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Research Article

Evaluation of utility of serological markers using rapid diagnostic test kits and investigating their correlation with peripheral blood count in a dengue fever outbreak

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Abstract

To study the seroprevalence in dengue and its correlation with hematological parameters. A retrospective analysis of 718 seropositive cases was done for one year from January 2021-December 2021. The rapid diagnostic test kit showing NS1 Ag, IgM Ab, and IgG Ab reactivity toward dengue was used. The dengue seropositive cases were divided into 7 serogroups (NS1Ag), (IgM Ab), (IgG Ab), (NS1 Ag IgM Ab), (NS1 Ag IgG Ab), (IgM Ab IgG Ab), (NS1Ag IgM Ab IgG Ab). The hematological data were analyzed. And the correlation between serological and hematological parameters was calculated using the ANOVA test. The dengue-positive cases were observed highest between 19-60 years of age (48.7%), along with male predominance and peak during October month (49.72%). The highest Seroprevalence was observed with NS1Ag (59.05%) followed by IgG Ab (22.42%) in outdoor patients and adults, whereas NS1Ag IgM Ab (6.55%) followed by IgM Ab (4.18%) was predominantly seen in indoor patients and children (0-12 yrs). The maximum affected hematological variable were platelet count (thrombocytopenia in 55.8%) and total leucocyte count (leucopenia in 38.0%) having a strong association with NS1Ag positivity followed by lymphocytosis (17.1%) and neutropenia (11.6%) which were in concordance with other studies. Both (NS1Ag and IgM Ab) detection in the active stage has a probable diagnosis of primary dengue infection and IgG detection in secondary infection. Thrombocytopenia and leucopenia are early markers of dengue in view of NS1Ag association. Thus, serological markers together with hemograms help in the early detection, treatment, and ascertaining prognosis of the disease.

Keywords: Dengue, Seroprevalence, Serological Markers, Hematological Parameters.

Introduction

Dengue is a mosquito vector-borne disease. It is caused by the ssRNA virus of the family *Flaviviridae* and genus *Flavivirus* (Rai et al., 2019; Fry et al., 2011). The four serotypes DENV1, DENV2, DENV3, and DENV4 are transmitted to humans via vector *Aedes aegypti* and lesser extent by *Aedes albopictus* mosquito (Fry et al., 2011). Dengue is a chief public health hassle, mainly in tropical and subtropical areas that are favorable for the growth of vector mosquitoes (WHO, 2022; Jenny et al., 2021).

The worldwide prevalence of dengue has increased. Approximately 100-400 million infections occur each year, with 80% generally mild and asymptomatic. According to WHO, 10 January 2022, dengue cases have grown 8-fold in two decades from 505,430 cases in 2000 to 2.4 million in 2010, and 5.2 million in 2019, and the number of deaths increased from 960 to 4032 between 2000-2015. Asia represents about 70% of the worldwide burden of disease (2022). According to National Vector Borne Disease Control Program (NVBDCP), 13 April 2022, a total of 1,23,106 dengue cases and 90 deaths were reported in India. In M.P.

State, 11,354 dengue cases and 0 death were reported in 2021 (NVBDCP, 2022).

In rural areas, the primary diagnosis of dengue is based on clinical symptoms due to limited access to diagnostic methods. In this situation, a fast, accurate, and cost-effective method of diagnosis can prevent the evolution of severe clinical cases (Jenny et al., 2021). The confirmatory diagnosis includes the detection of NS1 (Ag) antigen with corresponding IgM and IgG antibodies (Ab) by Enzyme immunoassay & Immunochromatographic test (EIA & ICT) and detection of viral genome by reverse transcription polymerase chain reaction (RT-PCR) (Joshi et al., 2018).

