Original Article

Prevalence of *Chlamydia trachomatis* and *Mycoplasma genitalium* in Patients with Benign and Malignant Ovarian Cancer by Nested PCR Method

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Received: 10 April, 2015; Accepted: 25 September, 2015

Abstract

Background: *Chlamydia trachomatis* (*C. trachomatis*) and *Mycoplasma genitalium* (*M. genitalium*) are considered factors in cervical and ovarian cancer and are associated with flaky cell carcinoma of the cervix. The role of steady infection, leading to chronic inflammation, in the of ovarian cancer has received very little consideration, although a background of pelvic inflammatory disease (PID) is in a case-control study associate to higher risk for ovarian cancer. *C. trachomatis*, the most common and important cause of PID in the developed world is the genital and cervical infectious agent. The aim of this study was prevalence of *C. trachomatis* and *M. genitalium* in patients with ovarian cancer who referred to Imam Hossein Hospital of Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Materials and Methods: In this descriptive study that was conducted from January 2014 to April 2015, 124 samples were studied which obtained from patients with ovarian cancer who referred to medical centers of Shahid Beheshti University of Medical Sciences. After obtaining samples from ovarian cancer tissue by the pathologist, for extraction DNA, samples were transferred to the laboratory of university. To confirm the presence of *C. trachomatis* in samples of ovarian cancer, specific primers for the Major Outer Membrane Protein (MOMP) genes of *C. trachomais*, were designed and used Nested PCR method for detection of *M. genitalium*. Sequencing was performed on the PCR and Nested PCR product to confirm the presence of *C. trachomatis* and *M. genitalium*.

Results: Out of 124 samples of ovarian cancer, 62 (50%) samples were malignant cancer and 62 (50%) were benign cancer as control group. From 65 malignant samples 14 (22.5%) were *Chlamydia trachomatis* positive. None of the tissue samples of benign cancer of ovary were positive for *C. trachomatis*. Notably, none of the 124 ovarian samples were positive in the *M. genitalium* standard PCR assay.

Conclusion: The results suggest that the spread of *C. trachomatis* in the female with ovarian cancer may be common. This finding reflects a possible role of *C. trachomatis* in the carcinogenesis of ovarian tumors. *C. trachomatis* infection may play a relative role in the pathogenesis of ovarian carcinomas or it could facilitate its progression.

Keywords: *Chlamydia trachomatis*, *Mycoplasma genitalium*, Ovarian Cancer, Nested PCR

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Please cite this article as: Dadashi M, Eslami G, Ghalavand Z, Goudarzi H, Fallah F, Owlia P, et al. Prevalence of *Chlamydia trachomatis* and *Mycoplasma genitalium* in Patients with Benign and Malignant Ovarian Cancer by Nested PCR Method. Novel Biomed. 2016;4(1):18-23.

Introduction

Ovarian cancer is the most common cancer of females and the malignancy with a 15–50% five-year survival rate globally[1]. The pathogenesis of ovarian carcinoma, the most lethal of gynecologic malignancies with 16-51% globally, is to a great extent still unknown. Infections by different microbes causing chronic disease have achieved increasing interest as possible promoters of various carcinomas, and infectious etiologies have been established for some carcinoma[2].

Chlamydia trachomatis (Ctr) is the most important common microbes of Sexual Transmitted Diseases (STD) worldwide, it can be detected from a large portion of women with tubal factor infertility (TFI) and high anti-*Ctr* antibodies can be detected in 70% of women with tubal occlusion[3]. *Ctr* Infection causes in man and woman urethral infections, which at worst can caused to infertility in the female through Fallopian tube injury. *Ctr* can also infect the eye and possible blindness as a result. Chlamydia infections count to the causing (STD) worldwide there are 600,000 reported cases every year. *Ctr* is the most common cause of PID and TFI in the world, and infertility is in itself a proposed risk factor for ovarian carcinoma[4-8]. Due to these associations, *Ctr* has been investigated as a common risk factor for ovarian cancer by studying *Ctr* plasma antibodies with refusing results. In women with ectopic pregnancies, women with TFI, and control women *Ctr* has also been isolate in ovarian tissues. *Ctr* is co-infections of the lower genital tract (LGT) play a significant role in carcinogenesis of the women upper genital tract (UGT), particularly epithelial ovarian carcinomas[9-11].

