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RESEARCH ARTICLE
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STUDY OF CORRELATION OF SEROLOGICAL MARKERS OF DENGUE VIRUS INFECTION WITH PLATELET COUNT AT A TERTIARY CARE HOSPITAL OF CENTRAL INDIA

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Abstract

Aim: -To confirm serologically, dengue infection in febrile patients who are clinically symptomatic by using ELISA to detect the presence of NS1 antigen and IgM antibodies.
-To correlate the platelet count with the serological markers of dengue virus infection.

Materials and Methods: This prospective study conducted for a period of one year in Department of Microbiology, Gajra Raja Medical College and J. A. Group of Hospitals (JAH), Gwalior, Madhya Pradesh and the study period extended from January 2018 to 31st December 2018 This study was performed among patients with febrile illness who were clinically symptomatic for dengue infection and referred to the Department of Microbiology, Gajra Raja Medical College, Gwalior for investigation of dengue virus infection during the study period of one year.The minimum blood samples required for the study were 350 samples. Blood samples from seronegative patients were taken as control for comparison of platelet count in fever patients.
A structured assessment form was used to obtain the clinical history regarding febrile illness including clinical symptoms and signs. Blood samples from clinically symptomatic IPD patients were collected by venipuncture under sterile precautions in different department of J.A. Group of Hospital and sent to the Department of Microbiology as soon as possible.

Results- A total number of 1,762 samples of clinically suspected dengue fever was processed for serological markers of dengue in department of microbiology during the period of one year from January 2018 to December 2018. Out of the 1,762, 568 cases were positive for serological tests, Prevalence of dengue seropositivity in clinically suspected dengue patients was 32.22%.

Conclusion- Dengue is presently regarded globally as the most important and rapidly spreading mosquito-borne viral disease. It is a cause of great concern to public health in India. Every year,

thousands of peoples are affected and contribute to the burden of health care. In the present study patients with febrile illness and clinically symptomatic for dengue were investigated during January 2018 to December 2018.

Key words: Seropositivity, Serological markers, Venipuncture, platelet count.

INTRODUCTION

Dengue fever is an acute febrile arbo-viral disease affecting the tropical and subtropical regions of the world. The incidence of this disease has increased over the last 50 years with 2.5 billion people living in areas where dengue is endemic.[1, 2]

The etiological agent of disease is dengue virus (DENV), a member of the family *Flaviviridae*. Although, a large number of potential vectors have been identified, *Aedes aegypti* and *Aedes. Albopictus* are responsible for the majority of dengue transmission.[3]

Depending on characteristic antigenicity feature virus has classified into four serotypes, namely DENV-1, DENV-2, DENV-3 and DENV-4.[4,5] While the fifth type was announced in 2013.[4] It affects 100 million people annually with 5,00,000 cases of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) and around 30,000 deaths mostly among children.[6]

Dengue and DHF is endemic in more than one hundred countries. South East Asia and Western pacific regions are severely affected bearing 75% of global disease burden. South East Asian countries are divided into 3 categories based on endemicity and India has been categorized in Group A where all the 4 serotypes are prevalent.[7,8] Due to rapid urbanization, lifestyle changes and deficient water management, the risk of dengue infection in India has increased in recent years.[9]

Infection with any of the DENV serotypes can be asymptomatic in a majority of the cases or may end in a large spectrum of clinical symptoms, ranging from a mild disease known as dengue fever (DF) to the most severe forms of the disease, which are characterized by coagulopathy, increased vascular fragility, and permeability known as dengue hemorrhagic fever (DHF). The latter may progress to hypovolemic shock known as dengue shock syndrom (DSS).[10, 11]

The combined case fatality rate is around 5% for dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).[12] It is known that early and specific diagnosis of DHF or DSS followed by supportive therapy reduces morbidity and mortality.[13, 14]

The ‘gold standard’ tests for the identification of dengue infection (DI) are not in the reach of peripheral and even most tertiary care laboratories. At present viral culture, viral RNA detection by reverse transcriptase polymerase chain reaction (RT-PCR) and serological tests such as an immunoglobulin M (IgM) capture enzyme linked immunosorbent assay (MAC-ELISA) for diagnosis and confirmation of dengue viral infection.[4] Virus isolation and molecular techniques cannot be used as routine diagnostic tests because they are laborious, time consuming and require specialized laboratory facilities.[15] Serological diagnosis by detection of antibodies is widely used, but antibodies appear only after 4 to 6 days of illness.[16] Secretory protein NS1 antigen is seen in high concentrations during acute phase of illness (1 to 5 days).[17] Combination of NS1 antigen detection along with antibody detection increases the diagnostic rates.[18] Immunochromatographic detection of these serological markers yield rapid results but have low sensitivity as compared to ELISA.[19] During first 3 days of illness platelet count is normal. Thrombocytopenia begins during febrile phase and platelet count is progressively reduced during hemorrhagic illness.[20] As per WHO guidelines, thrombocytopenia can be used as a simple diagnostic criteria for DHF.[21] The only accessory laboratory test which supports the diagnosis of dengue is platelet count and it can be roughly estimated by microscopy even in the peripheral laboratories.[10]

Hence the present study is designed to correlate the dengue serological markers with platelet count which, not only helps in identifying and categorizing the patient but also in planning management accordingly, thereby curtailing further progression of disease to its severe forms and thus increasing positive prognosis.

AIMS AND OBJECTIVES

-To confirm serologically, dengue infection in febrile patients who are clinically symptomatic by using ELISA to detect the presence of NS1 antigen and IgM antibodies.
-To correlate the platelet count with the serological markers of dengue virus infection.

