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DENGUE RAPID DIAGNOSTIC TESTS;
EVALUATION OF UTILITY IN A TERTIARY CARE HOSPITAL.

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ABSTRACT… Objectives: To determine the point of care role of dengue IgA and Dengue IgM / IgG rapid diagnostic tests (RDTs) in a tertiary care setting in terms of day of onset of illness at presentation and frequency of positive RDTs in dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). **Study Design:** Cross-sectional study. **Setting:** Abbasi Shaheed Hospital, Karachi. **Period:** August-2014 to January-2016. **Method:** Patients aged 13years and above with acute febrile illness, fulfilling the WHO case definition criteria of probable DF and DHF were included. Two immunochromatograpic (ICT) based RDTs, Assure dengue IgA and Panbio Dengue Duo Cassette (IgM / IgG) were used. Dengue IgA was employed in all patients from day 2 of illness whereas IgM / IgG was employed after day 4 of onset of fever. **Result:** Among 174 probable cases, 108 (62%) presented between 2 – 5 days of onset of fever, among whom 87 (80.5%) were found to be dengue IgA positive. Sixty-nine (39.65%) patients had DHF, among whom 97.1% were seropositive for IgA. Of 118 patients presented after 4 days of onset of illness, 59.3% were positive by IgM / IgG rapid assay. **Conclusion:** Considering the higher frequency of secondary dengue and DHF in dengue endemic-hyperendemic regions, IgA based ICT might be a helpful diagnostic assay for early diagnosis of dengue infection.

Key words: Point of care, Dengue IgA, Dengue IgM/IgG, Dengue hemorrhagic fever, Dengue shock syndrome.

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INTRODUCTION

Dengue virus infection, being endemic in more than 100 countries has emerged as the most important arthropod-borne viral infection globally. Based upon cartographic approach, Bhatt-et-al estimates there may 390 million (95% confidence interval 284-528) dengue infection per year among whom 96 million (67-136) exhibit symptoms.[1] WHO estimates approximately 500,000 people require hospitalization every year with a 2.5% mortality. Nearly 70% of population at risk of contracting dengue lives in South-East Asia and pacific regions.[2] Regular out breaks of dengue virus infection in South Asia Sub-continent countries carries significant impact on morbidity, mortality and economy of the developing countries.

Dengue infection being a systemic acute viral infection shows varying degree of clinical manifestation ranging from self-limiting acute febrile illness to life threatening dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) primarily characterized by increased plasma leakage, impaired haemostasis and endothelial activation.[3]

Hospitalization of suspected dengue cases is important not only for observation and monitoring but also to make an early diagnosis and ensure early and effective supportive care. At tertiary care setting, laboratory based IgM and IgG enzyme-linked immunosorbant assay (ELISA) is the most widely used diagnostic tool employed by the clinicians. However, MAC-ELISA may not be an ideal test for the clinicians as it takes several hours to complete and the facility is not frequently available in even most tertiary care hospitals of developing countries like Pakistan.

Also, as IgM antibodies to dengue virus circulates for up to three months or longer[3], a positive test might not represent recent infection which requires sero conversion with fourfold or greater rise in antibody titers in paired sera. Moreover MAC-ELISA is used five days after the onset of fever (sensitivity 90%, specificity 98%)[4] Whereas most patients usually seek medical advice earlier during febrile illness. Laboratory based non-structural protein 1 (NS1) ELISA has also been used during acute febrile illness[5] but again the facility is not available in most clinical settings. Immunochromatographic (ICT) rapid diagnostic test (RDTs) have been frequently employed by clinicians as the tests are rapid, easy to use and do not require trained staff and specialized equipments. Anti-dengue IgM/ IgG and IgA based RDTs and dengue NS1 based RDTs are commercially available and have been increasingly used not only in remote areas but also in main cities.

During past two decades seasonal outbreaks of dengue have been reported from all provinces in Pakistan. Karachi, and Lahore witnessed major out breaks.[6-7] During seasonal outbreaks large proportion of suspected dengue cases are referred to tertiary care hospitals in both public and private sectors. It has been found difficult to accommodate uncomplicated suspected dengue fever (DF) cases especially in public sector. Also significant number of complicated suspected cases of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) required intensive care monitoring and urgent supportive care.

