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HPV and cervical squamous intraepithelial lesions: clinicopathological study

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Abstract

The aim of our study is to determine the evidence of HPV infection, by either HPV-DNA test or histopathological examination, in patients with abnormal PAP-smear, for further clinical management. In 6-month period, we retrospectively selected a number of 103 patients in 16–54 years range of age that were investigated by complex diagnostic techniques supporting the evidence of HPV infection. Initially, these patients were evaluated cytological using the Bethesda system for PAP-smear interpretation along with colposcopic examination. A number of these patients were orientated to HPV testing (PCR) or to biopsy with histopathological diagnostic intent. The net predominance of LSIL over HSIL cytodiagnostic class parallels with that of condyloma and CIN1 over CIN2 and CIN3 histodiagnostic and suggests that progression to high-grade intraepithelial squamous lesion is rare and the HPV infection remain mainly transitory. On the other side, the incidence and distribution of HPV types in cervical infections are high variable and may change over time, the cytological screening of sexual active female population remaining the most practical tool in detection of genital HPV infection.

Keywords: HPV infection, squamous intraepithelial lesion, cytology, histopathology.

Introduction

Human papillomavirus (HPV) infection of female genital tract is one of the most common sexually transmitted diseases representing the main etiological factor of cervical cancer, with over 99.7% of these tumors positive for HPV DNA in recent studies. Accordingly, the *National Institutes of Health Consensus Conference on Cervical Cancer* concluded that the 'cervical cancer is unique in that it is the first solid tumor to be shown to be virally induced in essentially every case' [1, 2].

Prevalence of HPV infection in young women varies from 20 to 46 percent and as with other sexually transmitted diseases, the incidence of HPV infection is highest among young women [2].

Human papillomaviruses (HPV) belong to the *Papovaviridae* family and their genome consist in an circular double-stranded DNA of approximately 8 kb in length, which encodes for several regulatory and structural proteins known as early (E), E1, E2, and E4 to E7, and late (L), L1 and L2, proteins, involved in viral replication and having transforming (oncogenic) properties [3, 4]. There are more than 100 known HPV genotypes, grouped in cutaneous and mucosotropic. The mucosotropic HPVs group with at least 30 types that infect cervical mucosa, can be further divided into those that produce genital lesions with a very low risk of progression to cancer (low risk HPV, e.g.: 6, 11, 40, 42, 43, 44, 45, 54, 61, 70, 72, 81 types) and those with a moderate to high risk of progression to cancer (high risk HPV, e.g.: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82 and probably high risk HPV, e.g.: 26, 53, 66) [3, 5].

HPV6 and HPV11, classified as low-risk viruses are responsible for genital warts, and are almost never found in invasive anogenital cancers. The high-risk viruses (particularly HPV 16, 18, 45 and 31) are found in varying proportions in invasive cancer in different countries, HPV16 and 18 the two most common oncogenic types, being responsible for approximately 70% of all cervical cancers worldwide [5]. Wide world, approximately 1% of the population has genital warts and 4% of women have cervical precancerous lesions: low-grade squamous intraepithelial lesion (LSIL) or high grade SIL (HSIL); the HSIL lesion, preferentially observed in women aged 35–40 years, are at high risk of progression to invasive cancer [6].

The squamous cells of the transformation zone are particularly vulnerable to the adverse effects of persistent HPVs infection (especially high-risk viruses). The acute lesion appears after 6–12 weeks and can be totally asymptomatic. In this phase, the viral genome replicates only in the nucleus of an infected epithelial cell, independent of the host DNA and producing a large numbers of infectious virions as the cell matures; desquamation of mature epithelial cells with the release of large numbers of infective virions into the genital tract is followed by the further sexual transmission, completing the life cycle of the virus [7].

In the infected squamous cell, the viral protein product (E4) binds to and disrupts the cytoplasm keratin network, producing the cytological appearance of the koilocyte. Pre-malignant and malignant cells arise because of HPV DNA integration in the host cellular genome, with the subsequent overexpression of the viral E6 and E7 oncogenes. In this condition, the infected cells acquire a proliferate advantage by escaping growth control exerted by p53 and p105Rb tumor suppressor protein, both inactivated by E6 and respectively E7 viral products [6, 7].

