RESEARCH ARTICLE

Lack of Association between *Chlamydia trachomatis* Infection and Cervical Cancer - Taq Man Realtime PCR Assay Findings

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Abstract

Background: Cervical cancer is one of the most common cancers in developing countries and the second most common type of cancer in women globally. Several recent studies suggested a co factor role for *Chlamydia trachomatis* in pathogenesis of cervical cancer. This study aimed to evaluate existence of *C. trachomatis* DNA in pathologic blocks of patients with cervical cancer. Materials and methods: Seventy-six formaldehyde fixed paraffin embedded tissue specimens from patients with histologically proven history of cervical cancer as well as 150 blocks from healthy peoples were included in the present study. Thin slices were prepared from selected blocks followed by deparaffinization and DNA extraction; the presence of *C. trachomatis* DNA was examined by Taq Man real-time PCR. Results: Our TaqMan real time PCR assay with cervical specimens of patients with cervical cancer showed that there was no *C. trachomatis* DNA. Also, we found three positive specimens among our control group. Conclusion: It seems that based on results obtained from the specimens examined in the present study, there is no association between the presence of *C. trachomatis* DNA in cervical specimens and cervical cancer.

Keywords: *Chlamydia trachomatis* DNA - real-time PCR - cervical cancer

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Introduction

Cervical cancer is one of the most prevalent cancers in developing countries and the second common cancer among women in the world. Third fourth of patients are living in developing countries and 450,000 new cases of cervical cancer are reported annually in these areas (Anonymous, 2010). In 2008, the global estimation of cervical cancer was 473,000 with annual death 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 (Daling et al., 1996), multiple sexual partners (Jensen et al., 2011), history of human papilloma viruses (HPV) infection (Vidal et al., 2011; Carozzi et al., 2012; Magaldi et al., 2012) and infection with *Chlamydia trachomatis* (Kwasniewska et al., 2009; Simonetti et al., 2009). Published multi-centre reports indicated that detection of serum antibodies against *C. trachomatis* were associated with high risk of cervical cancer development (Paavonen et al., 2003; Smith et al., 2004; Naucler et al., 2007). Moreover, study on cytologic specimens of patients suffering from cervical cancer by PCR, confirmed existence of *C. trachomatis* DNA in these specimens (Klomp et al., 2010).

C. trachomatis may damage mucosal barrier and facilitate human papilloma virus infection or may interfere immune response and viral clearance and by this way support persistence infection of HPV (Steben et al., 2007). *C. trachomatis* belongs to Chlamydia genus, which includes obligatory intracellular pathogens. Chlamydiae are small, gram-negative bacteria and are characterized by a small genome with a limited capacity of biosynthesis. This bacterium causes several human diseases, including neonatal pneumonia, urethritis/cervicitis, trachoma and conjunctivitis (Saveleva et al., 2010). Epidemiological studies have shown that there is a relationship among cervical cancer associated with *C. trachomatis* antibodies, HPV type and HPV co-factors (Madeleine et al., 2007).

On the other hand, there are many studies in which the researcher did not find any association between contamination and infection with *C. trachomatis* and HSV cervical cancer (Paavonen et al., 2003; Naucler et al., 2007; Farivar et al., 2012).

As traditional PCR used in previous studies has a poor precision, low sensitivity, short dynamic range and low resolution in comparison with Real-time PCR (Naserpour et al., 2012), in this survey we used the latter sensitive and quantitative assay in evaluation of *C. trachomatis* prevalence in formaldehyde fixed paraffin embedded pathologic blocks of cervical cancer patients and control individuals.

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Materials and Methods

All the process on the patients samples were confirmed by Qazvin University of Medical Sciences Ethics Committee and patients were signed related forms.

Seventy-six formaldehyde fixed paraffin embedded tissue specimens from patients with pathologically proved history of cervical cancer referred to oncology departments at university hospitals, including Kosar hospital in Qazvin and Shohada Hospital, Imam Hossein Hospital and Mahdyeh Hospital of Shahid Beheshti University of Medical Sciences in Tehran were included to the study. Also, 150 blocks from healthy peoples referring to Qazvin University Hospitals for hysterectomy with different gynecological diseases other than cervical cancer were selected as control group.

