ORIGINAL RESEARCH PAPER

Pathology

CLINICOPATHOLOGICAL HEMETOLOGICAL AND MOLECULAR STUDY OF DENGUE SUSPECTED PATEINTS -A PROSPECTIVE STUDY IN TERTIARY CARE HOSPITAL

KEY WORDS: Dengue fever, NS1 antigen, IgM antibody, conventional PCR

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ABSTRACT

Background: Dengue is common mosquito born viral disease of human caused by dengue virus .Dengue virus belongs to family of Flaviviridae and there are 4 serotypes of virus which are DENV-1 to DENV -4. Across the world about 100 million infections are detected every year in which 5,00,000 manifest in hemorrhagic cases. By clinical presentation and laboratory test, infection with dengue can be diagnosed. **Method:** The study was from March 2020 to November 2021.Total 200 samples were taken . All the patients suspected for dengue were considered in this study and after ELISA only positive cases for NS1 were undertaken for molecular study. **Result:** A total 200 clinically suspected samples were taken . Out of 200 samples 120 (60.0%) were dengue positive , 80 (40.0%) were dengue negative . Out of 120 serologically dengue positive samples, 81 (67.5%) were positive for NS1 antigen and 39 were positive for IgM by ELISA. Out of 120 dengue posistive cases fever was the most common clinical presentation in 120 (100%) and thrombocytopenia was the most common hematological finding present in 102 (85.0 %) cases . Out of 81 (67.5%) dengue NS1 antigen positive samples on which molecular study was done showed, 22 (27.16%) positive for dengue virus RNA and 59 (72.84%) negative for dengue virus RNA by conventional PCR method. **Conclusion:** Awareness of clinical features, laboratory findings consisting of hematological and serological parameters as well as molecular study are important for diagnosis and management of dengue and dengue like illness.

INTRODUCTION:

Dengue is common mosquito born viral disease of human caused by dengue virus[1]. Dengue virus belongs to family of Flaviviridae and there are 4 serotypes of virus which are DENV-1 to DENV -4[2]. Across the world about 100 million infections are detected every year in which 5,00,000 manifest in hemorrhagic cases [3]. It may be result in nonspecific febrile illness like sudden onset of fever, severe headache, arthralgia, myalgia, bleeding manifestation, rash [4]. Dengue infection result in a disease continuum includes syndromes varying in severity and prognosis that are Dengue fever(DF), Dengue hemorrhagic fever (DHF), Dengue shock syndrome (DSS)[5] and among these all dengue hemorrhagic fever is most severe.Thrombocytopenia is most common feature of dengue fever. By clinical presentation and laboratory test, infection with dengue can be diagnosed[6] Laboratory diagnosis of dengue infection depends upon demonstration of specific antibodies in serum by hemagglutination inhibition, complement fixation, neutrilization test or ELISA. Other new test for diagnosis are reverse transcriptase PCR and hybridization probes for nucleic acid.

MATERIAL AND METHOD:

This Prospective study was carried out in the Department of Pathology, GAJRA RAJA Medical College & Hospital, Gwalior (M.P.) from 1st of March 2020 till 16th of November 2021. Total 200 samples were taken. All the patients suspected for dengue were considered in this study and pateints with fever but serologically negative were not included. Blood samples were collected from all 200 clinically suspected patients of dengue and Complete blood count were done by using fully automated analyser. Samples were screened for presence of dengue specific IgM antibodies using ELISA and dengue NS1 antigen detection were done using dengue NS1 Ag MICROLISA kit. Then molecular study was carried out only in NS1 positive samples by conventional PCR.

RESULT:

During the study period, total 200 clinically suspected samples were taken.

Out of 200 clinically suspected samples 120 were dengue positive (60.0%), 80 were dengue negative (40.0%).

Table 1: Distribution of the Participants in Terms of Dengue (n = 200)

Dengue	Frequency	percentage
positive	120	60.0%
negative	80	40 .0 %

Table 2 : Association Between Dengue and Age (n = 200)