Most HPV infections produce transient minor lesions (warts and low-grade squamous intraepithelial lesion) in majority of cases, while some infections with certain oncogenic HPV types may persist and progress to high-grade cervical intraepithelial neoplasia (CIN3) and then to invasive carcinoma, with an annual rate of progression to invasive cervical cancer of less than 1% [8, 9].

Materials and Methods

For the 6-month studied period, we retrospectively selected a number of 103 patients that were investigated by complex diagnostic techniques supporting the evidence of HPV infection, intending to make certain clinic pathological correlations and obtain information of therapeutically value.

Initially, these patients were evaluated cytological using the Bethesda system for PAP smear interpretation along with colposcopic evaluation, defining the following squamous cell abnormalities: NILM (Negative for Intraepithelial Lesions or Malignancy), ASCUS (Atypical Squamous Cells of Unknown Significance), ASCH (Atypical Squamous Cells cannot exclude HSIL), LSIL (Low grade Intraepithelial Squamous Lesion) and HSIL (High grade Intraepithelial Squamous Lesion). We excluded from our study those patients in NILM class, those in ASCUS class that revert to NILM after anti-inflammatory treatment and also those young patients (<35 years) in LSIL class and low-risk HPV positive that undergone PAP smear surveillance.

The HPV testing (using the PCR technique for AND HPV detection) was indicated to those patients in ASCUS class with suspect colposcopy, to the patients in LSIL class, and also for patients in HSIL cytodiagnostic, the HPV type detection (low and high risk) being useful for subsequent clinical management.

The oriented biopsy with diagnostic intent was performed for patients in ASCH and HSIL classes and also for patients in LSIL class HPV high-risk positive, as a choice method and the histopathologic diagnostic (processed by classical technique of paraffin embedding and Hematoxylin–Eosin standard staining) included: condylomas, cervical intraepithelial neoplasia (CIN) grade 1, 2 and 3 (*in situ* carcinomas) and also epithelial reactive changes associated in inflammations.

Results

In our study, the 103 patients selected in 6-month period were in 16–54 years range of age. The majority of these patients were in 31–35 years group totalizing 32 cases (31.06%). The distribution of patients was only slight variable between 21–25, 26–30, 36–40 and 41–45 years age groups (between 13 and 18 cases, 12.62% and 17.47% respectively), while <20 years age group and >46 years age group summed eight and respectively four cases (3.88%; Table 1).

Table 1 – ***Distribution of selected patients in age groups and cytodiagnostic and histodiagnostic classes***

Patients distribution by age group and cytological and histopathological diagnostics								
Distribution by age groups								
Age groups [years]	<20	21–25	26–30	31–35	36–40	41–45	>46	Total cases (%)
No. of cases	8	13	15	32	18	13	4	103
Distribution by cytodiagnostic class (Bethesda System)								
ASCUS	2	2	4	10	6	4	1	29 (28.15%)
ASCH	–	–	–	2	2	1	–	5 (4.85%)
LSIL	5	9	10	17	8	7	2	58 (56.31%)
HSIL	1	2	1	3	2	1	1	11 (10.67%)
Distribution by histopathologic squamous lesion type								
Condyloma	4	2	1	6	4	1	–	18 (17.47%)
CIN1	2	8	11	14	7	9	2	53 (51.45%)
CIN2	1	1	1	2	2	1	1	9 (8.73%)
CIN3	–	1	1	3	2	1	–	8 14.56%)
Reactive lesions	1	1	1	7	3	1	2	15 (14.56%)

From the 367 initial cytodiagnostics in studied period, we excluded 163 NILM cases, 75 ASCUS cases that revert to NILM after anti-inflammatory treatment and also 26 young patients (<35 years) in LSIL class but low-risk HPV positive that undergone PAP-smear surveillance.

The rest of 103 selected patients constituted four cytodiagnostic Bethesda classes as follow: ASCUS with colposcopic aspect suspect of HPV infection (29 cases, 28.15%); ASCH (five cases, 4.85%); LSIL (58 cases, 56.31%) and HSIL (11 cases, 10.67%; Table 1).

