High-risk human papillomavirus (HPV) types in patients with squamous intraepithelial lesions (SIL)

M. V. Perovic[1], *M.D. M.A.*; **M. J. Berisavac**[1], *M.D. Assoc. Prof.*; **N. Kuljic-Kapulica**[2], *M.D.*; **T. Jovanovic**[3], *M.D. Prof.*

[1]*Institute of Obstetrics and Gynaecology, University Clinical Centre of Serbia;* [2]*Medical Military Academy , Institute of Microbiology;* [3]*Institute of Microbiology and Imunology, Department of Virology, Medical University, Belgrade (Yugoslavia)*

Summary

The aim of the paper was to determine the probability of joined occurrence of certain HPV types, particularly those with high oncogenic risk and histopathological (HP) findings (LGSIL and HGSIL, i.e., micro invasive and invasive carcinoma) as well as to find out to what extent either presence or absence of HPV can be identified in HP findings.

The investigation was carried out on 48 patients. Based on a suspected colposcopy findings, Papanicolaou (Pap) smear and biopsy were performed and a histopathological analysis of the sample was carried out. A cervical smear was made on all the patients for HPV detection and typing. The results in the group with HPV, which showed histological diagnoses of LGSIL in 80% of cases, revealed numbers significantly higher with respect to the same summing up in the group where HPV was not detected (66.6%). HP findings of HGSIL in both groups were diagnosed in almost the same percentage and it should be taken into account that there were 6.7% of patients with Ca in situ in the group of HPV-positive patients. Therefore, it can be concluded that if a diagnosis of LGSIL or HGSIL, in particular, has been made on the basis of HP findings there is a great probability that it is a case of infection by one or more joined types of human papillomaviruses. The presence of virus specific genes in one of the stages of tumor development at the beginning of the infection indicates the viral etiology of tumors. The presence of HPV genome was not proved in 21% of patients with HGSIL. A multiple infection with different HPV types is more often found in patients with LGSIL than in those diagnosed with HGSIL. This demonstrates the selection of high oncogenic types and their persistence during the course of carcinoma progression.

Key words: Squamous intraepithelial lesion (SIL); HPV.

Introduction

The importance of clinical classification of HPV into three groups according to oncogenic risk has not yet been completely determined. At the time, it is considered that histology would completely comply with this classification, i.e., that low-grade squamous intraepithelial lesion (LGSIL) would generally be associated with the HPV types of low oncogenic risk [1]. LGSIL (CIN 1) is extremely heterogeneous as per associated HPV types. Using polymerase chain reaction (PCR) to detect certain HPV types in 278 CIN cervical biopsies of all grades, Lungu *et al.* (1992) showed that 22% of LGSIL (CIN 1) were coupled with more than one HPV type at the same time, 15% with the 6/11 types (low oncogenic risk type), 16% with type 16 and 3% with type 18 (high oncogenic risk type), 18% with type 31/33/35 (intermediary oncogenic risk) and almost 26% with others or yet undetermined HPV types. Other authors have reported similar results [2]. These studies have shown that histology does not correlate with HPV types for LGSIL (CIN 1).

On the contrary, there is a greater correlation between histology and associated HPV types in HGSIL (CIN 2 and 3). In the above-mentioned study by Lungu *et al.* only 7% of these lesions contained more than one HPV type, while even 88% were coupled with types 16, 18 or 33.

The aim of this paper was to determine the probability of joined occurrence of certain HPV types, particularly those with high oncogenic risk and histopathological (HP) findings (LGSIL and HGSIL, i.e., micro invasive and invasive carcinoma) as well as to find out to what extent either presence or absence of HPV can be identified in HP findings.

Material and Methods

The study was carried out at the Institute for Gynaecology and Obstetrics of the Clinical Centre of Serbia (IGA KCS) from January 1998 to September 2000. Forty-eight patients were included and formed a study group (Group I). Based on a suspected colposcopy finding a biopsy was performed and a histopathological analysis of the sample was carried out. A cervical smear test was made on all the patients for HPV detection and typing by using CREATECH – "Rembrandt" test (in situ hybridization – ISH) HPV screening/typing) Kreatech Diagnostics – DIANOVA, the Netherlands. After individual analysis of the studied parameters and their statistical processing, all parameters were investigated with respect to the presence of HPV infection. Patients in whom HPV infection was not detected formed a control group (C group).

For statistical analysis the X^2 test, Fisher test and Student's t-test was used.

Results

Screening of HPV infection

The result of HPV typing was negative in 68.8% of patients and positive in 31.2%. There was 14.6% of patients positive on one type, 12.5% on two, and 4.2% on three (Table 1.).

