Screening for adeno-associated viruses and human papillomaviruses in greek women with no cervical lesions

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ABSTRACT: In order to investigate the correlation between human papillomaviruses (HPV), causative agents of cervical cancer, and adeno-associated viruses (AAV), possible protective factor from this disease, we evaluated first the prevalence of cervical infection by these two viruses in asymptomatic Greek females (i.e. with normal cervices and no pathologic history). Our data indicates relatively low prevalence for both viruses (8.8% for HPV and 17.7% for AAV), compared to studies from other countries. This report is the first concerning prevalence of cervical AAV infection in Greece.

Key Words: HPV, AAV, Epidemiology, Cervix.

INTRODUCTION

Cervical cancer is the second leading cause of cancer deaths in women worldwide and it is of general acceptance that human papillomavirus (HPV) is the main causative factor. Adeno-associated viruses (AAV) of the parvoviridae family, which are also sexually transmitted viruses, in contrast, may play a protective role in HPV-mediated cervical carcinogenesis¹. Geographical differences of AAV and HPV prevalences of cervical infections have been reported²-⁴.

In order to investigate the possible interaction between these two viruses in Greece, we analyzed in a first step the prevalence of cervical infection with HPV and AAV in a cohort of asymptomatic («normal» cervix and no pathological history) women attending the gynecological outpatient clinic of a University Hospital in Northern Greece. This screening is the prerequisite for the assessment of the interaction of HPV and AAV in the development of cervical diseases.

MATERIALS AND METHODS

Cervical cellular material was collected from women, who underwent routine gynaecologic examination and Papanicolaou testing. In the present investigation 259 women (mean age 36.2 years, range 19-65 years) presenting with no pathological («normal») cytologic and colposcopic findings were included. From standard cervical smears collected with cytobrush, the remaining cells were placed in tubes with 0.9% saline and stored at -20°C. After being thawed, the cells were washed twice with PBS and DNA was extracted with standard methods. HPV DNA detection was based on PCR amplification of the L1 region using the MY 09/ MY11 primers (MY 09: 5’-CGT CCM ARR GGA WAC TGA TC- 3’, MY 11: 5’-GCM CAG GGW CAT AAY AAT GG - 3’). Us-

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ing RFLP-PCR the products were typed according to their fragmentation prototypes as high-risk types (hr: 16,18,31,33,35, 39,45,51,52,56,58) or low-risk types (lr: 5,6,8,11,30,32,34,40,42,47,53,57,66). AAV DNA detection was performed using primers Rep 78.1/ Pan3 for the first PCR and Rep 78.2 /Pan1 for the nested one ( REP 78.1 : 5’-CAT CGC GGA GGC CAT AGC CC-3’, REP 78.2 : 5’-ACG GGA GTC GGG TCT ATC TG -3’, PAN 1 : 5’-AAC TGG ACC AAT AAC TTT CC- 3’, PAN3: 5’-AAA AAG TCT TTG ACT TCC TGC TT -3’). Obtained PCR products were 380bp and 171bp, respectively. Using these primers, DNA of AAV types 2, 3, 5 and 6 is detected.

RESULTS

HPV infection of the cervix uteri was found in 23 out of 259 women (8.8%), and cervical AAV infection was detected in 46 out of 259 women (17.7%). An AAV/HPV co-infection was detected only in 7/259 (2.7%) of the samples. Table 1 shows the distribution of AAV and HPV DNA-positivity, as well as that of AAV/HPV co-infection, and of Hr and Lr HPV types, in the different age groups. From the 23 HPV-infected women, 14 (60.9%) were identified with high-risk subtypes. The HPV types detected were type 16 (7 samples), type 6 (5 samples), types 18 and 53 (3 samples) and types 11, 31, 33, 35, 58 (1 sample). We found only one woman with a dual infection with HPV types 6 and 16 and one infected with an unspecified HPV type. From the HPV/AAV co-infected women (n=7), one was infected with a low-risk HPV type, one with the unspecified HPV type and 5 with high-risk HPV types (Table 1).

**DISCUSSION**

Sero-epidemiological studies had shown that the majority of the general population has been exposed to AAV1. However, the incidence of (latent/persistent) AAV infection (viral DNA and viral particles) seems very variable [reviewed in 1,2]. High percentages of AAV DNA detection (50%) have been reported in studies examining cervical brushings from US American women with normal cervical cytology. An Italian study using standardized number of cervical cells also showed a high AAV positivity both in the presence (63.2%) and absence (45.3%) of HPV infection3. In contrast, other authors did not find any positivity for AAV DNA in smears from undiseased Jamaican women and female USA university students [cf., 2]. Discrepancies might be attributable to specific cultural, life-style or geographic influences, or to different sampling methods which yield fewer cells in the specimens, or different testing methods, since most of the studies are detecting antigens to the virus instead of viral DNA. In our cohort of «normal» greek women, the AAV DNA detection rate of 17.6% in brushings was lower than in other countries, a results

