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RESEARCH ARTICLE

DETECTION OF NS1 DENGUE ANTIGEN INSUSPECTED DENGUE FEVER CASES ATTENDING TERTIARY CARE HOSPITAL AND CORRELATE THE PRESENCE OF NS1 ANTIGEN WITH THE DURATION AND SEVERITY OF ILLNESS

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Abstract

Background: Dengue virus infection is one of the oldest diseases, and globally re-emerging mosquito-borne disease. Dengue viruses belong to the members of the genus *Flavivirus* within the family *Flaviviridae*. Patients presenting with acute dengue disease may be viraemic, but may not have developed antibodies. Early diagnosis depends mostly on detection of viral components such as the RNA. This study aims at determining NS1 positivity and correlation with the duration of illness.

Methods: A total of 489 fever cases consisting of both outpatient and inpatients with clinical presentation suggestive of "Probable Dengue" were included in the study. The blood samples were collected. NS1 antigen ELISA procedure was done for the serum samples using Panbio ELISA kit by strictly following the manufacturer's protocol. Descriptive analyses were performed to calculate means and standard deviation for continuous variables and relative frequency for categorical variables.

Results: The test showed 47 cases reactive for dengue NS1 among them, 29 cases were detected in 1-5 days, 17 cases were detected in 6-10 days and only one case was detected after 10 days. Applying a non-parametric test showed that the increased value of Panbio units is associated with the severity of disease.

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Introduction:-

Dengue virus infection is one of the oldest diseases, and globally re-emerging mosquito-borne disease [1,2]. Clinical presentation has a diverse range varying from self-limiting mild dengue fever leading to severe dengue and shock [2]. This diversity is due to the presence of four distinct serotypes namely Dengue virus 1, Dengue virus 2, Dengue virus 3 and Dengue virus 4 [3,4]. The initial signs and symptoms of dengue fever are similar to other viral fevers [5]. So a rapid laboratory diagnosis is indispensable and enables to initiate proper medical care which reduces the case fatality below 1%, as the estimated case fatality ratio is 2.5% [5,14]. As the treatment is symptomatic, its success is entirely based on an appropriate and early diagnosis of cases provided by laboratory confirmation [5].

Dengue viruses belong to the members of the genus *Flavivirus* within the family *Flaviviridae*. They are lipid-enveloped, positive-sense, single-stranded RNA viruses [7,8]. The genome of dengue virus is composed of three structural protein genes that encode the Nucleocapsid core protein, a membrane-associated protein, an envelope protein and seven non-structural proteins – NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 [7,8].

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Among these NS1 proteins that interact with the host immune system and evokes T-cell responses, NS1 induces the interleukin-10 production in the host by the monocytes^[9]. This contributes to the pathogenesis of dengue fever^[9]. Detection of Non-structural protein by NS1 based antigen capture ELISA correlates with severity of disease and is secreted from infected cells into the blood during the febrile phase^[9,10].

The laboratory diagnosis of dengue is mainly dependent on serology using IgM-capture enzyme-linked Immune-Sorbent assay^[11]. But the antigenic cross-reactivity between the members of Flaviviridae family reduces the specificity of this^[11] assay[]].

Patients presenting with acute dengue disease may be Viraemic, but may not have developed antibodies^[14]. Therefore, early diagnosis depends mostly on detection of viral components such as the RNA. This study aims at determining NS1 positivity and to correlate with the duration of illness.

Materials and Methods:-

This study was carried out in the Department of Microbiology, Department of Immunology, Department of General Medicine, Department of Social Pediatrics, Government Stanley Medical College and Hospital, Chennai during the period November 2017 to August 2018. A total of 489 cases selected by simple random sampling were included in this study. All cases of fever presenting with any two of the following symptoms of Headache, Myalgia, Arthralgia, Nausea, Vomiting and Rash suggestive of “Probable Dengue” were included in the study.

Both the cases admitted in the ward and those attending as out-patients of all age groups were included. Ethical and research clearance was obtained from Institutional Ethical Committee, Government Stanley Medical College, Chennai. The respective department authorities were sought for permission to conduct the study. Every patient included in the study were explained about the procedure of blood collection in their language and obtained an informed consent before enrollment into study.

Sample Collection and processing.

A detailed history was elicited and all information regarding personal identity, address, and biochemical parameters were noted. An informed consent was obtained from each patient. Under strict aseptic precautions 5ml of venous blood was collected in red cap Vacutainer with clot activator. The blood samples were centrifuged at 1100-1300 rpm for 15 minutes for serum separation. Each separated serum samples were Alliquoted into two Eppendorf and labeled. One set of serum samples were stored at -80° C freezer.

