COMPARISON OF DIAGNOSTIC EFFICACY OF RAPID DIAGNOSTIC DEVICES FOR DENGUE VIRUS INFECTION–A PIOLT STUDY

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Background: To compare the diagnostic efficacy of commercially available rapid diagnostic test devices for Dengue serology. To find out the sensitivity and specificity of rapid diagnostic devices with Elisa results as the Gold standard. **Methods:** During the dengue virus epidemic in Lahore a pilot study was conducted in order to evaluate the diagnostic efficacy of two most frequently used immunochromatographic rapid test devices in public sector hospitals. The results of both the kits were compared to each other. Sensitivity and specificity was calculated against results of ELISA as the reference gold standard. **Results:** Results of kit-A revealed a very high false negative rate when compared to ELISA where actual prevalence rate shown by ELISA was 96.0% compared to prevalence rate of 44.0% with rapid diagnostic test device kit-A. Similarly the results of rapid test device Kit-B showed high false negative results for dengue virus prevalence. Actual prevalence rate of dengue fever shown by ELISA was 96.0% where as it turned out to be 50% with the kit-B rapid test device. Comparison of Results of two kits revealed no significant difference of test positivity rates. **Conclusion:** Rapid test devices based on immunochromatographic method supplied in the public sector hospitals are not reliable diagnostic tools for screening for dengue virus infection Health authorities need to review their strategy for supply of more reliable tools during epidemics in order to avoid false negative results.

Keyword: Immunochromatographic rapid test, Dengue virus, ELISA, Screening tests

INTRODUCTION

Pakistan has experienced several outbreaks of Dengue fever in the past years including the outbreaks in Karachi in year 2007 claiming at least 22 lives. The present out break (Sept-Oct, 2008) in almost all provinces of the country is alarming. In Punjab more than 280 cases have been reported including 199 cases from District Lahore. The areas most effected City of Lahore are Kot Khwaja Saeed, Misri Shah, Sheran Wala , Gulshan Ravi, Shahdra , Band Road, Multan Road. These figures were provided by the ministry of Health based on the hospital admissions in tertiary care or some private hospitals. One can assume that actual number could have been far above as some never report to the hospitals. The health Department and Govt. authorities made huge efforts in launching public awareness programmes through media and conducting seminars at all the major public sector hospitals.

With such vast public awareness campaign the threshold for diagnosis became low. General public started seeking help even with non specific fever and headache. Since the key to proper management of Dengue fever remains an early diagnosis, the rapid diagnostic kits for anti-Dengue antibodies were the most readily available tool utilized in all public sector hospitals and the private labs. Commercially available kits based on Immuno-chromatographic method supplied by different vendors were used during this period of epidemic. No systematic study has been carried out in our country during any of the epidemics in the past years to evaluate the diagnostic efficacy of these kits. No data is available to document false positive and false negative results based on these readily available procedures. We undertook this pilot study during the recent epidemic in Lahore to compare the results of two of the commercially available rapid diagnostic devices for anti-Dengue IgM and IgG antibodies, verifying their results with Elisa as the Gold standard.

MATERIAL AND METHODS

This prospective study was carried out at Jinnah Hospital Lahore which was declared as the referral diagnostic centre for Dengue fever cases by the Govt. of Punjab. A total of 50 randomly selected cases suspected of Dengue fever referred through Emergency department and admitted in the Dengue ward of Department of Medicine were included. In view of the urgent nature of the crisis, the hospital administration supplied the pathology lab with diagnostic kits through direct purchase. The most readily available rapid diagnostic device available in the market was procured (Acon, Immuno-chromatographic anti Dengue antibody, IgG IgM antibody detection device). It is a qualitative membrane based immunoassay. Dengue antigen coated particles in the test strip are made to react with the patient's serum. The mixture then moves with capillary action to encounter the Anti human IgM and IgG in the test line area of strip. If anti Dengue IgM or IgG antibodies are present in patients' serum, and are captured by antigen coated particles, a coloured line will appear in the test area with anti human immunoglobulin of the respective class. In collaboration with the PMRC regional centre we could