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Typing of HPV infection

HPV typing was done in 19 patients for both partners (Table 2, Chart 1). The results show a high degree of agreement in HPV typing. There were 68.4% of equivalent results and 31.6% of inequivalent results (26.3% of women were positive while their male partners were negative and 5.3% of men were positive while their female partners were negative).

Correlation between virology and HP findings

The HP finding with respect to HPV typing showed that there were strictly defined variations. However, statistical processing showed that they were irrelevant (Table 3).

Discussion

A significantly large number of pathological findings of LGSIL (80%) and HGSIL (20%) in the study group occurring at the same time, clearly shows the clinical importance of colposcopy which indicated the need for biopsy (enabling a precise targeted biopsy) and histopathological examination. The results of the cervical smear test taken from these patients by applying a commercial method of an in situ DNA hybridization to detect

Table 1. — *The ratio between SIL findings and HPV typing.*

Finding	LGSIL	HGSIL	Total	
Negative	22	7	33*	68.8%
Positive	12	3	15	31.2%
6/11	3	0	3	6.3%
16/18	2	2	4	8.3%
6/11, 16/18	2	0	2	4.2%
16/18, 31/33	4	0	4	8.3%
6/11, 16/18 and 31/33	1	1	2	4.2%

* No findings in 4 patients.

Table 2. — *HPV typing of both partners.*

		Male Partner B				
		Negative	Positive			
			6/11	31/33	6/11 31/33	6/11 16/18 31/33
Female Partner A	Negative male	8	–	–	–	–
	Positive female					
	6/11	4	–	–	–	–
	16/18	1	–	1	–	–
	6/11, 16/18	–	2	–	–	–
	16/18, 31/33	–	1	–	1	–
Total		13	3	1	1	1

Table 3. — *The ratio between the HP and HPV findings in studied female patients.*

HP findings	HPV findings			
	ø		+	
	N	%	N	%
LGSIL	22	66.7	12	80
HGSIL	7	21.0	2	13.3
Ca in situ	0	0.0	1	6.7
No findings	4	12.1	0	0.0
Total	33	100.0	15	100.0

$X^2 = 2.037$; DF = 2; $p > 0.05$.

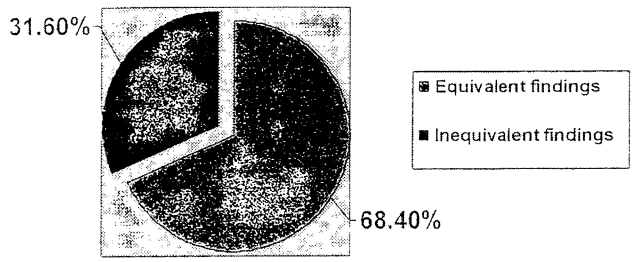

Figure 1. — HPV typing of both partners.

the presence of HPV infection showed the occurrence of HPV in 31.2% of women. Other researchers have reported even higher percentages, but they applied more sensitive detection methods such as PCR [3]. We were able to determine six types of HPVs classified in pairs by applying this method. The pair type 6/11 represents a group of HPV types with low oncogenic risk, the pair 16/18 represents the types with high oncogenic risk, whereas the pair 31/33 represents the types with medium oncogenic risk. The analysis of certain types of occurrence, i.e., the HPV pair types, on the overall sample showed that the occurrence of joined group types (two or all three pairs at the same time) was dominant, whereas, individually, the pair 6/11 was most frequently detected (Table 1).

For 19 patients (39.5%) the HPV typing of their partners was carried out as well (Table 2 and Chart 1). The results showed a high degree of equivalence in HPV typing. There were 68.4% of equivalent findings and 31.6% of inequivalent ones (26.3% of women were positive while their male partners were negative) and in 5.3% of cases the women were negative and their male partner positive.

Table 3 shows the HP finding in the group with HPV with respect to the control group (without HPV). The correlation analysis of the parameters for the group with HPV is to show to what extent the presence or absence of HPV can be detected on the basis of the HP finding. Furthermore, it is even more important to determine the probability of joined occurrence of certain HPV types and HP findings. The HP finding with respect to the HPV typing result showed that there were certain variations. However, the statistical processing showed that they were statistically insignificant ($X2 = 2.037$; DF = 2; $p > 0.05$). Summing up the results in the group with HPV, which showed histological diagnosis of LGSIL in 80% of cases, the number is significantly higher with respect to the same summing up in the group where HPV was not detected (66.6%). HP findings of HGSIL in both groups were diagnosed in almost the same percentage and it should be taken into account that there were 6.7% of patients with Ca in situ in the group of HPV-positive patients. Therefore, it can be concluded that if a diagnosis of LGSIL or HGSIL, in particular, has been made on the basis of HP findings there is a great probability that it is a case of infection by one or more joined types of human papillomaviruses.