MATERIAL AND METHODS

The present study was conducted in Department of Microbiology, Gajra Raja Medical College and J. A. Group of Hospitals (JAH), Gwalior, Madhya Pradesh.

Study Design

This was a prospective study conducted for a period of one year. The study period extended from January 2018 to 31st December 2018.

Study Subject

This study was performed among patients with febrile illness who were clinically symptomatic for dengue infection and referred to the Department of Microbiology, Gajra Raja Medical College, Gwalior for investigation of dengue virus infection during the study period of one year.

Inclusion Criteria:

Patients with febrile illness who were clinically symptomatic for dengue infection of all age and both sex whose blood samples were received in Department of Microbiology, Gajra Raja Medical College during the study period.

Exclusion Criteria:

• Patients who fail to give consent for the serological diagnosis.
• Patients with autoimmune diseases
• Samples which were exhibiting hemolysis, and whose serum is icteric or showing lipaemia,

Sample size:

It was assumed that the samples received from patients in Department of Microbiology, Gajra Raja Medical College, Gwalior during the study period were a representation of the population of Gwalior Chambal division of Madhya Pradesh. The minimum blood samples required for the study were 350 samples. Blood samples from seronegative patients were taken as control for comparison of platelet count in fever patients.

Study Procedure

1. Recruitment of Patients

All patients with either nonstructural protein antigen (NS 1 antigen) positive or dengue immunoglobulin M (IgM) positive were identified and data of febrile patients were collected from different outpatient and inpatient departments of J.A. group of Hospitals. The nature and purpose of the study was explained to the participants and written informed consent was obtained. Participation in the study was on a voluntary basis. Demographic characteristics of the participants and disease were recorded. Thorough history taking and physical examination were performed in all these patients.

2. Clinical and Demographic Data Collection

A structured assessment form was used to obtain the clinical history regarding febrile illness including clinical symptoms and signs.

3. Blood Sample Collection Procedure

Blood samples from clinically symptomatic OPD patients were collected by venipuncture under

sterile precautions as follows: The veins in the antecubital fossa or dorsum of the hand were identified and a tourniquet applied to make the veins visible. The area was then cleansed with 70% isopropyl alcohol swab and allowed to air dry; 5ml of blood was drawn using a disposable syringe. Blood samples from clinically symptomatic IPD patients were collected by venipuncture under sterile precautions in different department of J.A. Group of Hospital and sent to the Department of Microbiology as soon as possible.

In case of delay, samples were stored at 2 to 8^{o} C and transported in cold chain.

Laboratory Procedures

Out of the total 5 ml of collected blood sample, 2ml was kept in EDTA vial and 3ml in plain vial. Sample in EDTA vial was sent for platelet count by fully automated blood cell counter and blood sample in plain vial were allowed to clot at room temperature (20-25^{o}C) and centrifuged according to the standard serological procedures. The serum separated was stored in refrigerator at 2-8^{o}C and used for serological tests.

1. NIV DENGUE IgM Capture ELISA Kit

The kits were supplied to the department of Microbiology Gajra Raja Medical College Gwalior from NIV Pune and kept at 2-8^{o}C.

Principle:

IgM antibodies in the patient's serum are captured by anti-human IgM (mu chain specific) coated on to solid surface (wells). In the next step, Dengue antigen is added which binds to captured human IgM in the sample. Unbound antigen is removed during the washing step. In the subsequent step biotinylated flavivirus anti DEN monoclonal antibodies are added followed by Avidin –HRP. Subsequently chromogenic substrate (TMB –H_2O_2) is added, the reaction is stopped by 1N H_2SO_4.The intensity of colour/optical density is measured at 450 nm. The test was standardised and reported by NIV Pune in 1984. The performance of the test was evaluated by Christian Medical College Vellore.

Procedure

1. Select the samples to be assayed.
2. Dilute serum 1:100 in tubes or preferably in deep well plate using sample diluent for DEN IgM.
3. Remove required number of anti IgM coated strips
4. Wash the strips 3 times with 1x wash buffer.
5. Transfer 50µl of diluted samples from the deep well plate to respective wells as per the protocol.
6. Add 50µl of DEN IgM positive control& DEN IgM negative control to respective wells as per the protocol.
7. Cover the plate with aluminum foil to prevent evaporation of samples. Keep the plate in a closed humidified box inside the incubator and incubate the plate at 37^{0}Cfor 1 hour.
8. At the end of incubation, wash the plates five times with wash buffer. Tap the plate after last wash on a tissue paper.
9. Add 50 µl of DEN antigen to each well of plate. Take out the DEN antigen vial from the refrigerator, add 50µl to each well and put the vial back to the refrigerator.
10. Repeat step no.7 and 8.
11. Add 50 µl of Anti DEN monoclonal antibody HxB (Biotin labelled) to each well.
12. Repeat step no.7 and 8.
13. Add 50 µl of Avidin- HRP to each well.
14. Cover the plate with aluminum foil to prevent evaporation of samples. Keep the plate in a closed humidified box inside the incubator and incubate the plate at 37^{0}Cfor 30 minutes.
15. Repeat step no.8.
16. Add 100 µl of Liquid TMB substrate (TMB – H_2O_2) to each well.

17. Incubate at room temperature in dark for 10 minutes.
18. Stop the reaction exactly after10 minutes with 100 µl stop solution.
19. Measure the absorbance at 450 nm within 10 minutes after termination of reaction.

Quality Control

Each kit contains one vial of positive control and one vial of negative control. These work as marker of kit performance.

