USE OF DRIED BLOOD BLOTTED ON FILTER PAPER TO DETECT DENGUE IgM ANTIBODY AND DENGUE NS1 ANTIGEN

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Abstract. A cross sectional study was conducted at the Department of Microbiology, Maulana Azad Medical College, New Delhi, to determine the efficacy of capillary blood samples collected on filter paper compared with serum samples to detect dengue IgM antibody and dengue NS1 antigen. The serum and capillary blood samples were collected from 104 suspected cases of dengue fever at Lok Nayak Hospital, India. The blood eluted from the filter paper and the serum samples were tested for dengue IgM antibody and dengue NS1 antigen by commercial capture ELISA kits. Of the 104 patients, samples from 61 patients tested positive for dengue IgM antibodies with both the serum and filter paper. Samples from 38 patients were negative for both serum and filter paper. Five samples showed discordant results. The filter paper method had sensitivity of 96.8% and specificity of 97.4% for dengue IgM antibody compared to the serum samples. The positive predictive value (PPV) and negative predictive value (NPV) for the filter paper to detect dengue IgM were 95.31% and 95.0%, respectively. The Kappa value (>0.80) showed agreement between the filter paper and serum results for IgM antibody detection. NS1 antigen was detected 28 serum and 28 filter paper samples and was not detected in 72 serum and 72 filter paper samples. Discordant results were seen in 4 samples. The filter paper method had a sensitivity of 96.5% and a specificity of 96.0% compared to the serum for detecting dengue NS1 antigen. The PPV and NPV for the filter paper samples in detecting dengue NS1 antigen were 90.3% and 98.7%, respectively. The Kappa value showed agreement (>0.80) between the serum and filter paper results for detecting dengue NS1 antigen. The results show filter paper samples are a reasonable alternative to serum for detecting dengue infecting.

Keywords: dengue, IgM antibody, NS1 antigen, filter paper, India

INTRODUCTION

Dengue fever, a mosquito-borne viral infection, is a major public health problem. Dengue fever is found in urban and semi-urban tropical and sub-tropical regions throughout much of the world. The incidence of dengue has increased 30 times in last 50 years (WHO, 2009). It is estimated there are 100 million cases of dengue infection yearly and 250,000 deaths (Monath, 1994). The World Health Organization (WHO) has stated dengue fever is a disease that causes international

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public health problem with the potential to disrupt health security and spread beyond national borders (WHO, 2009).

In India dengue infection is endemic in suburban and urban areas. In recent years there have been more frequent outbreaks (Chakravarti *et al*, 2012) with increasing number of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) cases (Bharaj *et al*, 2008). All four dengue virus serotypes are prevalent in India (Chakravarti and Kumaria, 2005).

Dengue infection presents with wide range of signs and symptoms similar to other viral and bacterial infections. A reliable diagnosis can only be made by laboratory testing. Laboratory diagnosis of acute dengue infection can be made by direct detection of the virus in the patient's blood, through cell culture, which can take days, or by detecting viral nucleic acid by PCR, which is only available in specialized laboratories and requires an experienced technician (Sathish *et al*, 2002). Dengue infection can also be diagnosed by detecting dengue IgM/IgG antibody or by detecting dengue NS1 antigen, which is quicker, although has lower sensitivity. These techniques require collection of venous blood, for which the services of a trained venipuncturist are required. For epidemiological purposes, such as investigation of outbreaks, dengue surveillance and vaccine trials, an easier sampling method is needed, especially in remote areas. Venous blood requires care during transportation and storage under optimum temperature conditions.

Filter paper sampling would address these issues by offering a simpler, cost-effective method. It has been successfully used to detect several diseases, including viral infections such as hepatitis C (Abe and Konomi, 1998) and measles (Rawat *et al*, 2001).

Previous studies demonstrated the use of dried capillary blood on filter paper to detect dengue IgM antibody (Ruangturakit *et al*, 1994; Matheus *et al*, 2007). The use of dried capillary blood on filter paper to detect dengue NS1 antigen has not been reported. We determined to evaluate the efficacy of the filter paper method to detect dengue NS1 antigen compared with dengue IgM antibody.