Two hundred milligrams of tissues within the blocks were collected by 5 micrometer slices in a 1.5 ml Eppendorf microtube and after initial deparaffinization by Xylol and Ethanol (Farivar et al., 2012), their DNA were extracted using Roche High Pure Nucleic Acid Extraction kit (Roche, IRAN). Taq Man real-timePCR assay was done by *C. trachomatis* Primer Design kit (Primer Design, UK). Each Taq Man real-time PCR reaction consists of l0 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 Beta-actin gene (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 follows: 10 minutes in 95°C followed by 50 cycles of 95°C for 10 seconds and 60°C for 60 second and fluorescent accusation was done at the latter stage.

Statistical analysis

Data were analysed using SPSS software,version 17.0. The strength of the linear relationship between logarithms of mean number of bacteria and Ct values for probes were measured using the Pearson correlation coefficient.

Results

We did not find any positive *C. trachomatis* DNA in our patients but found three positive specimens among our control group.

The lowest and highest age range in patients and control groups were 21 and 78, 22 and 76 years, respectively. In this study our real-time PCR efficiency, regression coefficient and r2 were, 99.594%, 0.998, 35.432 respectively (Figure 1) and we could not find any *C. trachomatis* DNA in samples of our patients although in contrast there were 3 positive samples among our controls (Figure 2).

Our date showed that in our studied population there was no relationship between *C. trachomatis* infection and the types of cervical cancer ie. squamous cell carcinoma or adenocarcinoma (data were not shown).

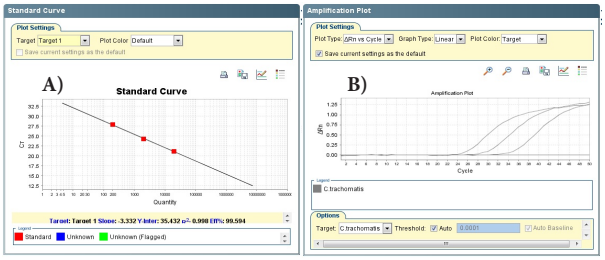

Figure 1. A) Standard Curve used to Calculate the Minimum Detection Limit for *C. trachomatis* DNA in Unknown Samples. The linear regression coefficient was 0.998 and efficiency of PCR was 99.594%. **B) Fluorescent Curves of the Standard Dilution Series.** From the left to the right $2X10^4$, $2X10^3$, $2X10^2$ and the negative control is presented by the horizontal straight line.

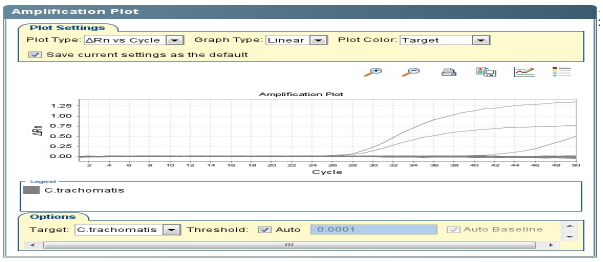

Figure 2. Amplification Plot of *C. trachomatis* Controls Specimens. As it is obvious in this figure, three samples showed raising amplification plot which was an indication of *C. trachomatis* DNA existence in those specimens.

Discussion

Studying the role of *C. trachomatis* in stimulating cervical cancer is one of the most interesting areas in bio-oncology researches. On the other hand, Taq Man real-time PCR in addition to offering more sensitivity and specificity than those of traditional PCR, provides us with the possibility of continuous studying of PCR products and hence, it is a favorable test in detection of *C. trachomatis* prevalence in cervical cancer.

In this study, our findings showed no specimens with *C. trachomatis* infection in patients and we found three positive specimens among our control group.There are numerous studies which confirm our results.

