The Sensitivity Comparison of Immunodiagnostic Assays for Diagnosing Dengue Fever

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Dengue fever is a vector borne disease caused by a dengue virus. It is an RNA virus of the family flaviviridae, with different serotypes. Herein, we report our attempt to carry out a sensitivity comparison of immunodiagnostic assays for dengue fever in dengue positive patients. Blood samples from 189 volunteers were collected. To determine the sensitivity of the NS1 test, two different types of tests—immunochromatographic tri-line test and rapid dengue test (RDT)—as well as IgM and IgG capture ELISA were performed. The result of RDT has shown that 59.7% of volunteers were IgM positive and 50.2% were IgG positive. Conversely, the results from capture ELISA shows 79.8% and 59.7% for IgM and IgG, respectively. The sensitivity of the capture ELISA test for IgM and IgG was higher than that of immunochromatographic tri-line rapid test, but the specificity was lower. Therefore, to confirm dengue fever, we recommend performing more detailed, investigative tests since a single test may not be sufficient.

Key words: Dengue, Rapid dengue test, IgM, IgG

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Received: October 5, 2016
Revised 1st: November 8, 2016
Revised 2nd: November 15, 2016
Accepted: November 15, 2016

Introduction

Dengue is a vector borne flavivirus having different serotypes. Rapid investigation is mandatory for dengue virus infection to properly manage the patient [1]. For the diagnosis of dengue infection various laboratory tests are employed to monitor circulatory serotypes [2,3]. These tests are performed by several methods such as isolation of virus from cell cultures, detection of nucleic acid by polymerase chain reaction (PCR), or viral antigen detection. Serological tests are being widely employed compared to other methods [4]. Non-structural protein (NS1) test and specific antibodies detection (serology) are mostly applied in diagnosis of dengue fever [5]. The isolation of virus and detection of nucleic acid are reliable and precise than finding of antigen tests, but the former tests are not generally accessible in every laboratory and due to their greater cost, majority of patients cannot

afford them. Detection claim of NS1 in blood sample during the primary infection and its sensitivity is 90% in febrile phase, though only detectable is to 60~80% in successive infection [6]. It is noteworthy, that most of the tests may fail in the early stages of the dengue disease to give result [5,7]. However detection tests through PCR and viral antigen provide precise result in the first seven days of infection [6].

Serological analysis is comprised of the identification of specific antibodies developed by complex immune system of the patient body such as IgG and IgM [8]. These tests are useful in confirming the dengue fever. Both IgG and IgM antibodies are formed in blood in a period of 5 to 7 days after appearance of fever. The highest concentration of IgM is noticed during the primary infection, but IgM is also formed in secondary infection. IgM becomes undetectable from 30 to 90 days after infection at primary phase [2]. By contrast, IgG arrives at climax value in the blood after 14 to 21 days and

remains measurable for about 60 years. The serum titers reach to climax and are typically elevated when infections re-occur in later stage. Both antibodies IgG and IgM grant defensive protection to the type of the virus that causing infection [5,8,9]. During analysis for IgM and IgG antibodies, the chance of cross-reactivity is possible with other flaviviruses, which may lead to a false positive result following current infections or giving vaccine to patient with virus of Japanese encephalitis or yellow fever. In patient that manifests common warning signs of the dengue fever, the determination of IgM antibodies are considered sufficient for the confirmation of diagnose [8].

In order to determine the sensitivity of NS1, IgM and IgG tests, 189 dengue infected volunteers were selected in the current study. All these volunteers were declared dengue patients in local hospital on the basis of NS1 findings. IgM and IgG tests were performed on rapid dengue test (RDT) and enzyme linked immune sorbent assay (ELISA).

Materials and Methods

1. Sample size and study design

All experiments were carried out according to the Scientific Procedures Issue-1 approved by the legal bodies of the University of Malakand Khyber Pakhtunkhwa, Pakistan. The ethical committee of the department of Biochemistry granted approval for conducting this study under the said protocols. Approval of the collection procedure was taken from University Review Board for the protection of human research prior to starting collection of blood samples from volunteers. And all participants in this study gave their written informed consent.

189 patients acutely infected with dengue virus (volunteers) were selected for serological tests out of 1,764 infected patients (as most of the infected peoples were reluctant to give blood samples). Samples of blood serum of dengue suspected volunteers were used for the comparison of efficiency of dengue IgG/IgM capture ELISA with that of lateral flow immunochromatographic test (ICG test) and NS1 findings. The age of dengue infected patients ranged between 11 to 80 years. A volunteer was considered a dengue patient

with NS1 positive according to hospital-based data. The test of the entire blood samples was performed by using lateral flow immunochromatographic test and dengue IgM/IgG capture ELISA. The sensitivity and specificity outcomes of the tests performed were established by comparing with NS1 findings.