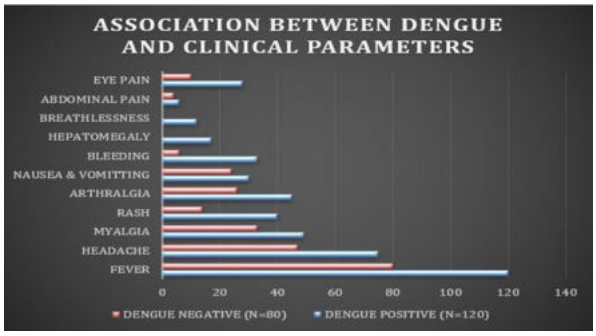
Age	Dengue	Fisher's Exact Test			
	Positive	Negative	Total	$\chi 2$	P Value
≤10 Years	10 (8.3%)	3 (3.8%)	13 (6.5%)	14.448	0.014
11-20 Years	46 (38.3%)	14 (17.5%)	60 (30.0%)		
21-30 Years	36 (30.0%)	34 (42.5%)	70 (35.0%)		
31-40 Years	13 (10.8%)	16 (20.0%)	29 (14.5%)		
41-50 Years	11 (9.2%)	11 (13.8%)	22 (11.0%)		
51-60 Years	2 (1.7%)	1 (1.2%)	3 (1.5%)		
61-70 Years	2 (1.7%)	1 (1.2%)	3 (1.5%)		
Total	120 (100.0%)	80 (100.0%)	200 (100.0%)		

Fisher's exact test was used to explore the association between 'Dengue' and 'Age' as more than 20% of the total number of cells had an expected count of less than 5.
In dengue positive group 8.3% of the patients were ≤10 Years of age, 38.3% were 11-20 Years, 30.0% were 21-30 Years, 10.8% were 31-40 Years of age. 9.2% were 41-50 Years, 1.7% were 51-60 Years, 1.7 % were 61-70 Years of age.

Table 3: Association Between Dengue and Gender (n = 200)

Gender	Dengue			Chi-Squared Test	
	Positive	Negative	Total	$\chi 2$	P Value
Male	86 (71.7%)	52 (65.0%)	138 (69.0%)	0.997	0.318
Female	34 (28.3%)	28 (35.0%)	62 (31.0%)		
Total	120 (100.0%)	80 (100.0%)	200 (100.0%)		

Chi-squared test was used to explore the association between 'Dengue' and 'Gender'.
Gender was not significantly associated with variable dengue.
In the dengue positive group 71.7% of patients were Male, 28.3% were Female
In the dengue negative group 65.0% were Male, 35.0% were Female.



Graph no. 1

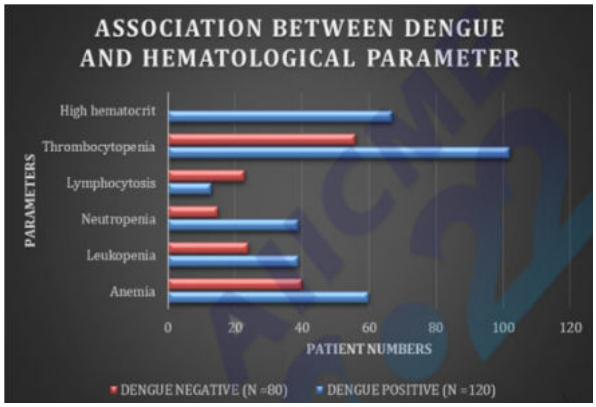
Table 4: Association between Dengue and clinical parameters (n= 200)

Parameters	Dengue		p value
	Positive (n = 120)	Negative (n = 80)	
Fever (Yes)	120 (100.0%)	80 (100.0%)	1.000[3]
Headache (Yes)	75 (62.5%)	47 (58.8%)	0.594[3]
Myalgia (Yes)	49 (40.8%)	33 (41.2%)	0.953[3]
Rash (Yes)***	40 (33.3%)	14 (17.5%)	0.013[3]
Arthralgia (Yes)	45 (37.5%)	26 (32.5%)	0.469[3]
Nausea/vomiting (Yes)	30 (25.0%)	24 (30.0%)	0.435[3]
Bleeding (Yes)***	33 (27.5%)	6 (7.5%)	<0.001[3]
Hepatomegaly (Yes)***	17 (14.2%)	0 (0.0%)	<0.001[3]
Breathlessness (Yes)***	12 (10.0%)	0 (0.0%)	0.002[2]
Abdominal Pain (Yes)	6 (5.0%)	4 (5.0%)	1.000[2]
Eye pain (yes)	28(23.3%)	10(12.5%)	0.056[3]
The following variables were significantly associated (p < 0.05) Presentation: Rash, Bleeding, Hepatomegaly, Breathlessness while other parameters are not significant.			

