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Study of laboratory markers of Dengue virus infection in pyrexia cases at a tertiary care centre.

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Abstract:
Background: *Pyrexia is defined as an elevated core body temperature of more than 98.9^{0}F. Dengue is recognized as world's major emerging tropical disease with potential fatal complications caused by single stranded RNA virus belonging to family Flaviviridae and transmitted by Aedes species mosquitoes.*
Purpose: *Study of the laboratory markers of dengue virus infection in pyrexia cases, for the early and specific diagnosis of dengue infection that help in definitive treatment & supportive therapy to reduce morbidity and mortality.*
Methods: *Blood samples collected from 400 acute pyrexia cases clinically suspected as Dengue in both sterile and EDTA tubes for ELISA tests and complete blood picture respectively. Serum separated and tested for Dengue NS1 & IgM using ELISA method. The haematological parameters (total leukocyte count, differential leukocyte count and platelet count) were recorded.*
Results: *Out of the 400 samples 78 were Dengue NS1 antigen positive, 64 were Dengue IgM antibody positive and 19 were positive in both tests. Luecopenia (< 4000/ µl) was observed in 38.75% and lymphoctosis in 25% . Thrombocytopenia with <100,000/µl was observed in 26.25% .*
Conclusion: *Early detection of Dengue can be achieved effectively by using NS1 & IgM capture ELISA and Haematological profile helps in diagnosis of complications of Dengue fever and used to monitor the prognosis.*
Key Words: *Dengue virus infection, pyrexia, NS1Ag, Capture ELISA, lab markers*.

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I. Introduction:

Dengue fever is one of the most common causes of viral pyrexia & is being recognized as the world's major emerging tropical disease. [1] Dengue is the most serious mosquito-borne viral disease and affects upto 100 million people each year, with a mortality rate of 20%. [2] Dengue virus is a single-stranded, positive sense RNA virus, belonging to family Flaviviridae & Genus-Flavivirus. It is mainly transmitted by female Aedes aegypti & Aedes albopictus mosquitoes. [3] Clinically, Dengue infection may resemble other infections like malaria, typhoid and leptospirosis, [4] but it is an acute viral infection with potential fatal complications like dengue haemorrhagic fever and dengue shock syndrome. Thus, lab investigations are required for early and definitive diagnosis[5,6,7].

Therefore, the present study was undertaken to study the lab markers of Dengue virus infection i.e NS1 Ag, IgM antibody (IgM Ab) and other haemotological parameters, which help in definitive diagnosis and proper management of the disease.

II. Materials and Methods:

A cross-sectional study was conducted in the Dept of Microbiology, at Sir Ronald Ross Institute of Tropical and Communicable Diseases (SRRITCD), Hyderabad, from September 2019 to December 2019. Institutional Ethics Committee approval was obtained prior to the study.
Sample size: 400 blood samples from pyrexia cases.
Inclusion criteria:

- Patients of all ages and both sexes, attending OPD with pyrexia of <7days duration & admitted for the same.
- Patients belonging to different socio-economic status.
- Patients giving informed, written consent.

Exclusion criteria:

- Pyrexia cases admitted for other than Dengue infection.
- Pyrexia cases with major co-morbidities.
- Pyrexia cases not giving consent.

Methodology: [8,9,]

A total of 4ml blood was collected from all the patients, who were included in the inclusion criteria, of which 2ml blood was put in plain vacutainers for serological testing & the remaining 2ml in EDTA tubes for testing haemotological parameters.

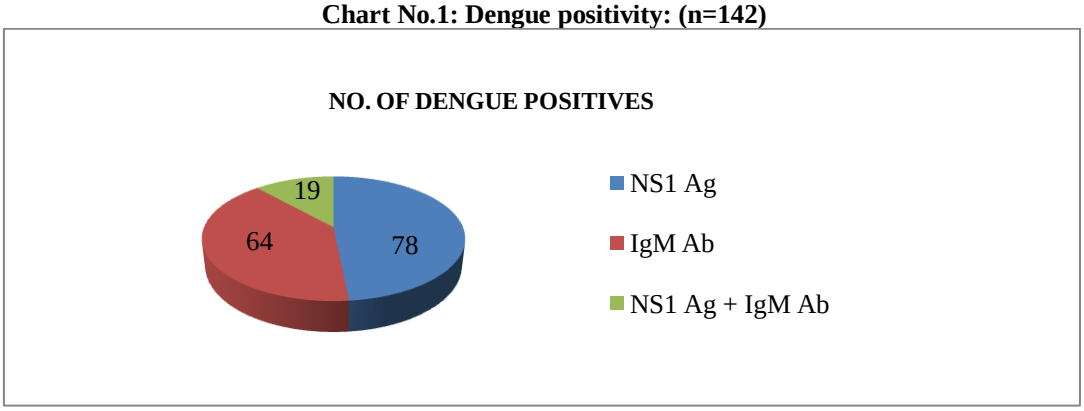
Serum obtained in plain tubes was tested for the presence of Dengue NS1 Ag (MERIL ELISA Kit), and also for the presence of IgM antibodies (MAC ELISA, NIV Pune), according to manufacturer's instructions.

