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ORIGINAL RESEARCH

Determination of NS1, IgG and IgM profile during an outbreak of Dengue at Tertiary Care Teaching Hospital, Doda, Jammu & Kashmir

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ABSTRACT
The present study is based on the assesment of fast of Immunochromatography test (ICT) over already existing ELISA test to detect and diagnose the dengue suspected cases. The antigen and antibodies identification criteria are used to detect the dengue specific NS1 antigen, IgM AND igG antibodies. A total of 635 patients' blood samples were collected from Government Medical College & Hospital in Doda, Jammu & Kashmir. Thepatients were showing the signs of fever for four days with established trombocytopenia (≤1,00,000 platelets/µL of blood). The similar samples were already subjected to be assessed by ELISA had given 26.0% positive rate. The similar detection pattern was reported in 98 samples checked by both the tests. Furthermore, NS1 antigen detection through ICT had displayed 57.6% sensitivity and 88.1% specificity. All the samples were detected against NS1, IgM and IgG to avoide the false positive results throughout. We propose that ICT rapid method is an excellent and ease to handle point of care method to be deployed for dengue cases screening in the outbreak areas.

Key words: Dengue, Immunochromatography, ELISA, NS1 Antigen, IgM and IgG Antibodies.

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INTRODUCTION

In tropical and subtropical areas of the world, dengue is a viral disease that spreads the fastest, carried by mosquito vector [1] and is a significant public health issue [2]. WHO reports 8 fold increase in dengue cases over the past two decades from 505,430 cases in 2000 to 5.2 million in 2019 with 960 reported deaths in 2000 to 4032 reported deaths in 2015 (3).India, Indonesia, Myanmar, Sri Lanka and Thailand are among the highly endemic countries in the world with epidemic risk in recent decades due to Dengue showing increase from 8 to 30 times. In India, over the past two decades, number of states where dengue is endemic has gone up from 8 to 35. Dengue is responsible for 16% of travel-related febrile illnesses.Dengue virus was first isolated in 1943, since then four serotypes have been identified (4) [genetically related but antigenically distinct] (DENV-1, DENV-2, DENV3, DENV-4) that belong to the Flaviviridae family. Lifelong immunity to a given serotype is provided after recovery from a single infection episode, but cross-immunity to other serotypes is only temporary and limited [5, 6].In 2009, WHO proposed revised classification of DENV infection as follows: 1) dengue; 2) dengue with warning signs (abdominal pain, persistent vomiting, fluid accumulation, mucosal bleeding, lethargy, liver enlargement, and increasing hematocrit with

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decreasing platelets); and 3) severe dengue (dengue with severe plasma leakage, severe bleeding, and/or organ failure) [8]. The ability to swiftly establish an acute dengue infection should aid in providing patients with the right care and management as soon as practicable [9]. Therefore, it is essential to treat dengue infected people as soon as possible to stop the development of severe illness types.

Transmission: The dengue virus is spread through a human-to-mosquito-to-human cycle of transmission, with the mosquito *Aedes aegypti* as the primary vector along with *Aedes albopictus*. For transmission to occur the female Aedes aegypti must bite an infected human during the viremia phase of the illness that manifests two days before the onset of fever and lasts 4–5 days after onset of fever. After ingestion of the infected blood meal the virus replicates in the epithelial cell lining of the midgut and escapes into hemocoel to infect the salivary glands and finally enters the saliva causing infection during probing. The extrinsic incubation period (EIP) lasts from 8 to 12 days and the mosquito remains infected for the rest of its life. The intrinsic incubation period covers five to seven days [7]. The virus also gets passed on to the egg of the female Aedes mosquito. Such mosquitoes carry the virus by birth (4).