Table no. 5 -Association between Dengue and hematological parameters (n=200)

Parameters	Dengue		p value
	Positive (n = 120)	Negative (n = 80)	
Anemia (Yes)	60 (50.0%)	40 (50.0%)	1.000[1]
Leukopenia (Yes)	39 (32.5%)	24 (30.0%)	0.709[1]
Neutropenia (Yes)***	39 (32.5%)	15 (18.8%)	0.032[1]
Lymphocytosis (Yes)***	13 (10.8%)	23 (28.7%)	0.001[1]
Thrombocytopenia (Yes)***	102 (85.0%)	56 (70.0%)	0.011[1]
Raised Hematocrit (Yes)***	67 (55.8%)	0 (0.0%)	<0.001[1]

The following variables were significantly associated (p< 0.05) with the variable 'Dengue':, Neutropenia, Lymphocytosis, Thrombocytopenia, Raised Hematocrit, while other parameters are not significant.



Graph no. 2

Table 6: Distribution of dengue with serology

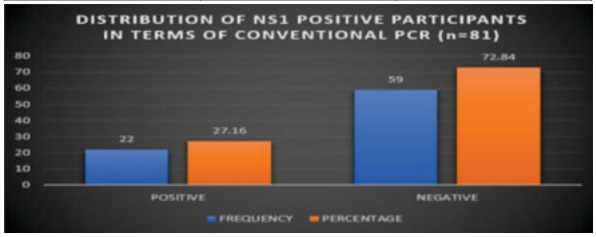
Serologically tested confirmed	Number (percentage)
NS1 positive only	56(46.67%)
IgM positive only	39(32.5%)
Positive for both serological markers	25(20.83%)
Total dengue positive	120(100%)

Table 7: Distribution of the Participants in Terms of NS1 (n = 200)

NS1	Frequency	Percentage
Positive	81	40.5%
Negative	119	59.5%

Table 8: Distribution of NS1 positive Participants in terms of Conventional PCR (n = 81)

Conventional PCR	Frequency	Percentage
Positive	22	27.16%
Negative	59	72.84%



Graph no. 3

DISCUSSION:

Dengue is an important emerging disease of the tropical and subtropical region today. Global incidence of dengue has grown dramatically in recent decades. In our study which was conducted in the department of Pathology, GRMC Medical college, Gwalior during the period from March 2020 to November 2021, total 200 dengue suspected cases were studied, out of which 120 (60.0%) were serologically confirmed cases of dengue infection and 80 (40.0%) were serologically negative patients.

In our study out of 120 (60.0%) serologically positive cases 56 (46.67%) are NS1 antigen positive, 39 (32.5%) are dengue specific IgM antibodies positive and 25(20.83%) are positive for both the seromarkers (NS1 and IgM) of dengue.

In the study done by **Rubina Paul et al** [7] on 3306 patients clinically suspected for dengue fever were tested for seromarkers of NS1 and IgM. It was found 60.6% (2005/3306) patients were serologically positive for dengue virus among which 59% (1181) of total samples (3306) were tested positive for NS1 antigen, 41% (824) were IgM positive.

In the study conducted by **Manoj et al** [8`] the maximum cases belongs to age 13- 18 yrs. Numerous authors detected that the maximum number of cases were seen in age group of >11 years(34.02%) and the least affected age group as infants.[9]

In the present study out of 120 dengue positive cases maximum number of cases belong to 11-20 years age group 46 (38.3%) followed by 21-30 years age group 36 (30.0%), followed by 31-40 years age group 13(10.8%), followed by 41-50 years age group 11 (9.2%), followed by < 10 years of age group 10 (8.3%) followed by 51`- 60 years of age group 2 (1.7 %) and 61- 70 years had also age group 2 (1.7 %) .

So age is found to be significantly associated with dengue (p<0.05).

In this study out of 120 dengue positive cases, 86 (71.7%) patients are males and 34 (28.3%) are females. Similar study which was conducted **by Bashir et al** [10] in which male patient were 63.4 % and female patient were 36.6% and in the study **of Rubina et al** there was also male preponderance. In our

study out of 80 dengue negative patients, 52 (65.0%) patients are male and 28 (35.0%) are females. There is no significant difference between the various groups in terms of distribution of Gender ($\chi 2$ = 0.997, p = 0.318). Gender variation is not significantly associated with dengue.

Out of 120 cases fever is the most common clinical presentation in 120 (100%) followed by headache 75(62.5%), myalgia 49 (40.8%), arthralgia 45 (37.5%), rash 40 (33.3%), bleeding manifestations 33 (27.5%), nausea/vomiting 30 (25.0%), eye pain 28 (23.3%), Hepatomegaly 17 (14.2%) breathlessness 12(10.0%) ,abdominal pain 6 (5.0%).