1. If OD of negative control is more than 0.8.

OR

2. If OD of positive control is less than 6 times the OD of negative control.

In both the situations, the test was considered as invalid.

Interpretation of Results:

1. If OD of sample tested is less than OD of negative control by a factor of 2.0 –sample should be considered as negative.
2. If OD of sample tested is more than OD of negative control by a factor of 3.0- sample should be considered as positive.
3. If OD of sample tested exceeds OD of negative control by a factor of 2.0 but is less than OD of negative control by a factor of 3.0 – sample should be considered as equivocal.

Performance Characteristics

1. Diagnostic characteristics -

Sensitivity – 98.53%

Specificity – 98.84%

2. Reproducibility –

Table. 2 : Precision measures of NIV dengue IgM capture ELISA Kit

Sample Pool	Standard deviation (SD)	% Coefficient of variation (% CV)
Positive control	0.159	9.22%
Negative control	0.008	10.21%
Dengue strong positive	0.177	12.77%
Dengue weak positive	0.087	16.69%
Dengue negative	0.009	9.52%

EUROIMMUN Dengue Virus NS1 ELISA kit

Principles of the test: The test kit contains microtiter strips each with 8 break-off reagent wells coated with monoclonal anti-dengue virus NS1 antibodies against the serotypes 1,2,3,4. In the first reaction step, diluted patient samples are incubated in the wells. If samples are positive, the dengue virus NS1 binds to specific ant-dengue NS1 antibodies. To detect the bound antigens, a second incubation is carried out using an enzyme-labelled anti-dengue virus NS1 antibody (enzyme conjugate) catalyzing a colour reaction.

Preparation and stability of the patient samples

Samples: Human serum or EDTA or heparin plasma.

Stability: Patient samples to be investigate can generally be stored at + 4^{o}C for up to 14 days. Diluted samples should be incubated within one working say.

Sample dilution: Patient samples are diluted were diluted 1:2 in sample buffer.

NOTE: The calibrators and controls are prediluted and ready for use, do not dilute them.

For semi quantitative analysis incubate calibrator 2 along with the positive and negative controls and patient samples.

(Partly) manual test performance

1. Sample incubation (1st step): Transfer 100 µl of the calibrators, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol. For manual processing of microplate wells, cover the finished test plate with the protective foil. When using an automated microplate processor for incubation follow the recommendations of the instrument manufacturer. Incubate for 60 minutes at +37°C 1°C

2. Washing :

Manual: Remove the protective foil, empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.

Automatic: Remove the protective foil and wash the reagent wells 3 times with 450 µl of working strength wash buffer.

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle,

Then empty the wells. After washing (manual and automated tests). thoroughly dispose of all liquid from the microplate by taping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

3. Conjugate incubation (2nd step) : Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-dengue virus NS1 antibody) into each of the microplate wells. For manual processing of microplate wells. Cover the finished test plate with the protective foil. When using an automated microplate processor for incubation follow the recommendations of the instrument manufacturer.

4. Incubate for 60 minutes at+37°C 1°C.

5. Washing: Empty the wells. Wash as described above.

6. Substrate incubation (3rd step) : Pipette 100 µl of chromogen /substrate solution into each of the microplate wells. Incubate for 15 minutes at room temperature (+18°C to +25°C, protect from direct sunlight).

7. Stopping: Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

8. Measurement: Photometric measurement of the colour intensity should be made at a wavelength of 450 nm and a reference wavelength between 620 nm and 650 nm within 30 minutes of adding the stop solution. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Calculation of results

Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction of the control or patient sample over the extinction of the calibrator 2. Calculate the ratio according to the following formula:

$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator 2}} = \text{Ratio}$$

Ratio <0.8	:	negative
Ratio >0.8 to <1.1	:	borderline
Ration > 1.1	:	positive

In case of a borderline result, test systems for the detection of specific antibodies against dengue virus can help with the diagnosis. In some cases, also PCR diagnostics may be helpful.

Test characteristics

Calibration: As no international reference preparation exists for dengue virus NS1, the calibration is performed in relative units (RU/ml).

For every group of tests performed, the extinction readings of the calibrators and the relative units and/or ratios determined for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should

be repeated.

The binding activity of the antigens and the activity of the enzyme used are temperature-dependent. It is therefore recommended using a thermostat in all three incubation steps. The higher the room temperature (+18^{o}C to +25^{o}C) during the incubation steps, the greater will be the extinction. Corresponding variations apply also to the incubation times. However, the calibrators are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the results.

Antibody: The wells of the microtiter plates are coated with monoclonal anti-dengue NS1 antibodies (mouse). These antibodies are specific against dengue virus NS1 of serotypes 1,2,3,4.

Linearity: The linearity of the dengue virus NS1 ELISA was determined by assaying at least 4 serial dilutions of different patients samples. The dengue virus NS1 ELISA is linear at least in the tested concentration range 1 RU/ml to 100 RU/ml.

Detect limit: The lower detection limit is defined as the mean value of analyte-free sample plus three times the standard deviation and is the smallest detectable dengue NS1 concentration. The lower detection limit of the Dengue Virus NS1 ELISA is 0.8 RU/ml.

Cross reactivity: The quality of the antibodies used guarantee a high specificity of the ELISA. Sera from patients with acute infections or recent vaccinations of, or against different Flaviviruses, respectively were analysed with the vengue virus NS1 ELISA. All 33 samples were found to be negative.

Interference: Haemolytic, lipaemic and icteric samples showed no influence on the result up to a concentration, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA. All 33 samples were found to be negative.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 samples. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 3 determinations performed in 10 different test runs.