To illustrate the epidemiological associations of Chlamydial infections ovarian cancer development it has been supposition that the pathogen perhaps triggers epigenetic variation in host DNA repair pathways or that it changes host cell survival pathways by disrupting DNA damage signalling pathways associated with tumorigenesis[12-15]. Endocervical epithelial cells are the primary site of chlamydial infections. It has been hypothesized which the host immune response by *Ctr* infection is responsible for the injury rather than the infection itself[16-20].

Mycoplasma genitalium is other one of the important microorganisms to develop of genital infection that has evolved as an important sexually transmitted infectious agent leading PID, possibly with negative effects on fertility[21,22]. *M. genitalium* was also studied as ovarian carcinoma agent was found. The presence of *Mycoplasma* DNA (polymerase chain reaction [PCR]) has been found in 59% of patients with ovarian cancer.We sought to study and analyze the presence of the microorganisms *Ctr* and *M. genitalium* in ovarian cancer of women with ovarian carcinomas, benign conditions cancer and borderline tumors in patients with ovarian cancer who refferred to Imam Hossein Hospital of Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Methods

Samples collection: This descriptive study was conducted from January 2014 to April 2015. In this survey, 62 ovarian cancer (OCa) and 62 non OCa pathologycal specimens was collected from patients aged between 22-60, referred to Imam Hossein hospital, Tehran, Iran from 2004-2014.

Demographics data including histological type of cancer, pathological stage of cancer, OIN (Ovarian Intraepithelial Neoplasia), were recorded. Then formalin–fixed and paraffin-embedded tissue section of open ovarectomy, core niddle biopsy and TURO (Trans Urethral Resection of the Oval) samples were examined from with OCa and from patients with BOH.A single pathologist experienced in urogenital pathology performed microscopic evaluation of the microscopic slides to determine the cancerous and non-cancerous tissue differentiation. The best paraffin-

embedded block containing cancerous tissue of the patients with ovarian cancer was selected for examination.Samples were transported to department of Microbiology, Medical School, Shahid Beheshti University of Medical Sciences for further analysis.

DNA extraction: DNA was extracted from formalin-fixed and paraffin-embedded tissue blocks by G-spin TM total DNA extraction Kit (iNtRON Biotechnology Co. Korea). First, the paraffin blocks were sliced into thin pieces using a sterile razor bladder and were placed in a 1.5ml tube (not more than 25mg). According to the manufacturer's instruction, xylene was used to removal of paraffin and then the bacterial DNA was extracted from tissue and measuring of concentration their stored at -20°C.

Standard PCR: PCR assay for detection of *Ctr* and *M. genitalium* designed and used specific primers of *Ctr* and *M. genitalium* PCR detection Kit (Pars Tous CO. Iran), respectively. Primers within the *Ctr* PCR mix and *M. genitalium* PCR Mix were specific for conserved 23srRNA and 16srRNA coding region in the *Ctr* and *M. genitalium* genome respectively. The PCR reaction mixture contained 14.6 µl of *M. genitalium* PCR mix, 0.4 µl of HS-Taq DNA polymerase and 5 µl of DNA template was used for adjusting to a final volume of 20 µl. For negative and positive controls and 5 µl of PCR grade water and 5 µl positive controls, respectively. Nested PCR mixture for *Ctr* and *M. genitalium* were cycled under the following thermal conditions (Table 1 and Table 2). The PCR products were analyzed on 2% agarose gel electrophoresis and the gel was stained with ethidium bromide (0.5 µg/ml) and viewed by UV transilluminator. The presence of 250 bp and 255 bp fragments were positive for *Ctr* and *M. genitalium* respectively.

Statistical analysis: Demographics data was analyzed using Statistical Package for Social Sciences (SPSS) software (version 16).

