Iranian Journal of Clinical Infectious Diseases
2008;3(1):19-23

ORIGINAL ARTICLE

Study of human papilloma virus in anogenital condylomas by PCR method

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ABSTRACT

Background: Anogenital condyloma is a relatively prevalent disease diagnosed in about 1.3 million Americans, annually. This disease is seen in 1% of sexually active adults. Aimed to evaluate anogenital condylomas with respect to human papilloma virus by PCR Method, present study was conducted in Skin Research Center, Shaheed Beheshti University of Medical Sciences.

Materials and methods: This cross-sectional study was conducted on 32 patients suffering from anogenital condyloma confirmed by pathological examination. Biopsy samples obtained from anogenital lesions of this disease were examined by PCR Method for existence of human papilloma virus as well as determination of virus type.

Results: In 12 patients (37%), human papilloma virus was detected by PCR Method. In addition, there was no difference in distribution of patients with respect to affliction with human papilloma virus between high-risk and low-risk groups. There was no relationship between clinical manifestation and type of papilloma virus.

Conclusion: This study is recommended to be repeated with more samples under more controlled condition.

Keywords: *Anogenital condyloma, Human papilloma virus, Polymerase chain reaction.*
(Iranian Journal of Clinical Infectious Diseases 2008;3(1):19-23).

INTRODUCTION

Wart is a benign proliferation of cutaneous cells and mucous, which appears as a result of infection with human papilloma virus. This virus grows slowly and can remain subclinically for long periods of time. Anogenital wart (Condyloma) is a sexually transmitted disease affecting both genders, which has become a serious clinical problem particularly in women (1). Condyloma is a relatively prevalent disease diagnosed in about 1.3 million Americans, annually (2). This disease is seen in 1% of sexually active adults and in 2% of cases, genital condyloma is observable using acetic acid. In 20 to 50% of population, DNA of human papilloma virus is detectable in genital tract (3).

More than 70 types of human papilloma virus are identified and among the most important types generating anogenital wart in both genders, types 6, 11, 16, 18, 31 and 33 can be named. Types 6 and 11 induce low risk warts with respect to malignancy. In contrast, types 16, 18, 31 and 33 have potential ability to become malignant and are classified in high risk category (4, 5).

The most prevalent human papilloma virus types involved in appearance of condyloma are types 6 and 11, but infection with types 18 and 16 is expected (6, 7).

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Infection with human papilloma virus is not limited to anogenital area and can extend to upper genital tract in women. Also, perianal lesions can involve anal canal in both genders. The disease induced by high risk types (16, 18, 31, 33) increases likelihood of malignancy in upper genital area (cervix) (1).

Infection of cervix with human papilloma is associated with premalignant and malignant lesions in this area (1). Using PCR, virus DNA has been detected in cervical carcinoma as well as in vulvar carcinoma, with type 16 being the most important one in cervical carcinoma and types 6 and 11 being main ones in cervical and vulvar carcinomas (8).

Using PCR, total prevalence of human papilloma virus DNA in penile cancer of men is the same as vulvar cancer and is reported to be 40% (9).

At present, due to low incidence of warts induced by potentially malignant (high risk) types of human papilloma virus, it is not recommended to follow up the patients for malignant disease (cervical carcinoma and penile cancer), but given the seriousness of the mentioned malignancies, it seems reasonable to follow up the patients due to the above-mentioned reason or at least in case of encountering high rate of anogenital condyloma induced by high risk types, such an action (following up the patients) may be deemed necessary.

In this study, all patients suffering from anogenital condyloma who referred to the clinics affiliated to Skin Research Center of Shaheed Beheshti University of Medical Sciences were studied for existence of human papilloma viruses of type 6, 11, 16 and 18.