NS1 antigen ELISA procedure was done for the serum samples using Panbio ELISA kit by strictly following the manufactures protocol

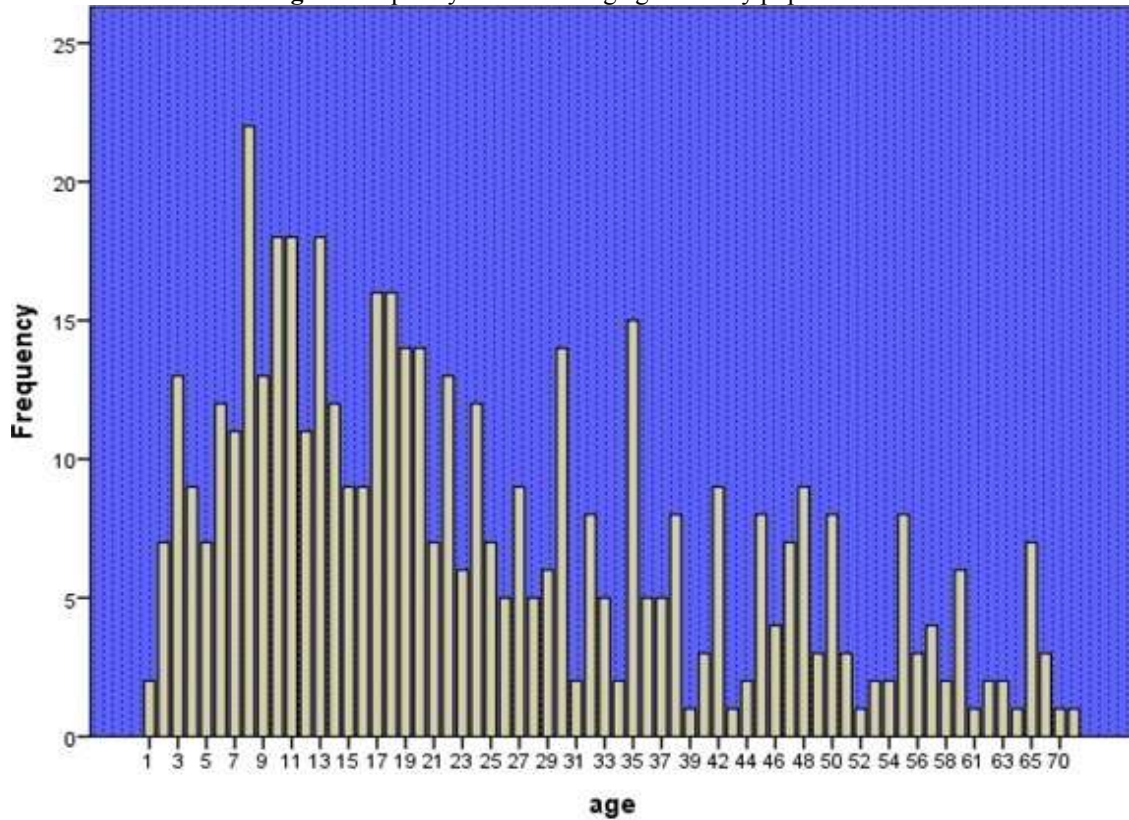
Statistical analysis

Descriptive analyses were performed to calculate means and standard deviation for continuous variable and relative frequency for categorical variable. Statistical analysis was performed using SPSS version 16.0. Chi-Square tests with Yates correction were performed for statistical comparison and correlation coefficient was calculated for bivariate data. An Anova test was checked for the significance of association the three categories with the Panbio units of detected NS1 antigen.

Observation and Results:-

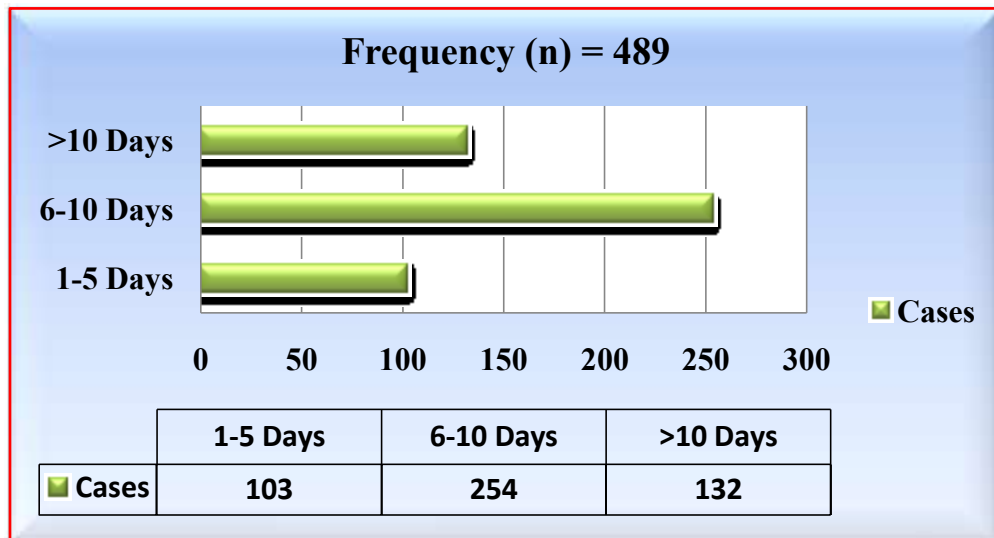
General characteristics of the study population

Fig.1:- FrequencyChart showing age of study population.