Many authors have stated that in cases where a SIL diagnosis had been made the HPV infection of the uterine cervix could be detected with significant probability

accompanied by other epidemiological [4] and molecular findings [5]. When HPV types were classified into three groups according to oncogenic risk it was considered that this classification would comply with the SIL histological grade. As a matter of fact, it was considered that HPV types with low oncogenic risk would prevail in LGSIL and the types with high oncogenic risk in HGSIL [1]. Today, it is known that HP results do not correlate with the HPV types in cases of LGSIL [3]. On the contrary, there is a greater correlation between the HP results and associated types of HPV in HGSIL [3] and it has been confirmed in this paper. HP findings of LGSIL (34 patients) showed the occurrence of HPV infection in approximately 35.29% of cases, which was not as significant as the fact that HPV had not occurred. The analysis of HPV types pointed out the heterogeneity of detected types. It means that the most frequent finding was of joined groups of virus types (combinations were detected in 58.3% of cases), which was a big number with respect to the overall virus detection. However, the pair 6/11 of HPV types was most frequently detected in individual cases (one quarter of all cases). This is also a significant number, but only among individually detected types. The pair 6/11, which represents a group of benign, i.e., low oncogenic risk HPV types, also dominated in the combinations of detected viruses (57.14%). HPV types with individual high and medium oncogenic risks were detected in about 16.6% of LGSIL cases. Both Herrington and Evans [6] *et al.* found the types of high and medium oncogenic risk (16/18, 31/33) in 15% of LGSIL. Similarly, applying a sensitive PCR technique, Lungu [3] detected combination types in 22% of LGSIL, 6/11 types in 15%, 16/18 high risk oncogenic types in 19%, 31/33/35 types of intermediary oncogenic risk in 18%, and other yet unknown HPV types in even 26% of LGSIL. Chabaud *et al.* [7] detected more often 6/11 low risk oncogenic types in LGSIL (43%) than in HGSIL (17%) as well as 16/18 high oncogenic risk types in both SIL stages in a high percentage (over 70%). Using the PCR method, these authors detected type 45 which belongs to the high oncogenic group in 10% of cases. The determination of certain HPV types in LGSIL is important for it is considered that the lesions containing high oncogenic risk types more likely carry a higher potential toward progression [8]. The overall review of the studies showed that there were more studies, which had investigated the natural course of LGSIL. It was found that approximately 12-15% of LGSIL progressed into the severe stages of the disease [9].

The same process was carried out in HP findings of HGSIL (10 patients). The occurrence of HPV infection was detected in 30% of cases with the same probability of HGSIL occurrence even in the absence of HPV infection.

Studying the distribution of HPV types in HGSIL with detected HPV infection an increased presence of viruses in combination, i.e., joined pairs simultaneously, was noted, which was irrelevant with respect to the detection of individual pairs of HPV types. When a pair of HPV types is detected, then 16/18 evidently dominates as the representative of the high oncogenic risk HPV group (66.6%). This pair actually dominates even in the combinations of HPV types. There is a high correlation between the detection of high oncogenic risk HPV types on the uterine cervix in cases of higher grades of HGSIL as well as on the samples used in this study. This is in agreement with other authors' results. Bergeron *et al.* detected types 16 and 18 in 61% of HGSIL by applying the method of Southern blot hybridization. Franquemont *et al.* [10] obtained the same results in 70%. Lungu [3] identified types 16, 18 and 33 in HGSIL in 88% of cases by applying the PCR method. Herrington *et al.* [6] detected types 16, 18, 31, 33, and 35 in 90% of HGSIL cases by applying the in situ hybridization method. Chaubaud *et al.* [7] also indicated that there was an important correlation between HGSIL and HPV types with high oncogenic risk.

The presence of HPV genome was not proven in 21% of patients with HGSIL. A study should be carried out on a larger number of patients in order to confirm the initial role of viruses in transforming a normal cell into a malignant one.

A multiple infection with different HPV types is more often found in patients with LGSIL than in those diagnosed with HGSIL. This demonstrates the selection of high oncogenic types and their persistence during the course of carcinoma progression.

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Address reprint requests to:
M. PEROVIC, M.D.
Nemanjina, 13
11080 Zemun (Yugoslavia)