2. Collection and preservation of blood samples

All 189 patients were visited at their houses. For collecting blood samples from volunteers, services of an expert were hired for taking blood samples. The volume of blood required for tests was 5 mL. The patients were visited at different times according local needs. Special arrangements were made after receiving blood samples from volunteers for its preservation. After collecting blood sample with help of sterilized syringe, they were poured into jell tubes and stored in ice. After collection and safe handling the samples were brought to laboratory where they were kept below 4°C in refrigerator.

3. Serological tests

The blood samples were centrifuged to separate serum from blood cells. The serum was subjected to different procedures. The anti-dengue IgM and IgG antibodies were analyzed by using one step lateral flow immunochromatographic tri-line test card (Haimen Shengbang, Jiangsu, China) and cap-ELISA and anti-dengue IgM and IgG antibodies (Diagnostic Bioprobes, Milan, Italy).

4. Anti-dengue IgM and IgG tri-line card

The main rapid diagnostic device, Immunochromatographic tri-line card was used for detection of anti-dengue IgG/IgM antibodies which was easily available on the market. It consists of a membrane that captures anti-dengue IgG/IgM antibodies. The test strip on which dengue antigen material is loaded, combine with the patient's serum from sample well to react. The serum sample was diluted by the diluents, introducing the diluents into wells. The reacting mixture then ascends with help of action of capillary to come across the anti-dengue IgM and IgG in the line of test of region of device. If patient's serum is adulterated with anti-dengue IgM or IgG antibodies, and are detected by antigen covered material, a

red streak will become visible in the region of trial with anti-dengue antibodies of the relevant category. The results of rapid tests were noted and recorded. The positive test of both IgM and IgG was recognized by appearance of red lines against IgM and IgG regions respectively. About 1 mL serum was applied to the commercially available RDT strip for the determination of IgG and IgG antibodies. After passing specific time duration, the strips were visually examined for positive or negative results. This type of analysis took 10 to 15 minutes.

5. Dengue IgM and IgG antibodies cap-ELISA

The IgG-cap ELISA and IgM-cap ELISA were performed to investigate antibodies for dengue in blood serum in according to guidelines provided by manufacturing companies. In short, 100 μ L per well of patient sera was diluted 1:100. At the same time control agents were also supplemented to the analyzing plate covered with any anti-dengue IgG antibodies or anti-dengue IgM antibodies to detain the IgG or IgM antibodies. The two plates were kept warm for 60 minutes at temperature of 37°C. Then the plates were washed and assay plates were loaded with 100 μ L per well of enzyme conjugate excluding the empty wells. The response was stopped by adding 100 μ L per well of 1M H₃PO₄ passing interval of 10 minutes. The values of optical density (OD) were calculated at 450 nm. The test was declared as positive when ratio between absorbance of sample and measured value of cut-off was greater than 1 and considered negative for cut-off value less than 1.

Results

1. Immunochromatographic tri-line rapid test and cap-ELISA

As presented in Table 1, on cap-ELISA test, out of total 189 patients 151 (79.8%) were positive for IgM and 113 (59.7%)

were IgG positive as compared to 113 (59.7%) and 95 (50.2%) for IgM and IgG respectively on immunochromatographic tri-line rapid strip.

2. Efficiency of rapid test and cap-ELISA in dengue infection

The efficiency of immunochromatographic tri-line test and capture ELISA regarding antibodies IgM and IgG are presented in Table 2. The efficiency of IgM and IgG capture ELISA is found higher 79.8% (151/189) and 59.7% (113/189) respectively as compared to 59.7% (113/189) and 50.2% (95/189) of the respective results of immunochromatographic tri-line test card. The investigation of both anti-dengue IgG and IgM of infected patients by dengue cap-ELISA was drastically higher than dengue immunochromatographic tri-line tests and which in turn is higher than hospital based data of patients.

3. The sensitivity and specificity of rapid test and cap-ELISA in dengue infection

The comparison of sensitivity and specificity of rapid test and cap-ELISA is given in Table 3, 4. The sensitivity and specificity of the rapid test for IgM is 59.7% and 40.2%, respectively while of cap-ELISA test it is 79.8% and 20.1% respectively. Similarly the sensitivity and specificity of rapid test for IgG is 50.2% and 49.7% as compare to the IgG result of cap-ELISA which is 59.7% and 40.2%, respectively.