Different biomarkers can be used to make the diagnosis. They include the separation of the virus from mosquitoes or culture, detection of viral genomic RNA, capture and detection of viral products (NS1 protein), or the evaluation of the host immune response to viral infection (IgM and IgG antibodies specific to the virus) [10].Primary infection is indicated by a considerable rise in IgM levels 3–5 days following the onset of symptoms. This may last for one tothree months. IgM can also be found in secondary infection, and high levels of IgG are present 6–15 days after the onset of symptoms [11]. Two blood samples must be collected following the World Health Organization's (WHO) definition of a dengue case in an acute febrile illness. The first sample was taken between 1 to 5 days following the start of symptoms, and the second sample was taken between 6 and 14 days later, during the convalescent phase [12]. In this context, this study was carried out to detect Dengue specific NS1 antigen, IgM and IgG antibodies by Enzyme Immunosorbent Assay (ELISA) and rapid Immuno-chromatographic (IC) card test, respectively. There is no treatment for dengue at present and the only available vaccine is not capable of preventing the disease or reducing its severity in the infected (4).

AIM

To compare the immuno-chromatographic test and ELISA for NS1 antigen, so as to improve the early detection of dengue.Dengue seroprevalence research has never been conducted in the Doda district of Jammu and Kashmir. Hence, this study is first of its kind to estimate the prevalence of Dengue in Doda district of Jammu province.

MATERIAL AND METHODS

Place and time period of study: The cross sectional hospital based study was conducted at the Department of Microbiology, Govt. Medical College, Doda Jammu and Kashmir. Doda is the largest district (Geographical Area-2758.95 Sq Km) in the Jammu province having population of 409,936 (Male: 213,641, Female: 196,295), located at 33.13°N 75.57°E, at an altitude of 5000 feet above the sea level (13). The study was conducted from June 2022 to November 2022.

Inclusion criteria: Serum samples of patients with acute onset of fever of >4 days, Patients suspected of viral fever with temperature more than 37.5°C with or without rashes, and a positive tourniquet test followed by lab investigations such as low platelet counts.

Exclusion criteria: Those febrile patients with normal platelet count/haematocrit level or those who were positive for malaria after peripheral smear examination were excluded from the study.

Method of processing

Collection and transport of samples: A total of 635 serum samples from patients with a possible dengue infection were obtained in red-capped vacutainer for the present study. The serological detection of dengue IgM and IgG as well as the detection of the dengue NS1 antigen were used to assess the severity of the dengue infection in these patients. All samples were examined using an immune-chromatographic test, which is available commercially, (J. Mitra Company Pvt. Ltd. New Delhi, India) for NS1, IgM, and IgG antibodies test) and an Enzyme linked Immunosorbent assay, (ELISA) using RecombiLISA™. Samples were centrifuged and plasma separated. Samples were processed according to the manufacturers instruction. Those samples not processed within 6 hours were preserved at -20°C and were processed within 3 days were collection.

RICT (Dengue NS1Ag + Ab Combo)

Dengue day 1 rapid test

Two devices are included in the Dengue Day 1 Test kit; one is for the differential detection of Dengue

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IgM/IgG antibodies in human serum/plasma, and the other is for the detection of Dengue NS1 antigen. Three lines make up the Dengue IgM/IgG test device: Control line "C," IgM test line "M," and IgG test line "G." Anti-human IgM and anti-human IgG monoclonal antibodies, respectively, are coated on the IgM and IgG test lines. As instructed by the manufacturer, the test was conducted. After 20 minutes, the results were read (positive results could show up as soon as 2–10 minutes). Confirmation of negative results was possible only after 20 minutes.

Specimen processing

The more accurate results from dengue ELISA tests will come from using fresh samples that have not been frozen and thawed. In this investigation, only a few samples were run immediately, while the remainder was stored at a temperature of -20°C. The kit and parts were kept between 2 to 8°C. (The kit's expiration date specifies the time after which it should not be used)

Data analysis: Data was analysed in Microsoft Excel Sheet. Continuous variables were analysed in the form of mean and standard deviation while categorical variables were summed up as frequency and percentages. Chi-square test was used to obtain results.