Table. 3) Reproducibility in EUROIMMUNE dengue virus NS1 ELISA kit

Intra-assay variation, n=20		
Sample	**Mean value (RU/ml)**	**CV (%)**
1	9	3.1
2	29	3.2
3	37	2.8

Inter-assay variation, n=20		
Sample	**Mean value (RU/ml)**	**CV (%)**
1	12	9.6
2	34	10.6
3	43	7.9

Sensitivity and specificity: 39 clinically characterized patients sample (INSTAND) were investigated with the EUROIMMUN Dengue Virus NS1 ELISA. The sensitivity amounted to 100%k, with a specificity of 100%.

Table. 4) Sensitivity and specificity in EUROIMMUNE dengue virus NS1 ELISA kit

n=39		**INSTAND**		
		Positive	**Borderline**	**Negative**
EUROIMMUNE Dengue Virus NS1 ELISA	Positive	14	0	0
	Borderline	0	0	0
	Negative	0	0	25

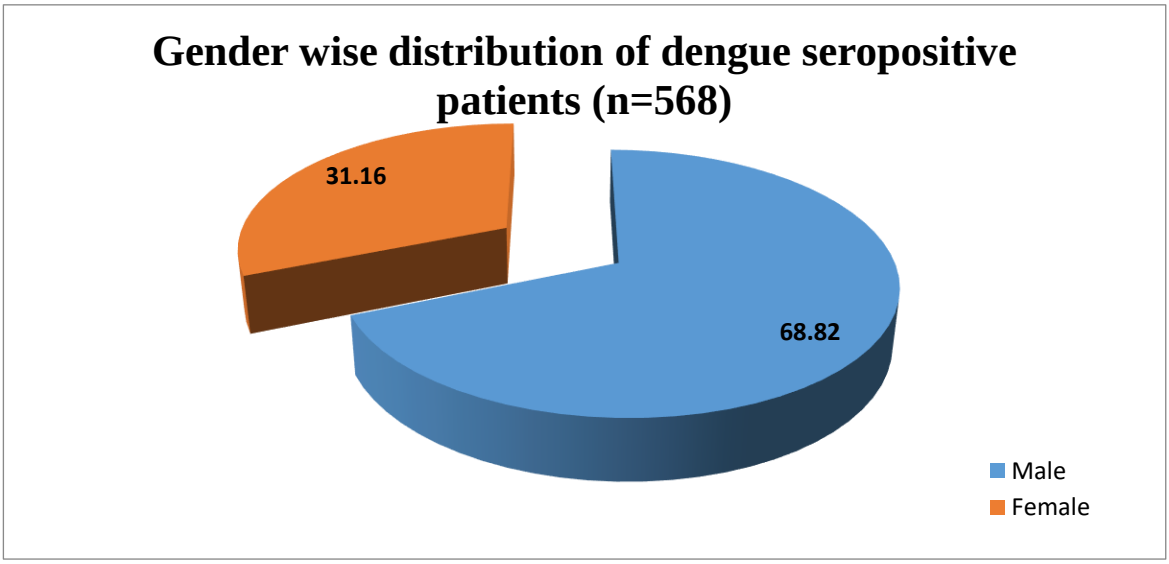
RESULTS AND OBSERVATION

A total number of 1,762 samples of clinically suspected dengue fever was processed for serological markers of dengue in Department of microbiology during the period of one year from January 2018 to December 2018. Out of the 1,762, 568 cases were positive for serological tests, Prevalence of dengue seropositivity S in clinically suspected dengue patients was 32.22%.(Table 5)

Table 5 : Prevalence of dengue seropositive cases

Total Patients	Total Dengue seropositive (IgM, NS1)	Prevalence
1,762	568	32.2%

The present study showed that among dengue seropositive patients, male patients (68.82%) were more common compared to female patients (31.16%). The male: female ratio was 2.2:1. (Table. 6)



Graph 1 : Pie chart showing gender wise distribution of dengue seropositive patients.

The present study revealed that patients of all age groups were affected by dengue virus infections. The most common affected age group was 11-20 years followed by 0-10 years, 21-30 years, 31-40 years, 41-50 years, 51-60 years and >60 years respectively. (Table.7)

Table 7: Age wise distribution of dengue seropositive patients

Age (years)	**Male**	**Female**	**Total**
0-10	**124 (21.83%)**	**52 (9.15%)**	**176 (30.98%)**
11-20	**168 (29.57%)**	**59(10.38%)**	**227 (39.96%)**
21-30	**58 (10.21%)**	**29 (5.10%)**	**87 (15.31%)**
31-40	**27 (4.75%)**	**16 (2.81%)**	**43 (7.57%)**
41-50	**06 (1.05%)**	**12 (2.11%)**	**18 (3.16%)**
51-60	**04 (0.70%)**	**05 (0.88%)**	**09 (1.58%)**
>60	**04 (0.70%)**	**04 (0.70%)**	**08 (1.40%)**
Total	**391 (68.83%)**	**177 (31.16%)**	**568 (100%)**

Our study showed that out of total 568 dengue seropositive patients, 176 patients were from rural area and 392 patients were from urban area.(Table. 8)

Table 8 : Area wise distribution of dengue seropositive patients

Area	**Number of patients**	**Percentage**
Rural	**176**	**30.98**
Urban	**392**	**69.01**
Total	**568**	**100**

The present study revealed that dengue positive cases started appearing in month of may and maximum number of dengue positive cases occurred during october(47.8%) followed by november and september and minimum (0%) cases in the month of january february march and april .(Table 9)