The blood in EDTA tubes was used for testing hematological parameters like Hematocrit(PCV), Total Leukocyte count (TLC), Differential Leukocyte count(DLC), and platelet count.

III. Results:

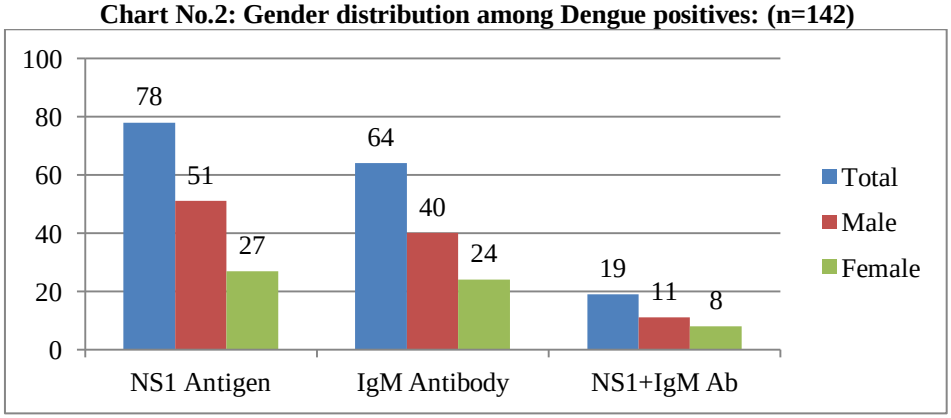
Out of the 400 samples tested, 142(35.5%) were dengue positives, out of which, 78 were NS1 Ag positives and 64 were IgM antibody positives. 19 samples were positive for both NS1 Ag and IgM antibody.

Chart No.1: Dengue positivity: (n=142)



Out of the 142 Dengue positives, 91 were males & 51 were females. Among the 91 males, 51 were positive for NS1 Ag, 40 were positive for IgM Ab and 11 showed both NS1 Ag & IgM Ab. Among the 51 females, 27 showed NS1Ag positive, 24 were IgM Ab positive & 08 members were both NS1 Ag & IgM Ab positive. The present study showed that males were predominantly affected than females, with a male:female ratio of 1.78:1. The predominant lab marker detected in both males & females was NS1 Ag (54.92%).

Chart No.2: Gender distribution among Dengue positives: (n=142)



Among the 91 Dengue positive males, the most affected age group was between 11 to 20 yrs (43 positives) followed by 21 to 30 yrs (30 positives). The least affected age group was >50 yrs, with only 02 positives. Among all the age groups, the predominant marker detected was NS1Ag.

Table No.1 : Age – wise distribution among Dengue positive Males (n=91)

AGE	NO. OF MALES	NS1 AG POSITIVES	IgM AB POSITIVES	BOTH POSITIVES
< 10 yrs	05	02	03	01
11 – 20 yrs	43	29	18	05
21 – 30 yrs	30	12	14	03
31 – 40 yrs	06	04	02	01
41 – 50 yrs	05	03	02	01
> 50 yrs	02	01	01	00
TOTAL	91	51	40	11

Among the females, the most common age group affected was between 21 to 30 yrs (22 positives), followed by 11 to 20 years age group, showing 14 positives. The least affected age group was >50 yrs, with only 02 positives.

Table No. 2: Age – wise distribution among Dengue positive Females (n=51)

AGE	NO.OF FEMALES	NS1 AG POSITIVES	IgM AB POSITIVES	BOTH POSITIVES
< 1O yrs	03	01	02	01
11 – 20 yrs	14	07	07	01
21 – 30 yrs	22	12	10	04
31 – 40 yrs	07	05	02	01
41 – 50 yrs	03	01	02	00
> 50 yrs	02	01	01	01
TOTAL	51	27	24	08

The haemotological parameters detected during the present study were: Total leukocyte count (TLC), Differential Leukocyte count(DLC), Platelet count and Haematocrit. Among the 142 Dengue positives, 101 patients had a TLC of <4000/µl, indicating leukopenia, which is a marker of dengue severity. 106 of them had lymphocytosis of >40%. And 97 patients showed a haemotocrit of >45%, indicating severe dengue. Thrombocytopenia, which is a marker of dengue severity, was seen in 103 /142 positives, out of which 93 patients had a platelet count of 50,000-1.5lakh/cu.mm , whereas 10 patients had a platelet count of <50,000/cu.mm.