Frequency Chart showing age of study population .The mean age of the patients is 24 with a range of 1 to 70 years and the standard deviation 17.09 (Fig.1)

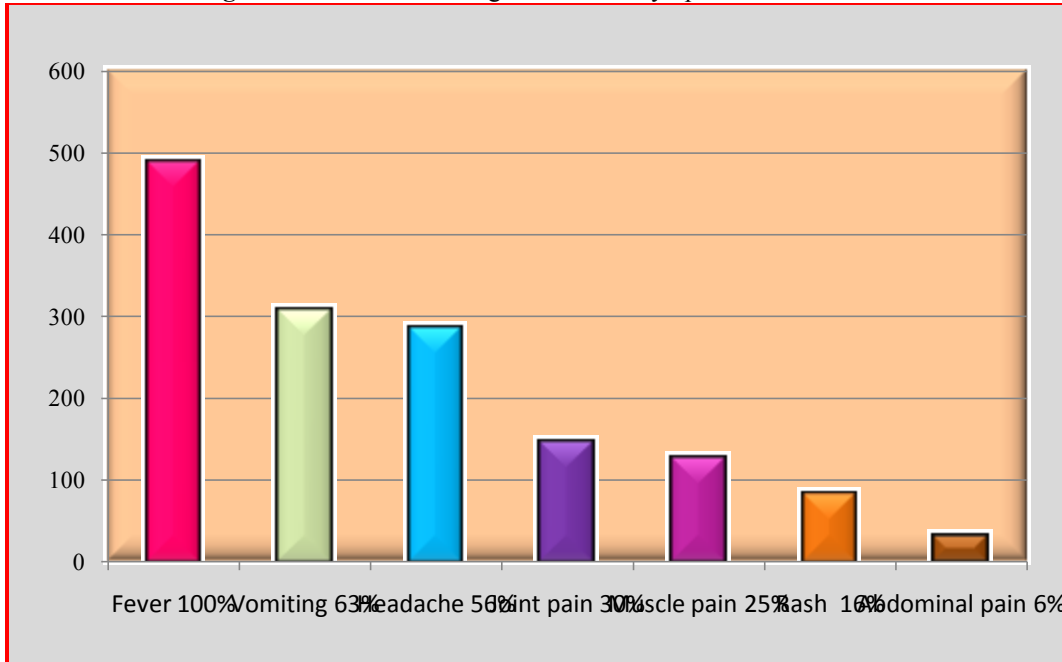
Fig.2:- Bar Chart showing the frequency of all fever cases based on duration of fever.



There were 103 cases with fever of 1 to 5 days duration, 254 cases with fever of 6 to 10 days and 132 cases with fever of more than 10 days as shown in Fig:3. There were comparatively more number of cases in fever of 6 to 10 days.

Common Symptoms of Study Population

Fig.3:- A Bar chart showing the commonsymptoms of all cases.

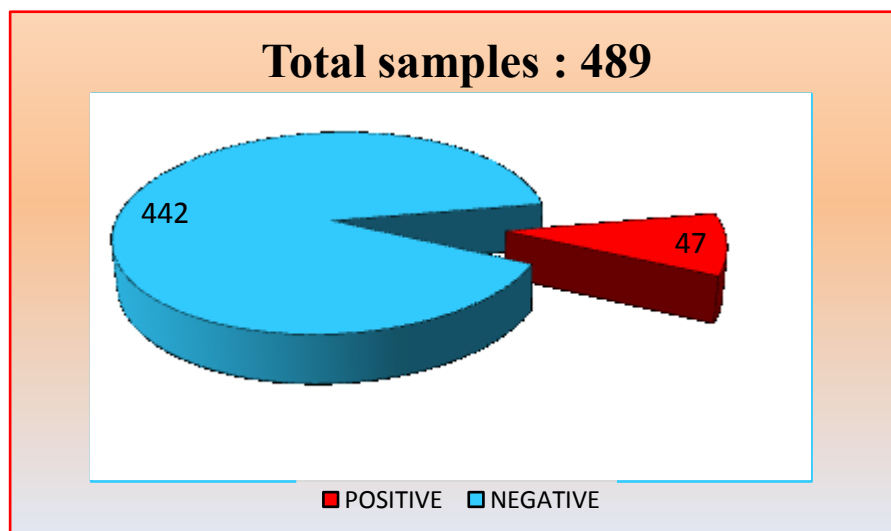


All cases of fever had the above associated symptoms suggestive of “probable dengue” such as nausea /vomiting 308(63%), headache 286 (56%), joint pain 147 (30%), muscle pain 126 (25%), rash 83 (16%) and abdominal pain 31 (6%).

