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OPTIMIZATION OF REVERSE TRANSCRIPTASE-PCR BY USING SPECIFIC PRIMERS IN DETECTING DENGUE VIRUS

[1]Dr. Amina Ilyas, [2]Dr Affaf Ahmed, [3]Dr Abdul Hanan Ghumman

[1]MBBS, King Edward Medical University, Lahore, Pakistan.

[2]Services Hospital Lahore

[3]Lahore General Hospital

Abstract:

Background: *Dengue virus belongs to positive stranded encapsulated RNA viruses. In Pakistan species infecting people are not known. The methods used for detection of virus in Pakistan are ELISA, Dot blot immunoassay and compliment fixation test.* ***Objective:*** *to detect dengue virus by using specific primers like DEN-1, DEN-II, DEN-III, DEN-IV cloning.* ***Methods:*** *15 samples were collected from Mayo Hospital Lahore and Allied Hospital Faisalabad through non-probability convenient sampling. RNA was extracted and optimization of RT-PCR was done by using specific primers like DEN-I, DEN-II, DEN-III, DEN-IV cloning. Reverse transcriptase PCR can detect dengue virus with more accuracy in less time. Difference among four dengue virus serotypes can be detected by using RT-PCR. Detection in early phase of infection is possible by using RT-PCR.*

Key Words: *reverse transcriptase PCR, dengue virus, primers, detection.*

*** Corresponding author:**

Dr. Amina Ilyas,
MBBS,
King Edward Medical University,
Lahore, Pakistan

QR code

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INTRODUCTION:

Dengue virus is RNA enveloped virus. It is transmitted into human through mosquito bite, *Aedes aegypti[1]*. The vector growth and development occurs in rainy season that is why the dengue fever outbreaks occur most commonly during vector breeding season. The virus replication inside vector also depends on many factors like temperature [2].
Dengue fever also called break bone fever or hemorrhagic fever. It has incubation period of 10 to 12 days. It usually starts with flue like symptoms, high grade fever. The disease is associated with myalgias, joint pains, headache and retro-orbital pain. In dengue hemorrhagic fever, epistaxis, hematuria, hematemesis etc. occurs. The exact mechanism through which virus cause disease is still unknown [3].

In Pakistan dengue outbreak occurred in Lahore, Punjab in 2011. More than 5000 people died of dengue fever in 2011. The tests used to detect dengue virus were ELISA, dot blot immunoassay. This study aims to detect the efficacy of RT-PCR in detection of different serotypes of dengue virus [4].

METHODS:

World health organization classified dengue virus on the basis of disease severity and symptoms, in 2011. Presently classification is done on basis of clinical administration, however, the mechanism of infection of dengue virus is still unknown. Dengue virus is produced more on hot, humid and damp places or where stagnant water is present[5]. Transmissibility and infectivity along with growth rate of dengue virus depends greatly on temperature and seasonal variation. Population density also affects the transmission rate of dengue virus. Immune level of human host and self-resistance are other major factors. Dengue virus mutations occurred due to changes within cells also determines intensity of infection [6]. Dengue outbreak in different parts of world like Thailand, Cambodia, Vietnam has clearly shown the specie life cycle changes. Quantification of RNA during symptom onset is necessary, in addition time period for implication of immunoglobulin M between 4 to 6 days [7].

Techniques used for separation of RNA are kit methods, virus culture, nucleic acid, NSI or IgM. Any test used for confirmatory diagnosis of dengue fever is not approved yet. WHO and Disease control organization is working in establishing guidelines regarding diagnosis of dengue fever.

Over population and increasing temperature are directly proportional to larvae growth, its development into adult form, biting rate [8]. On the other hand increased temperature decreases the virus replication inside vector. Thus temperature ranges have been defined in form of mild, medium and high in order to detect the growth rate of *Aedes aegypti.* Vector prefers the rainy season, fresh and clean water collected in open indoor and outdoor containers for its growth and development. Annual vapour pressure has been selected as an important determining factor responsible for vector growth and replication and this has been approved by many scientists [9].

RESULTS:

Blood samples of dengue fever patients were collected from Mayo Hospital and Allied Hospital Faisalabad. Sample collection was done only after following the standard guidelines for sterilization and aseptic techniques were strictly followed. Blood sample was collected in EDTA vials, sample inside these vials was inverted twice or thrice for homogenization, total RNA was isolated. Strict precautionary measures were taken while handling the sample to avoid infection transmission to healthcare providers or laboratory persons.

Total RNA isolation was done by using Leuko lock™, total RNA method, tempus RNA tube, Trizol method. After collection it was run in agarose gel for confirmation. 3µg/ml, 9µg/ml and 90µg/ml RNA was collected using Leuko lock™, Tempus™ blood RNA tube, Trizol method, respectively. The agarose gel has three band upper, middle and lower for 28S rRNA, 18S rRNA and 5S rRNA, respectively. The gel was run on 100volts for an hour.

Table1: RNA collection through different methods.

Samples	Leuko lock™ total RNA method		tempusTM blood RNA tube method		Trizol method	
	RNA concentration (µg/ml)	A_{260}/A_{280}	RNA concentration (µg/ml)	A_{260}/A_{280}	RNA concentration (µg/ml)	A_{260}/A_{280}
1	3	.7	6	.01	40	1.5
2	2	.6	5	.02	60	1.6
3	1	.5	4	.03	70	1.7
4	2	.4	8	.04	80	1.8
5	1	.3	7	.05	90	1.9
6	2	.2	9	.06	50	2

Table 2: Four sets of primer sequences along with their temperature used in multiplex PCR.

Primers	Primer sequence 5′forward 3′ 5′ reverse 3′	Serotypes	Temperature in celcius.
S1	CAAACCATGGAAGCTGTACG TTCTGTGCCTGGAATGATGCT	DENV1	51.8 52.4
S2	CAAACCATGGAAGCTGTACG TTCTGTGCCTGGAATGATGCT	DENV2	51.8 52.4
S3	GAGTGGAGTGGAAGGAGAAGGG CCTCTTGGTGTTGCTCTTTGC	DENV2	58.6 54.4
S4	CAGACTAGTGGTTAGAGGAGA GGAATGATGCTGTAGAGACA	DENV1	52.4 49.7
S5	ATATGCTGAAACGCGTGAG CATCATGAGACAGAGCGAT	DENV3	48.9 48.9

DISCUSSION:

Aedes aegyti is the vector for transmission of dengue virus in humans. The favorable environment for breeding of vector is from month of July to September in subcontinent [10]. Stagnant water in containers helps larvae to grow and convert into adult form. In addition hot temperature also enhances the growth and development [11,12].

Use of mosquito topical repellents, nets and sprays help in prevention from mosquito bite. Several studies have been done so far in order to find the rapid and effective diagnostic test for detection of dengue virus. RT-PCR has been approved as better test in comparison with ELISA and dot blot to detect dengue virus[11,12,13]. Understudy title was conducted to compare efficacy of RT-PCR with other tests. Samples were taken from Pakistani population, from two cities where dengue outbreak occurred.

CONCLUSION:

Dengue virus is an encapsulated RNA virus from arbovirus group. It occurs most commonly in tropical and subtropical region. It has 11 Kb length. It consists of a nucleocapsid or core protein , membrane associated protein and an envelope, 7 non structured genes. Previously in Pakistan, dot blot immunoassay, ELISA and complement fixation tests were used. RT-PCR is used for rapid and accurate detection of dengue virus serotypes.

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