Quint et al. in their study on 71 cervical adenocarcinoma biopsy by PCR assays, reported that none of their cases were positive for *C. trachomatis* (Quint et al., 2009). Naucler et al. in a nested case control study on 13595 Taiwanese women by serologic assay, found that in their studied individuals *C. trachomatis* was not associated with cervical cancer (Naucler et al., 2007). Paavonen in a case-control study on serum banks found that there was no materially difference interaction among HPV16 DNA-positive squamous cell carcinomas and there was an antagonism in the carcinogenic effects of *C. trachomatis* and HPV6/11 with HPV16 (Paavonen et al., 2003). Safaeian and colleagues in their study on 182 women with prevalent and 132 women with incident histological cervical neoplasia, reported that by assessing DNA or IgG and after controlling for carcinogenic HPV-positive status, there was not any association between *C. trachomatis* status and risk of cervical premalignancy (Safaeian et

al., 2010). Zereu and colleagues in their study on 206 paraffin-embedded cases of adenocarcinoma of the uterine cervix by PCR assay found that all samples were negative for HSV-2 DNA and *C. trachomatis* DNA (Zereu et al., 2007). Castle and colleague reported that they were unable to find any association between severity of HTLV-I, *C. trachomatis* and/or HSV_2 in Jamaican women with cervical neoplasia (Castle et al., 2003). All these studies like ours, confirm that there is no association between existence of *C. trachomatis* DNA and cervical cancer.

On the other hand, Wallin and colleagues in their study on 118 women who after having a normal Pap smear had a cervical cancer diagnosis, showed that a prior cervical *C. trachomatis* infection was associated with an increased risk for development of invasive cervical cancer (Wallin et al., 2002).

Samoff and her colleagues reported that the association between Chlamydia infection and cervical cancer may be due to an effect of Chlamydia infection on persistence of high-risk HPV (Samoff et al., 2005). Deluca and colleague in a study on 189 sexually active women, with cytological abnormalities in cervical epithelium by PCR technique, found that women infected with *C. trachomatis* showed a higher risk for viral infection than non-infected ones (Finan et al., 2006). Also, Finan and colleagues in their study on 86 HPV-positive and 213 HPV-negative women by PCR assay, found that a higher prevalence of *C. trachomatis* was found in HPV-positive samples (Finan et al., 2006).

Bosch and de Sanjose in their study on the epidemiology of human papilloma virus infection and cervical cancer found that previous exposure to other sexually transmitted diseases such as *C. trachomatis* and Herpes Simplex Virus type 2 (Bosch et al., 2007) was a risk factor for cervical cancer in their studied patients and Madeleine and colleague in their study in a population-based study reported that the risk of squamous cell carcinomas associated with antibodies to *C. trachomatis* will be increased. Also they reported an association between specific serotypes of *C. trachomatis* and squamous cell carcinomas.

Du and colleagues in a study on about 20,000 cervical cancer deaths, found that there was a high Chlamydia infection rate and association between *C. trachomatis* and the cervical cancer mortality rate (Du et al., 2010). Dalstrom and colleagues reported that previous exposure to *C. trachomatis* had a strongly increased risk for cervical cancer (Dahlstrom et al., 2011). Also, Valadan in a case-control study reported that serum antibodies to *C. trachomatis* were associated with an increased risk for Cervical intraepithelial neoplasia (CIN).

There was also a significant association between presence of inclusion bodies for *C. trachomatis* and CIN. These results indicate a strong association between CIN and Chlamydia cervicitis (Valadan et al., 2010).

Clearly these studies do not confirm our results. The differences between obtained results in these studies may lay back in the selected evaluating assay. While molecular methods evaluate present infection with *C. trachomatis*, seroepidemiological techniques evaluate previous encountering with this micro-organism. Finally, it seems that there is some significant methodological issues, which cloud interpretation of the results in some previous studies regarding the role of *C. trachomatis* in cervical cancer (Miller et al., 2011).

Using Taq Man real-time PCR assay which has high sensitivity and specificity with capacity of determining two hundred copies of viral genome per micro liter of sample provide us a very good reliability for assessing our obtained results.

With respect to the absence of any positive *C. trachomatis* DNA in our patients and also the presence of three positive specimens among our controls specimens, 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 *C. trachomatis* DNA and cervical cancer.

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