Table 9 : Seasonal variation of dengue seropositive patients

Month	Dengue seropositive patients (N=568)	Percentage
January 2018	0	0
February 2018	0	0
March 2018	0	0
April 2018	0	0
May 2018	01	0.22
June 2018	0	0
July 2018	02	0.26
August 2018	20	3.5
September 2018	101	17.7
October 2018	271	47.8
November 2018	155	27.2
December 2018	18	3
Total	568	100

Table 10 : Clinical profile of dengue positive patients

Clinical feature	Number of cases	Percentage
1.Fever	**568**	**100**
2.Headache	**354**	**62.32**
3.Retro_ orbital pain	**162**	**28.52**
4.Arthralgia / Myalgia	**198**	**34.85**
5.Abdominal Pain	**222**	**39.08**
6.Nausea/ Vomiting	**306**	**53.87**
7.Rash	**73**	**12.85**
8.Bleeding Manifestations	**34**	**5.98**
9.CNS Symptom	**42**	**7.3**

In our study out of the 1728 sample tested a total of 568 samples were tested positive for either one or both markers (NS1, IgM) tested. Of the 568 positive serum samples, 249 (43.83%) patients were positive for NS1 only and 211 (37.15%) positive for IgM only,. Both the markers was detected in the remaining 108 (19.01%) samples. (Table. 11)

Table 11 : Prevalence of serological markers of dengue.

Dengue markers	Total positive cases	Percentage
Only NS1 Ag	249	43.83
Only IgMAb	211	37.15
NS1 Ag + IgMAb	108	19.01
Total	568	100

The present study showed that 77.29 % of dengue seropositive patients were having platelet count below 100000/cc while 22.71% dengue seropositive patients were having platelet count above 1,00,000/cc. In patients having thrombocytopenia 49 patients had platelet count below 20000, 124 patients had platelet count between 21000-51000 and 266 patients had platelet count between 51000-100000 (Table 12).

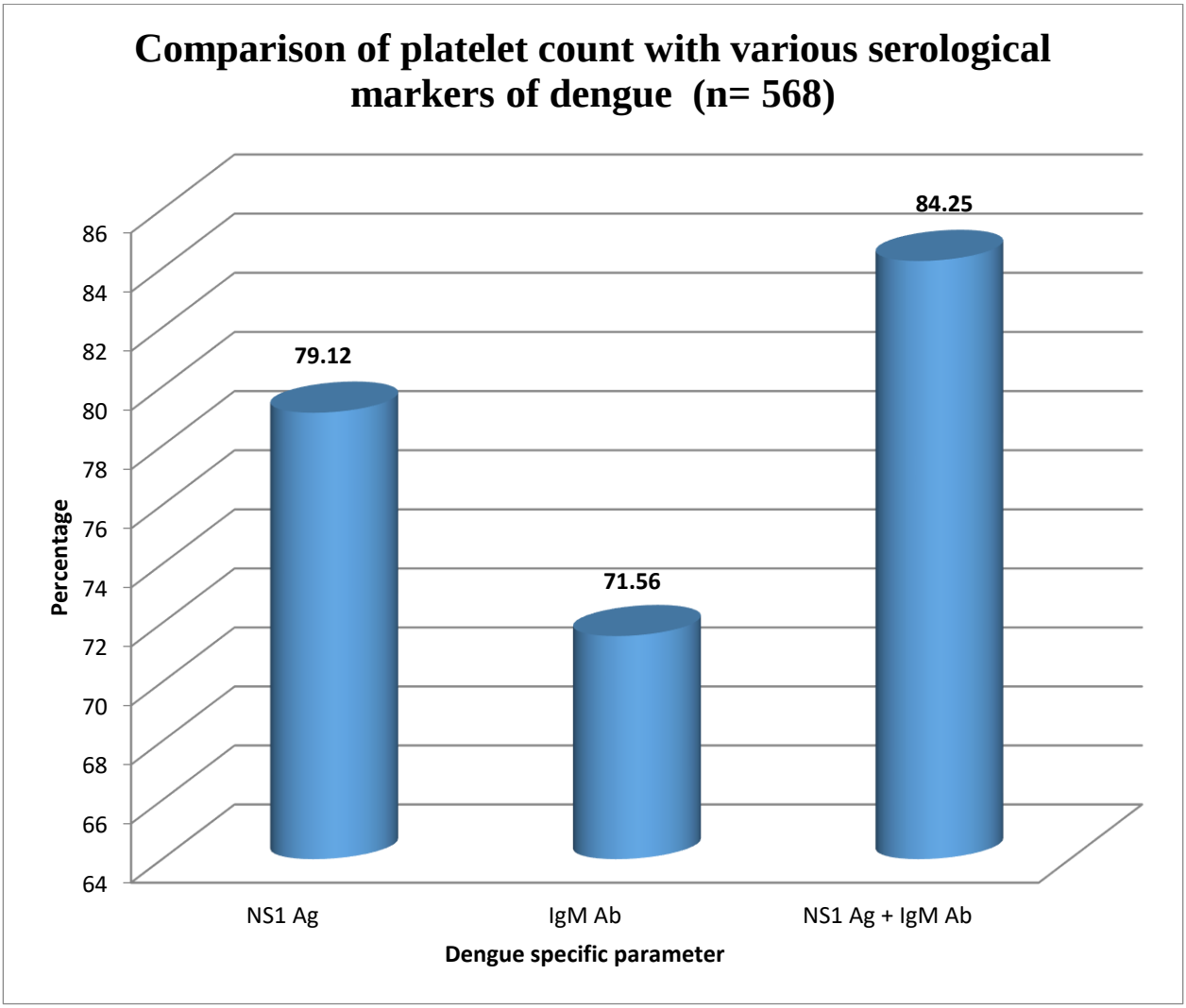
Table :12 Platelet count in dengue seropositive cases