Table 2. The efficiency (%) of rapid test and cap-ELISA in dengue infection (n=189)

Nature of test	IgM	IgG
Rapid strip	113/189=59.7%	95/189=50.2%
Cap-ELISA	151/189=79.8%	113/189=59.7%

Table 1. Test results of IgM and IgG of hospital based data, rapid strip and cap-ELISA

Nature of test	IgM Positive (%)	IgM Negative (%)	IgG Positive (%)	IgG Negative (%)	IgM not performed (%)	IgG not performed (%)
Hospital based-data	11.1%	8.4%	1.5%	14.8%	80.4%	83.5%
Rapid strip	59.7%	40.2%	50.2%	49.7%	–	–
Cap-ELISA	79.8%	20.1%	59.7%	40.2%	–	–

Table 3. The sensitivity and specificity of rapid test for IgM and IgG (n=189)

Type	Positive (n)	Negative (n)	Sensitivity (%) [*]	Specificity (%) [†]
IgM	113	76	59.7	40.2
IgG	95	94	50.2	49.7

*Sensitivity (%)=[true positive/(true positive+false negative)]x100.

†Specificity (%)=[true negative/(true negative+false positive)]x100.

Discussion

Sensitivity is the capability of the test to recognize rightly the subjects who have the infection. It is the ratio of the patients who have positive test infected with illness to number of all persons who have the infection using the formula; Sensitivity (%)=[true positive/(true positive+false negative)]x100. On the other hand, specificity is the capability of the test to recognize rightly the subjects who do not have the infection. It is the ratio of the patients who show negative test and do not suffer from illness to all number of persons who have not the infection of illness; Specificity (%)=[true negative/(true negative+false positive)]x100 [10]. The high specificity of test manifests a small number of false negative outcomes. Sensitivity and specificity are the distinctiveness of test which is very valuable when evaluating a test used to monitor a free-living population. These test features of test are co-dependent too. When sensitivity increases, specificity decreases accordingly and vice versa [11].

In the current study, the sensitivity and specificity of rapid test and cap-ELISA in dengue infection was compared in the connection with detection of anti-dengue IgM and IgG. The blood serum of suspected dengue patients was subjected to IgM and IgG tests on two different devices. Only 21 patients were IgM positive and rest of cases were left undetermined according to the hospital data. The purpose was to determine sensitivity of NS1, IgM and IgG tests for diagnosis of infection of dengue as well as to compare the sensitivity of two test devices i.e. routine dengue- immunochromatographic tri-line test and IgM and IgG capture ELISA. The comparative positive and negative test results of hospital-based data and analyzed blood serum of dengue suspected patients were evaluated

Table 4. The sensitivity and specificity of cap-ELISA test for IgM and IgG (n=189)

Type	Positive (n)	Negative (n)	Sensitivity (%) [*]	Specificity (%) [†]
IgM	151	38	79.8	20.1
IgG	113	76	59.7	40.2

*.†: 'See Table 3.'

along with the sensitivity and specificity of rapid test and cap-ELISA for anti-dengue IgM and IgG. The results show that the sensitivity of cap-ELISA is higher both for IgM and IgG than that of immunochromatographic tri-line test. On the contrary, specificity of cap-ELISA is lower for both IgM and IgG as compare to specificity of immunochromatographic tri-line test. Serological investigation for the finding of dengue IgG and IgM antibodies as well as dengue antigen NS1 tests are performed while diagnosing the dengue fever. The combinations of these tests have far-reaching consequences to diagnose dengue properly. IgM cap-ELISA assay was more efficient than immunochromatographic tri-line rapid test as former is more efficient (79.8%) than later (59.7%). Similarly, IgG cap-ELISA test was more effective (59.2%) than immunochromatographic tri-line rapid test (50.2%). The sensitivity of cap- ELISA both for antibodies IgM and IgG was greater in dengue infection diagnosis than immunochromatographic tri-line rapid test. In light of the current findings, NS1 test has low accuracy as compared to RDT, which in turn was inferior to IgM and IgG cap-ELISA assay in diagnosing dengue infection. Therefore, it is not appropriate to depend on use of single test while diagnosing the dengue infection. The dengue IgG cap-ELISA and IgM cap-ELISA test has superior intensity of performance over the immunochromatographic tri-line rapid strip as well as the test result mentioned in the hospital-based data of the patients suffering from dengue fever.

Acknowledgements: All the authors are thankful to Higher Education Commission of Pakistan for providing funds and research facilities to our laboratory.

Funding: None

Conflict of interest: None

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