The most large cytodiagnostic class was LSIL (Figure 1; 56.31%), the majority of patients being in 31–35 years group of age (17 cases, representing 29.31% from LSIL category). This cytodiagnostic class was more rare in the other groups with a slightly decrease for 26–30 and 21–25 years groups (from 10 to nine cases respectively) and also for 36–40 to 41–45 years (from eight to seven cases respectively). LSIL were rather rare in patients under 20 and above 46-year-old (with five and respectively two cases; Table 1).

The LSIL class was followed as frequency by the

ASCUS cytodiagnostic category (28.15%), the majority of these patients with a number of 10 cases being also in 31–36 years age group (34.48% from ASCUS category; Table 1).

Patients in HSIL class (Figure 2) summed only 11 cases (10.67%), three of these affecting women in 31–35 years group of age (27.27% of HSIL category). Even of high-grade, this lesion was also found in young patients (under 20-year-old) (one case) at same frequency as in 26–30, 41–45 and >46 years group of age (9.00% of HSIL category). Patients in 21–25 and 36–40 years group of age summed two cases each (18.18% of HSIL category; Table 1).

The most rare was the ASCH cytodiagnostic category with five cases (4.85%) that affected patients in 31–35 and 36–40 years group of age with two cases each (40.00% of ASCH category) and in 41–45 years group of age, with one case (20.00% of ASCH category; Table 1).

Follow the biopsy, the histopathological diagnostic was: condyloma, in 18 cases (17.47%); CIN1, in 53 cases (51.45%); CIN2, in nine cases (8.73%) and CIN3 /*in situ* carcinoma, in eight cases (7.76%). In other 15 cases (14.56%), the histopathological diagnostic was epithelial reactive changes in inflammation, affecting some of the patients in ASCUS with suspect colposcopy (Table 1).

The condyloma lesions (Figures 3 and 4) represented 18 cases (17.47%) while CIN 1 (Figure 5) was the most frequent histological diagnostic summing 53 cases (51.45%). These two types of lesion corresponded to LSIL and ASCUS (majority of cases) cytodiagnostic classes and similarly affected most frequently the 31–35 years group of age, with six (33.33% of condylomas) and respectively 14 (26.41% of CIN1 lesions) cases. Condyloma was also more frequently seen in <20 years and 36–40 years group of age, with four cases in each (22.22% of condylomas) while LSIL affected more frequent 26–30 and 41–45 years age groups, with 11 (20.75% of LSILs) and respectively nine cases (16.98% of LSILs; Table 1).

CIN2 and CIN3 lesions were rare (Figure 6), representing nine (8.73%) and eight (7.76%) cases respectively. These lesions corresponded to ASCH and HSIL cytodiagnostic classes and affected predominantly patients in 31–35 and 36–40 years group of age, with a total of four (44.44% of CIN2) and five (62.50% of CIN3) cases respectively. Even high-grade lesions, CIN2 was found in patients <20 years group of age (one case, 11.11% of CIN2) and CIN3 affected one patient (12.50% of CIN3) in 21–25 years group of age (Table 1).

HPV test with HPV typing was performed in only 41 cases, as the cost of this investigation was rather high (Table 2).