Platelet Count	Number of cases	Percentage
<20000/cc	49	8.62
21000-50000/cc	124	21.83
51000-100000/cc	266	46.83
>100000/cc /cc	129	22.71
Total	568	100

The present study revealed that out of the 568 total positive cases of dengue, 439 (77.29%) showed thrombocytopenia. In 249 cases that were positive for NS1 Ag, thrombocytopenia was evident in 197 (79.12%) cases. and when IgM antibodies is found to be positive thrombocytopenia was noted in 151out of 211 (71.56%) cases. when both the markers NS1 Ag + IgMAb was .positive, thrombocytopenia was observed in 91 out of 108 (84.25%) cases. In our study significant association found between serological markers of dengue infection and platelet count. The findings were statistically significant (p value < 0.05) χ^2, DF, p value 7.40, 2, 0.024688 (Table 13).

Table 13 : Comparison of platelet count with various serological markers of dengue.

Dengue specific parameter	Total positive serum sample	Platelet count <100000	Plate Count >100000	
NS1 Ag	249	197 (79.12%)	52	
IgMAb	211	151 (71.56%)	60	
NS1 Ag + IgMAb	108	91 (84.25%)	17	
TOTAL	568	439 (77.29%)	129	χ^2, DF, P Value 7.40, 2, 0.024688



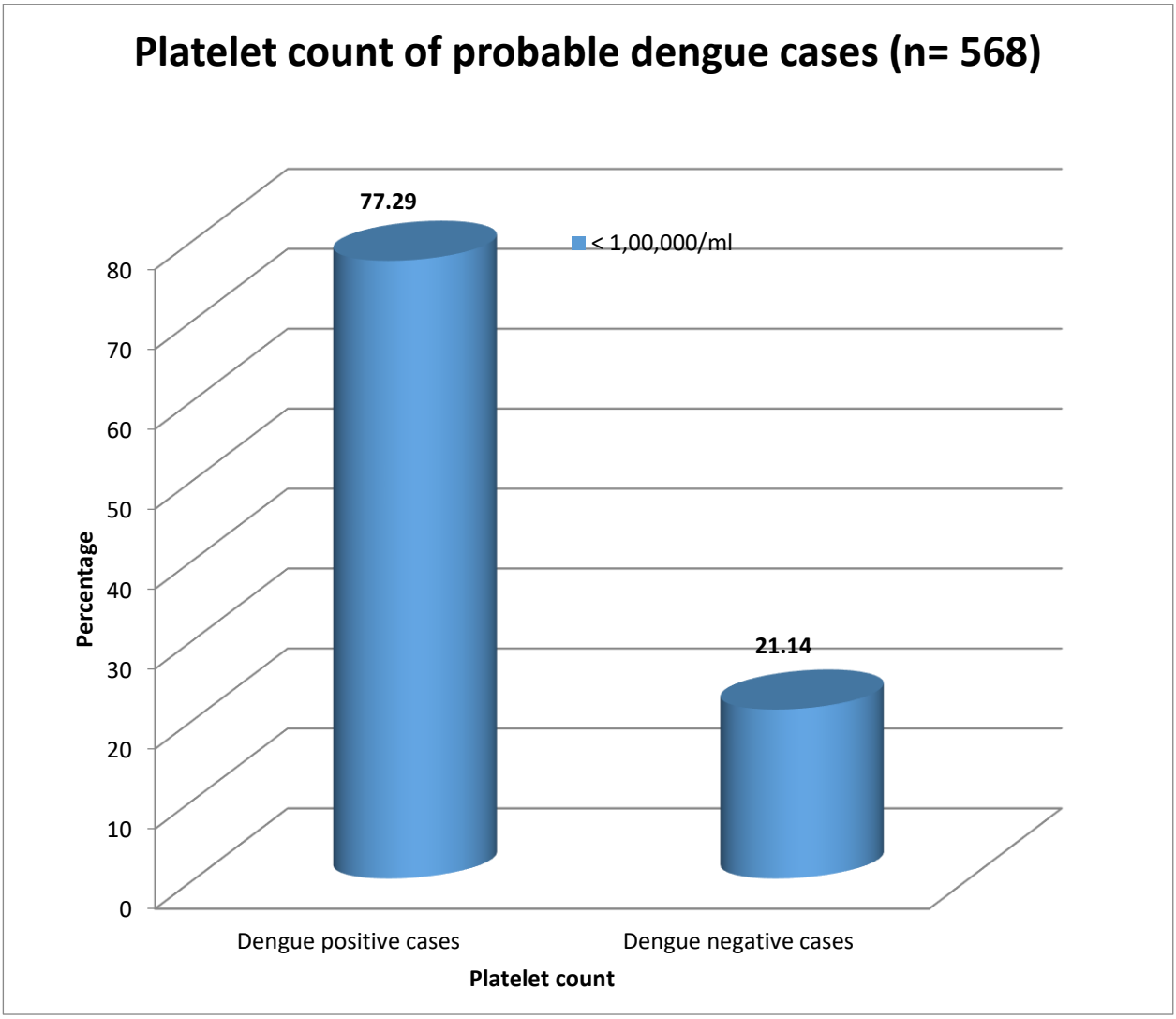
Graph 4 : Column chart showing comparison of platelet count with various serological markers of dengue.