Detection of NS1 Antigen By ELISA

	Frequency	Percent	Valid Percent	Cumulative Percent
Positive	47	47	10	10
Negative	442	442	90	100
Total	489	489	100	

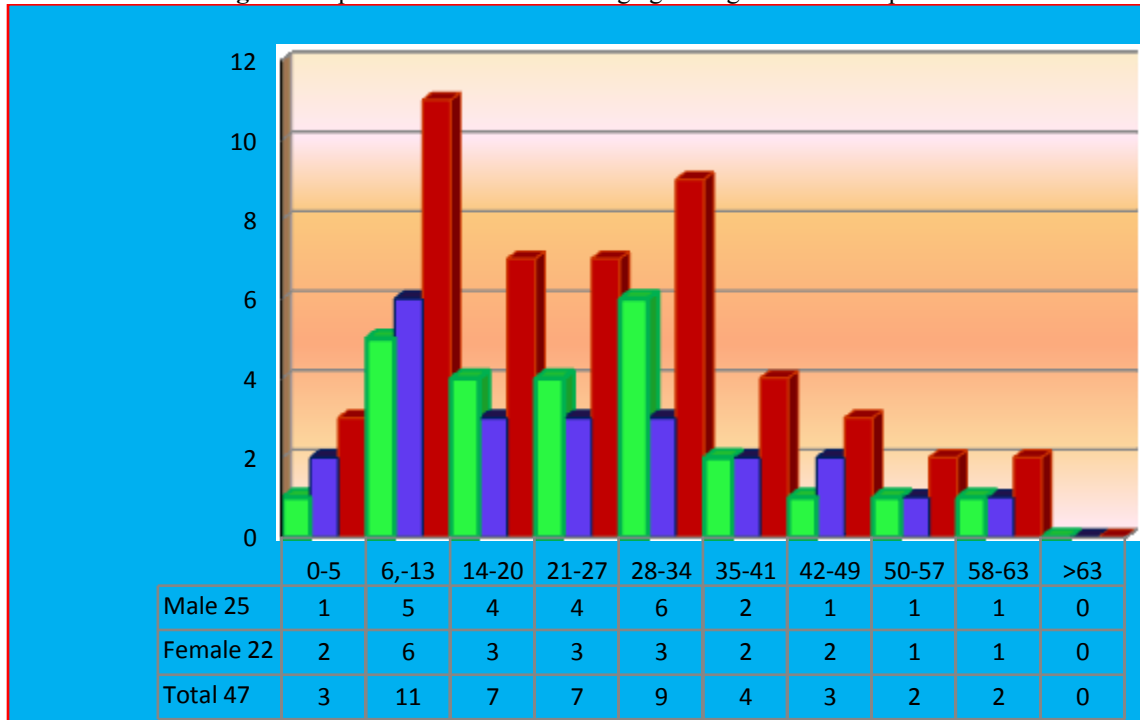
Fig 4:- Pie chart showing NS1 antigen detection.



Out of 489 patients tested for dengue fever by NS1 ELISA 47 (9.6%) cases were found to be reactive

Age and Gender Wise distribution of NS1 Positivity

Fig. 5:- Proportional bar chart showing age and gender of NS1 positive cases.



Among the total 47 cases positive for dengue NS1, 25 (53%) were males and 22 (47%) were females. This chart clearly reveals that the highest number of cases belonged to the age group 6 to 13 years (23%) with predominance in female children. This was followed by the age group of 28-34 years (19%) with higher prevalence in males.

Ns1 Positivity and Duration of Fever

Fig.6:- Line chart of NS1 positivity and duration of fever.

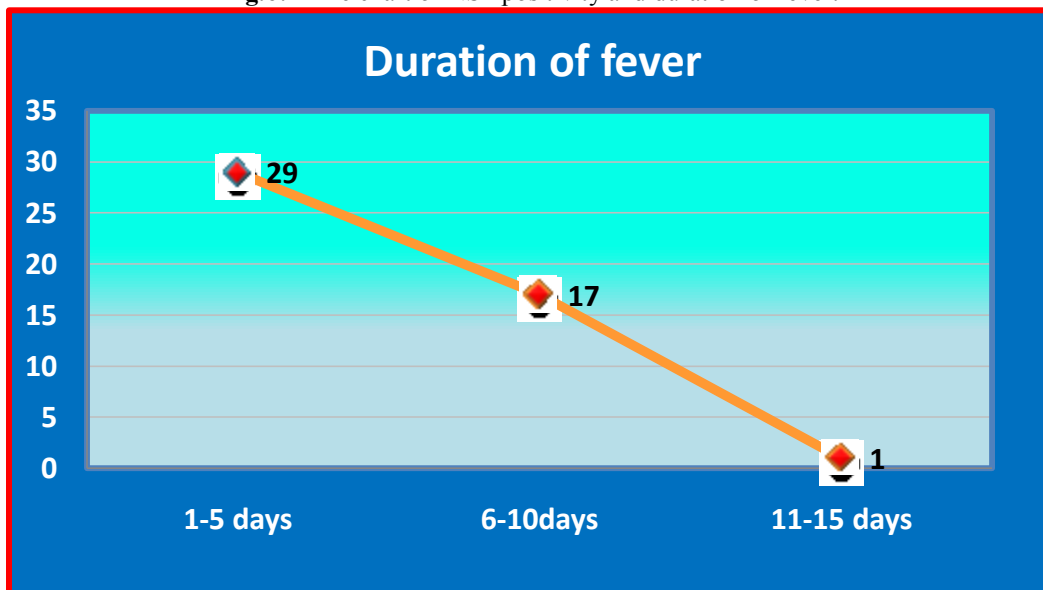


Fig.6: Shows that 29 cases were detected in 1-5 days, 17 cases were detected in 6-10 days and only one case was detected after 10 days.

