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Analysis of Human Papillomavirus and Herpes Simplex Virus Genus -2 from Patients with Cervical Cancer in Isfahan, Iran

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ABSTRACT

Background: Although cervical uterine cancer is a second form of cancer among women, but it occupies fifth form among all cancer types. ***Methods:*** In the present study, human papillomavirus (HPV) and herpes simplex virus 2 (HSV-2) in cervical cancer patients by using real time polymerase chain reaction (PCR) technique and the relation between their viral loads were investigated. 156 cervical carcinoma tissues were collected from married women in health centers in Isfahan, Iran. ***Results:*** The results showed that among 156 specimens, 58.97%, 45.51% and 7.05% were positive for HPV DNA, HPV-16 and HPV-18 respectively. Only in 2.3% specimens, HSV-2 and HPV-16 were positively detected where viral load HSV-2 in conjunction with HSV-16 dramatically increased. ***Conclusion:*** Thus the present study not only confirmed that viral load of HPV-16 is more than other HPV types, but also in possible conjunction with HSV-2, both rates will significantly increase.

Key words: HPV, HPV16/18, cervical cancer, HSV-2, real time PCR

1. INTRODUCTION

In the present scenario, cervical uterine malignancy is the second form of cancer in women, (1) and fifth deadliest cancer in women. It influences about 16 per 100,000 women a year wherein about 9 per 100,000 die every year. Nearly 80% of cervical cancers were reported in developing countries around the world where 473,000 cases were involved in 2008 (2) and 225,000 people died in 2010 (3).One of the main important causes of cervical cancer is some kind of human papillomavirus (HPV) contamination. More than 100 kinds of various HPV have been recognized, and about 30- 40 infect the anogenital tract. Moreover, 15 types are oncogenic (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 (4) and HPV 16 and 18 have the highest percentage among cervical cancer patients. Nevertheless, 40-90% of primary cervical carcinomas have been reported worldwide (5, 6). Herpes simplex virus genus (HSV) is one of the members of the Herpesviridae family, which causes some diseases in human (7). 60%-95% of adults are either carrying HSV viruses or influenced by related diseases. Generally, they are detected in the patients with infections and it affects their immune system (8, 9). Many epidemiology researches have proven the relationship among HPV and HSV-2 in cervical cancer (10-14).

On the other hand, the cervical cancer's peril in HPV-16 or HPV-18 is less than infection with HPV and HSV- 2. However, according to other research, there isn't any relationship between HSV-2 and cervical cancer (15) but HSV-2 DNA is detectable in a high percentage of neoplasia. Besides, the helping role for HSV-2 in cervical cancer is assumable (16). Possibly HSV-2 is a beginner for cervical cancer and HPV continues its way to carcinogens (17). The viable reason is that HSV is able to break the chromosomes (18, 19), affect amplification of DNA (20), and possibly the double minute chromosomes are formed with it (21-23). In the present study, we have used real time PCR technique to determine not only the rate of HPV and HSV-2 in cervical cancer patients in Isfahan, but also compare the rate of HSV-2 prevalence with HPV in these patients.

2. MATERIAL AND METHODS

Total number of 156 tissues of paraffin blocks pertained to cervical carcinoma were gathered from married women in health centers in Isfahan, Iran. DNA tissues were extracted from paraffin blocks by High Pure Viral Nucleic Acid Kit (Roche). Almost all tumors were categorized as squamous cell carcinomas (24). Generic primers GP 5, GP 6, and genotypes 16 and 18 were utilized in HPV real time PCR. Besides, there persisted ability to increase the L1 gene fragment from the genital HPV types (25,26). In addition, the methodology of Alexander *et*

al. (1999) to detect HSV-2 (27) was referred. However, its amplification and detection was used as an internal control of the testing processes. Eventually, b-globin amplified was used as an internal control in these processes.

Primers and PCR protocol

During this investigation, the primer pairs GP 5, GP 6 for HPV and the primers *viz*. HPV-16 and HPV-18 were utilized. Thermal cycle conditions included primary denaturation at 95°C for 10 minutes followed by 50 cycles at 95°C incubation for 10 seconds, 50°C for 10 seconds and 72°C for 30 seconds respectively. Afterwards per 72°C extension, fluorescence was cleared and real time PCR was carried out from copies/reaction in standard curves of 2×10^1 to 2×10^6(28).

Moreover, different primes and probes were utilized to recognize two viral subtypes according to the glycoprotein G (gG) gene of HSV (27). The forward primer HSV-2 was 5'-TCCTG/CG TTCCTA/CACG/TGCCTCCC-3', and reverse primer was 5'-GCA GIC AC/TA CGTAACGCACGC T-3'and its label probe (probe HSV-gG2-P) was 5'-FAM-CGACCA GAC AAACGAACGCCGCCG T-3'-TAMRA. Each 50 µl-PCR mixture contained 5 µl of purified DNA, 833nM density per primer, and 100 nM probe. After 2 min of incubation at 95°C for denaturation, the 45 cycles of PCR were performed where every cycle was 95°C for 20s and 58°C for 1 min respectively. The fluorescent intensities per reaction were read during PCR cycling in a Corbett Rotor-Gene 6600 thermo cycler. All viruses were detected in the range of 10^1 to 10^7 copies/reaction.