also procure the BIAS-3 (MB Millennium Biotechnology) Rapid test device. It is a rapid membrane based screening test to differentially detect anti Dengue antibodies which is a newer generation lateral flow immunochromatographic assay. The test uses two antibody binding proteins conjugated to colloidal gold particles and a combination of Dengue antigens immobilized on a membrane. Patient's serum is added to the sample pad, it passes through two antibody binding protein/Gold complex which bind immunoglobulins in the serum. As this complex passes over the immobilized antigens, on the membranes, any anti Dengue antibodies present is captured by the antigen. The positive test is seen as a coloured band. Patient's serum was tested with both the devices and the results of both the devices were compared. It was a blind study and the personnel involved had no information about the results with the other device.
Results of each device were verified with ELISA technique and the Sensitivity and specificity of each device was calculated.

RESULTS

A total of 50 cases were tested in this pilot study. Rapid test devices of two manufacturers were used for serological diagnosis of dengue virus infection. Results obtained with kit-A (Acon) and Kit-B (BIAS-3 MB Millenium Biotechnology) were compared .The case positivity rate of the two kits are depicted in Table-1. There was no significant difference of test positivity rates between the two kits. Results obtained with both rapid devices were verified for accuracy with Elisa as the reference standard. Validation results of kit-A and Kit-B are depicted in Table -2 and Table-3 respectively. Extremely high percentage of false negative results as compared to ELISA were obtained with both the kits. Kit-A revealed prevalence rate of dengue viral infection to be 44% compared to 96.0% with ELISA indicating a low accuracy for kit-A results. . With Kit-B once again low accuracy results were obtained compared to ELISA where only 50% prevalence for dengue virus was revealed against 96% prevalence results with ELISA. Test positivity rate of kit-A and kit-B compared to ELISA was also calculated. With both the kits test positivity rate was significantly higher with ELISA.

Table-1: Comparison of case positivity rate of kit A and B

Tests	Positive		Negative	
	Freq	%	Freq	%
Kit A	22	44	28	56
Kit B	25	50	25	50

Chi-square= 0.3 6 p=0.5

There was no significant difference of test positivity rates between kit A and B.

Table-2: Validation results of kit A

Parameters	Findings	95% Confidence limits
Sensitivity	45.8%	31.6–60.7%
Specificity	100.0%	19.8–100.0%
Predictive value +	100.0%	81.5–100.0%
Predictive value -	7.1%	1.2, 25.0%
False positive rate	0.0%	-
False negative rate	92.8%	75.0–99.12%
Accuracy	48.0%	33.8–62.4%
Prevalence by kit	44.0%	30.2–58.6%
Prevalence by ELISA	96.0%	85.1–99.0%

Kit A is showing extremely high percentage of false negative results as compared to ELISA that leads to very low accuracy of the kit A. Actual prevalence rate of dengue fever shown by ELISA is 96.0% as compared to the prevalence depicted by the kit as 44.0%.

Table-3: Validation results of kit B

Parameters	Findings	95% Confidence limits
Sensitivity	52.1%	37.4– 66.5%
Specificity	100%	19.8–100%
Predictive value +	100%	83.4–100%
Predictive value -	8.0%	1.4 to 27.5%
False positive rate	0	-
False negative rate	92.0%	72.4–98.6%
Accuracy	54.0%	39.4–67.9%
Prevalence by Kit	50.0%	35.7–64.2%
Prevalence by ELISA	96.0%	85.1–99.0%

Kit B is showing extremely high percentage of false negative results as compared to ELISA that leads to very low accuracy of the kit B. Actual prevalence rate of dengue fever shown by ELISA is 96.0% as compared to the prevalence depicted by the kit as 50.0%.

DISCUSSION

During an epidemic, rapid diagnosis of Dengue fever is the key to proper management of the patient. Diagnosis depends on a high index of suspicion during the epidemic season supplemented by lab results. Clinically the Dengue virus infection may remain asymptomatic or become symptomatic as Dengue fever, Dengue Haemorrhagic fever (DHF) and Dengue shock syndrome (DSS). Last two syndromes are diagnosed if the WHO described criteria are fulfilled. The relatively benign Dengue fever presents classically with high grade fever accompanied by headache, retrobulbar pain, muscle and bone pains and generalized peticheal rash. With these classical signs a high suspicion of Dengue fever is warranted which then needs the laboratory confirmation. Over and above these clinical manifestations the DHF is characterised by haemorrhagic manifestations in the form of spontaneous bruising and bleeding from mucosal surfaces, supported by lab findings of thrombocytopenia (<100,000) and high haematocrit (more than 20% higher than expected, or drop in haematocrit of 20% or more from baseline following I/V fluid). The Dengue shock syndrome in addition will have features of clinical shock. Laboratory diagnosis is mandatory before labelling the case as of Dengue virus infection. Virus remains in the