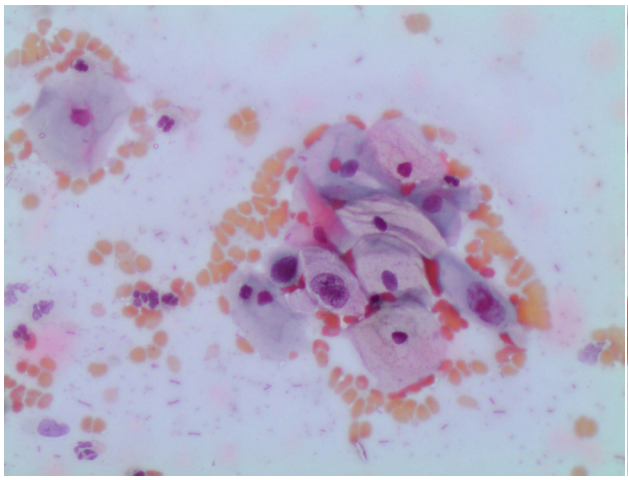

Figure 1 – ***LSIL (PAP stain, ×200).***

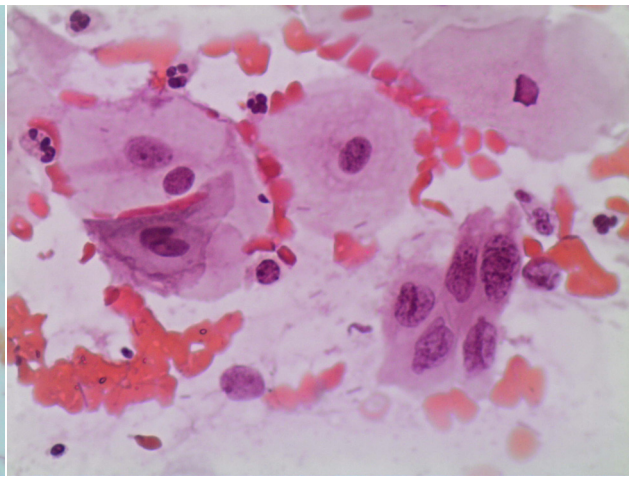

Figure 2 – ***HSIL (PAP stain, ×400).***

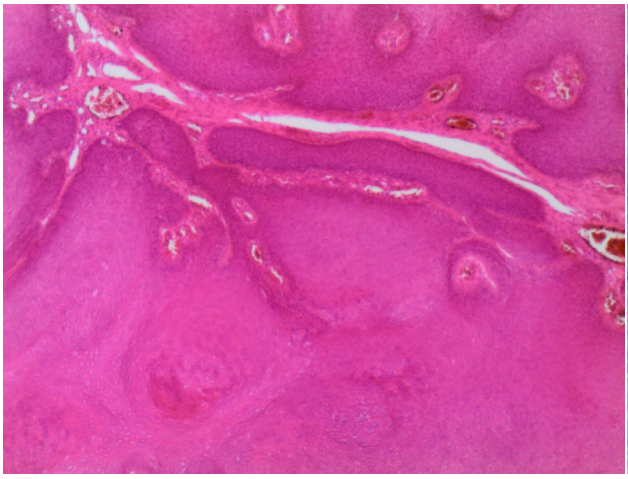

Figure 3 – ***Condyloma acuminata (HE stain, ×40).***

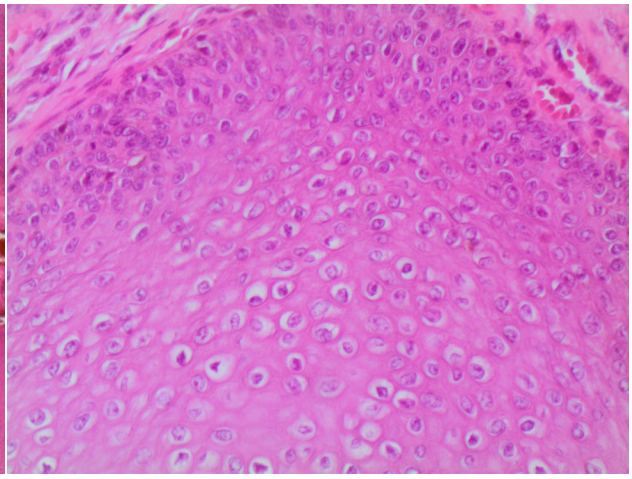

Figure 4 – ***Koilocytosis (HE stain, ×200).***

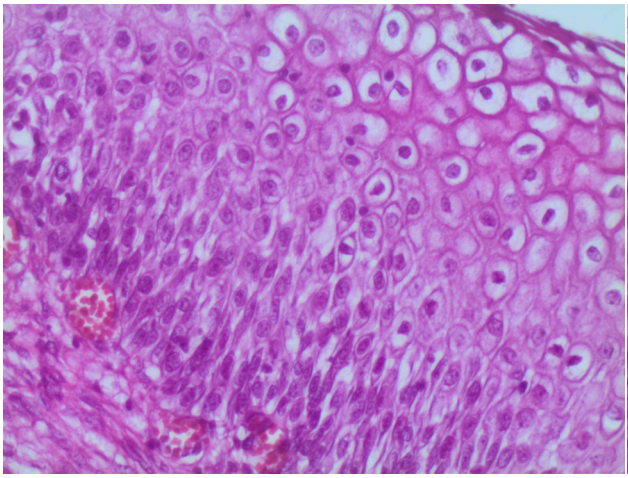

Figure 5 – ***Koilocytosis, CIN 1 (HE stain, ×200).***

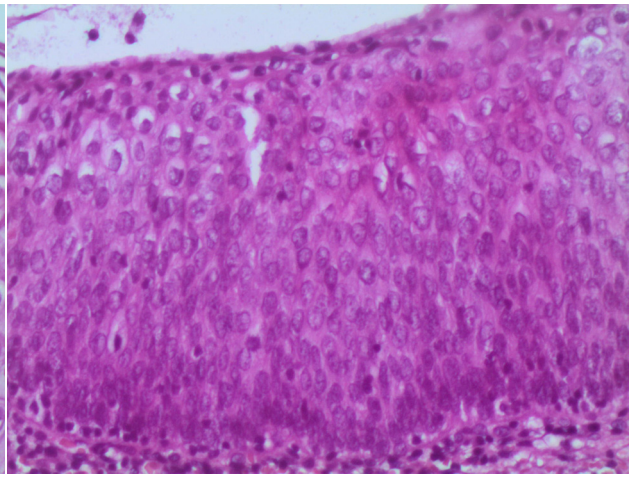

Figure 6 – ***CIN 3 (HE stain, ×200).***

There was identified a number of 18 HPV types grouped in two categories: low-risk HPV summing 21 (38.88 %) of HPVs and high-risk and probably high-risk HPV including the rest of 33 viruses (61.11%; Table 2).

In the low-risk HPV category, the most frequent were 61 type (seven cases, 33.33% of low-risk HPVs) followed by 6 type (five cases, 23.80% of low-risk HPVs), 81 type (with three cases, 14.28% of low-risk HPV), 54 type (two cases, 9.52% of low-risk HPV) and 42, 45, 70 and 72 types (found in one case/4.76% of low-risk HPV, each; Table 2).

Table 2 – ***The distribution of HPV types in category of risk***

HPV types	Low-risk								High-risk and probably high-risk									
	6	***42***	***45***	***54***	***61***	***70***	***72***	***81***	***16***	***18***	***31***	***33***	***39***	***52***	***56***	***68***	***53***	***66***
No. of cases	5	1	1	2	7	1	1	3	9	4	1	5	1	3	4	3	2	1
Total (%)	21 (38.88%)								33 (61.11%)									

