IJPPS 8(3) 2016

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Submission date: 26-Sep-2019 10:08AM (UTC+0800)
Submission ID: 1180199363
File name: IJPPS_8_3_2016.pdf (228.71K)
Word count: 2201
Character count: 10350

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 3, 2016

Short Communication

APPLICATION OF DESIGNED MPCR PRIMER FOR E6 GENE OF HPV TYPE 45 AND 52 IN CERVICAL CANCER PATIENTS

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Received: 13 Nov 2015 Revised and Accepted: 13 Jan 2016

ABSTRACT

Objective: Cervical cancer is the third disease that causes death in the world. The main cause of cervical cancer is *Human papillomavirus* (HPV). HPV have E6 and E7 oncogenes that, responsible for cancer incidence. One of molecular biology techniques for HPV identification is PCR. The aimed of this study is to applicate the multiplex PCR primer design for HPV type 45 and 52 identification.

Methods: DNA was isolated from 31 cervical cancer biopsy and cervical smear samples. DNA was amplicated with MY09/MY11 primer. Line probe array used to genotype of HPV. Application of primer design use MPCR methods with conditions was hot start 95 °C (3 min), denaturation 95 °C (15 s), annealing 58 °C (30 s), extension 72 °C (15 s) and final extension 72 °C (1 min). The amplified products were analyzed on a 1.5% agarose gel, stained with Gel Red and visualized in-gel documentation system.

Results: Result of HPV identification of HPV shown 19 of the 31 samples in this study was positive HPV. HPV genotyping was obtained the HPV type of the samples were HPV type 16, 18, 45 and 52. Application of design primer gives positive result. The band on S1, S2, S3 and S4 lane indicated the presence of gen E6 of HPV type 45 in J.1, J.3 and J.14 sample code and HPV type 52 in J.22 sample code.

Conclusion: MPCR primer design for E6 gene of HPV type 45 and 52 was applicated for identification of HPV type 45 and 52 in cervical cancer patients.

Keywords: Cervical cancer, Primer design, HPV type 45, HPV type 52, Multiplex PCR.

Cervical cancer is a malignant tumor that begins with the growth of abnormal cells in the cervical epithelium [1]. Cervical cancer is the third disease that causes death in the world with estimated 35,673 of deaths and 83,195 new cases of cervical cancer, while in Indonesia, cervical cancer is the second disease cause of death in women, especially in 15-44 y women [2].

World Health Organization (2007) stated that the main cause of cervical cancer is *Human papillomavirus* (HPV) [3]. HPV can be transmitted through sexual intercourse. HPV was classified into high-risk HPV and low-risk HPV. High risk HPV (HPV type 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 8, 59, 66, 68, and 82) is the group can cause cancer, especially cervical cancer [4]. ICO HPV Information Centre (2014) states that the prevalence rate of cervical cancer in Indonesia is 7.4% for HPV types 45 and 8.3% for HPV type 52 [2]. Based on Aldi (2015) research the prevalence of HPV type 45 for the region Sumatra and Riau were 60.47 % [5].

HPV have E6 and E7 oncogenes responsible for cancer incidence. E6 and E7 genes play a role in encoding an oncoprotein that will help replication of the virus, immortality, and cell transformation. Identification of HPV types required specific gene in the virus that causes cervical cancer, so the E6 and E7 genes is used to determine the HPV type [6]. HPV identification uses molecular biology techniques, like polymerase chain reaction (PCR). Identification of HPV using multiplex PCR method. The procedure of identification with multiplex PCR use more than one pair of primer, so we can identification many types of HPV only on a PCR process. This technique is more efficient than conventional PCR

The most important part in PCR is a primer. Primer is a series of proteins that will amplified the target of DNA. In this study, identification of genes E6 HPV type 45 and 52 use multiplex primer design [7]. The aim of this study is to applicate the multiplex PCR primer design for HPV type 45 and 52 identification in cervical cancer patients using multiplex PCR method.

The samples used in this study is 24 cervical cancer biopsy and 7 cervical smears derived from M. Djamil General Hospital, Padang and Arifin Achmad, Pekanbaru [5]. The tissue samples were isolated using DNA Extraction Kit gSYNCTM and cervical smear samples were isolated using Genomic DNA Mini LinkTM Pure Kit (Invitrogen TM). Isolation of DNA was done according to the protocol of the kit being used [5].

HPV identification used MY09/MY11 primers. The PCR conditions were hot start 94 °C (5 min), denaturation 94 °C (45 s), annealing 55 °C (45 s), extension 72 °C (45 s) and final extension 72 °C (5 min). The amplified products were analyzed on a 1.5% agarose gel, stained with gel red and visualized in-gel documentation system.

Line probe array used to genotyping of HPV. DNA was amplicated with MY09/MY11 primer by giving biotin as a label on one of the primer. Genotype-specific probe stopped on the membrane. Amplicon and the genotype-specific probe were mixed. Hybridization washed, and the expected band would stay. Streptavidin added in an alkaline environment (alkali posphatase), substrates was added and the band on the strip was detected [8].

The primers used in this study were HPV type 45 E6 gene primer (forward primer 5'-TGC GGT GCC AGA AAC CAT TGA-3' and reverse primer 3'-TTT CTT GCC GTG CCT GGT CA-5') with product length 129 bp and HPV type 52 E6 gene primer (forward primer5'-CAC GAA TTG TGT GAG GTG CTG-3' and reverse primer3'-GGT CAC AGG TCG GGG TCT-5') with product length 402 bp [7].