RESULTS

All 635 samples were tested by both rapid ICT and ELISA for dengue parameters (NS1 antigen, IgM, and IgG antibodies). Out of this ELISA was positive for 26.80% of samples while 24.10% were positive by ICT (Table 1). Detection of Dengue-positive cases by rapid immunochromatographic card test was evaluated for its sensitivity and specificity against ELISA as a reference test for NS1 antigen, IgM and IgG antibody detection. Sensitivity=57.64%, Specificity=88.17%, Positive predictive value=64.05%, Negative predictive value=85.06% were observed (14) (Table 2).

Among 153 ICT positive cases detected, 12 (7.8%) showed positive for NS1 only, 38 (24.83%) for IgM only, 18 (11.76%) for IgG only, 15 (9.80%) for NS1+IgM+IgG, 49 (32.02%) for NS1+IgM, 9 (5.88%) for NS1+IgG and 12 (7.84%) were positive for IgM + IgG. Among 170 ELISA-positive cases, 9 (5.29%) showed positive for NS1 only, 42 (24.70%) for IgM only, 16 (9.41%) for IgG only, 23 (13.52%) for NS1+IgM+IgG, 10 (5.88%) for NS1+IgM, 0 (0.00%) for NS1+IgG and 70 (41.17%) were positive for IgM + IgG (Table 3).

The 635 cases (170 male and 465 female) reported were analysed based on age and sex parameters. Among males, highest number of patients (42) were in the age group of 11-20 years while lowest number of patients (4) were in the age group of >70 years age. Similarly, among females, highest number of patients (132) were in the age group of 21-30 years while lowest number of patients (15) were in the age group of 61-70 years age (Table 4). Patients were showing various symptoms of dengue including vomition (182), headache (168), myalgia (159), bleeding disorder (79) and abdominal pain (47) (Fig 1).

Laboratory findings reveal haematocrit (Hct) was found to be elevated in 72 (11.33%) patients, while 93 (14.64%) and 49 (7.71%) patients represented leukopenia and elevated liver enzymes, respectively. Thrombocytopenia was present in all the study groups (Fig 2).

Table 1: Detection of Dengue cases by rapid ICT and ELISA detection of dengue

Test	Sample Tested	Positive		Negative	
		Cases	%	Cases	%
ELISA	**635**	170	26.80%	465	73.20%
ICT		153	24.10%	482	75.90%

Table 2: Comparison of ICT and ELISA

ICT	ELISA		
	Positive	**Negative**	**Total**
Positive	98 (True Positive / a)	55 (False Positive / b)	**153**
Negative	72 (False Negative /c)	410 (True Negative / d)	**482**
Total	**170**	**465**	**635**

Table 3: Detection of positive cases by ICT

Parameters	ICT	ELISA
NS1 Only	12	9
IgM Only	38	42
NS1+IgM+IgG	15	23
NS1+IgM	49	10
NS1 +IgG	9	0

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IgM + IgG	12	70
IgG Only	18	16
Total	**153**	**170**

Table 4: Age and sex-wise distribution in a study group

Age (in years)	Male		Female		Total	
	No	%	No	%	No	%
0-10	24	14.11%	40	8.60%	64	10.07%
11-20	42	24.70%	82	17.63%	124	19.52%
21-30	22	12.94%	132	28.38%	154	24.25%
31-40	28	16.47%	88	18.92%	116	18.26%
41-50	19	11.17%	46	9.89%	65	10.23%
51-60	21	12.35%	37	7.95%	58	9.13%
61-70	10	5.88%	15	3.22%	25	3.93%
>70	4	2.35%	25	5.37%	29	4.56%
Total	**170**	**26.7**	**465**	**73.2**	**635**	**100%**