In present study we determined the point of care role of two dengue RDTs (Dengue IgM/IgG Cassette and Dengue IgA rapid test) as a diagnostic aid in a tertiary care setting in terms of day of onset of illness at presentation and frequency of positive RDTs in patients presenting with DHF/DSS. Early diagnosis will help tertiary care physician making decision, especially in over-burden settings, to admit or follow the uncomplicated suspected dengue cases as outpatients or urgently transfer the suspected dengue patients with warning signs to special or intensive care settings. So, it may help setting out guidelines for the admission criteria in both uncomplicated and complicated dengue infection.

PATIENTS AND METHODS

This cross-sectional study was conducted at Abbasi Shaheed Hospital, Karachi from August-2014 to January-2016. Informed consent was taken from all enrolled cases. Enrollment criteria were all patients aged 13-years and above presenting with acute febrile illness (fever for 2-7 days) and fulfilling the World Health Organization (WHO) cases definition criteria of DF and DHF. Patients were enrolled as probable DF cases as per WHO criteria of probable DF i.e acute febrile illness with two or more of the following manifestations: headache, retro-orbital pain, myalgia, arthralgia, rash, hemorrhagic manifestation, leucopenia and supportive serology i.e a positive immunoglobulin (IgM) antibody test on serum samples collected five or more day after the onset of fever, or occurrence at the same location and time as other confirmed cases of DF. DHF is defined as probable cases of dengue and hemorrhagic tendencies, thrombocytopenia and evidence of plasma leakage i.e 20% rise in haematocrit or signs of plasma leakage (pleural effusion, ascites or hypo-proteinemia).

Patients with sepsis, malaria, enteric fever, bleeding diathesis and haematological malignancies were excluded. .Complete blood count (CBC), peripheral thick and thin smear for malarial parasite, blood cultures, liver function test (LFTs), urea, creatinine and serum electrolyte were performed in all patients, whereas prothrombin time (PT), Internal Normalised Ratio (INR), Activated partial thromboplastin time (APTT) and D-diamers were performed in selected cases.

Two RDTs, were used for the serological test, the Panbio Dengue Duo Cassette (IgM/IgG) and ASSURE® dengue IgA rapid test. The rapid ICT is a qualitative membrane based immunoassay for the detection of dengue specific antibodies (IgM, IgG and IgA) in whole blood, serum or plasma. Dengue due IgM/IgG cassette (sensitivity 92.1%, specificity62.1%)[8] is a lateral flow based ICT for the

qualitative detection of dengue IgM and IgG. The IgG cutoff in the test has been set to detect high IgG levels characteristic of secondary infection. The primary dengue infection in defined by visible IgM band without visible IgG band whereas secondary infection is defined by a positive IgG band with or without positive IgM bands. Dengue IgA RDT (sensitivity 86.7%, specificity 86.05%)[9] is reverse flow technique ICT rapid test for detection of anti-dengue IgA.

At enrollment whole blood samples for two RDTs and other laboratory parameters were taken. Strict adherence to is manufacture advice was employed for RDTs. Acute phase sera of all enrolled patients were also sent for the reference IgM and IgG capture ELISA.

Demographic profile of enrolled patients, detailed clinical history, clinical characteristics such as duration of fever, bleeding manifestations (petechiae, epistaxis, melena, haematemesis, hemoptysis and hematuria), gastrointestinal manifestations (nausea, vomiting and diarrhea) and neurological manifestations (drowsiness, altered behavior and seizure), clinical examination, laboratory parameters and follow up was obtained. For statistical analysis SPSS 20 was used. Frequencies were computed for qualitative variables and mean and standard deviation were computed for quantitative variables, statistical significance was considered at $P<0.05$.