In dengue fever, non-structural protein (NS1 Ag) is identified as an early marker in serum at high concentrations in dengue patients typically between 1-9 days of onset of clinical manifestations (Joshi et al., 2018). The primary infection is characterized by the presence of IgM Ab in 3-5 days after the commencement of infection that persists for 1-3 months. Whereas, secondary infection is characterized by raised IgG Ab with a peak at 6-15 days after the onset of symptoms (Jenny et al., 2021). Other than these, there is an alteration in diverse hematological parameters in the course of dengue fever. Platelet count, total leucocyte count, neutrophils, and lymphocytes, that too aid in the diagnosis and prognosis of

infection. Thus, the study aimed to investigate seroprevalence with a correlation among serological and peripheral blood count of dengue. Monitoring both parameters will be helpful in the timely accurate diagnosis and prognosis of the disease.

Method and materials

The present study is a retrospective cross-sectional study and approved by the Institutional ethics committee of Government Bundelkhand Medical College Sagar (M.P). Our study included 718 positive dengue cases presented at a tertiary care hospital, Sagar, for 1 year from Jan 2021-Dec 2021.

The laboratory diagnosis was done based on solid phase Immuno-Chromatography method using a rapid diagnostic test kit (Standard Q Dengue Duo) with NS1 Ag, IgM Ab, and IgG Ab reactivity toward dengue fever (Jang et al., 2019). The positive cases were divided into 7 serogroups i.e. (NS1Ag), (NS1Ag, IgMAb), (IgMAb), (NS1Ag, IgGAb), (IgMAb, IgGAb), (NS1Ag, IgMAb, IgGAb), and (IgGAb). The hematological parameters were analyzed by an automated hematology analyzer. Fig. 1 Shows a flowchart of the methodology of the serological test of dengue by a rapid diagnostic test kit.

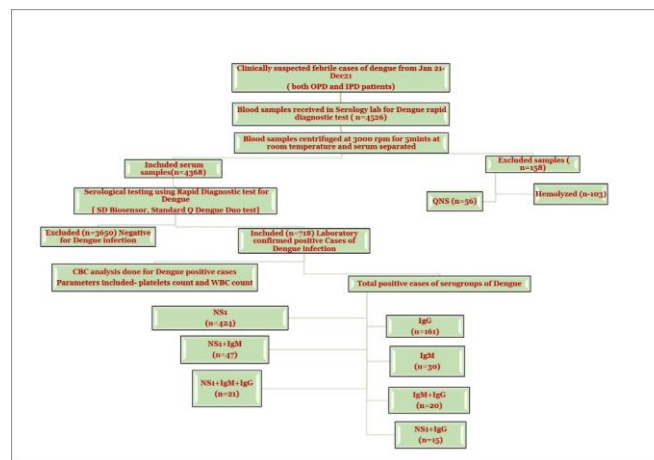


Fig. 1 Methodology of serological testing of dengue by a rapid diagnostic test kit.

Statistical analysis

Statistical evaluation was carried out on MS Windows. The statics were first keyed into a Microsoft Excel spreadsheet and then analyzed with the usage of SPSS, version (12.0.1. or 20.0). The mean standard deviation/standard error of the mean and 95% confidence interval for the mean was calculated. Differences between groups were compared using the Pearson chi-square test for categorical variables. Correlation analysis between independent parameters was done by ANOVA test. (P value ≤ 0.05 were considered significant).

Results and discussion

Overall, 718 laboratory-confirmed dengue cases were analyzed from Jan 2021-Dec 2021. The highest number of cases occurred in the month of October at 49.72%. Young adults (19-30yr) were most commonly affected, with 48.7% of cases within 19-60 years. Table 1. The age and gender distribution of serum markers of dengue showed a higher proportion of NS1 Ag in both adult males and females compared to other serogroups and age groups. The maximum number of cases was present in males with a male: female ratio of 1.5. Fig. 2 Shows the distribution of dengue-positive

Table 1. The age and gender distribution of serum markers in dengue-positive cases.

