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Original article

Prevalence of adeno-associated virus and human papillomavirus DNA in Iranian women with and without cervical cancer

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ABSTRACT

There is plenty of substantial evidence to support anti-tumor activity of viruses. Adeno-associated virus (AAV) may interact with human papillomavirus (HPV) to modify the risk of cervical neoplasia. The seroprevalence of AAV among women with cervical cancer has been reported to be lower than healthy ones. In spite of this finding, detection of AAV DNA in cervical biopsies does not entirely support the inverse association between AAV seropositivity and cervical cancer. This association is still controversial and requires more thorough evaluation in different countries. The aim of this case–control study was to find the prevalence of AAV and HPV DNA sequences in Iranian women with and without cervical cancer to assess the probable association of AAV infection and cervical cancer. In this study, paraffin-embedded tissue samples of 61 cervical cancer cases and 50 healthy controls (HCs) were investigated for AAV and HPV DNA by semi-nested and nested PCRs respectively. AAV DNA was detected in 7 cases (14%) of HCs and 9 specimens (14.8%) of case group. According to the branching in the phylogenetic tree, AAV2 was the only type detected in this study. Moreover, HPV DNA was detected in 8 cases (16%) of HCs and 44 specimens (72.13%) of case group. In conclusion, a low proportion of cervical biopsies from Iranian women contained AAV-2 genome. No significant difference in correlation between HPV and cervical cancer in presence or absence of AAV genome in cervix was found.

1. Introduction

Cancer is one of the most important causes of death worldwide then understanding the etiology, pathogenesis and treatment of cancer is a major challenge in medicine. Different environmental and genetic factors increase the risk of neoplasm development [1]. Among environmental factors, infections are associated with almost 17.8% of all cancers [2]. Overall around 15% of all human malignancies can be attributed to viral infections [3]. In developing countries, this percentage is even higher because certain infections are more common in these countries [4]. In this issue viral infections have two divergent faces, some viruses can be oncogenic, however some have anti-tumor activity which specifically kill tumor cells but leave the normal ones undamaged [5]. Adeno-associated viruses (AAVs) are ubiquitous human helper-dependent parvoviruses with some evidence of antitumor activity particularly in cervical cancer [6]. This malignancy ranks as the third cause of death in the gynecologic cancers and 14th among all cancers affecting women in the United States (American cancer society). Moreover, human papillomavirus (HPV) infection has been recognized as the main cause of cervical cancer [4]. AAV may interact with HPV to modify the risk of cervical neoplasia [6].

AAVs are members of genus Dependovirus from the *Parvoviridae* family, which require a helper virus to facilitate their replication. In the absence of a helper virus within the cell, AAVs establish a latent infection [7]. It is well-known that the primary AAV infection commonly occurs in childhood [7]. In human, AAV infection has not been associated with any disease regardless of its ability to integrate into genome of the cell. Serological studies in human suggest that AAV infection is prevalent [8].

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Table 1
AAV and HPV DNA presence in biopsies of Iranian women with and without cervical cancer.

DNA detection results		Type of samples	
		Healthy Control biopsies	Cervical cancer biopsies
AAV +	HPV +	0	7
	HPV –	7	2
	Total NO.	7	9
AAV –	HPV +	8	37
	HPV –	35	15
	Total NO.	43	52
Mantel-Haenszel Test		P-value >0.05	

The seroprevalence of AAV among women with cervical cancer has been reported to be lower than healthy women [4]. In spite of this finding, detection of AAV DNA in cervical biopsies does not entirely support the inverse association between AAV seropositivity and cervical cancer [9].

This association is still controversial and needs to be evaluated by further epidemiological studies in different countries.

The aim of this case–control study was to estimate the prevalence of AAV and HPV DNA sequences in Iranian women with and without cervical cancer to assess the probable association of AAV infection and invasive cervical cancer.

2. Materials and methods

Paraffin-embedded tissue samples of 61 cervical cancer cases and 50 healthy controls (HCs) were provided from archives of Pathology Department, Imam Khomeini hospital, Tehran, Iran. All histological slides from cervical biopsies were retrieved from patients' medical records. All of histological slides from cervical biopsies were re-evaluated before laboratory analysis and the samples with no pathological changes were classified as HCs and the samples with pathological changes related to cervical cancer as case group, were included in this study.