As detection of NS1 decreases with duration of fever, the study population cases were sorted based on duration of fever into 3 groups. A chi-square test is applied with help of (2*3) contingency table.

Biochemical Parameters

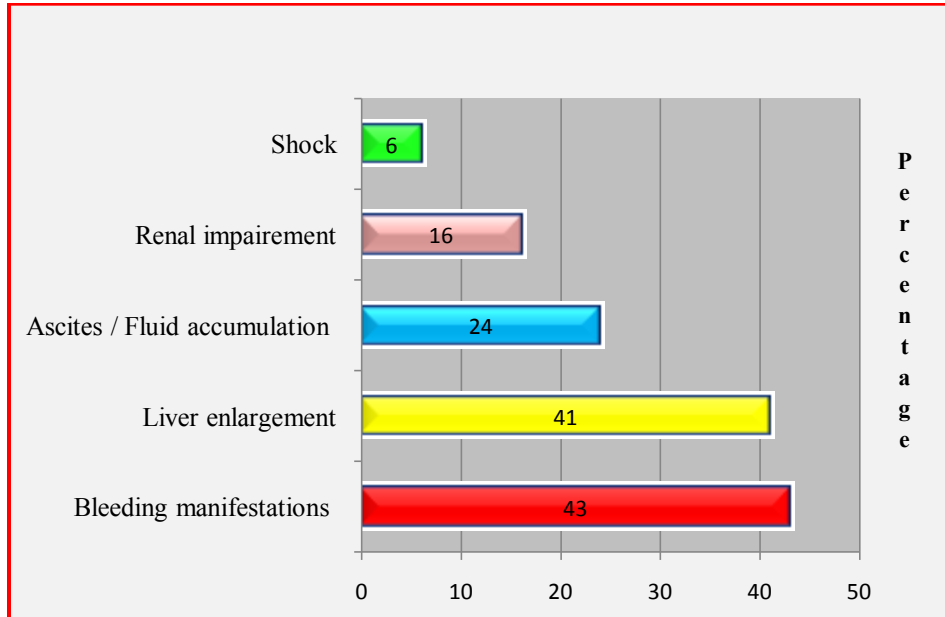


Fig:7, Shows the clinical complications present in cases, bleeding manifestations seen in 43, liver enlargement seen in 41, fluid accumulation 24, renal impairment 7 and shock 6.

Thrombocytopenia In Dengue Positive Case

Out of the 489 cases detected, 43 cases had thrombocytopenia and among these 21 were NS1 positive cases.

Degrees of Thrombocytopenia

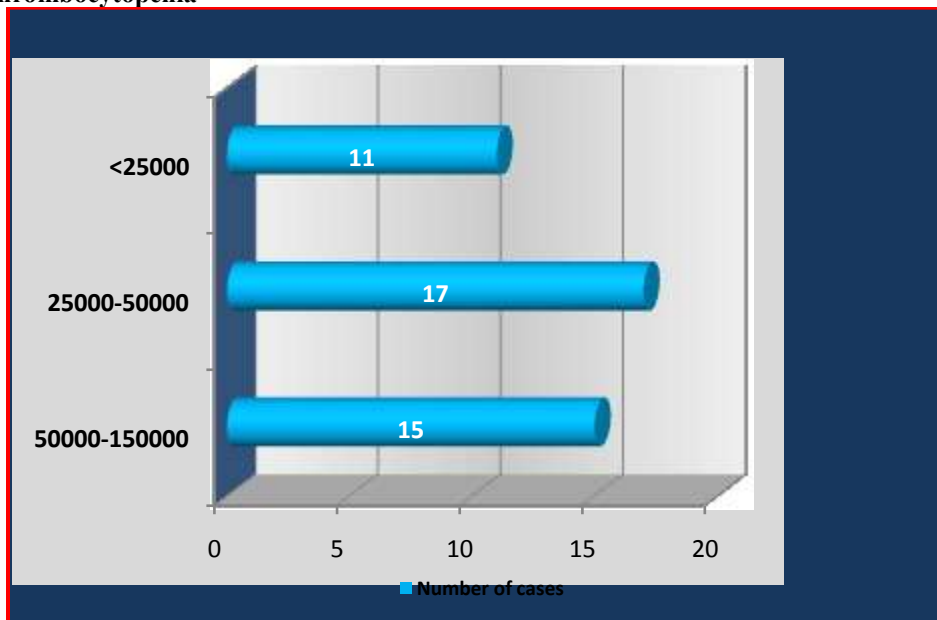


Fig 8:- Reduced platelet count n=43.