Similar study was conducted by **Mehta et al** [11] in which fever was present in all the patients of dengue infections.

In the study by **Raj et al** [12], most common feature was fever followed by headache and equal number of patients presenting with vomiting and abdominal pain .

Among the 80 controls fever is the most common symptom 80 (100%), followed by headache 47 (58.8 %), myalgia 33 (41.2 %), arthralgia 26 (32.5%), nausea/vomiting 24 (30.0%), eye pain 10 (12.5%), bleeding 6(7.5%), abdominal pain 4 (5.0%), hepatomegaly and breathlessness 0 (0%). So the clinical features of serologically dengue negative patients are similar to that of serologically dengue positive patients and so the symptomatology has been aptly grouped under umbrella term Dengue like illness (DLI) .

Skin Rash, Bleeding, Hepatomegaly, Breathlessness were significant variables found to be associated with dengue ($p < 0.05$). while other clinical parameters are not significant.

Present study show thrombocytopenia as the most common hematological finding present in 102 (85.0 %) cases, followed by raised hematocrit 67 (55.8%), anemia 60 (50.0%), leukopenia 39(32.5%), neutropenia 39(32.5%), raised SGPT 33 (27.5 %), Lymphocytosis 13 (10.8%) .

Similar findings were also found **in Getachew et al** [13] in which thrombocytopenia was the most common hematological feature 61 (59.8 %) out of 102 cases, anemia 45 (44.1%) and leukopenia 27 (26.5 %).

Thrombocytopenia might be due to decreased production of platelet due to suppression of the bone marrow by a virus and also due to binding of dengue antigen to platelet and increased antibody mediated immunological destruction of platelets. [14]

Raised hematocrit occurs due to hemoconcentration due to increased plasma permeability which is the basic pathophysiological change in dengue. [15]

Among the 80 dengue negative patients thrombocytopenia is present in 56 (70.0%), anemia 40(50.0)%, leukopenia 24(30.0%), lymphocytosis 23(28.7%), neutropenia 15 (18.8%). Raised hematocrit is not present in these patients.

Our study show variables found to be significantly associated ($p < 0.05$) with the 'Dengue' are Neutropenia, Lymphocytosis, Thrombocytopenia, raised Hematocrit .

While other parameters do not show significant association .

Out of 120 serologically dengue positive samples, 81 (67.5%) are positive for NS1 antigen and 39 are positive for IgM by ELISA where the sample collection was done in Acute phase of illness.

Out of these 81 (67.5%) dengue NS1 antigen positive samples on which molecular study was done showed, 22 (27.16%) positive for dengue virus RNA and 59 (72.84%) negative for dengue virus RNA by conventional PCR method.

A study conducted by **Chua et al** [16] tested 558 patients with clinical diagnosis of dengue, the sensitivities were 91.6%, 58.9%, 48.4%, 40.5% for NS1 ELISA, real time RTPCR, conventional PCR and virus isolation respectively .

Another study conducted by **Shashi et al**,[17] also found that dengue complex specific RTPCR(conventional PCR), were seen to miss few cases as compared to the real time RTPCR method and Real time PCR is highly sensitive and promising tool than other molecular diagnostic methods.

CONCLUSION:

In current study we got 120 (60.0%) serologically confirmed cases of dengue viral infection by ELISA out of 200 patients admitted in JAH with symptoms of Dengue and Dengue like illness. Majority of the positive patients are in 11-20 years age group. There are male preponderance in our study. On the basis of this study we can say that, the most common presenting symptoms in dengue positive patients is fever followed by headache, rash, bleeding, hepatomegaly, breathlessness, myalgia, arthralgia, nausea/vomiting, abdominal pain.

Thrombocytopenia is the important hematological findings found in majority of patients followed by raised hematocrit.

These findings should prompt physician on the probability of dengue infection in the study area so as to take immediate majors for crisis management. Out of 120 serologically confirmed dengue cases 81 (67.5%) are positive for NS1 antigen by ELISA which are further evaluated by conventional PCR method. By the conventional PCR 22 (27.16%) samples have confirmed RNA virus .

By this study we can say that awareness of clinical features, laboratory findings consisting of hematological and serological parameters as well as molecular study are important for diagnosis and management of dengue and dengue like illness.

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