In the age group 16 to 25 years, male patients recorded N-65%, L-35% and E-4% whereas female patients recorded N-57%, L-36% and E-8% respectively (Table 5).

In a study by Chaloe Wong *et al.* [16] found that dengue patients had higher neutrophil percentage predominately in

the first 5 days of the fever then this was reversed and the percentage of lymphocytes increased as well as reversed neutrophil to lymphocyte ratios occurred on day 6 to 9 of fever.

Table 5: Differential count of the dengue patients.

Age group (Years)	Male	Female
5 to 15	N-75%	N-61%
	L-25%	L-35%
	E-7%	E-5%
16 to 25	N-65%	N-57%
	L-35%	L-36%
	E-4%	E-8%
26 to 35	N-54%	N-83%
	L-44%	L-10%
	E-2%	E-7%

3.6. Total platelet count of the dengue patients: Platelets are the parts of cells that the body uses for clotting and helps to promote other blood clotting mechanisms. These cells secrete procoagulant (clotting factors) to promote blood clotting and also secrete chemicals that attract neutrophils and monocytes to sites of inflammation. The average platelet count in the male patients in the age group 5 to 15 years recorded 1.7 lakhs respectively whereas; female patients also recorded 1.6 lakhs respectively. In the age group 16 to 25 years, male patients recorded 3.7 lakhs whereas female patients recorded 2.8 lakhs respectively. The high value of platelet count was found in the age group between 16 to 25 years in male patients (Table 6). A study [17] found that decreased platelet count was recorded in patients diagnosed by dengue fever. Decreased levels of platelets, a clinical condition called as thrombocytopenia is an important diagnostic parameter in identifying dengue patients [18, 19].

Table 6: Total platelet count of the dengue patients.

Age group (Years)	Male	Female
5 to 15	1.7 lakhs	1.6 lakhs
16 to 25	3.7 lakhs	2.8 lakhs
26 to 35	2.1 lakhs	3.8 lakhs

4. Conclusion

In this study it was found that high level of IgM was recorded in the age group between 5 to 15 years and low level of IgM was found in the age group between 26 to 35 years and In the age group between 16 to 25 years and 26 to 35 years, only male patients recorded high levels of NS1 and in the same age, female patients recorded as low level of NS1. The present study shows that dengue fever most commonly affect male population compared to females especially in the age group of 16 to 35 years. The haematological parameters tested in this study showed normal levels both in male and female dengue patients. From the study it may be concluded that there is no specific medicine to treat dengue infection. If we feel dengue fever we can avoid taking analgesics like Acetaminophen and Aspirin which can increase bleeding. Some precaution measures like taking rest, drinking plenty of water and consulting the doctor at right time may keep us away from dengue. Dengue virus can be prevented keeping our surrounding clean by covering stagnant water bodies and covering the body with appropriate clothes and also using mosquito repellents to avoid being bitten.

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