Fig.8:Shows that 43 cases had platelet count less than normal value. Among these 43 patients 15 patients had platelet counts between 100000-150000, 17 patient had platelet counts 100000- 50,000 and 11 patients had platelet counts less than 50,000

As reduced platelet count were seen in other viral fever, malaria and scrub typhus fever, an association was seen with dengue fever and reduced platelet count using Pearson Chi-square Test.

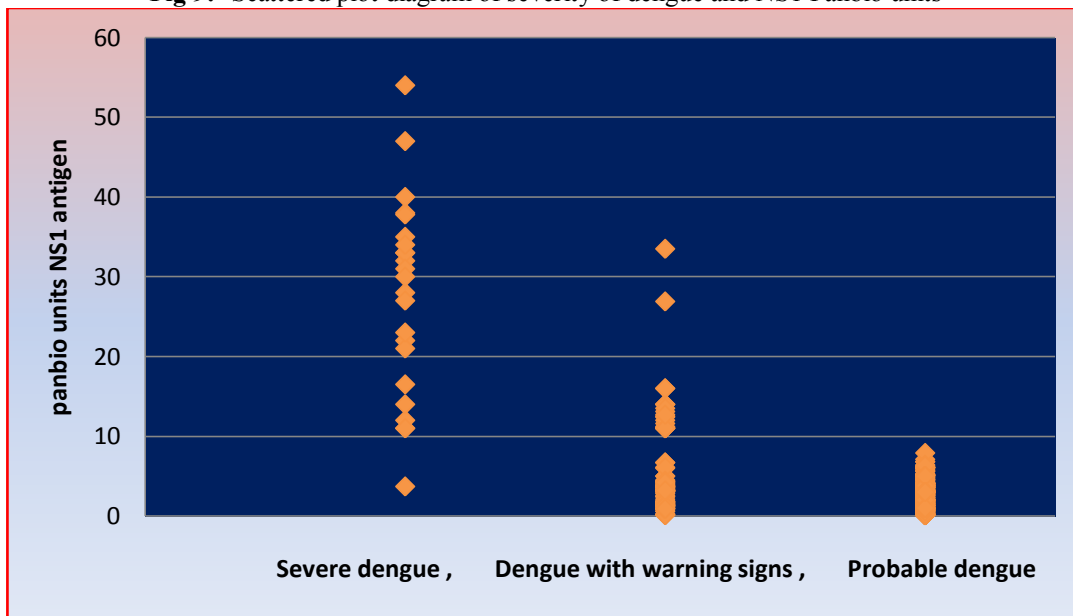
Assessment Of Severity Of Dengue With Ns1 Antigen

Based on the clinical and biochemical parameters the cases were classified into

1. Severe dengue,
2. Dengue with warning sign and
3. Probabledengueas per WHO’s definition.

The NS1 panbio units detected by ELISA of all 489 cases were plotted against the 3 categories in scattered diagram. An Anova test was checked for the significance of association the three categories with the Panbio units of detected NS1 antigen.

Fig 9:- Scattered plot diagram of severity of dengue and NS1 Panbio units



The scattered diagram showing higher values of NS1 Panbio units are found to be present on the plots of severe dengue. The maximum value detected was 56 units. The PanbioUnitsupto 33 were seen in category of dengue with warning sign. Some lower values of Panbio are also seen in severe dengue. So test of significance showing that increased value of Panbio units is associated with severity of disease is tested with Anova test.

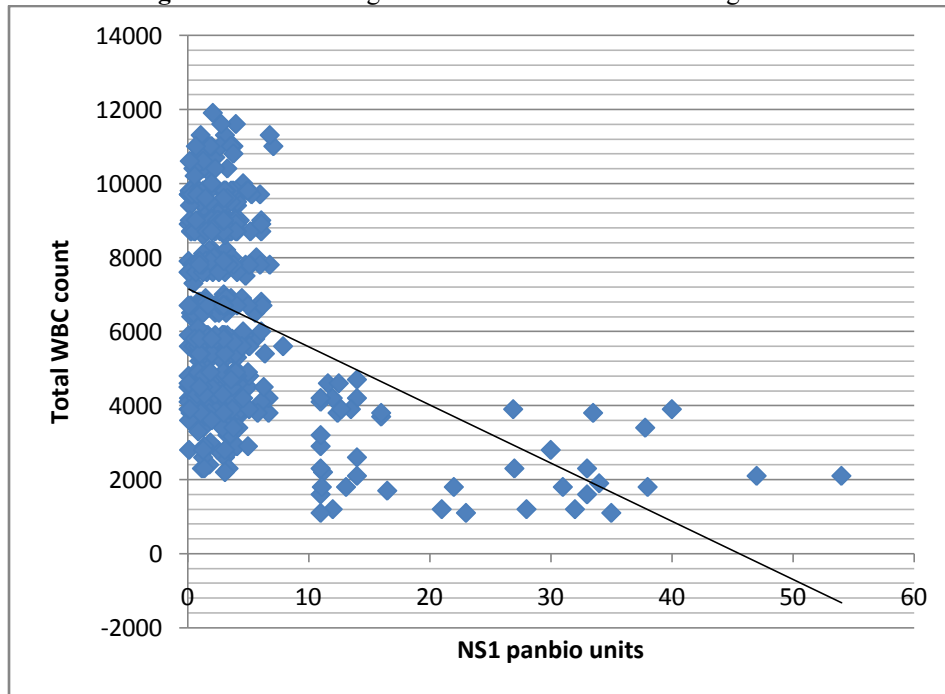
Table.4:- Mean,SD of NS1 Panbio units and Severity of dengue.