PATIENTS and METHODS

In this study, which was conducted on a cross-sectional basis, all patients referred to the clinics of Skin Research Center of Shaheed Beheshti University of Medical Sciences, who were clinically suspicious to anogenital condyloma and declared their readiness to be included in the study, were studied. After clinical examination and registering related data, biopsy sample was prepared from the lesion suspicious of wart, and part of the sample was sent to Loghman Hospital Laboratory for pathological examination.

In those cases in which diagnosis of wart has been confirmed in the above sample, other part of the sample was sent to Cellular Molecular Biology Research Center of Shaheed Beheshti University of Medical Sciences for determining existence of human papilloma virus and its type using PCR. Biopsy samples were stored in the lab in the media containing formalin and normal saline, respectively. PCR was performed as follows:

1- DNA Extraction: Each sample was divided into some parts, washed by special buffer, boiled with the help of 5 mM $MgCl_2$, 1% SDS, 0.32 M Sucrose and 10 mM Tris, and then DNA was released. Then, protein components were isolated from DNA by phenol- chloroform and released DNA was concentrated by alcohol and then precipitated.

2- Design of Primers: L_2 gene of HPV (of various types) was searched from gene bank and designed and synthesized by DNA sis Software for identification of primer virus.

3- PCR: Appropriate quantities of DNA (the sample suspicious of virus), dNTP, special buffer for polymerase enzyme, magnesium chloride, Tag DNA Polymerase Enzyme, both reverse and forward primers were mixed inside PCR special tube under desirable conditions and placed in normal cycler, so that PCR reaction is initiated according to the following schedule:

Predenaturation	50 minutes
Denaturation 94 C	30 seconds
Annealing 50-60 C	30 seconds
Extension 72 C	30 seconds
Post extension 72 C	5 minutes

4- Electrophoresis of PCR Product: PCR product underwent electrophoresis on Agarose Gel and stained by Etidium Bromide, so that DNA bands

can be reproduced and visible with the help of UV transilluminator. It is noteworthy that some pictures have been prepared from the above gels.

After they have been gathered, data were analyzed using chi-square test and adopting significance border on $p< 0.05$.

Human papilloma virus (types 6,11,31,33) primers are as followes:

HP 6133F 5'- TGG ATT ATA AAC AAA CAC A-3'

HP 6133 R 5'- GTG GTA TCT ACC ACA GTA ACA3'

Human papilloma virus (types 16,18,35) are as follow:

HP 168 F5'- GAA TAT GAT TTR CAG TTT ATT TT3'

HP 168 R 5'- TCT YKA GAA AAC TTT TCC TTT-3'

RESULTS

In this study, 32 patients (7 male and 15 male) suspicious of affliction with anogenital condyloma have been studied. Mean age of these patients was 29.5±8.1 years (min. 16 and max. 46). Five patients were single, 25 were married and 2 were divorced. Mean duration of disease was 6.1± 6 months (at least 1 and at most 24 months).

In 13 patients, the same lesion was found in sexual partner. Eleven patients have been previously treated by cryotherapy and 2 by Podophiline for the lesion under study.

Genital area in 25 patients and perinanal area in 4 patients were involved. In 3 patients, both areas were involved. Extent of involvement was mild (below 10 lesions) in 11 patients, moderate (10 to 20 lesions) in 15 patients and severe (more than 20 lesions) in 3 patients. Clinically, it was diagnosed that 20 patients were infected with condyloma acuminatum, and 12 with flat condyloma. No patient was infected with bowenoid papulosis. No patient had a history of taking immunosuppressors during last 6 months. In all cases, pathological findings correlated to koilocytes with mild atypical changes and clinical diagnosis for affliction with verrucose lesion was confirmed by pathological findings.

In 12 patients (37%), PCR findings were indicative of human papilloma virus in lesions. In skin lesions, papilloma virus types 6 and 11 were present in 4 patients and types 16 and 18 were present simultaneously in 3 patients. Papilloma virus type 11 was identified in 1 patient and type 18 in 4 patients.