serum of the patient during acute phase and can be isolated on cell culture or the viral RNA can be detected with RT-PCR. Antibodies to virus appear as the viremia starts declining, IgM anti-Dengue antibody followed by IgG anti-Dengue antibody. Serological diagnosis remains the mainstay of diagnosis during the epidemic since viral isolation is laborious, expensive and is only available in reference laboratories. For serological diagnosis several commercially available rapid diagnostic kits are used which are based on Immunochromatographic method. These vary in their sensitivity and specificity. These have the advantage of giving quick results and providing information about both IgM and IgG anti Dengue antibodies thus helping differentiate between Primary and secondary Dengue infection. No systematic study has been done in our country yet to test the diagnostic efficacy of these various commercial devices available. We conducted a pilot study during the recent epidemic in the city of Lahore to evaluate the sensitivity and specificity of two of the most commonly used rapid diagnostic devices. The sensitivity and specificity of both the kits was calculated. There was an agreement amongst the results of both the devices to some extent. However when the results of individual device was compared to the results of ELISA as the reference standard, it gave alarmingly high false negative results with a sensitivity of 45.8% and 52.1% for the two devices respectively. We also observed that with increased work load the efficiency falls. For the technician performing a large number of tests it was difficult to pick up the weak positive reactions since the colour developed was less intense and only with great concentration such cases could be picked up. Day light is usually not available in the labs and in tube lights the colour reading needs an experienced sharp technician to pick up the weak positive cases.

Several studies have compared different immunoassay methods to detect IgM Dengue antibodies including ELISA, dot ELISA, dipstick assay, dot blot assay and immunochromatograghic test devices.[1,2] Some studies have tested the diagnostic accuracy of various commercially available rapid test devices.

The results of our study tally with the observation of Blacksell *et al*[1] who conducted an elaborate prospective study where they compared 08 commercially available immunochroamtographic rapid device tests (RDT) using a panel of reference samples. The results revealed very low sensitivity where 6/8 RDT had sensitivity of less than 50% (6–50%). However specificity in this study was higher. The authors advocate that currently available RDT for the detection of IgM antibodies for the diagnosis of acute Dengue infection are unlikely to be useful for patient management. The same group (2) carried out meta analysis of published peer reviewed studies which had attempted to find dengue Immunochromatographic test device (ICT) diagnostic accuracy and evaluated eleven studies. They reported highly heterogenous results with sensitivity ranging between 45–100% and specificity range of 57-100%. In another prospective study carried out in Laos, Blacksell *et al*[3] compared eight commercially available Dengue ICT devices. Poor diagnostic accuracy was reported with only 2/8 showing sensitivities >50%. Contrary to our findings and above mentioned studies however, the study by Vaughn *et al*[4] report a 100% sensitivity of rapid test device (Pan-Bio, Brisbane, Australia) for diagnosis of Dengue fever when they compared the results to Haemagglutination assay and Enzyme immunoassay. Sang *et al*[5] compared the results of a rapid diagnostic test device in 98 cases of primary and secondary Dengue fever cases with Haemagglutination assay as the reference standard. The result revealed 98% sensitivity for the Immunochromatographic device suggesting it as a useful diagnostic tool. Using same reference standard of Haemagglutination assay Kittigul *et al*[6] reported sensitivity of 79% for the immunochromatographic rapid test device but a higher specificity of 95%.

CONCLUSION

In conclusion the results of our study are highly alarming. Health authorities and the clinicians need to consider this important aspect of diagnostic tools for Dengue virus infection so as to plan well ahead of time and arrange for either more reliable rapid diagnostic test devices or direct the health care workers to use Elisa test in all the cases with a high index of suspicion for Dengue fever.

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