Results

A total of 206 patients were enrolled, of them 31 patients were excluded owing to malaria, 22 patients had plasmodium virus and 9 had falciparum malaria. Among 31 patients with malaria (vivax and falciparum), who presented with fever and thrombocytopenia, IgM dengue ICT was positive in 3 patients whereas dengue specific IgA was found to be negative in all cases. In 11 patients MAC ELISA was missing so they were also excluded. The study population, as such, comprised 174 patients of whom maximum number of patients were admitted in September (n: 42, 24%) and October (n: 65, 37.3%). One hundred and eighteen (67.8%) were admitted through emergency department and 56 (32.1%) were admitted through out- patient department. Overall 119 (68.39%) were male and 55 (31.6%) were female with mean age of 26.7 ± 9.8 (range 14-70 years).

Table-I shows the clinical and laboratory characteristic of patients on presentation. One hundred and eight (62%) patients presented with fever of 2 – 5 days duration while 66 (37.9%) presented between 6 - 9 days of fever.

Fever (duration)	N (%)
Fever : 2 – 5 days	118 (62%)
Fever : > 5 days	66 (37.9%)
Myalgia	126 (72.4%)
Arthralgia	114 (65.5%)
Bleeding manifestation	111 (63.8%)
Rash	73 (42%)
Epistaxis / gum bleeding	82 (47%)
Melena / haematemesis / hematuria	63(36.2%)
Abdominal pain	149 (85.6%)
Nausea / vomiting / diarrhea	98 (56.3%)
Drowsiness / altered behavior	9 (5%)
Leukopenia (< 4,000/mm3)	85 (48.8%)
Platelet count <100,000/mm^3– 50,000/mm^3 <50,000/mm^3– 20,000/mm^3 <20,000/mm3– <10,000/mm3	 24 (13.7%) 73 (42%) 77 (44.25%)
SGPT (>2times normal)	96 (55%)
Torniquete test	42 (24.13%)
Comorbidities *	10 (5.7%)
Length of stay < 5 days >5days	 136 (78%) 38 (21.8%)

Table-I. Clinical and Lab characteristics of enrolled patients at presentation (n = 174)

Table-II shows results of two RDTs performed on days of presentation. Among 108 (62%) patients who presented with 2 – 5 days of fever, 87 (80.5%) were dengue IgA positive. Our results show that 17 patients who presented with acute febrile illness and thrombocytopenia and enrolled as probable cases of dengue had positive dengue IgA antibodies but negative IgM / IgG capture ELISA. All patients had three sets of malarial parasite smear and culture results negative. All patients were managed with I/V fluid and antipyretic and were discharged as cases

of probable dengue infection (Table-III). Three patients were managed as DHF. Repeat IgM / IgG serology on convalescent sera using MAC-ELISA were found to be positive in six patients, rest were discharged as probable cases of dengue infections. Sixty-nine (39.65%) patients had DHF, among whom 67 (97.1%) were seropositive for IgA at the time of presentation. Overall 163 cases were positive by MAC-ELISA among whom 129 (79%) patients had secondary dengue infection and 34 (20.85%) had primary dengue infection. Overall bleeding magnification were observed 111 (63.8%) of patients. One patients died of DSS and was positive for both dengue IgA and IgM / IgG RDTs.

Days of fever (no. of cases)	Dengue IgA RDT (n=174)		Dengue IgM/ IgG ICT (n=118)	
	Negative n (%) 41 (23.5%)	**Positive 133 (76.4%)**	**Negative 48 (40.6%)**	**Positive 70 (59.3%)**
Day two (2)	1	1	-	-
Day three (12)	5 (41.6%)	7 (58.3%)	-	-
Day four (42)	7 (16.6%)	35 (83.3%)	-	-
Day five (52)	8 (15.3%)	44 (84.6%)	23 (44.2%)	29 (55.7%)
Day six (40)	10 (25%)	30 (75%)	14 (35%)	26 (65%)
Day seven (11)	4 (36.6%)	7 (63.6%)	5 (45.5%)	6 (54.5%)
Day eight (14)	5 (35.7%)	9 (64.2%)	6 (42.5%)	8 (57.1%)
Day nine (1)	1		-	1