In table 1, distribution of papilloma virus positive patients has been shown by clinical type of wart and type of virus obtained. By chi-square test, it was revealed that there was no relationship between clinical manifestations and type of papilloma virus.

Table 1. Distribution of patients infected with anogenital wart by clinical type of wart and type of human papilloma virus

Clinical manifestation of wart	Type of virus				Total
	6	11	16	18	
Acuminatum	3	3	1	4	11
Flat	1	2	2	3	8

With respect to involvement area, genital area was involved with types 6 and 11 (low risk viruses) in 7 cases and with types 16 or 18 (high risk) in 8 cases. In anal area, involvement with types 16 or 11 and 16 or 18 was observed in 2 cases for both cases ($p>0.05$).

DISCUSSION

In present study, 32 patients suffering from anogenital wart were studied. Mean age of these patients was 29.5 years. Age distribution of patients correlates with age of maximum sexual activity, which is indicative of role of virus transmission through sexual relationship that is the major way of disease transmission (2). However, 5 patients were single and as they alleged, they had no history of sexual relationship. It suggests that non-sexual transmission should be considered in some patients, as mentioned in other references (10, 3).

In 13 patients, there was a similar lesion in their sexual partner. Given high rate of infection with

anogenital wart through sexual relationship, therefore, it seems reasonable to recommend patients not to have sexual relationship until improvement of the lesions or for 6 to 12 months after infection or use condoms (2).

Anatomically, the most common affected area is genital area (in 28 patients). Clinically, it was diagnosed that 20 patients were infected with condyloma acuminatum, and 12 with flat condyloma. In many previous studies, genital area was the most common area, and clinically, condyloma acuminatum was more prevalent (11, 9).

Of 20 patients infected with condyloma acuminatum, 19 patients had genital involvement and in more patients, number of lesions was varied. In a study by Skerlev in 2002, most cases of condyloma acuminatum were reported to be on mucogenital surfaces and the lesions were mainly multifocal and their number varied between 5 and 15 (9).

In all biopsy samples, pathological findings were indicative of koilocytes with mild atypical changes. In fact, pathological manifestation of various lesions was the same in clinical examination. This result has been obtained from previous studies (12, 1). On this basis, light microscope seems to have limited role in classification of the lesions.

In 12 patients (37%), virus DNA was detected in the lesions by PCR Method. In another study on 171 male patients with genital wart, PCR and RFLP (Restriction Fragment Length Polymorphism) were used for identification of human papilloma viruses of types 6, 11, 16 and 18. 80% of the samples were positive for existence of human papilloma virus.

In a study conducted by Syrjanen et al. in 1987, 44.4% of anogenital wart samples contained human papilloma virus. In addition, in a study by Handley in 1992, 53.3% of the samples were positive for existence of human papilloma virus (14), the reason for this small figure was reported to be relatively low sensitivity of in situ hybridization (the method used in their study). Using very sensitive and molecular-specific PCR method, our study may determine this low percentage in lab error during work in lab.

Types 6 or 11 (low risk viruses with respect to malignancy) were identified in 5 patients (41.6%), and types 16 or 18 (low risk viruses with respect to malignancy) were identified in 7 patients (58.3%).

In previous studies, in most anogenital wart samples, low risk viruses have been reported with very much percentage than types 16 and 18 (14-16, 8, 1). Furthermore, in a study conducted by Skerlev et al. in Zagreb in 2002 (12), virus types 6 or 11 were identified in 79% of the samples and types 16 or 18 in 21%. In a study by Grce, 80% of identified viruses were of low risk type. Human papilloma virus type 16 was found in 4.6% of the samples, and type 18 in only 1.7% (14).

According to the findings of this study, which are in obvious contradiction with other researches, it seems necessary to repeat the research with more samples under more controlled condition.

ACKNOWLEDGEMENTS

Efforts made by Dr. Bahram Kazemi and esteemed personnel of Cellular Molecular Biology Research Center of Shaheed Beheshti University of Medical Sciences for PCR tests are appreciated.

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