Classification	Total number	Mean NS1panbio units	Standard deviation
Severe dengue	24	27.56	12.71
Dengue with warning signs	98	5.16	6.13
Probable dengue	367	2.5	1.6

Applying Anovaparametric test $F=346.84$, with degree freedom 2 and significance= 0.0001 . This shows significant association between the categories of dengue fever and NS1 Panbio units. The inference is that for every 5times increase in Panbio units for NS1 antigen there is a shift of category 2(Dengue with warning sign) to category3(Severe dengue).

Correlation of NS1 Units and Total White blood Count

Fig 10:- Scattered diagram of Total WBC and NS1 antigen units.



The Pearson’s correlation coefficient r is -0.4469. It signifies a negative correlation between NS1 antigens detected in Panbio units and total counts. The reduced total counts were seen with increased Panbio units.

Fig.11:- Scattered diagram showing increased hematocrit value and NS1Panbiounits.

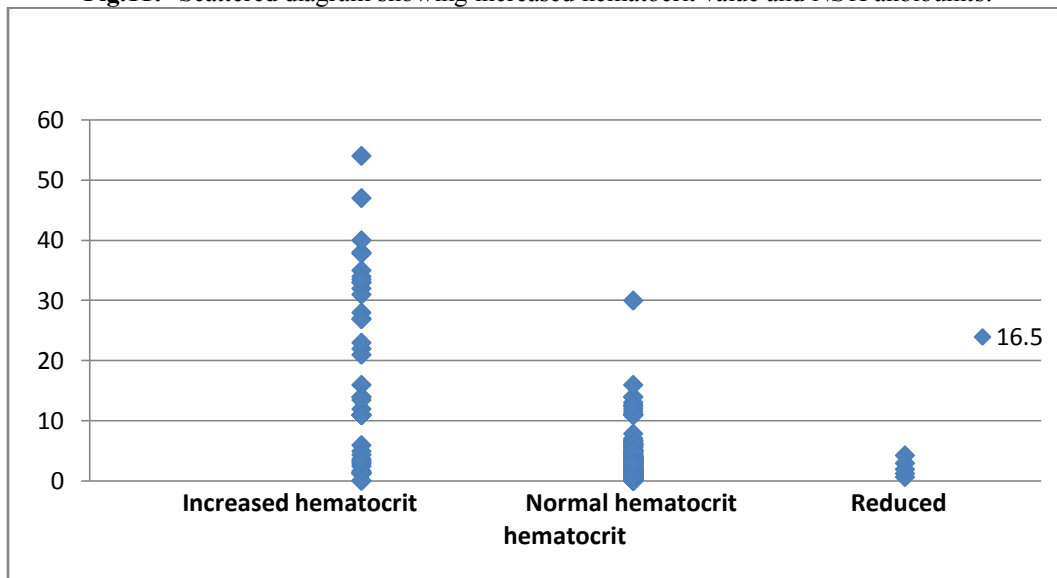


Fig 11: shows Cases with increased Hematocrit values have higher Panbio units of NS1 antigen and lower values in normal hematocrit.

Discussion:-

Dengue fever is a major public health problem in tropical and subtropical regions of the world especially in countries like India, due to its nature of widespread outbreak causing morbidity and mortality. The morbidity and mortality can be prevented by early diagnosis and management. Diagnosis is mainly serological involving detection

of IgM antibody in most health care facilities. But IgM appears in the blood only after 4 to 5 days. Although virus isolations and PCR procedures are the gold standard test of diagnostic procedure for early infection, NS1 ELISA can still be used as an alternate tool for early diagnosis.

This study was conducted at Stanley Medical College, Chennai during the period November 2017 to August 2018. Blood samples were taken from 489 patients with febrile illness with signs and symptoms suggestive of “Probable Dengue” as described by World Health Organization. The age, gender, duration of fever, blood platelet count, Total count, Hematocrit, Liver enzymes and renal function test were recorded for each patients. NS1 antigen was detected by ELISA procedure following the kit instructions.

The study population consisted of patients of all age group with a range of 1 to 70 years as shown in Fig 1. Although the samples were collected by simple random method, based on the duration of fever the total cases were grouped into 3 categories for the purpose of comparing percentage of case detection in each group. There were 103 cases in fever of 1-5 days, 234 cases in 6-10 days and 132 in 11 -15 days shown in Fig 2.