Table-II. Dengue IgA and IgM / IgG RDTs results at presentation

S. No	Sex	Fever (Days)	Bleeding Manifes.	Neurological Symptoms	GI symptoms	Labs						Dengue Serology IgM		Dengue Serology IgG		Dengue IgA Rapid Test
						Hb	Hct	Plat	Tlc	SGPT	MP	ICT	ELISA	ICT	ELISA	
1	M	5	No	No	No	13.1	42	28	2.4	45	Negative	Negative	Negative	Negative	Negative	Positive
2	M	5	No	No	Yes	13	41.9	72	3.4	30	Negative	Negative	Negative	Negative	Negative	Positive
3	M	3	No	No	yes	12.4	55	30	5	72	Negative	Negative	Negative	Negative	Negative	Positive
4	M	5	No	No	Yes	14	42.2	10	3.7	148	Negative	Negative	Negative	Negative	Negative	Positive
5	M	5	No	No	Yes	13.8	52	15	3	90	Negative	Negative	Negative	Negative	Negative	Positive
6	M	5	No	No	Yes	12.7	41.5	21	3.3	160	Negative	Negative	Negative	Negative	Negative	Positive
7	M	5	No	No	No	13.4	41.5	133	3.2	85	Negative	Negative	Negative	Negative	Negative	Positive
8	F	5	No	No	Yes	9.8	32	26	3.2	67	Negative	Negative	Negative	Negative	Negative	Positive
9	M	4	No	No	Yes	12.8	38	23	2.9	47	Negative	Negative	Negative	Negative	Negative	Positive
10	M	7	Yes	No	Yes	15.6	35.5	19	3.7	96	Negative	Negative	Negative	Negative	Negative	Positive
11	M	5	Yes	No	Yes	10.2	51	25	3.4	63	Negative	Negative	Negative	Negative	Negative	Positive
12	F	7	Yes	No	No	14.4	49	25	4.5	85	Negative	Negative	Negative	Negative	Negative	Positive

Table-III. Characteristics of Probable Dengue Cases with Positive IgA and Negative IgM / IgG Capture ELISA on Presentation

S. No	Sex	Fever (Days)	Bleeding Manifes.	Neurological Symptoms	GI symptoms	Labs						Dengue Serology IgM		Dengue Serology IgG		Dengue IgA Rapid Test
						Hb	Hct	Plat	Tlc	SGPT	MP	ICT	ELISA	ICT	ELISA	
13	M	7	Yes	No	Yes	11.2	48	9	11.2	120	Negative	Negative	Negative	Negative	Negative	Positive
14	F	7	Yes	No	Yes	10.2	30.6	25	5	79	Negative	Negative	Negative	Negative	Negative	Positive
15	M	5	Yes	No	Yes	13.4	35.5	30	4.2	86	Negative	Negative	Negative	Negative	Negative	Positive
17	F	6	No	No	Yes	10.5	38	85	2.8	45	Negative	Negative	Negative	Negative	Negative	Positive

Discussion

Among undifferentiated acute febrile illness, dengue fever and malaria account for the significant number of referrals to tertiary care hospital in endemic countries. Point of care RDTs have been increasingly used in many clinical setting for early diagnosis of dengue fever. In present study utilization of two ICT based RDTs, Dengue IgA and IgM/IgG rapid tests have been evaluated at presentation with acute febrile illness in a tertiary care setup. Our study shows that considerable proportion of patients (62%) presented within first five days of onset of high grade fever. Increasing trend to seek medical advice at tertiary care level might attribute to the increasing awareness to dengue fever in our city where regular outbreaks have been occurring since more than a decade. We also observed that significant proportion (53%) presented with thrombocytopenia during early febrile days (3 - 5days) which might be another factor for tertiary care referrals.

Thrombocytopenia is also a common hematological finding in both falciparum and vivax malaria. Considering the non-specific clinical features of dengue infection and malaria, reliable and accurate point of care dengue RDTs might prove helpful for clinicians to differentiate dengue from malaria and initiate prompt supportive therapy.

Concurrent infection of dengue and malaria are very infrequent even in endemic countries(10,11). Present study also reports only 2 cases of concurrent dengue and malaria infections. In contrast to our findings a study from Lahore, Pakistan reported high proportion of dengue-malaria co-infections (17/52) during 2012 outbreaks of Dengue infection(12). Both RDTs were found to be negative in isolated malaria infections in present study. As IgM/IgG assay is being done after first 5 days of onset of illness, a positive IgA with negative smear for malarial parasite will help treating physician initiate early supportive therapy.