Fig 1: Clinical features in the study group

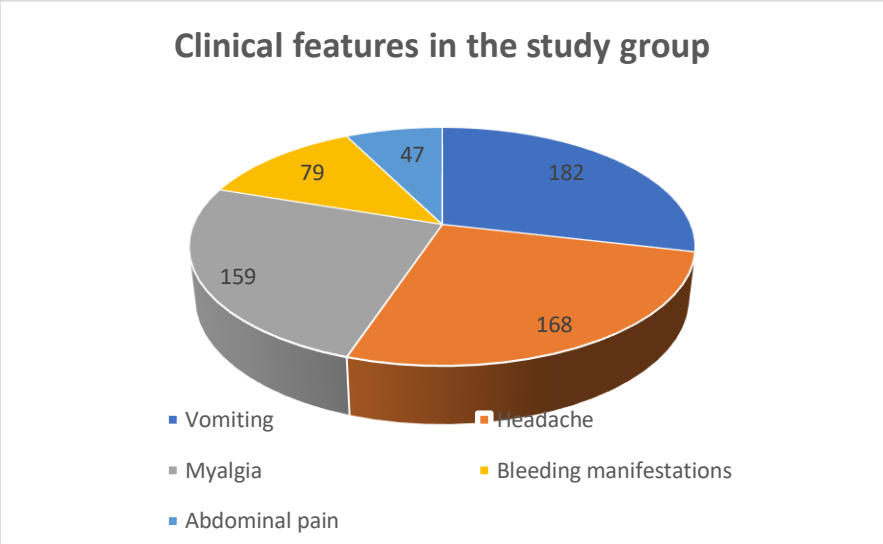
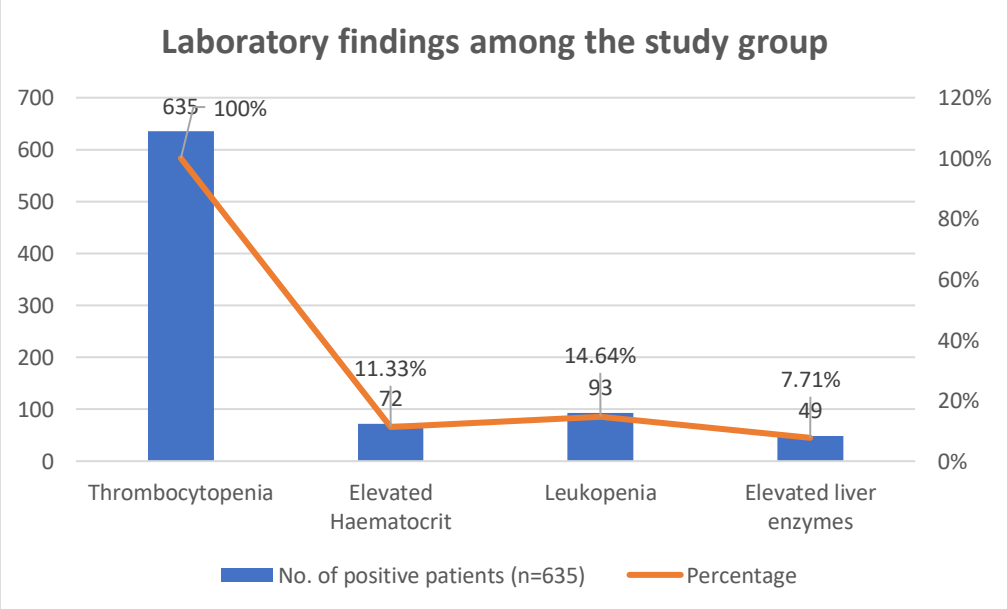


Fig 2: Laboratory findings among the study group



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DISCUSSION

The techniques of dengue serological diagnosis which have been widely used are based on the detection of dengue-specific IgM/IgG antibodies by ICT and ELISA technique. The dengue-specific antibodies begin to appear only around fifth day of fever in primary infection (15). Even in most secondary infections, both the IgM and IgG type antibodies cannot be recorded before third day (16). So, there is a window period, both in primary and secondary dengue infection when no antibodies are detected. This window period can be reduced by using a highly specific and extremely reliable viral marker i.e. dengue NS1antigen, which is detectable in blood from the first day after the onset of fever, both in primary and secondary infections (17).