Serum markers	Number	AGE GROUP					
		0-12 Years		13-18 Years		19-60 Years	
		Male	Female	Male	Female	Male	Female
NS1	424	78	58	72	40	103	73
IgG	161	20	10	10	12	69	40
NS1+IgM	47	12	10	7	3	9	6
IgM	30	4	4	3	4	7	8
NS1+IgM+IgG	21	4	1	2	2	10	2
IgM+IgG	20	3	0	5	1	8	3
NS1+IgG	15	1	0	3	1	6	4

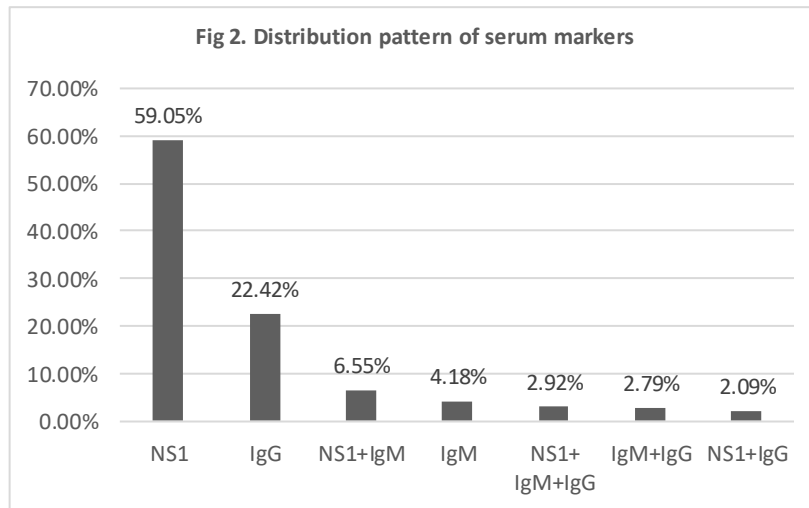


Fig. 2 The distribution pattern of serum markers in dengue-positive cases.

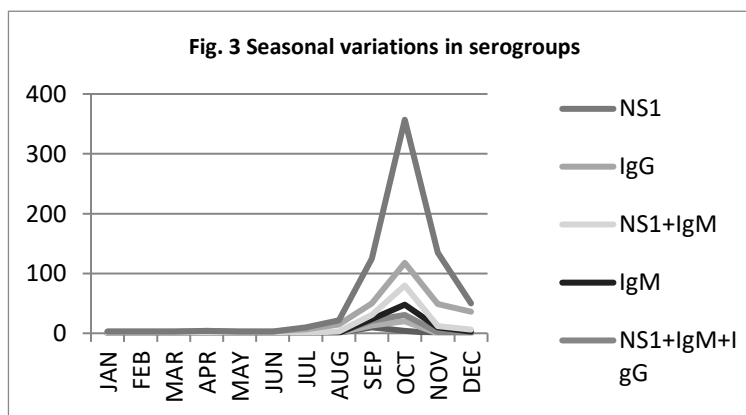


Fig. 3 The seasonal variations of serum markers in dengue-positive cases.

cases into 7 serum markers and showed maximum cases of NS1 Ag followed by IgG Ab positive cases Fig. 3. Seasonal variations in serogroups of dengue are shown by plotting the graph. The graph shows a peak in dengue-positive cases in October month with a predominance of NS1 Antigen. Fig. 4 Shows the evaluation of peripheral blood parameters (Hemogram). Our study shows thrombocytopenia (55.8%), leucopenia (38.7%), neutropenia (11.6%), and lymphocytosis (17.1%). Table 2. Shows the correlation between serological

and hematological parameters tested by the Annova test. The mean value was calculated and observed a significant association of platelet count, WBC, neutrophil, lymphocyte, monocyte, and eosinophil with serological groups. Also, there is a correlation of serum markers with indoor and outdoor patient status.