Simultaneous or sequential circulation of four dengue virus serotypes (DENV1 – DEN4) lead to increasing number of secondary dengue infection in our population.[13,14] Present study also reports high frequency (79%) of secondary dengue cases. Although secondary dengue infection shown to be associated with more severe illness i.e DHF / DSS most probably due to antibody dependent enhancement, primary dengue infection has also been reported to be associated with DHF. Bachal et al reported higher levels of dengue virus specific IgG and IgA during pre-defervescence in primary dengue infection with DHF.[15]

Multiple studied showed variable kinetic of DENV specific IgM, IgG and IgA during primary and secondary dengue infection. Anti-dengue IgM, the predominant antibody in primary infection, detectable by MAC-ELISA is found to be positive in nearly half of the patients during febrile phase and in rest it is detectable within 2 – 3 days of defervescence and may remain detectable up to 3 – 8 months. Low levels of IgG appear shortly after IgM. Secondary dengue infection is characterized by higher levels IgG which appears before or simultaneously with IgM but levels of later is remain significantly lower or undetectable.[3-4,16]

For treating physician a major limitation of using

IgM / IgG serological assay by either capture ELISA or rapid ICT, is that there test can only be used after first five days of onset of illness thus prompt initiation of supportive care rely on high degree of clinical suspicion. Dengue NS1 antigen and Dengue specific IgA antibody based assays have been shown to be useful during early febrile days.[5-17] ELISA and ICT based NS1 assay have been used as a diagnostic tool during febrile phase till defervescence (1 – 9 days of fever onset) however, due to presence of virus – IgG immunocomplexs this assay has shown to be less sensitive in secondary infection as compared to primary infections[18,19] and may give false negative results in dengue endemic and hyperendemic areas. Studies comparing sensitives and specificities of IgA and NS1 reported dengue IgA to be more sensitive during early febrile phase of both primary and secondary dengue infection.[13,20] Shorter persistence of Dengue IgA in serum (approximately up to 40 days) and higher sensitivity for secondary infections could help early detection of dengue infection in endemic and hyperendemic regions.

In present study 17 patients with probable dengue who presented within 3 – 7 days of onset of fever were found to be negative for IgM/IgG MAC-ELISA, had positive IgA test among whom three patients had DHF. As MAC-ELISA has low sensitivity in first 4 – 5 days of onset of fever[21], detection of dengue specific IgA using ICT might be a helpful diagnostic tool in suspected dengue cases. In present study significant proportion of patients (80.5%) were found to be IgA positive within 5 days of onset of illness. A meta-analysis by Alagarasu et al also reported higher accuracy of IgA based assay when acute phase sera were collected 4 days after the onset of symptoms.[22]

Higher percentage of dengue specific IgA have found to be positive in secondary infection as compared to primary infection.[23-24] Levels of serum IgA has also shown to be correlated with severity of disease, investigations reported higher percentage of IgA positive cases in secondary DHF or DSS than in primary cases.[25-26] Our study also reports significantly higher cases of DHF (39.65%) who were positive for IgA (97.1%).

Conclusion

In dengue endemic-hyperendemic areas with high frequency of secondary DHF cases dengue IgA based RDT might be helpful as a rapid point of care diagnostic tool during early febrile phase and defervescene and might help clinician to keep patient under strict monitoring and start early supportive care. However negative results should be carefully monitored both clinically and by laboratory parameters including hematocrit, platelet count, SGPT, sets of malarial parasite smear and by repeat dengue serology.

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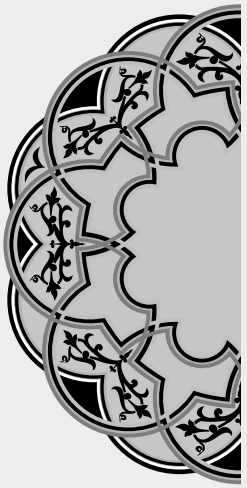

"Love all, Trust a few,
Do wrong to none."

Shakespeare

AUTHORSHIP AND CONTRIBUTION DECLARATION

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