The high-risk HPV category included HPV 16 as the most frequent the type, found in nine cases (27.27% of HPV in this category). Other types in this category were HPV 33 (with five cases, 15.15% of HPV in this category), HPV 18 and 56 (four cases/12.12% of HPV in this category, each), HPV 52 and 68 (three cases/9.09% of HPV in this category, each) and HPV 31 and 39 (one case/3.03% of HPV in this category, each). The 53 and 66 types, which are probably high-risk HPVs, were also included with two (6.06%) and one case (3.03%) respectively (Table 2).

Nineteen of the HPV tested patients (46.34%) has associated two or more HPV types either of low and high risk, as for example 6 and 54 (low-risk), 18, 39 and 53 (high-risk) types in one of the cases.

Discussion

The addressability to gynecologic investigation among the patients selected in our study was more pronounced between those in 31–35 years group of age (31.06%) and it decreased progressively to 7.76% in <20 years patients and 3.88% in >46 years age group.

According to the *Bethesda 2001 System*, our 367 initial cytodiagnostics included 163 NILM cases (44.41%) and 104 ASCUS (with and without suspect colposcopy) cases (28.33%) that summed the most cases among the atypical ones (*vs.* LSIL with 22.88%, *vs.* HSIL with 2.99% and *vs.* ASCH with 1.36%) as there is mentioned in several similar studies.

The atypical squamous cell category (ASC) is considered the most frequent cytological epithelial abnormality by the most laboratories [10] even there is considerable interobserver variability [7] related to patient population and risk factors, being reported an ASC/SIL ratio is also variable, with a value of 2.9 or less in majority of the laboratories and a predominance of ASCUS (>90%) *vs.* ASCH (around 5%) lesions [11].