MATERIALS AND METHODS

We conducted this cross sectional study at Lok Nayak Hospital and Department of Microbiology, Maulana Azad Medical College, New Delhi, India. This center is one of 80 dengue infection sentinel surveillance sites in India under the National Vector Borne Disease Control Program (NVBDCP). The study was carried out from September 2010 to April 2012. Delhi state has the highest prevalence of dengue in India.

One hundred four patients with clinical symptoms of dengue infection based on WHO criteria (WHO, 1997) were included in the study after obtaining informed consent. If routine lab tests or clinical features during the first 48 hours after admission/presentation suggested a different diagnosis, the case was excluded. Patients who refused to participate were also excluded from the study. The study was approved by the ethics committee of the institute.

Two samples of 3 ml venous blood were collected aseptically from each patient. A few drops of capillary blood were collected by finger prick and blotted directly on a 15 mm circle printed on No. 3 Whatman filter paper. The Whatman filter paper samples were then left to dry out of direct sunlight, placed in a sealed bag with desiccant and stored at 4°C. The

serum samples were separated, placed in Eppendorf vials and immediately transferred to a refrigerator and kept at -20ºC until used.

Tests performed

Both the serum and elute from the filter paper were tested for dengue IgM antibody and NS1 antigen by commercially available ELISA kits. For IgM antibody detection, IgM dengue ELISA captures test kits, supplied by National Institute of Virology (NIV), Pune, were used. For NS1 antigen detection, Panbio Dengue Early ELISA kits (Panbio Diagnostics, Brisbane, Australia) were utilized. Samples were declared positive or negative based on the ratio of absorbance of the sample to that of the negative per the manufacturer's instructions.

The blood on the filter paper was reconstituted following the method of Tran *et al* (2006).

IgM antibodies

One 15 mm circle of filter paper was cut and soaked in 0.2 ml phosphate-buffered saline supplemented with 2% fetal bovine serum and kept at room temperature overnight. The sample was then shaken on an automatic shaker, centrifuged and the supernatant collected. One 15 mm circle of filter paper contained 40 to 50 l of blood or 20 l of serum. The supernatant was considered to have a dilution of 1:10. This was then further diluted ten times to reach the kit recommended dilution of 1:100.

NS1 antigen

One circle of filter paper was cut and soaked in 150 l of sample dilution buffer included in the kit. This was kept at room temperature overnight. The next morning, it was shaken on an automatic shaker and centrifuged. One hundred microliters of supernatant was collected and added to the ELISA well.

Statistical analysis

All analyses were performed using SPSS, version 17.0 (SPSS, Chicago, IL). The Kappa agreement statistic was used to analyze the sensitivity, specificity, positive predictive value (PPV), and the negative predictive value (NPV) of the filter paper sample compared with the serum as a gold standard.

RESULTS

Of the 104 patient samples tested, 75 were positive for either NS1 antigen and/or IgM antibody by dengue IgM ELISA. Of these 75 patients, 29 were females and 46 males; the M:F ratio was 1.5:1. The mean age of the patients was 27.0 years (range 2-60 years). Anti-dengue IgM antibodies only were detected in 63 of 104 samples (Table 1). NS1 antigen only was detected in 29 of 104 samples (Table 2). Seventeen samples were positive for both NS1 antigen and anti-dengue IgM antibodies.

From the filter paper samples the sensitivity was 96.8%, the specificity 97.4%, PPV was 95.3% and NPV was 95%. The Kappa value (0.89) showed perfect agreement between the filter paper and serum results for IgM antibody detection.

The filter paper method gave a sensitivity of 96.6% and a specificity of 96.0%, for detecting dengue NS1 antigen compared to serum. The PPV and NPV of the test were 90.3% and 98.7%, respectively. The Kappa value (0.902) showed perfect agreement between the filter paper and serum for detecting NS1 antigen.

DISCUSSION

Epidemiological studies require preservation of the integrity of the serum sample, which requires proper collection

Table 1
Dengue IgM antibody detection comparing serum and filter paper samples.

Parameter	Serum positive for dengue IgM	Serum negative for dengue IgM	Total
Filter paper positive for dengue IgM	61	3	64
Filter paper negative for dengue IgM	2	38	40
Total	63	41	104

Table 2
Comparison of filter paper and serum samples to detect dengue NS1 antigen.

Parameters	Serum positive	Serum negative	Total
Filter paper positive for dengue NS1 antigen	28	3	31
Filter paper negative for dengue NS1 antigen	1	72	73
Total	29	75	104

Table 3
Comparison of filter paper and serum samples to detect dengue IgM antibodies by number of days after onset of fever.

