Lack of Association between Herpes Simplex Virus Type 2 Infection and Cervical Cancer - Taq Man Realtime PCR Assay Findings

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Abstract

Background: About one third of the human population suffer cancer during their lifetime and more than 20% of total morbidity is related to neoplasia. Cervical cancer is generally the most common cancer in developing countries and the second most common in women globally. The role of human papilloma viruses viruses in its induction is clear. However, the involvement of hepres simplex virus type 2 (HSV-2) is controversial. Therefore a survey was conducted of the prevalence of HSV-2 in patients with cervical cancer and also healthy people with sensitive and quantitative Taq Man real-time PCR assay.

Materials and methods: Seventy six formaldehyde fixed paraffin embedded tissue specimens from patients with histologically proven history of cervical cancer as well as 150 control blocks were sectioned for deparaffinization and DNA extraction.

Results: There was no HSV-2 DNA in our patient specimens but four control samples were positive, all with a history of hysterectomy.

Conclusion: Considering the absence of any positive viral HSV-2 DNA in our patients and also the presence of four positive specimens among our controls, we did not find any relationship between the presence of HSV-2 DNA and cervical cancer.

Key words: HSV-2 - realtime PCR - cervical cancer - Iranain women

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Introduction

Cervical cancer is one of the most prevalent cancers in developing countries and the second common cancer between women in the world. Third fourth of patients are living in developing countries and 450,000 new cases of cervical cancer are reported in these areas, annually (Disease Control & Prevention, 2010). In 2008, the global estimation of cervical cancer was 473,000 and with an annual death rate of around 253,000 (Menczer, 2003).

Many etiological agents have been reported to be involved in cervical cancer among those are: sexual relationship in early ages, multiple sexual partners, history of HPV infection, poor immune system function, recurrent sexual infection diseases, smoking, contact with diethyl sterol (DES) within embryonic period, history of intra-epithelial neoplasia, poor personal hygiene, use of contraceptive pills for more than 5 years and most important and controversial, HSV-2 (Announcement the society of Gynecologic oncologists’, 2006).

Epidemiological studies have shown that there is an interaction between HPV and HSV-2 in induction of cervical cancer (Lulitanond et al., 1994; Yamakawa et al., 1994; Kanerva et al., 2008). Also, these studies indicate that simultaneous infection with HPV and HSV-2 increases the risk of cervical cancer induction when compared to infection with either HPV-16 or HPV-18 alone. On the contrary, other studies have suggested there is no relationship between infection with HSV-2 and the emergence of cervical cancer (Szostek et al., 2009). Meanwhile almost 30% of genital tumors have sequences of HSV-2 genome and Bam HIE fragments which both of them have the capacity of transformation of human and mice cells (Newcomb et al., 2009). Also HSV-2

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DNA may be found in higher degrees of neoplasia which suggests a potential role for HSV-2 in cervical cancer (Linda, 2002). It has been assumed that HSV-2 is a starter for cancer and infection with HPV occurs later (Partridge & Koutsky, 2006). Indeed herpetic lesions can facilitate entrance of HPV in basal cells and so inflammatory responses to HSV-2 infection may decrease T helper capacity to produce immunity response efficiently and also facilitate HPV persistent infection and induce cancer (Smith et al., 2002).

Synergisms between HPV, HSV-2 and induction of cervical cancer have been confirmed by laboratory experiments (Di Paolo et al., 1998). Others studies have been shown that in infected cell HSV-2 can affect human genome without any traces by the Hit and Run mechanism (Smith et al., 2002).

As different molecular methods in detection of HSV-2 DNA have different sensitivity, different reported findings in published studies maybe affected by these sensitivity limitations. So, in this survey we used sensitive and quantitative Taq Man real time PCR assay in evaluation of HSV-2 prevalence in formaldehyde fixed paraffin embedded pathologic blocks of cervical cancer patients and control individuals (Naserpour et al., 2011).

Materials and Methods

Seventy six formaldehyde fixed paraffin embedded tissue specimens from patients with pathologically proved history of cervical cancer referring to oncology department of Kosar University hospital hospital in Qazvin and Shohada, Imam Hossein and Mahdyeh Hospitals of Shahid Beheshti University in Tehran and 150 blocks from healthy peoples referring to Qazvin University Hospitals for hysterectomy with different gynecological diseases other than cervical cancer were selected as control for our study.

Two hundred milligrams of tissues within the blocks were collected by 5 micrometer slices in a 1.5 ml Eppendorf microtubes and after initial deparaffinization by Xylol and Ethanol (Sawamura et al., 2011) their DNA were extracted by Roche High Pure Nucleic Acid Extraction kit (Roche, IRAN). Taq Man real time PCR assay was done by HSV-2 Primer Design kit (Primer Design, UK). Each Taq Man real time PCR reaction consists of 10 µl of ABI 2X Master mix, 1µl of HSV-2 probe/primer mixture , 1µl of internal extraction control , 3 µl of RNase/DNase free DDW (provided in the kit) and 5 µl of extracted DNA or standard (provided in the kit). Endogenous ACTB control which was used for qualifying biological samples amplified by a mixture of 10 µl of Master mix ,1 µl of ACTB probe/ primer , 4 µl of DNase/RNase free DDW and 5 µl of patient or control extracted DNA samples. PCR program as recommended by manufacturer was as follow: 10 minutes in 95º followed by 50 cycle of 95º C for 10 seconds and 60ºC for 60 second and fluorescent accusation was done at the latter stage.