Among selected patients the majority of 56.31% were in LSIL cytodiagnostic class, followed by the ASCUS category associated with suspect colposcopy in 28.15% cases, HSIL and ASCH classes with 10.67% and 4.85% cases, respectively. Irrespective of lesion class, the most affected patients were in 31–35 years group of age. Both of LSIL and HSIL were found in patients <20 years (ratio LSIL/HSIL being 5/1) and also >46 years (ratio 2/1).

The net predominance of LSIL over HSIL cytodiagnostic class suggests that progression to high-grade intraepithelial squamous lesion is rare and the HPV infection remain mainly transitory, as it also comes out from other similar studies [2, 12, 13]. In that, Schlecht NF *et al.* (2003) found that most LSIL regresses over short periods with only a small minority of LSIL that progress [8].

Follow the histopathological examination of bioptic fragments, the CIN1 was the most frequent lesion (51.45% cases), and in association with condylomas (17.47% cases) occurred in women with LSIL cytodiagnostic and also in almost half of the women in ASCUS class with suspect colposcopy (14/29 cases, 48.27%). In different studies, the incidence of CIN after an ASCUS Pap smear was between 7% and 58% [14].

The rest of ASCUS patients were found with epithelial reactive changes in inflammation. The CIN1 lesions were more frequent in 31–35 years and 26–30 years groups of age (47.16%) as the condylomas that affected predominant patients between 31 and 35-year-old (33.33%).

Relatively equal as frequency (8.73% and 7.76% respectively), the CIN2 and CIN3 lesions were much more rare lesions (16.50% all together) with a LSIL/ HSIL ratio appreciatively of 4/1. These lesions overlapped to HSIL and ASCH cytodiagnostic classes and affected most frequently patients in 31–35 years and 36–40 years group of age. As in other similar studies, the highest incidence of intraepithelial squamous lesions irrespective of grade, was among patients in 31–35 years group of age, with the high-grade lesions typically diagnosed in women of 25 to 35-year-old [15, 16].

HPV test (PCR) used in our study identified 18 different HPV types classified as low-risk and high-risk (including probably high-risk HPV) with net predominance of the last category (61.11%).

In different epidemiological studies, the most frequent HPV types found in relation with genital warts are 6 and 11, while the 16, 18, 45 and 31 types are the most involved in high-grade squamous intraepithelial cervical lesions with high malignant potential [5, 17, 18].

The incidence and distribution of HPV types in cervical infections are variable and these may change over time [19]. In our study, among low-risk types, the most frequent were HPV 61 (33.33%) and 6 (23.80%) followed by the HPV 81 and 54 (14.28% and 9.52% respectively), while the high-risk category included HPV 16 (27.27%) as most frequent type, and also the HPV 33 (15.15%), 18 and 56 (12.12% each), 52 and 68 (9.09%, each). Particularly, in low-risk category, the most frequent was type 61, while type 11 has not been detected. It is also to point out that the occurrence of infection with two or more HPV types either of low and high risk, described in other similar studies [20] was not rare (46.34%).

Conclusions

According to the *Bethesda 2001 System*, the majority of selected patients were in LSIL cytodiagnostic class, followed by the ASCUS category associated with suspect colposcopy, HSIL and ASCH classes. Irrespective of lesion class, the most affected patients were in 31–35 years group of age. The histopathological examination of bioptic fragments, revealed the CIN1 as the most frequent lesion, followed by the condylomas, both lesions occurring in women with LSIL cytodiagnostic and also in almost half of the women in ASCUS class with suspect colposcopy. The histopathologic diagnostics of CIN2 and CIN3 altogether confirmed the suspicion of HSIL cytologic abnormality and were also found in ASCH class patients.

As low-risk HPV types, the most frequent were HPV 61 and 6 while in high-risk category predominated HPV 16. It is to mention that infection with two or more HPV types either of low and high risk was relatively frequent.

The most practical tool in detection of genital HPV infection remains the cytological screening of sexual active female population. As additional diagnostic methods are HPV typing test and biopsy in very carefully selected cases in which the colposcopy has been proven its utility.

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