On comparison of platelet count with dengue seropositivity, it was revealed that thrombocytopenia [platelet count less than 1 lakh, as per WHO guidelines for DHF] were present in higher number of dengue positive 439 out of 568 [77.29%]cases than dengue negative cases 74 out of 350 [21.14%]

cases. These findings were statistically significant (p value < 0.05) (Table. 14) χ^2, DF, p value. 509.69, 1, 0.000003.

Table 14: Platelet count of probable dengue cases

Platelet count	Dengue positive cases	Dengue negative cases	χ^2, DF, p value
<1,00,000/ml	439[77.29%]	252[21.14%]	509.69, 1, 0.000003
>1,00,000/ml	129[22.71%]	942[78.86%]	
Total	**568**	**1194**	

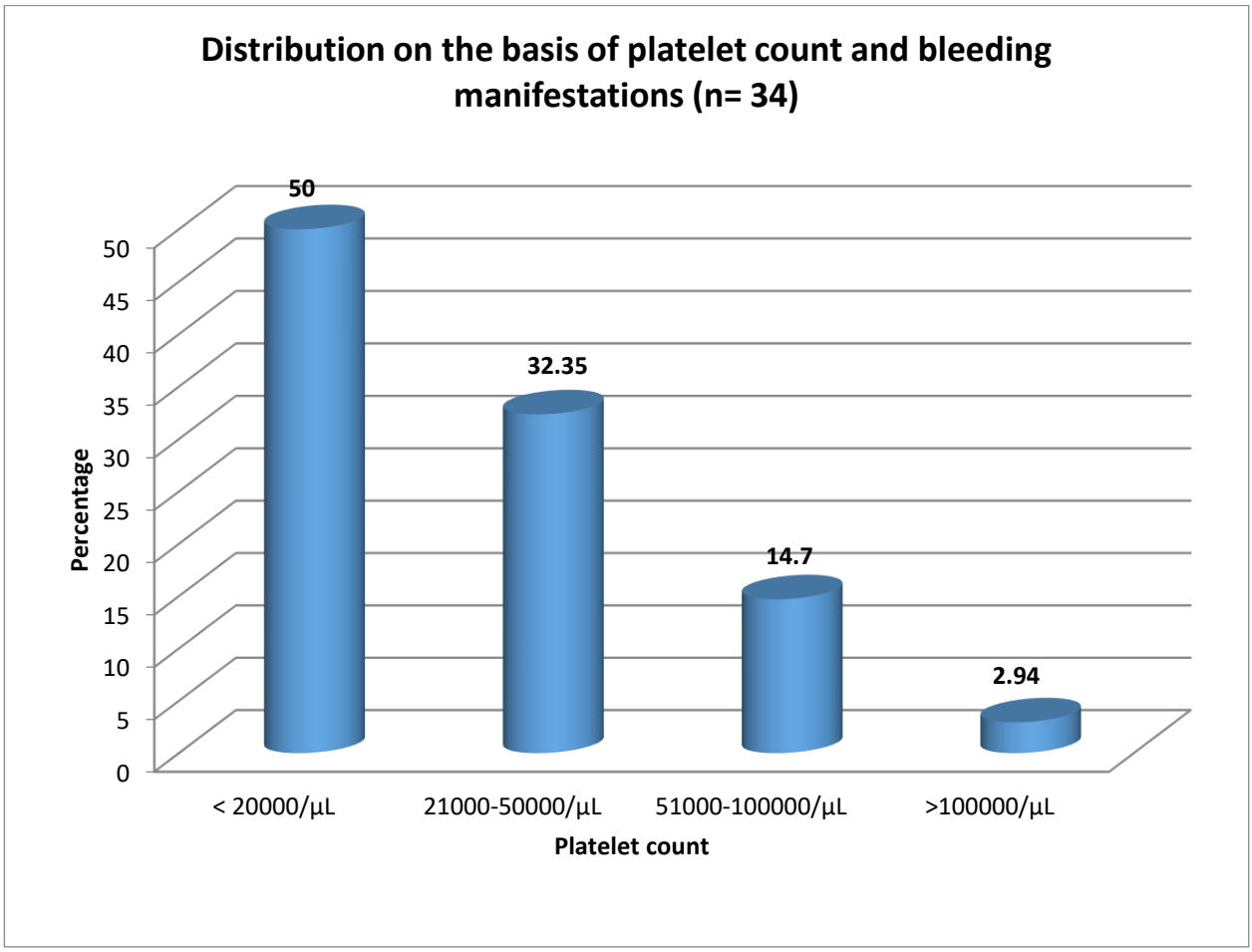


Graph 5: Column chart showing platelet count of probable dengue cases

In our study it was found that out of the 34 cases who had bleeding manifestations, 17 patients had platelet count below 20000/µL, 11 patients had platelet count between 21000 to 50000/µL, 5 had between 511000/µL to 100000 /µL, 01 had platelet count above 100000/µL (Table 15).

Table (15). Distribution on the basis of platelet count and bleeding manifestations

Platelet count	**Patients having bleeding manifestations**	**Percentage %**
< 20000/µL	**17**	**50.00**
21000-50000/µL	**11**	**32.35**
51000-100000/µL	**05**	**14.7**
>100000/µL	**01**	**2.94**



Graph 6 : Column chart showing distribution on the basis of platelet count and bleeding manifestations

Among the 34 cases which had a bleeding manifestations, petechiae/ purpura, was the most common presentation with 15 cases (%) followed by ecchymosis 06 cases, melena 06 cases gum bleed 4 (%) cases, epistaxis 02 (2%), 01 (%) case had hematemesis (Table 16).