(add one para here depicting findings of table 1,2,3)

The present study shows that out of 635 suspected cases, females are found to be predominant than the males in the ratio of 2.7:1. However, Sex wise distribution of dengue positive cases in the present study shows that males are more affected than the females. Out of 170 positive cases confirmed by ELISA, 98 (55.6%) were male and 72 (42.3%) were female in the ratio of 1.3:1. These findings are in consonance with [18] and Gargi Ghosh *et al.* (19) who reported similar results where males are more affected than the females. In contrast, a study [20] reported that females are more predominant than the males. High prevalence amongst males is probably due to more outdoor activities by males in comparison to females which results in more exposure to day biting mosquitoes.

Majority of the suspected cases 154 (24.25%) who were seeking medical attention belongs to the age group of 21-30 years in the present study. The present study shows that the majority of positive cases are in the age group of 11-30 years. Out of 170 dengue positive cases shown by ELISA, 58 (34.1%) were in the age group of active adults 11-20 years. Manisha Patankar *et al*(17) also reported the same results in which active adults forms the majority of the positive cases. As active adults are doing more outdoor work activities, there are more chances of them being infected. People of all age groups were affected as also reported by Asima Ajaz, et al., 2023 (21). However, average age group affected is between 21-30 years age. These findings are in consonance with (21),(22) .

The current study shows that among the 635 cases studied presented with fever associated with vomiting 28%, headache 26% had myalgia, 25% had myalgia,12% had bleeding manifestations, 7.4% had abdominal pain. Lab findings shows that haematocrit was found to be elevated in 11% and 14% presented with leukopenia and 7.7% had liver enzymes elevated which was similar to (23) and in contrast to (19)Ghargi Ghosh *et al.* [14].

Prevention: Prevention depends on control of and protection from the bites of *A. aegypti* . The primary method of controlling *A. aegypti* is by eliminating its habitat (24). This is done by getting rid of open sources of water, or by adding insecticides or biological control agents to these areas. People can prevent mosquito bites by wearing clothing that fully covers the skin, using mosquito netting while resting and/or the application of insect repellent (DEET being the most effective) (25). The virus also gets passed on to the egg of the female Aedes mosquito. Such mosquitoes carry the virus by birth. Therefore, vector control strategies should cover adult mosquitoes as well as the larvae and eggs (4). In vector control, by using guppies (Poeciliareticulata) or copepods (Doridicolaagilis) in stagnant water and infecting the mosquito population with bacteria of the Wolbachia genus the mosquito transmission can be controlled (26).

Advantages and disadvantages of Rapid RICT kit over conventional ELISA kits:

Compared with conventional ELISA which needs 4 hours, RICT results are available within 20 min. This will be very helpful in initiating immediate treatment and minimizing the serious complications and mortality of dengue. Conventional ELISA isused for batch testing of large samples (92 samples or twice with 44 samples each time) while RICT can be performed for single or small number of samples. Further performing three conventional ELISA for NS1 antigen, IgM and IgG, for each patient would face lot of practical difficulties. The limitations of Rapid kit are their relatively lower sensitivity and specificity (90%). They are quite susceptible to unfavourable storage conditions and can give false results. RICT which is an easy test can be used in primary care centres so that early diagnosis can be made and morbidity and mortality could be significantly brought down. Since dengue is a notifiable disease, introduction of RICT combination packs in all levels of health care system for speedy and accurate diagnosis and confirmation of the clinical suspicion would be the need of the hour.

CONCLUSION

The commercially available rapid immunochromatographic test device can be used as a screening device during dengue outbreaks but not as a diagnostic tool. It should not be used as a standalone device for diagnosis of dengue. All samples should be subjected to both antigen (NS1) and antibody (IgM and IgG) testing to increase the positivity rate and to prevent missing of positive cases. Cases with higher degrees of suspicion are to be subjected to diagnostic tests with higher sensitivity & specificity like ELISA and PCR.Further molecular studies are essential to know the accurate information of Dengue serotypes

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which will be helpful in formulating vaccines in future.

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