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Determination of Herpetic keratitis frequency using Loop Mediated Isothermal Amplification (LAMP) Method in Isfahan, Iran

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ABSTRACT

Herpetic keratitis which is resulted from herpes simplex virus type I, is considered the most common cause of corneal blindness that needs being diagnosed and treated in order to prevent irreversible eye effects. The best method to diagnose it is molecular method which has high sensitivity and exclusivity. The current study was done on 25 eye samples collected from patients referred to the Feyz hospital of Isfahan who were diagnosed with herpetic keratitis disease. After cornea sampling and extracting DNA from eye samples, LAMP-PCR technique was done to search genome of herpes simplex virus type I. Among 25 suspected cases of herpetic keratitis, 5 samples were confirmed to have genome virus of HSV1 and herpetic keratitis frequency was determined by 20%. The results of this investigation showed a relative high frequency of herpetic keratitis in this zone using LAMP-PCR diagnostic method compared with similar studies which indicates high sensitivity of this technique. Therefore, using such rapid, accurate and cheap molecular method is suggested.

Keywords: Herpes simplex virus type I, Herpetic keratitis, LAMP-PCR

INTRODUCTION

In herpetic keratitis, the person suffers from corneal ulcers and dendrites ulcers or some vesicles on eyelid. When keratitis recurrent, Sometimes the context of corneal tissue is progressively trapped that leads to permanent turbidity and blindness (Kaye and Choudhary, 2006). In most cases, keratitis is a unilateral disease, but in 1.3-10.9% of the patients, it is bilateral (Hyashi et al, 2006). Infection by herpes simplex virus type I is the most common factor of corneal blindness. Hence, rapid and accurate diagnosis of the disease is important (Brooks et al, 2010). Diagnostic methods include immunological and

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serological methods and virus cell culture (Solomon, 1998). The disadvantages of these methods include: being time consuming, capable to detect the early stages of the disease and the probability of obtaining false positive or negative responses (Conraday et al, 2009). Therefore, use of molecular diagnosis method is necessary (Conraday et al, 2009). A molecular method that has been applied widely in recent years in rapid diagnosis of pathogens is PCR technique (Deguo et al, 2008). Although PCR method has high accuracy and wide applications, it has some limitations in diagnostic centers due to need for expensive devices and using dangerous material such as ethidium bromide (Notomi et al, 2000). LAMP is another molecular diagnosis method in which genome duplication is conducted by loop. This method was optimized for the first time in 2000 by Notomy et al. In LAMP technique, whole the amplification reaction is carried out under single-temperature as continuous form, and there is no denaturation of double-stranded nucleic acid to single-stranded to begin polymerization reaction (Kaneko et al, 2006). The reaction is easily done in this method even without initial denaturation, amplification efficiency is very high since, there is no time loss and the reaction is done continuously in optimum temperature for enzyme activity (Mori and Notomi, 2009). The reaction is amplified within 30 to 40 minutes, total cost is significantly reduced since, LAMP method does not need specific chemical material with expensive devices (Yang et al, 2011). The objective of this study is to determine herpetic keratitis frequency in Isfahan city by LAMP methods.

MATERIALS AND METHODS

The current study has been carried out on 25 eye samples collected from the people who had referred to Feyz hospital of Isfahan. These people were diagnosed by ophthalmologists and based on clinical criteria to have herpetic keratitis. DNA extraction: HSV1 virus was extracted by phenol – chloroform method. At first, 200 microliters of sample was poured in a micro tube and boiled for 10 minutes. 200 microliters phenol was added and was centrifuged for 15 minutes at 10000 g. three fuzzes were formed. 200 microliters of chloroform was added to the upper fuzz and was centrifuged for 10 minutes. The upper fuzz was separated. Absolute alcohol was added by twice the separated upper fuzz volume and was put in a -20 ^{0}C freezer for 24 hours. Then, it was centrifuged at 12000 g for 20 minutes. Micro tube was put in a 65 ^{0}C benmurray for 10 minutes. 50 microliters was added to the lower sediment. Preparation of primer: primers proper for gene ORF62 which are specific of HSV1 were produced. Its sequence is in accordance with Table 1.

Table 1. Sequence of applied primers to search virus HSV1

Primer name	sequence ۳ → ۵	Tm
F3	CAGCCACACACCTGTGAA	49/3
B3	TCCGTCGAGGCATCGTTAG	55/8
FIP	CCAGACGTTCCGTTGGTTGGTAGGTCTTTTACTTTGACTGTTCG CGCACC	80/7
BIP	CCATCATCGCCACGTCGGACTTTTTCGGCGTCTGCTTTTTGTG	83/9
LFP	AAATCCTGTCGCCCTACACAGCGG	66/8
LPB	CACCCCGCGACGGGACGCCC	73/8

LAMP reaction mixture

LAMP reaction was done in total volume of 25 microliters containing primers F3, B3 with concentration by 10 Pm, FIP and BIP with concentration by 20 Pm, LFP and LPB with concentration of 10 Pm, Dntp with concentration of 10 Mm, 8 units of enzyme Bst polymerase and enzyme buffer of polymerase.

LAMP test

Reaction mixture was put in a 62 ^{0}C benmurray for 30 minutes. In order to control the test, DNA of virus HSV1 was used as positive control and sterile distilled water was used as negative control.

LAMP reaction evaluation

1 microliter of SYBR 10% was added to each micro tube and was observed under UV light.

Electrophoresis of LAMP products

Lamp product was electrophoresed on 1.2% agarose gel in buffer TBE 0/5 X.