Days after onset of fever	No. of samples tested	Positive with filter paper only	Positive with serum only	Positive with both serum and filter paper (%)
1	2	0	0	1 (50)
2	4	0	0	2 (50)
3	11	0	0	4 (36.3)
4	11	1	0	4 (36.3)
5	20	1	1	10 (50)
6	17	1	1	12 (70.5)
7	30	0	0	20 (66.7)
8	9	0	0	8 (88.8)
Total	104	3	2	61 (58.6)

and storage of the sample. Many dengue endemic countries in the developing world lack infrastructure making it a challenge to conduct such studies. Filter paper has several advantages over serum. Compared to serum collection, filter paper requires a lower volume of blood. Hence, it can be collected from finger prick samples, making it convenient, especially in children and newborn babies. One study found using capillary blood collection increased the number of volunteers willing to participate in research programs (Rodríguez-Pérez *et al*, 1999). The filter paper method also simplifies collection of samples in the field, obviating the need for ice boxes to maintain an optimum temperature. Li *et al* (2005) found HIV p24 antigen to be stable at room temperature for two weeks, suggesting capillary blood

Table 4
Comparison of filter paper and serum samples to detect dengue NS1 antigen by days after onset of fever.

Days after onset of fever	No. of samples tested	Positive with filter paper only	Positive with serum only	Positive with both serum and filter paper (%)
1	2	0	0	1 (50)
2	4	0	0	1 (25)
3	11	0	0	6 (54.5)
4	11	1	0	3 (27.7)
5	20	0	0	12 (60)
6	17	1	1	5 (29.4)
7	30	1	0	0 (0)
8	9	0	0	0 (0)
Total	104	3	1	28

collected on filter paper may not require freezing like serum samples. This is an advantage in tropical countries where maintaining a cold chain is difficult. There are potentially fewer risks with filter paper than with glass collection vials for breakage or transmission to others.

The present study demonstrates the feasibility of filter paper sampling to detect dengue IgM antibodies and NS1 antigen using commercially available ELISA kits. Both dengue IgM antibodies and NS1 antigen on filter paper may be detected simultaneously (Tables 3 and 4). The filter paper method had 96.8% sensitivity, missing only 2 positive serum samples. The specificity was also high (97.4%), with three samples positive by filter paper method but were negative by serum testing (Table 1). These three samples were taken from patients 4 to 6 days after the onset of fever (Table 3). Of the 3 samples positive by filter paper and negative by serum, two were NS1 antigen positive, indicating they were true positives. This yielded a PPV of 95.3%. The NPV of 95% was also good. Previous studies (Matheus *et al*, 2007, 2008) using filter paper samples to detect dengue IgM antibodies yielded varying sensitivities (81-98%) and specificity (90-98%).

Dengue NS1 antigen can detect dengue infection in the first few days of infection, unlike dengue IgM antibodies to detect outbreaks early (Matheus *et al*, 2008). In this study, filter paper sampling compared favorably to serum samples in detecting dengue NS1 antigen with a high sensitivity and specificity of 96.6% and 96.0%, respectively. The positive predictive value for detecting dengue NS1 antigen with the filter paper method was 90.3% compared to serum, but all 3 discordant samples positive with the filter paper method and not the serum (Table 2) indicating these were true positives (Table 4). In actuality, the specificity and PPV with the filter paper method for detecting NS1 antigen were 100%. A possible reason for greater detection of dengue NS1 antigen with the filter paper method could be a higher stability of NS1 antigen on filter paper or the persistence of viral particles in capillary blood for longer time (Matheus

et al, 2007). The NPV was good (98.7%). The overall agreement between the serum and filter paper samples was good since the Kappa value for dengue IgM antibody and NS1 antigen were 0.89 and 0.902, respectively.

The filter paper method appears to be a reliable alternative to serum samples to detect dengue IgM antibodies and NS1 antigen. Its simplicity and ease of use encourage widespread applicability of this method in dengue endemic areas in developing tropical countries where transport and storage problems associated with venous blood samples occur.

Further studies are needed to determine the long term storage stability and compare the quantitative measurement of NS1 antigen and IgM antibodies in serum and capillary blood blotted on filter paper.

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