Our study analyzed the presence of serum markers with age distribution and reported the maximum number of positive cases were young adults from 19-60 years of age with a prevalence of 48.7%. and with a male predominance of 1.5:1 concordance with the study results reported by Rai, Azad, Nautiyal, and Acharya (2019). This study shows a correlation

between serogroup distribution with age but no correlation with gender. In the current study, the maximum number of cases reported in the post-monsoon period in October month at 59.8%, which is in concordance with the study done by Patil et al. (2020).

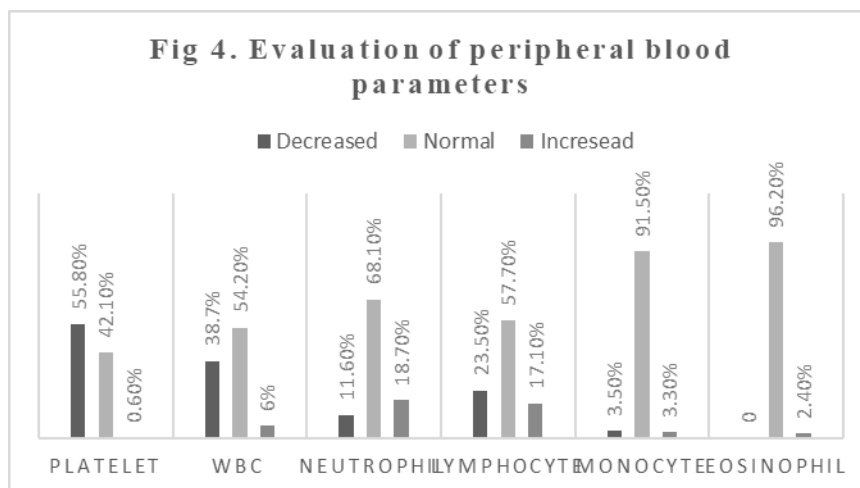


Fig. 4 Evaluation of peripheral blood parameters (Hemogram).

The seropositivity pattern showed NS1 antigen predominance followed by IgG antibody showing concordance with study results by Joshi et al. (2018). Whereas (NS1Ag, IgM Ab) and (IgM Ab) patterns are similar to Rai et al. (2019) study. And had a higher proportion of (NS1Ag, IgMab) serum markers in children than in adults. Thus, this study shows a significant relationship between age and serum markers, whereas no significant differences between genders. A similar analysis was reported in a study done by Joshi et al. (2018). NS1 Antigen is the earliest marker detected in blood between 1-9 days during the acute phase of dengue fever. IgM is the earliest antibody to be detected within 3-5 days or after day 5 and not detected after 2-3 months of infection (Rai et al., 2019; Joshi et al., 2018) indicating active/recent infection. As IgM is nonspecific, they can cross-react with other Flaviviruses. Therefore, (NS1Ag, IgM Ab) is a more specific serological marker compared to IgM Ab only. Detection of (NS1Ag, IgGAb), and (IgM Ab, IgG Ab) indicates the seroconversion stage of infection. IgG antibodies will be detected at the end of the first week of the onset of symptoms indicating past infection, secondary infection, or cross-reaction with other *Flavivirus* antigens. and remain lifelong in some patients. There is an increased risk of severe diseases like dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) occurring during secondary infection due to antibody-dependent enhancement (ADE) of infection (Narayan and Tripathi, 2020).

It is important to distinguish primary infection from secondary dengue infection early in the course of illness. It helps predict prognosis and decide whether a patient needs admission and close monitoring or could be managed at home (Changal et al., 2016). Therefore, in our study, we also analyzed serological parameters with patient OPD and IPD status. And observed the predominance of NS1 Ag (59.05%) followed by IgG Ab (22.42%) in outdoor patients and adults. In contrast to (NS1Ag, IgM Ab) (6.55%) followed by IgM Ab (4.18%) predominantly in indoor patients and children.

Our study also evaluates peripheral blood parameters (Hemogram) and their correlation with serological parameters by the Anova test. The mean value in different serogroups was obtained and a confidence interval of 95% and P-value <0.05. The hemogram was compared with 7 serogroups. NS1, IgM, IgG, (NS1, IgM), (NS1, IgG), (IgM, IgG), (NS1, IgM, IgG), and observed significant correlation with platelet count, TLC(WBC), Neutrophil, Lymphocyte, Monocyte, and Eosinophil.