Results

In a total of 124 study patients, there were 62 OCa patients and 62 controls with BOH. Study population charactistics and GS (grade and stage) information are summarized. *Ctr* was detected in 14 (22.5%) samples of 62 OCa patients and there was no *Ctr* in the 62 control with BOH. Notably, none of the 124 ovarian samples were positive in the *M. genitalium* standard PCR assay.

Table 1: Amplification protocol for detection of *Ctr*.

Cycle	Time	Temprature
1	10 Min	94
	30 Sec	94
30	40 Sec	55
	30 Sec	72
1	5Min	72

Table 2: Amplification protocol for detection of *M. genitalium*.

Cycle	Time	Temprature
1	10 Min	94
	30 Sec	94
40	30 Sec	56
	30 Sec	67
1	3 Min	72

The results of PCR: Nested PCR assay for detection of *C. trachomatis* was used. Overall, 22.5% (14%) of ovarian cancer samples for the presence of *C. trachomatis* were positive. So that 22.5% (14 cases) of cancer samples and 0% of BOH (control group) were positive for CT. The results of this study showed that in the experimental group (ovarian cancer) and control group (BOH) there are positive cases of *Chlamydia trachomatis* [P value <0.001, OR=10.07, 95% CI (2.81-36.00)]. Among patients with positive CT Location, clinical symptoms, stage of tumor progression, tumor type, tumor region in ovarian cancer pathological degree, OCP use and age, there was no significant relationship (P>0.05).

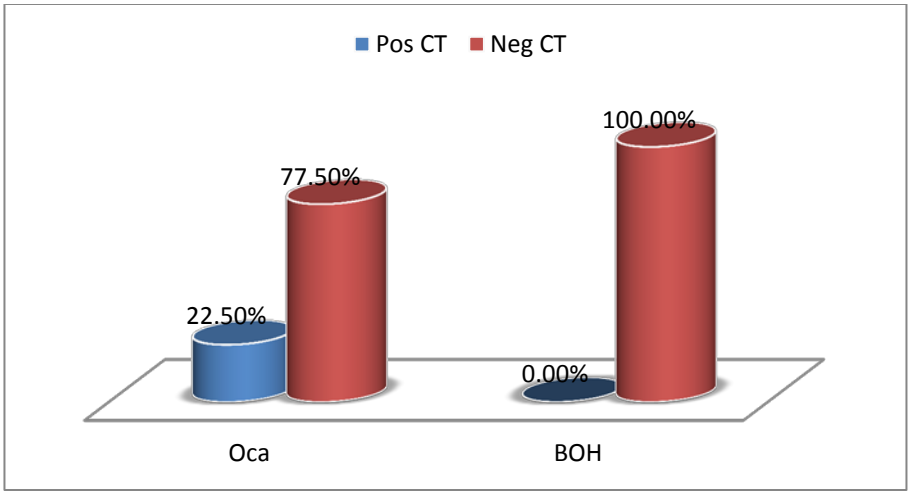

Figure 1. Distribution of Chlamydia trachomatis in paraffin group (ovarian cancer) and controls