Avoiding any cross-contamination between samples, 5–10 μm thick sections were prepared from each specimen. The sections were deparaffinized with xylene then digested with digestion buffer containing proteinase K. DNA was extracted with phenol/chloroform method and its quality was evaluated by PCR using specific primers for human β –globin gene [10]. All β -globin-positive samples were subjected to both nested PCR for HPV using the consensus MY09/MY11 primer pair (outer primers) and the GP5+/GP6+ primer pair (inner primers), targeting about 150 bp fragment of HPV L1 gene [11] and semi-nested PCR for AAV (Rep gene) detection. AAV semi-nested PCR was carried out using following forward and reverse primers (designed in this study): In the first round: AAV F1: 5′- ACC AAC ATC GCG GAR GCC AT-3′, AAV Ra: 5′- GGC TGC TGG TGT TCR AAG GT-3′ and AAV Rb: 5′- GGC TGC TGA TGC TCG AAG GT-3′ and in the second round AAV F2: 5′- AAC TGG ACC AAT GAR AAC TTT CCC T-3′ with the same reverse primers were used. As the positive control, the *in vitro* synthesized PUC57 plasmid enclosing desired fragment was used for AAV and a positive cervical sample was used for HPV.

The resulting amplicons of the second round of PCR for AAV amplification were sequenced at the National Influenza Center, School of Public Health, Tehran University of Medical Sciences.

BioEdit version 7.0.0 DNA analysis software [12] was used to align the sequences. To identify AAV types phylogenetic tree was constructed by TREECON package version 1.3 b [13], applying Kimura's two-parameter method and the neighbor-joining method with 1000 replicates bootstrap analysis.

Statistical analysis was performed using IBM® SPSS® Statistics V22.0. The Chi square and Mantel-Haenszel tests were used to measure the differences. P values <0.05 were considered statistically significant.

3. Results

In this study a total of 111 paraffin-embedded cervical biopsies from Iranian women diagnosed with and without cervical cancer (case and control) were analyzed for presence of AAV and HPV DNAs.

Age distribution in both case and control groups showed, the most samples belonged to women aged from 41 to 60. The mean age in case and control groups was 49.47 ± 10.33 years (range: 26–80 years) and 48.32 ± 10.62 years (range: 26–77 years) respectively. No significant difference in age was found between case and control groups in this study (P value = 0.695).

AAV DNA was positive in 14% (7/50) of HC group and in 14.7% (9/61) of case group (p = 0.91). Moreover, HPV DNA was positive in 16% (8/50) of HC group and in 72.13% (44/61) of case group. In HC group 87.5% (7/8) of detected HPVs were type 16 and one was type 33. In case group type 16, 18 and 31 were detected with 75% (33/44), 4.6% (2/44) and 2.2% (1/44) frequencies. Simultaneous infections with two or three types were found in 18.2% (8/44) of case group.

No significant difference in correlation between HPV and cervical cancer in presence or absence of AAV genome in cervix was shown by Mantel-haenszel test (Table 1). All 16 AAV positive samples were subjected to sequencing. According to the branching in the phylogenetic tree, AAV2 was the only type detected in this study (Fig. 1) (GenBank Accession Numbers: KT906216-KT906231)

4. Discussion

Serological documents suggested that AAV infections have been prevalent in human [8] with seroprevalence from 30% to 60% [14]. However 11 different types had been described for AAV, only AAV1–5 and AAV7–9 can be classified as true serotypes and AAV6, 10, and 11 are just different variants. Among these, AAV2, 3 and 5 are the most prevalent serotypes, respectively [7,15].

Several epidemiological and experimental studies suggested that AAV might have anti-oncogenic activity and it was recognized as an oncolytic agent. It might interact with HPV and modify the risk of cervical cancer [6]. The reverse association between AAV seropositivity and cervical neoplasia has been stated in different studies [4,15,16], moreover in vitro researches have revealed that Rep78 gene of AAV can interfere with HPV-induced cell transformation [17]. Nevertheless, DNA-based analyses on cervical specimens regarding AAV role in cervical cancer, showed inconsistent results [9].

The present study is the first to assess the prevalence of AAV and HPV DNA simultaneously in Iranian women with and without cervical cancer. Here no reduction in cervical cancer risk in the presence of AAV DNA was found.