RESULTS

After the end of reaction, following addition of SYBR, positive microtube was seen in bright green and negative microtube was seen in very pale orange (Figure 1). LAMP reaction product was confirmed by electrophoresis of 1.2% agarose gel (Figure 2). After applying LAMP technique on herpetic keratitis samples, it was found that, 5 samples out of 25 samples was reported positive using LAMP technique and herpetic keratitis frequency was determined by 20%.

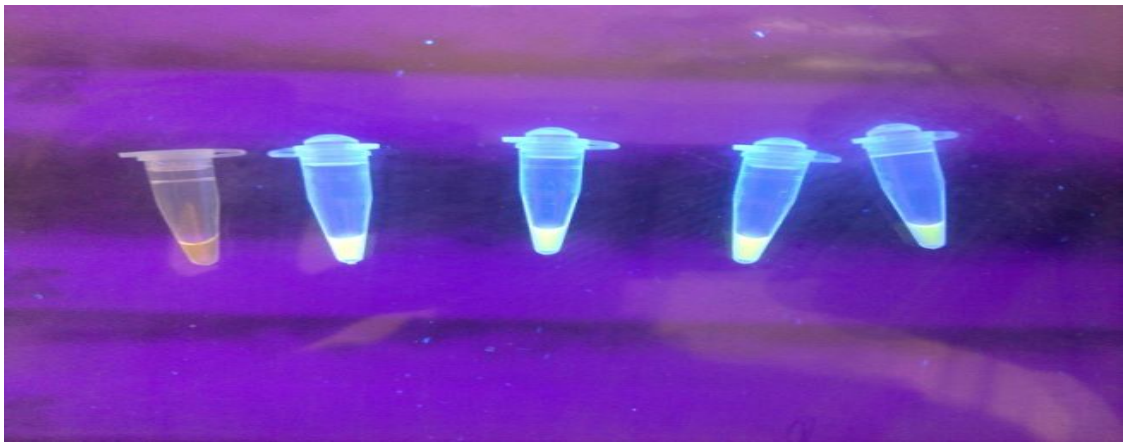

Figure 1. Results of LAMP reaction

Microtube 1- negative control Microtube 2- Positive control Microtube 3, 4, 5- Samples

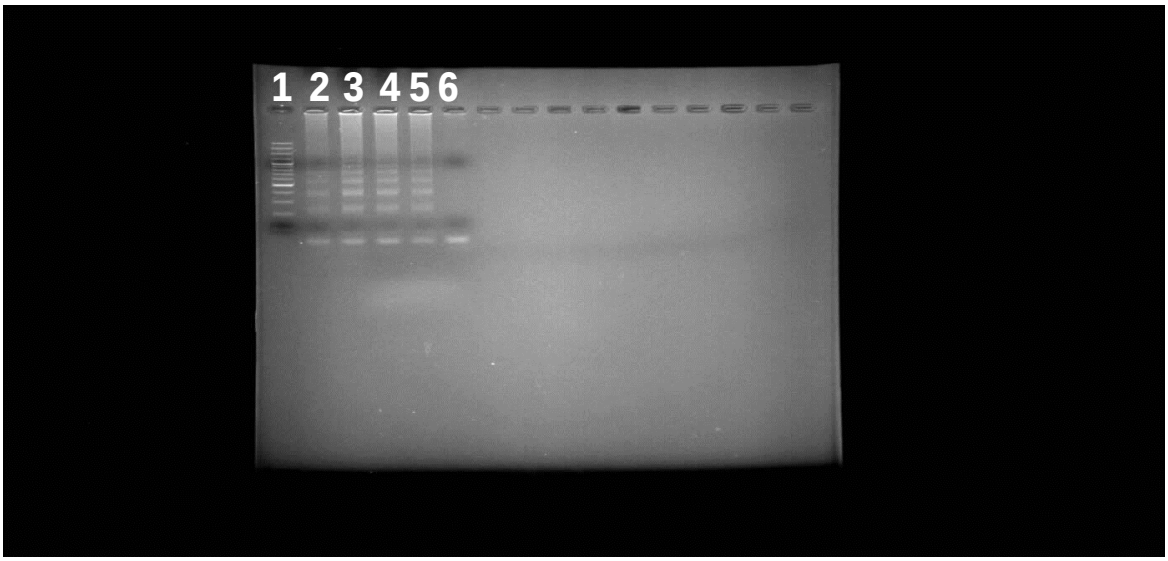

Figure 2. Electrophoresing the LAMP product of polluted samples to HSV1

1- DNA markers 2- Positive control 3, 4, 5- Samples 6- Negative control

DISCUSSION

Infectious keratitis is a real ophthalmology emergency and requires prompt diagnosis and treatment to prevent irreversible visual effects. Accurate identification of the pathogen type and its treatment is one of the most important problems in clinical ophthalmology (Mirdehghan et al, 2008). In this study, herpetic keratitis frequency was determined by 20%. In an investigation conducted by canko et al. herpetic keratitis frequency was determined by 55% using LAMP technique (Kaneko et al, 2005). Noormohammadian (2009-2010) determined the herpetic keratitis frequency by cell culture methods of virus and PCR. Herpetic keratitis frequency was determined by 2% cell culture and 9% PCR (Noormohamadian, 2011). Therefore, according to previous studies and obtained results from current studies, it can be mentioned that, LAMP technique is more sensitive in determination of herpetic keratitis frequency compared with applied techniques.

Conclusion

HSV1 virus detection existing in samples of herpetic keratitis by LAMP technique is highly sensitive and specific, and LAMP method can be used for rapid, accurate and cheap detection in diagnosis of infectious agents.

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