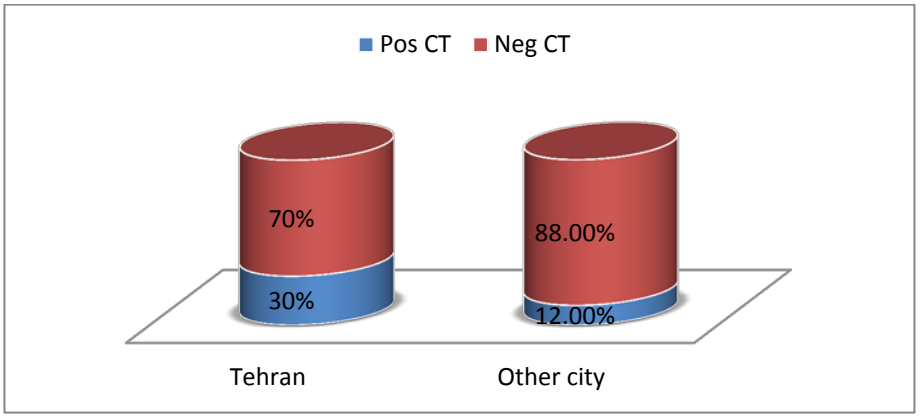

Figure 2. Distribution of Chlamydia trachomatis by Location

Discussion

The results of this study, indicating the rate of *C. trachomatis* in paraffin blocks ovarian cancer, with a prevalence of 22.5% compared with the control group (BOH) with a prevalence of 0%. These showed that the *C. trachomatis* is one of the factors in the development of ovarian cancer [p value <0.001, OR=10.07, 95% CI (2.81-36)]. *Mycoplasma genitalium*, *C. trachomatis* and *papillomavirus* are known worldwide as a risk factor for cancer of the genital area.

Iran has known as one of the countries that have low prevalence of ovarian cancer among world. Recent years, it has reported that the rate of ovarian cancer from 192,000 in 2005 has increased to 20,000 in 2008 has increased[23]. In 2005, 24,498 cases of cancer in the female population reported that in 1923 cases the cancer related to the ovary (Gynecological) so that 793 cases are about ovarian cancer that included 41.2% of all gynecological cancers.

Ovarian cancer ranked first with 41.2% and cancer of endometrium and cervical respectively ranked second and third in gynecological cancers[24]. Ness and his colleagues studied in Pennsylvania showed that a strong association between Chlamydia species and ovarian cancer[25]. Significant increase in the species *C. trachomatis* infection in cancer tissue has been reported in their study.

In this study, increased IgG against heat shock protein of *C. trachomatis* in patients with ovarian cancer suggests that the bacteria associated with ovarian cancer. These results confirmed the findings of the coming study. In another study by Idahl and colleagues in Sweden in 2010, ovarian tissue of 186

cases examined for the presence of *C. trachomatis*, *Neisseria gonorrhoeae* and *Mycoplasma genitalium*. As a result, none of the above organisms were found in the ovarian tissue of patients with ovarian cancer in other words Idahl and colleagues demonstrated that none of the *C. trachomatis*, *Mycoplasma genitalium* and *Neisseria gonorrhoeae* bacteria is considered as a risk factor for ovarian cancer[26], so this finding is contrary to the results of the present study. The reason for this discrepancy in the results of these two studies may be the lack proper isolation and low quality and DNA extraction accuracy of ovarian biopsy samples in Idahl and colleagues research.

Due to the fact that the prevalence of micro-organisms in ovarian tissue is relatively low if the extraction and isolation of bacterial DNA not be accurate and good quality increased the probability of bad isolation and therefore a negative test result for the presence of studied bacteria. The average age of patients with ovarian cancer in the study of Idahl and colleagues was 58.9 and by analogy with present study 9.7 years increase in age of patients seen. In other words, the average age of patients with ovarian cancer examined in our study was 49.2 registered. This may indicate that ovarian cancer in our study was 9.7 years earlier than studied population in Sweden. In studies conducted in 1993.1995 and 2005, *C. trachomatis* was identified as a common cause of having the PID[27-29]. In another study conducted by Haggerty and colleagues in 2008, *C. trachomatis* was isolated from ovarian tissue of patients with ectopic pregnancies. The results of this study showed that *C. trachomatis* is associated with malignant and genital area disorders[30]. Another study conducted in 2008 by Carvalho JP *et al.* The results of this study suggest that *C. trachomatis* and *Mycoplasma genitalium* are the most important risk factor for chronic inflammation and ultimately Aytla ovarian cancer[31].

Conclusion

Due to the global prevalence of genitourinary infections and anatomic location of the ovary, this matter that infectious agents may play a role as a risk factor for ovarian cancer, it is not surprising. The results of this study support the hypothesis of relation between sexually transmitted infections and ovarian cancer and it can be concluded that the inflammatory effects caused by this organism, together with other risk factors, can be effective in ovarian cancer. Therefore, early diagnosis and early treatment of *C. trachomatis* infections can be used as a common method of prevention and treatment of ovarian cancer.

Acknowledgement

Hereby appreciated from assistance of Shahid Beheshti University of Medical Sciences Cancer Research Center to achieve to this study.

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