Common presenting symptoms of all cases of fever were nausea and vomiting 63%, headache 56%, joint pain 30%, muscle pain 25%, rash in 16% and abdominal pain in 6% Fig 3.

It was found that 47 cases were positive for NS1 antigen by ELISA procedure out of the total 489 cases of acute febrile illness which was about 10% as shown in Fig 4, consistent with previous study of **Sudipta Poddar et al** which was 16% (139/824). But in their study sampling was done in strata of which more than 600 cases were with period of illness less than 5 days whereas in this study cases less than 5 days of illness were only 103 similar to another previous study by **Amol Hartalkar et al**. The percentage positivity of NS1 with the same duration of illness in study population is 25%.

The peak prevalence of dengue was in the age group of 6 to 13 of either gender and followed by adult males of 14 to 20 years Fig 5. The previous study in India by **Arvind Neralwar et al** shows peak prevalence among young adult males which ranks only second place in this study. But in another study by **Bhaswati Bandyopadhyay et al** in the year 2013 conducted in India which has recorded highest number cases in the age group of 11-30 years. This study gives an even more precise information about the exact age group who are more affected. The reason for children with highest prevalence can be the following reasons. This age group are mostly children of school going, who are more exposed to bite of mosquitoes and also due to the fact that Aedes mosquito being a day time biter. Also the study population itself had more of children of this age group Fig 1.

The age and gender distribution of NS1 antigen showed similar distribution with a peak in children and reduces with age as shown in Fig. 5 similar to **Bhaswati Bandyopadhyay et al**. Also explains that detection percentage is based on occurrence which dependent on exposure of infection.

The percentage positivity of NS1 antigen is 25% in with duration of illness less than 5 days of fever, 6.6% in fever cases of duration 6 to 10 days and 0.7% in cases with duration of illness more than 10 days as shown in Fig: 6.

Platelet counts were less than 1,50,000 in 43 of the total 116 (37%) dengue positive cases. Among these patients 15 patients had platelet counts between 100000 to 1,50,000/Microlitre, 17 patients had counts between 50000-100000 / Microlitre and 11 patients had platelet counts less than 50,000 / Microlitre as seen in Fig 10. All these patients had Petechiae, Melena and Bleeding tendency. This finding has a robustness that antigens were still present even at the time when complications had set in and NS1 was present along with antibodies. The significant association of platelet count decrease with seropositive dengue case were tested with chi square test and found to be significant.

It was observed in this study that 17 cases of dengue NS1 test negative also had thrombocytopenia. Among them 8 cases turned out to be malaria positive as this region is endemic to malaria, 3 cases was leptospirosis and the rest of the cases could be other viral fever infections or it could still be false negative dengue.

It is found in this study as shown in the Fig 12: that as the duration of fever increases there is a decrease in antigen detection. So this association was tested using chi-square test and the test was significant. An increase in duration of illness increases with antibody detection shown in fig. 13 and proved by chi square test, consistent with the previous study **Arvind et al**.

It was found in this study 24 out of 27 (88%) NS1 positive cases were categorized as severe dengue based on clinical findings. This shows that Viraemia was present even after the appearance of antibodies showing the severity of the disease.

All the cases categorized as “Severe Dengue” based on clinical and biochemical parameters. As an attempt, Panbio units obtained by NS1 ELISA for all the 489 cases were compared with the severity of disease. The test of significance was tested by Anova test. This is shown in Fig 11. All cases of severe dengue had high values of NS1 Panbio units. These findings are similar to previous study by ShiranAjithParanavitane et al.

It was also found that NS1 Panbio units of severe dengue cases were 5 times more than that of dengue with warning signs and this was 2 times more than that of cases of suspected dengue. So Panbio units can be used as marker for assessing the severity of disease. Anova was tested for Panbio units and total WBC counts. A negative correlation as shown in Fig 12 was obtained using correlation coefficient. Scattered plot shows as viral antigens increase there is reduction in WBC count. So as Viraemia increases there is more destruction of white blood cells leading to leucopenia. These findings were consistent with the previous study by ShiranAjithParanavitane et al.

Similarly a scattered diagram was plotted with the value of Panbio units and hematocrit. There was increase in Panbio units with all cases with elevated Haematocrit value Fig.12. Similar findings were seen in study by ShiranAjithParanavitane et al.

Conclusion:-

Dengue virus described as the “Deadly virus and is the most rapidly spreading mosquito borne disease faster than that of Malaria or West Nile Virus” by World Health Organization has now emerged onto the global stage infecting about half of world’s population. Primary prevention involving vector control measures are the main stay of disease control, the secondary prevention including early diagnosis and treatment is utmost important after an outbreak of an epidemics as there is no specific treatment and vaccines against dengue still under trial. NS1 antigen detection by ELISA is useful in early phase would be a good guide to predict the recurrence of outbreak and for efficient prevention and management.

Conflict of interest:

None declare.

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