Our study noted Leucopenia in 273/705 (38.7%) cases, in concordance with studies by Joshi 2019; Rai 2020; where Leucopenia has been reported in 36%, and 37.8% respectively. It is a major finding in dengue caused by bone marrow suppression by a virus (Rai et al., 2019; Joshi et al., 2018). A small number of patients were detected with Leukocytosis (6%) can be seen secondary to superadded bacterial infections

Table 2. Statistical analysis of the correlation between serological and hematological parameters.

Parameter		NSI	IgG	NSI+ IgM	IgM	NSI+IgM +IgG	IgM+ IgG	NSI+ IgG
Platelet	N	415	159	47	30	20	20	15
	Mean	137860	206422	113765	172400	90250	128000	86700
	SD	88257	126709	61352	172400	96351	87822	93499
P-value .000								
WBC	N	415	159	47	30	20	20	15
	Mean	4912.5	7695.5	4297.8	8300.0	5210.0	7950.0	4400.9
	SD	4249.0	7476.1	2282.2	58.37.0	4056.0	3268.9	2387.4
P-value.000								
Neutrophil	N	415	159	47	30	20	20	15
	Mean	63.25	58.62	47	55.53	53.10	58.35	58.00
	SD	16.466	14.281	15.723	17.610	14.535	19.599	12.851
P-value .000								
lymphocyte	N	415	159	47	30	20	20	15
	Mean	28.83	32.26	43.28	35.20	38.10	32.30	31.40
	SD	15.311	13.041	15.321	16.142	13.611	17.541	10.829
P-value .000								
Monocyte	N	415	159	47	30	20	20	15
	Mean	5.14	5.69	6.13	5.90	5.75	5.50	6.80
	SD	2.631	2.381	2.542	2.510	2.552	3.187	2.757
P-value .015								
Eosinophil	N	415	159	47	30	20	20	15
	Mean	2.78	3.33	3.32	3.37	3.05	3.35	3.80
	SD	1.986	1.806	1.520	1.542	1.731	1.137	2.242
P-value .011								

or in the presence of hemorrhagic shock (Rai et al., 2019). Neutropenia is the decrease in granulocytes in bone marrow in the early phase as well as marked degeneration of mature neutrophils during the febrile illness observed in 18.7% of the cases. In the current study, neutrophilia was noted in only 11.6% and Lymphocytosis in 17.1% of cases. Monocytes are predominant targets of dengue viruses and were noted in only 3.5% of cases showing concordance with the study by Rai (2019) with 4.9%. Eosinophilia was present in 2.4% of cases in concordance with a few studies. Thrombocytopenia was noted in 55.8% of cases. It is the immune-mediated clearance of platelet by anti-dengue antibody-mediated platelet destruction. Thus, thrombocytopenia is a major finding in the differential diagnosis of dengue (Joshi et al., 2018). Therefore, early diagnosis of dengue is important for timely clinical intervention and disease control (Jang et al., 2019). Limitation of the study: Hemoglobin and hematocrit variables were not included in this study. And very few similar studies compared data and derived conclusions.

Conclusion

The current study observed the utility of simple, rapid, and cost-effective rapid diagnostic test based on immunochromatography and hematological parameters in the diagnosis and prognosis of dengue, especially in rural and peripheral areas during the limited resources in the public healthcare system. This becomes important in the course of epidemics and outbreaks when hospitals are flooded with

patients and early triage is necessary. Thus, evaluating the correlation between serological and hematological parameters will be helpful in diagnosis and monitoring the disease course.

Conflict of Interest

The author hereby declares no conflict of interest.

Consent for publication

The author declares that the work has consent for publication.

Ethical Considerations

The study was approved by the institutional ethical committee.

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