<table>
<thead>
<tr>
<th>Viral DNA-positive</th>
<th>All women</th>
<th>19-29y</th>
<th>30-39y</th>
<th>40-49y</th>
<th>50-65y</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV (all types)</td>
<td>23*(8.8)</td>
<td>10 (15.3)</td>
<td>7 (6.7)</td>
<td>4 (5.5)</td>
<td>2 (10.0)</td>
</tr>
<tr>
<td>HrHPV</td>
<td>14 (5.4)</td>
<td>7 (10.7)</td>
<td>3 (2.9)</td>
<td>2 (2.7)</td>
<td>2 (10.0)</td>
</tr>
<tr>
<td>LrHPV</td>
<td>8 (3.1)</td>
<td>3 (4.6)</td>
<td>3 (2.9)</td>
<td>2 (2.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>AAV</td>
<td>46 (17.7)</td>
<td>15 (23.0)</td>
<td>14 (13.7)</td>
<td>10 (13.8)</td>
<td>7 (35)</td>
</tr>
<tr>
<td>AAV&amp;HPV (all types)</td>
<td>7*(2.7)</td>
<td>3 (4.6)</td>
<td>2 (2.0)</td>
<td>1 (1.4)</td>
<td>1 (5.0)</td>
</tr>
<tr>
<td>AAV&amp;hrHPV</td>
<td>5 (1.9)</td>
<td>2 (3.1)</td>
<td>1 (1.0)</td>
<td>1 (1.4)</td>
<td>1 (5.0)</td>
</tr>
<tr>
<td>AAV&amp;lrHPV</td>
<td>1 (0.4)</td>
<td>1 (1.5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

*One woman infected with unspecified HPV type (excluded from Hr/Lr calculations).
that has to be confirmed by studies using cervical biopsies. HPV DNA detection rate was also found to be lower in our samples [8.8% (5.4% for Hr HPV)] compared to other countries confirming previous observations in Greek women [4]. It is noteworthy, that the peak in HPV and AAV DNA prevalence is observed in the lowest age group (19-29 years), declining until age 49 for both viruses. The highest age group includes women from 50-65 years. Here, an increased prevalence of the two viruses is observed.

Our report is the first concerning prevalence of cervical AAV infection in Greece, i.e., in a country with very low prevalence of cervical HPV infection, as well as with one of the lowest cervical cancer incidence in Europe [4]. The data will be the basis for studies on HPV-AAV interactions to evaluate if co-infection with the two viruses might have consequences for the development of cervical neoplasia, especially in low risk populations.

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**Πληθυσμιακός έλεγχος ασυμπτωματικών γυναικών για την παρουσία θηλωματικών και αδενοεξαρτωμένων ιών**

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**ΠΕΡΙΛΗΨΗ:** Ο καρκίνος του τραχήλου της μήτρας είναι μια από τις συχνότερες νοσολογικές αλλεργίες του γυναικείου πληθυσμού και η δεύτερη σε βαθμό θνησιμότητα. Οι κύριοι αιτιολογικοί παράγοντες της νόσου είναι η μόλυνση με ένα ή περισσότερα από τους καρκινογόνους υπόπτους του ιού των ανθρωπίνων θηλωμάτων (HPV). Από την άλλη πλευρά, οι αδενοεξαρτώμενοι ιόι (AAV, που είναι επίσης σεξουαλικά μεταδοτικοί), σύμφωνα με πρόσφατες έρευνες εμφανίζουν ομοιόμορφες ιδιότητες και δρουν προστατευτικά εναντίον των θηλωματικών.

Βασική προϋπόθεση για την διερεύνηση της σχέσης των δύο ιών είναι η ύπαρξη δεδομένων για τα ποσοστά εμφάνισης τους στο φυσιολογικό πλήθημα, δηλαδή σε γυναίκες, οι οποίες έχουν φυσιολογικά κολποσκοπικά και κυτταρολογικά ευρήματα. Με μεθόδους μοριακής ανίχνευσης εξετάσαμε 259 δείγματα. Ανιχνεύθηκε AAV DNA σε ποσοστό 17,6% και HPV DNA 8,8% (5,4% υψηλού κινδύνου υπόπτου). Τα ποσοστά και της κατανομής των δύο ιών είναι χαμηλά σε σχέση με αυτά που έχουν παρατηρηθεί σε άλλες χώρες και σε τι αφορά τους θηλωματικούς συμπτώματος με άλλες μελέτες στον Ελληνικό πλήθημα. Είναι σημαντικό να αναφέρετε ότι το μέγιστο της επικράτησης τους εμφανίζεται στην μικρότερη ηλικιακή ομάδα (19-29 ετών), μειώνεται μέχρι την ηλικία των 49 ετών και για τους δύο ιό ουσιαστικά αρχίζει να αυξάνεται στην ηλικία 50-65 ετών. Η έρευνα μας είναι η πρώτη που μελετά το ποσοστό επικράτησης των αδενοεξαρτώμενων ιών στο Ελληνικό πλήθημα, σε μια χώρα με τα χαμηλότερα ποσοστά μόλυνσης από θηλωματικού και εμφάνισης τραχηλικού καρκινού.

*Λέξεις Κλειδιά: HPV, AAV, Επιδημιολογία, Τράχηλος.*
REFERENCES


