Iranian Journal of Pathology (2008)3 (4), 183- 185

Original Article

Prevalence of Human Papilloma Virus among Women with Cervical Intraepithelial Neoplasia III and Invasive Cervical Cancer from 2001 to 2006 in Bandarabas

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ABSTRACT

Background and Objective: **To estimate the risk of human papilloma virus (HPV) infection for cervical malignancies, we conducted a case-control study in southern Iran (Hormozgan province).**

Materials and Methods: **For this purpose, 52 paraffin embedded blocks with exact diagnosis of cervical carcinoma(50 carcinomas and 2 carcinomas in situ) from 2001 to 2006 and 52 praffin embedded blocks of cervical tissue specimens with normal histopathology as the control group were tested for the presence of HPV DNA using PCR based assay.**

Results: **HPV DNA was found out in 16 out of 52 patients (30.7%), while it was not detected in any of the control group samples.**

Conclusion: **Considering the fact that unrestrained sexual behavior increases risk of becoming infected with HPV, our finding is in favor of the concept of low frequency of HPV infection and thus its less important role in women with cervical cancer in islamic countries.**

Key words: Cervix Cancer, Papilloma Virus DNA Probes, Polymerase Chain Reaction

Introduction

Cervical cancer is one of the most prevalent forms of carcinoma among women worldwide and accounts for about 12% of all cancer cases among women and having an incidence of more than 400/ 000 cases per year (1). Some studies have shown that cervical cancer is the fifth most frequently seen malignant neoplasm and the second most common cancer in women worldwide (2). The association between certain human papilloma viruses (HPVs) and cervical cancer is well documented and research since past 2 decades has revealed that HPVs are etiologically related to the development of most cases of cervical cancer (3;4). There is no exact statistical data on cervical cancer prevalence among Iranian women, however cervical cancer like other types of genital cancer is more prevalent in the northern parts of Iran including Mazandaran province (5;6), but generally the association between HPVs and cervical cancer has not been studied among Iranian women.

The main goal of our case-control study was PCR

Received: 29 May 2008
Accepted: 13 Augest 2008
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based assay of the association of HPV DNA with cervical cancer in patients admitted to Shariati hospital (Bandarabas) in Hormozgan province (southern Iran) from 2001 to 2006.

Materials and Methods

Specimens

We extracted paraffin embedded blocks of cervical tissue specimens with histopathologic diagnosis of cervical carcinoma from 2001 to 2006 from archive of the pathology department of Bandarabas Shariati hospital. After revision of corresponding slides and assurance of consistency, 2 cases were excluded and finally 52 paraffin embedded blocks with exact diagnosis of cervical carcinoma (50 carcinomas and 2 carcinomas in situ) were included in our study. In parallel, we selected 52 blocks of paraffin embedded cervical tissue specimens with normal histopathology as the control group. We tried to age match the control group with the study group.

DNA extraction

Sections at a thickness of 5-10 micrometer were prepared from each specimen, avoiding any cross contamination between samples (using separate disposable items such as gloves, blades and tubes). Sections were subsequently deparaffinized by xylene and digested using digestion buffer containing proteinase K, followed by extensive extraction with phenol/chloroform (7). The extracted DNA was stored at 4 °C until being tested.

PCR

DNA quality was evaluated by PCR using primers G73/G74 that amplify a 268 bp product from the human b-globin gene. Then, b-globin positive samples were subjected to HPV PCR by GP5+/GP6+ primers for L1 open reading frame (ORF) that amplifies a 150 bp product from the HPV L1 ORF (8).

-G 73: 5'-GAAGAGCCAAGGACAGGTAC
-G 74: 5'-CAACTTCATCCACGTTCACC
-GP5+: 5'-TTGTTACTGTGGTAGATACTAC
-GP6+: 5'-GAAAAATAAACTGTAAATCATATTC

PCR was performed according to the procedure described by Yi Ting et al (8). Samples were subsequently subjected to agarose gel electrophoresis (2% agarose) and stained with ethidium bromide.

Results

HPV DNA was found out in 16 out of 52 cases (30.7%), while HPV DNA was not found in any of the control group (Table 1). Out of 52 cases of cervical carcinoma, 38 cases (73%) were squamous cell carcinomas, 4 cases (7.6%) were adenocarcinomas, 6 cases (11.5%) were poorly differentiated carcinomas and 4 cases (7.6%) were carcinoma in situ (CIN III). At the same time, 10 out of 38 (26.3%) squamous cell carcinomas, 2 out of 4 (50%) adenocarcinomas, 2 out of 6 (33.3%) poorly differentiated carcinomas and 2 out of 4 (50%) carcinoma in situ were positive for HPV DNA (Table 2).

Table 1: PCR results in case and control groups

Group	Positive (%)	Negative (%)	Total
Cases	16 (30.7)	36 (69.2)	52
Controls	0	52 (100)	52

Table 2: PCR results in various carcinoma types

Various carcinoma type	Positive (%)	Negative (%)	Total
SCC	10 (26.3)	28 (73.6)	38
Adenocarcinoma	2 (50)	2 (50)	4
Poorly differentiated carcinoma	2 (33.3)	4 (66.6)	6
Carcinoma in situ	2 (50)	2 (50)	4

Discussion

Many epidemiological studies have shown that human cancer is a multifactorial disease which can develop through different molecular biologic pathways. At present there is compelling evidence that the development of human cervical cancer without the involvement of specific types of human papilloma viruses is exceptional or impossible (9).

The international biological study on cervical cancer demonstrates that 92.9% of cervical cancers from 22 countries contained HPV DNA ranging from 75 to 100% (4). In our study, we found that the frequency of HPV DNA in our cervical carcinoma patients was 30.7%. This figure is much lower than what was reported from Brazil (76%) (10), Mozambique (92%) (11), and several other countries

in Latin America(12;13) and also lower than what was reported in the international biological study on cervical cancer from 22 countries (75 to 100 %).

Since some studies have reported some difficulties in reproducing PCR results with formalin fixed paraffin embedded tumor tissues, therefore, it is possible that if fresh frozen tumor tissues had been examined, HPV positive cases might have been higher because amplification step could be hampered by formalin (10).

On the other hand, our finding is much closer to the study performed in an Islamic country like Malaysia in which 45.1% of specimens were positive for HPV and the conclusion was that maybe HPV is not a crucial risk factor in the cases of cervical cancer there (14).

Considering the fact that unrestrained sexual behavior and having unprotected sex with multiple sexual partners increases risk of becoming infected with HPV (15;16), in Islamic countries due to common presence of strict religious laws and beliefs among women in whom free and extramarital sexual activities are severely prohibited, these risk factors are expected to be greatly reduced or minimal. In our study, none of our cases had previous history of having multiple sexual partners and rather all were married women having only one sexual partner.

Conclusion

Thus, our finding can be in favor of the concept of less important role and low frequency of HPV infection in women with cervical cancer in Islamic countries. However, we believe that before generalizing this theory, a larger number of patients from other Islamic countries be examined.

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