Table 16.) Distribution on basis of pattern of bleeding tendency.

Pattern of bleeding	No. of cases	Percentage %
Petechiae/ Purpura	**15**	**44.11**
Ecchymosis	**06**	**17.64**
Melena	**06**	**17.64**
Gum bleed	**04**	**11.76**
Epistaxis	**02**	**5.88**
Hematemesis	**01**	**2.94**

DISCUSSION

Among the 1,762 clinically suspected dengue patients, 568 (32.2%) were tested positive for either one or both the serological markers for dengue virus infection. The seropositivity reported in various studies ranges from 11.9% Jyothi.p *et al* to 40% Shindhnai.V *et al*.[7,10] Seropositivity found in our study is more than the studies done by Jyothi.p *et al* and Kulkarni RD *et al* and less than the studies done by Krunal D Mehta *et al* and Dutta P *et al*.

The present study showed the male preponderance in the dengue virus infection. Various studies done by Krunal D Mehta *et al* Saha K Ashis *et al* and Chakravarti A *et al* also showed similar pattern for gender wise distribution of dengue virus infection. So this finding of our study is in accordance of the above studies. The male predominance can be due to their more involvement in outdoor work and

travelling. A more probable explanation in favor of our findings may be the underreporting of females at healthcare systems in our country. However some studies as done by Kabra SK *et al*. showed female dominance which is also seen in the study done by Mittal H *et al.*[50]

In our study the most commonly affected age group by DENV infection was 11-20 years followed by 0-10 years and then 21-30 years. Studies done by Mahesh Kumar *et al* Saha K Ashis *et al* Krunal D Mehta *et al* also found that the commonest age group affected was 11-20 years and 11-30 years.The maximum (39.96%) number of cases in the children and young adult age group indicates that the disease is endemic in these regions. Adults manifest with disease less in endemic areas because they become immune to the infecting virus.

However, in the study done by Neerja M *et al*, more numbers of cases were found in the adult age group. [153] This implies that the virus had been introduced to a non-exposed population and disease was not endemic. True endemicity is said to be reached when the adult infection decreases and only the children are infected more by the causative agent.

The present study revealed that the more number (69.01%) of serologically positive cases was belonging to urban area and less number of cases (30.98%) from rural area. Since long time dengue had been remain the disease of urban areas due to the availability of favorable habitat for dengue vector. Study done by Chakravarti A *et al* supports the finding of present study. [149] However, this disease is progressively spreading to rural areas. The spread of dengue from urban to rural areas may be related to socio-economic, human ecological and lifestyle changes, like increased transport, mobility and urbanization of rural areas. These changes contribute to the proliferation of man-made larval habitats of the mosquito vector. So the disease which was primarily an urban problem has now become a widespread health concern in India. The study done by Jagtap MB *et al* showed the shifting trends of dengue to semi urban and rural area.

The present study showed that maximum dengue positive cases occurred during october (47.8%) followed by november (27.2%) and september (17.7%). Dengue positive cases started appearing in month of May reached the peak in November and tapered thereafter with minimum (0%) cases in the month of january, february, march and april. The seasonal pattern found in our study is in accordance with various studies done in India. Studies done by Mahesh Kumar *et al* and Pruthvi D *et al* also found the similar seasonal pattern as of present study. The high rates of dengue virus transmission occur during the warm and humid conditions of the rainy season which coincides with monsoon and post-monsoon period in India. During this period the conditions remains much favorable for abundant growth of the vector i.e. *Aedes* mosquito. Interaction between rainfall, temperature and relative humidity is associated with the occurrence of dengue cases, which peaked at post-monsoon period during the months of October and November. Chakravarti A. *et al*. However, few studies reported highest dengue transmission during summer months, a study done by, Chouhan GS *et al*. report an outbreak occurring during April and May in Rajasthan. Outbreaks of dengue occurred from May to June and from March to May Risbud AR *et al* in Maharashtra.

CONCLUSION

Dengue is presently regarded globally as the most important and rapidly spreading mosquito-borne viral disease. It is a cause of great concern to public health in India. Every year, thousands of peoples are affected and contribute to the burden of health care. In the present study patients with febrile illness and clinically symptomatic for dengue were investigated during January 2018 to December 2018.

A total of 1762 samples were tested and 568 samples are found positive for either one or both the serological markers of dengue virus infection indicating the seropositivity of 32.2% with maximum cases identified during month of November and October in post-monsoon period. Mostly male children and young adults were infected by the virus belonging to urban and rural areas.The most common clinical presentation was fever followed by headache ,nausea and vomiting, abdominal pain and arthralgia myalgia and retro-orbital pain. Rashes(12.85%) and bleeding manifestations (5.98%) also found in some cases. out of the total of 568 samples tested positive 249 (43.83%) patients were

positive for NS1 only, 211 (37.15%) positive for IgM only and both the markers was detected in the remaining 108 (19.01%) samples. Among the total positive cases of dengue, 77.29% showed thrombocytopenia. In cases that were positive for NS1 Ag, thrombocytopenia was evident in 79.12% cases. and when IgM antibodies is found to be positive thrombocytopenia was noted in 71.56% cases. When both the markers NS1Ag + IgMAb was .positive, thrombocytopenia was found in 84.25% cases. Potential fatal complications were mostly observed in cases having platelet count below 20000. The study will serves as the baseline data about circulation of Dengue viruses in Gwalior and Chambal region. The study will also help clinicians to monitor the progression of dengue fever.

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