



Molecular Identification of Human Papiloma Virus (HPV) in Cervical Neoplasia and the Risk Factors Affecting Cervical Cancer in Isfahan

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ABSTRACT

The rate of cervical cancer in Asians is deferent, based on the risk factors affecting and the rate of papiloma virus infection among females. Cervical cancer is one of the important cancers between Iranian women. In the present study the rate of cervical neoplasia was detected in Isfahan and some risk factors affecting the incidence of cervical cancer was analyzed. The human papiloma virus infection was also detected in cervical neoplasia via PCR method after DNA extraction. 15000 pap smear tests results achieved from patient documents for detection of the rate of squamous cell neoplasia, based on at least one report in pap smear test in a random and distributed sampling. We have also studied the effect of some risk factors on the incidence of cervical cancer in 100 patient and 100 females without any neoplasia report as a control group. The results analyzed by Chi2 and T tests. The rate of human papiloma virus (HPV) infection detected in pap smear specimens using specific primers for amplification of HPV genome. At least one neoplastic report was shown in 0.91% of females and 0.61% of which had at least one ascus report. 55.4% of patients with neoplastic pap tests were infected with HPV in PCR test. Teenage marriage, high parities, lower education and smoking increases the risk of cervical neoplasia. It is concluded that molecular methods including genome analysis are useful method in identification of HPV.

1. Introduction

Cervical cancer is a disease which results from malignant growth of squamous cells in the cervix (Cuzick et al., 1998). Cervical cancer is the second cancer incident in women in the world. Screening of squamous cells via pap smear test in continuous periods is the best way for detection of this type of cancer (Guyton et al., 2000).

If the transformation of cells diagnosed on time, the disease will be controlled controlled in 90% of incidences (Guyton et al., 2000). The present study performed in two steps: one was the detection of incidence of cervical cancer in pap smear test and polymerase chain reaction (PCR) amplification. The second was the study about the effect of some risk factors in the cervical cancer incidence.

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2. Materials and Methods

2.1. Specimens

Pap smear tests in 15000 women achieved from patient documents for cervical cancer neoplasia in Isfahan and 156 of positive specimens randomly analyzed for papiloma virus infection by PCR method.

2.2. DNA extraction and PCR reactions

DNA extracted by phenol and chloroform and PCR performed using general primers: 5'-GAA AAA TAA ACT GTA AAT GA-3', 5'-TTT GTT ACT GTG GTA GAG ATA C-3' PCR reaction mix for 100 nano grams DNA contained: Primer 200 pM, MgCl₂ 1.5 mM, Taq DNA polymerase 1 unit, dNTP 200mM. PCR thermal cycle contained: 95°C 1 min, 3 cycles; 95°C 1 min, 56°C 2 min, 72°C 1.5 min, 40 cycles.

2.3. Detection of risk factors

A questionnaire was given to 100 patients with cervical cancer and a questionnaire of 100 women without any cervical neoplasia, then the data compared for the effect of risk factors in cervical neoplasia.

3. Results

3.1. The incidence of cervical cancer

137 out of 15000 women had at least one positive cervical neoplasia report in pap test. The data shown in table 1 revealed that 55.4% of pap smear positive women showed PCR positive results for HPV infection.

3.2. The effect of risk factors

There were significant associations between the teenage marriage, high parities and the low levels of education with cervical cancer involvement. Also the employed persons had a 67.5% lower risk for cervical cancer. Smoking increased the risk of the disease up to 10 percent. Normal delivery increased the risk of cervical cancer by 86% compared to cesarean.

0.91% of females had at least one report for cervical neoplasia in Iran. Teenage marriage, high parities, lower education and smoking were

increased the risk for cervical neoplasia involvement. Other results are shown in table 2.

4. Discussion

In the present study, about 0.91% of women in Isfahan had at least one cervical neoplasia report and 0.61% of them had at least one ascus report in the pap test. The PCR test revealed that in 55.4% of patients with a positive pap test, the human papiloma virus genome is present. Other studies have shown different prevalence in different areas. For example in United States it increases up to 5% in some areas. The prevalence of cervical cancer in different parts of world is 5 to 40 cases in every 100000 women, but it is important to know that all positive pop smear tests don't lead to cancer (Pits and Clarke, 2002; Sirovich et al., 2004). The prevalence of cervical cancer is 20 times lower than the prevalence of abnormal cells in pap smear test (Beral et al., 1994; Bares et al., 2008). This finding doesn't affect the importance of pap smear test, because this test is the first and most important step for diagnosis of cervical neoplasia (Beral et al., 1994; Quinn et al., 1999; Hidalgo et al., 2003; Perovic et al., 2009).

In the present study, significant associations detected between the teenage marriage, high parities and the low levels of education with cervical cancer involvement. Also the employed persons had a 67.5% lower risk for cervical cancer. Notably, smoking increased the risk of the disease up to 10 percent. Normal delivery increased the risk of cervical cancer by 86% compared to cesarean. In the other studies, low educational level, teenage marriage, human papilomavirus infection and the factors which increase the rate of this infection (such as smoking and high parities) have been reported as risk factors increasing cervical cancer incidence (Cain et al., 2000; Eileen, 2003; Pett et al., 2006; Perovic et al., 2009). Ibrahim et al., 2011 reported that women with uterine cervix laceration, assisted vaginal delivery, female genital mutilation or episiotomy showed an increased the risk of cervical cancer (Ibrahim et al., 2011). It is concluded that molecular methods including genome analysis are useful method in identification of HPV.

Table 1. Data extracted from the cases analysis

Total number of pap test results	Total number of patients with positive pap test	Patients with PCR positive test
15000	137	76

Table 2. Mean and standard deviation of some agents in control and case groups

Agent	Healthy group (control)	Patients (case)	P value
Age	36.03±8.57	52.35±14.18	0.0001
Weight	67.29±9.04	64.26±2.12	0.23
Marriage age	20.17±3.94	11.79±6.22	0.0001
Parity	2.17±1.17	4.06±2.85	0.0001

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