3. RESULTS

The results of the research were collected for abnormal cervical biopsy diagnosis of 156 patients. The patients' age ranged from 26–64 years with an average of 40±5 years. All biopsies were Squamous Cell Carcinomas (SCC). 92 of 156 patients (58.97%) with cervical specimens utilizing real time PCR were HPV-DNA among which HPV genotypes 16 was the most common and 18 followed it. HPV types 16 and 18 were represented in 71 (45.51%) and 11 (7.05%) of cervical specimens respectively.

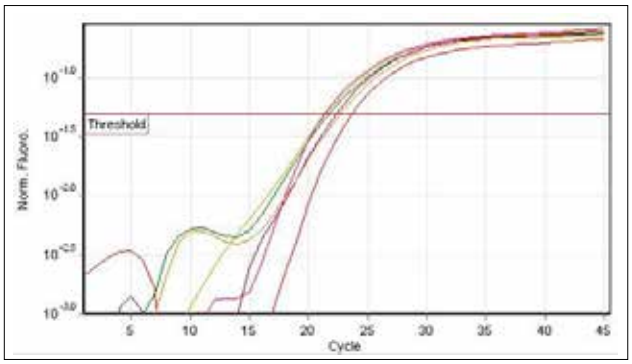

Figure 1. Quantitative data for b-globin assay

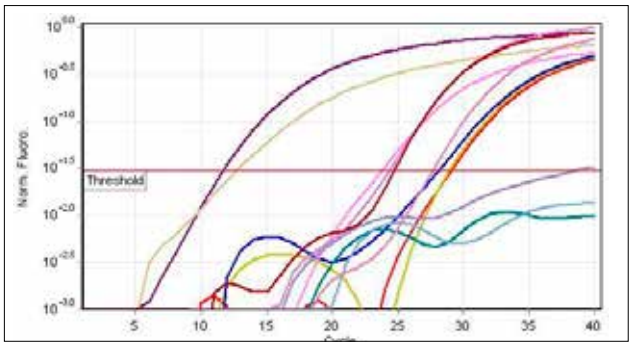

Figure 2. Quantitative data for HPV DNA virus levels. Real-time amplification with fluorescence detection. The plasmid controls for two species, water blank, and negative human control DNA are indicated. The remaining curves are patient specimens with HPV DNA virus levels.

According to our research, HPV 16 and 18 were detected in 65 and 5 samples separately. Besides, both were found in 6 samples. Finally, there wasn't any high-risk genotype of 16 and 18 in 10 samples. The viral load of HPV16-positive was more than 10^6copies/reaction. On the other hand, over half of the HPV18 specimens contained less than 10^4copies/reaction.

The entire samples were tested for HSV-2 among which only 3 samples were positive for HPV-16 and HSV-2 (% 2.3) and 2 samples indicated negative HPV and positive HSV-2 respectively. The viral load of HSV-2 in cases with positive HPV was much higher than negative ones. In addition, about 10^3 -10^4copies/reaction in positive HPV and 10^2 in negative HPV samples were reported.

4. DISCUSSION

The existence of HPV 16 and 18 oncogenes is the main risk factor for high-grade dysplasia and invasive cancer (29). This risk for HPV16 and 18 is more than other HPV types (30).

According to previous research, viral load has direct relation to disease intensity. Based on the research with real time, high-risk and low-risk HPV PCR have illustrated much higher viral load in HPV-16 than the other types, and the severity of cervical disease has a direct correlation with increasing HPV-16 viral loads (31).

During the recent years, the following prevalence of HPV was reported in different parts of Iran: 64% in 2006 in northwest Iran, 78.6% in 2002 in Mazandaran province, 87.1% in 2003 for southern Iranian patients, and 85.5% in 2002 and 73% in 2006 in Tehran, Iran (32) and between 29%-37.9% in Zabol, Iran in 2011.

Also, the previous researches showed the different role of HSV-2 in cervical cancer where in some of them were not related to any increasing risk of cervical carcinoma (33). On the other hand, in some researches it was proposed that genital HSV-2 infection with HSV-2 is unable to create cervical cancer, itself, but it can increase the peril of cervical cancer with conjunction of HPV (34).

Nevertheless, the purpose of the present study was to determine the existence and amount of viral load HSV-2 and HPV 16 – 18 in the abnormal cervical biopsy (SCC) in Isfahan, Iran. We found nil relationship between HSV-2 infections in the cervical carcinoma samples. But 45.51% of cancer samples were HPV 16 positive. To sum up, it is a high risk factor. Additionally, we determined that all the HSV-2 positive carcinoma cervices were infected with HPV-16 and the rate of HSV in these samples were higher than others thus confirming the cooperation HSV-2 and HPV-16 in squamous cell carcinomas. Nevertheless, we couldn't find any correlation between HPV-18 and HSV-2. Thus, it can be safely concluded that viral load of HPV-16 is higher than other HPV types and if in conjunction with HSV-2, the rate of both will significantly increase.

CONFLICT OF INTEREST: NONE DECLARED.

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