Chart No. 3: Platelet count of Dengue positive patients: (n=142)

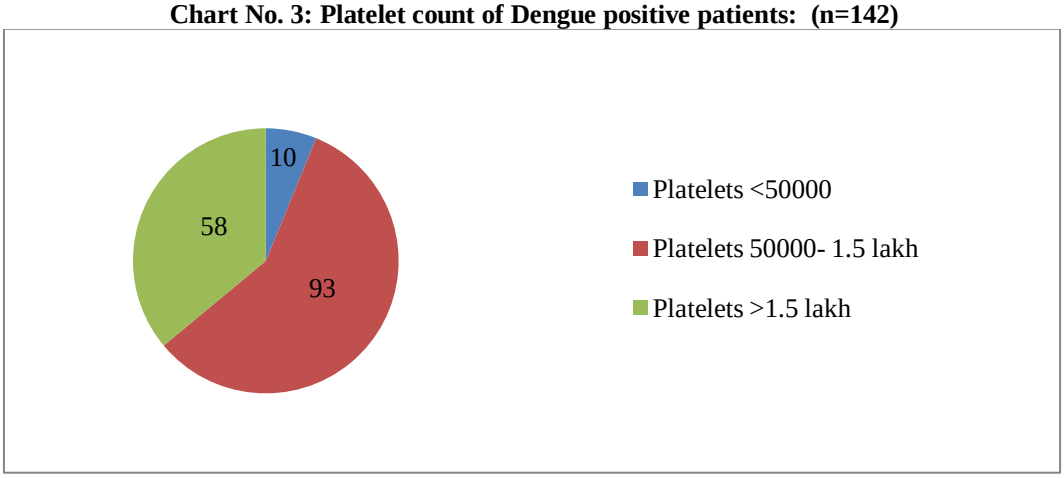


Table No. 3: Haemotological Parameters in Dengue positive patients: (n=142)

PARAMETER	NS1 +VE (78)	IgM +VE(64)	BOTH +VE (19)
LEUCOCYTE COUNT			
< 4000/µl	**50 (64.1%)**	**40 (62.5%)**	**11 (57.89%)**
> 11000/µl	**28**	**24**	**08**
LYMPHOCYTES			
< 40%	**26**	**23**	**06**
> 40%	**52 (66.66%)**	**41 (64%)**	**13 (68.42%)**
HAEMATOCRIT			
< 45 %	**31**	**26**	**07**
> 45 %	**47(60%)**	**38(59.37%)**	**12(63.15%)**

IV. Discussion :

Dengue is a tropical and subtropical mosquito-borne infection that can cause severe illness and death. During the past 30 years, dengue fever has dramatically expanded its geographical range and shortened its epidemic cycle in many places. According to the World Health Organization (WHO), dengue is endemic in over 100 countries and approximately two-fifths of the world's population is currently at risk for dengue fever with an estimated 50 million infections annually.[10] Among the estimated 2.5 billion people at risk globally for dengue, about 1.8 billion (i.e. more than 70%), reside in Asia Pacific countries. [11]

In order to provide timely information for the management of patients and early public health control of dengue outbreaks, it is important to establish the diagnosis of acute dengue virus infection during the first few days of manifestation of the clinical symptoms. [12]

The NS1 antigen is a highly specific marker of dengue infection, as there is no cross-reaction of the dengue NS1 protein, with those of other related flaviviruses. Detection of NS1 has been a promising test to diagnose dengue in its early febrile stage, due to its long half-life in blood. [13] In the present study, dengue positivity was 35.5%, which closely correlated with the study done by R.D.Kulkarni et al [14] , i.e 32.4%. and the percentage of NS1 Ag positives in the present study (54.92%), correlated well with the studies done by Juthatip et al [15] i.e 57.79% and Patel Bhavika et al [16] i.e 57.1%. This observation clearly indicates that NS1Ag is a highly specific marker and is a very useful tool in the early diagnosis of dengue infection. Thrombocytopenia in the present study was 62.5%, which correlated well with the study done by R.D.Kulkarni et al i.e 63.7%. Raised haematocrit(>45%) was seen in 60% of the positive cases in the present study, which correlated with the study done by Juthatip et al, where 61.65% of the positives showed raised haematocrit. This suggests that there is a strong correlation between dengue seromarkers and hematological parameters in dengue positive cases.

The reasons for male predominance in the present study, could be due to the greater exposure of males to Aedes mosquitoes during the daytime, either at workplace or while travelling to & from work. This observation too was seen by R.D.Kulkarni et al and Patel Bhavika et al, in their respective studies.

Table No. 4: Comparison of Dengue parameters with other studies:

Study	Dengue Positivity	NS1Ag positives	Thrombocytopenia (<1.5 lakhs/c.mm)	Raised Hematocrit (>45%)	Gender Predominance (M:F)
RD Kulkarni et al [14] (Jul 2010-Jan 2011)	32.4%	29.68%	63.7%	--	1.7:1
Juthatip et al [15] (Sept 2013-Jul2015)	15.2%	57.79%	59.7%	61.65%	0.9:1
Patel Bhavika et al [16] (Jan2016-Dec2016)	11.52%	57.1%	52.9%	49.5%	1.9:1
Present Study (Sept 2019-Dec2019)	35.5%	54.92%	62.5%	60%	1.78:1

V. Conclusion :

Early diagnosis of dengue can be done effectively by performing ELISA to detect NS1 Ag and IgM antibody. Haematological parameters like thrombocytopenia, leukopenia, and raised hematocrit help in provisional diagnosis and act as markers of disease severity & complications. Prompt diagnosis and immediate specific treatment, with maintenance of platelet count, gives good recovery in dengue cases.

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