Results

In this study, 76 pathologic blocks from cervical cancer patients and 150 blocks from control individuals, were examined for HSV-2 DNA. The lowest and highest age range in patients and control groups were 21 and 78 and 22 and 76 years respectively. There were different kinds of cancer between our patients groups and the major reason for referring in control group was hysterectomy. In this study our real time PCR efficiency, regression coefficient and R² were 99.433%, 0.999 and 32.717 respectively (Figure 1) and we could not find any HSV-2 DNA in samples of our patients while we found 4

![Figure 1. A) Standard Curve used to Calculate the Minimum Detection Limit for Herpes Simplex Virus DNA in Unknown Samples. The linear regression coefficient was 0.999 and efficiency of PCR was 99.43%; B) Fluorescent curves of the standard dilution series. From the left to the right 2x10⁴, 2x10³, 2x10² and the negative control is presented by the horizontal straight line.](image1)

![Figure 2. Amplification Plot of HSV-2 Control Specimens. Four samples showed raising amplification plot which was an indication of HSV-2 DNA existence](image2)
HSV-2 DNA positive among our control group (Figure 2). Also, in our study mean age of HSV-2 positive control individuals were 38±2 years.

Discussion

Studying the role of viruses in stimulating cancer is one of the most interesting areas in biooncology researches and for many tumors this association has confirmed. On the other hand, Taq Man real_time PCR in addition to offering more sensitivity and specificity than traditional PCR, provide us possibility of continuous studying of PCR products and hence it is a favorable test in detection of HSV-2 prevalence in cervical cancer. In this study, our findings showed that there were no specimens with HSV-2 DNA contamination in patients group although there were four HSV-2 DNA positive specimens in our control group. Moradi in his study (Moradi et al., 2005) in 2004 used PCR assay for evaluation of HSV-2 contamination in patients with different levels of cervical neoplasia. He reported that 28.7% of patients had positive test results for HSV-2. Similar study in Greece in 2001 (Sapountzi-Krepa et al., 2001) showed that 70.3% of cervical intraepithelial neoplasia patients infected with HSV-2. Also, Uribe-Salas Study (Uribe-Salas et al., 2003) indicated that the prevalence of HSV-2 in sex workers was more than students group and this finding shows that there is an association between sexual contacts and contamination with HSV-2. In Poland, Kwansniewska study (Kwansniewska et al., 2009) showed that the prevalence of co-infection of HPV with Chlamydia trachomatis and HSV-2 in cervical cancer patients was more than control individuals. Also, Kjaer study (Kjaer et al., 2006) on 800 women referred to a healthcare center by ELISA test which was targeted on a HSV-2 specific antigen showed that the prevalence of HSV-2 was 76% and 26.2% in Greenland as a high incidence cervical cancer and Denmark as a low incidences one, respectively. These results suggested that there is a significant relationship between cervical cancer and infection with HSV-2 and differ from our results.

On the other hand, Zereu study (Zereu et al., 2007) on etiologic backgrounds and risk factors of cervical adenocarcinoma by routine PCR assay showed that all their tested patients had no HSV-2 contamination. In 2009, Szostek and his colleague study (Szostek et al., 2009) on 125 specimens of cervical cancer patients with different levels of neoplasia by PCR and nested PCR showed that none of the studied specimens were infected with HSV-2 viruses. These results were in accordance with our results.

Epidemiologic studies showed that during initiating and development of cervical cancer there is an interaction between HSV-2 and HPV-16 or HPV-18 viruses. This studies (Kjaer et al., 2006; Kwansniewska et al., 2009; Jalouli et al., 2010) indicated that development of cervical cancer depends on contamination with HPV although many of women with HPV infection never develops cervical cancer and HSV-2 infection plays a cofactor role.

The reason for such a great differences in reported results of involvement of HSV-2 in the cervical cancer may lay back in Hit and Run mechanism (Jones , 1995; Smith et al., 2002; Hoenil & Weon, 2005). By this mechanism, HSV-2 can induce cervical cancer without leave any traces in genome of cancer cells. Inactivated HSV-2 can alter human and rodent cell growth characteristics and starts tumorogenesis and entrance of HPV-16 or HPV-18 will complete the cancerogenic process (Di Paolo et al., 1998; Smith et al., 2002; Szostek et al., 2009). Using Taq Man real_time PCR assay which has high sensitivity and specificity with capacity of determining two hundred copy of viral genome per microliter of sample provide us a very good reliability for assessing obtained results.

In conclusion, with respect to the absence of any positive viral HSV-2 DNA in our patients and also the presence of four positive specimens among our controls, it seems that based on results obtained from the specimens examined in the present study we did not find any association between the presence of HSV-2 DNA and cervical cancer.

References


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