The result of HPV identification of HPV shown 19 of the 31 samples in this study was positive HPV (Table. 1). HPV genotyping was obtained the HPV type of the samples were HPV type 16, 18, 45 and 52 (table 1).

Primer MY09/MY11 is a general primer that has been used in many studies. MY09/MY11 primer commonly used in environmental epidemiology north and south America and Asia. Based on Weimin, *et al.*, (1997) research, MY primer detects more than GP primer with ratio 90%: 46.7 [9]. In this study, positive results indicate with a band on 450 bp [10]. All of the positive HPV samples were cervical cancer biopsy samples, and no cervical smear had HPV DNA in this study. The prevalence of HPV in this study was 61.29% (n=19) of 23

samples, compared with previous research using GP primer, the prevalence of HPV was 60,47% (n=26) of 43 samples [5]. From this data known that no significant result between GP and MY primer in the identification of HPV DNA.

High risk HPV, in general, is more often present in patients with cervical cancer are HPV 16, HPV 18, HPV 45, HPV 52, HPV 31, HPV 33, and HPV 35 and so on. The results linear array probe found type of positive DNA samples was high-risk HPV such as HPV 16, HPV 18, HPV type 45 and HPV type 52. The result of this study shown that the designed primer gives positive result (Fig.1).

The thin band on S1, S2, and S3 line still indicated the presence of gen E6 of HPV type 45 in sample 1,3 and 14. E6 gene has a role in encodes an oncoprotein that will help replication of the virus, immortality and cell transformation which is the host of HPV DNA [6]. Based on this results in proof that the multiplex primer design can be used to identification of HPV type 45 and 52.

Table 1: Shows identification of *Human Papillomavirus* using line probe array

S. No.	Samples code	Result of HPV identification	Result of HPV
1	J.1	+	HPV 45
2	J.2	+	HPV 18
3	J.3	+	HPV 45
4	J.4	-	-
5	J.5	+	HPV 16
6	J.6	-	-
7	J.7	-	-
8	J.8	+	HPV 18
9	J.9	+	HPV 18
10	J.10	+	HPV 18
11	J.11	-	-
12	J.12	+	HPV 16
13	J.13	+	HPV 16
14	J.14	+	HPV 45
15	J.15	+	HPV 16
16	J.16	+	HPV 18
17	J.17	-	-
18	J.18	+	HPV 18
19	J.19	+	HPV 18
20	J.20	+	HPV 18
21	J.21	+	HPV 18
22	J.22	+	HPV 52
23	J.23	+	HPV 16
24	J.24	+	HPV 16
25	A.1	-	-
26	A.2	-	-
27	A.3	-	-
28	A.4	-	-
29	A.5	-	-
30	A.6	-	-
31	A.7	-	-

Sample code J.1-J.24 is code for cervical cancer biopsy samples, samples code A.1-A.7 is code for cervical smear samples.

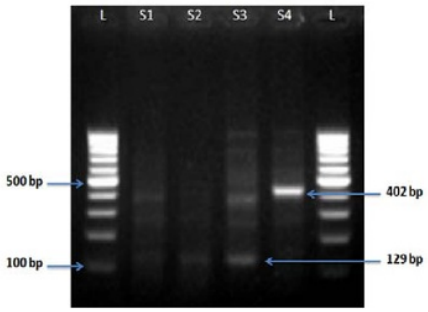

Fig. 1: Multiplex PCR Tests HPV type 45 and HPV type 52

Fig. 1 shown that HPV type 45 appear a band at 402 bp while HPV type 52 is appear a band at 129 bp. Molecular diagnosis of HPV infection is very important for cervical cancer screening. Commonly methods, such as hybrid capture (HC2), in situ hybridization and PCR, is very good in terms of sensitive and specificity. Nowaday, PCR most widely used in a wide range of molecular diagnostic because of its ability to detect small fragments of deoxyribonucleic acid (DNA) [11, 12].

In this study, HPV type 45 and 52 was identification used multiplex PCR method. This method can detect some types of HPV in one tube at one time. Multiplex PCR is very advantageous because minimizing the use of materials, time and costs. The most important materials in PCR are a primer and DNA templates, but multiplex PCR have a different composition with conventional PCR. Multiplex PCR requires more than a pair of primers, template DNA, the molecule of water and dimethyl sulfoxide (DMSO). DMSO on multiplex PCR used to inhibit the activity of taq polymerase thus reducing amplification product. DMSO can increase amplification and enzyme interaction.

MPCR primer design for E6 gene of HPV type 45 and 52 was applicated for identification of HPV type 45 and 52 in cervical cancer patients. There are three positive samples HPV type 45 (J.1, J.3, J.14) and 1 positive samples HPV type 52 (J.22) from 31 samples from the cervical tissue and smears. MPCR primer testing showed positive results with the band that came out in the size of 129 bp and 402 bp.

ACKNOWLEDGEMENT

We gratefully acknowledge Dr. M. Djamil General Hospital, Padang and Arifin Achmad General hospital, Pekanbaru for providing us cervical cancer tissue biopsies and cervical smears. We would like to thank university superior research grant from the Ministry of Research, Technology and Higher Education of Republic Indonesia for supporting this study.

CONFLICT OF INTERESTS

Declare none

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IJPPS 8(3) 2016

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