European Journal of Obstetrics & Gynecology and Reproductive Biology 170 (2013) 501–506

Contents lists available at SciVerse ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Progression of cervical low grade squamous intraepithelial lesions: in search of prognostic biomarkers

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ARTICLE INFO

Article history:
Received 21 February 2013
Received in revised form 18 June 2013
Accepted 6 July 2013

Keywords:
LSIL
HPV
p16
Ki-67
p53
pRb

ABSTRACT

Objective: It has been reported that approximately 10% of low grade squamous intraepithelial lesions (LSIL) progress to high grade squamous intraepithelial lesions (HSIL) within a 2-year follow up. The factors related to lesion progression are currently unknown. The aim of the study was to identify prognostic markers of the course of LSIL. This retrospective study was designed to correlate regression, persistence and progression of biopsy-proven LSIL with patients' age, HPV genotypes and immunohistochemical expression of the main cell cycle regulating proteins: p53, pRb, p16, and Ki-67.
Study design: A total of 584 consecutive patients with biopsy proven LSIL and 2-year follow-up were included in the age analysis. HPV genotyping was performed in 328 LSIL cases using the SPF10 PCR-LiPA25 (version 1), 238 LSIL cases were immunostained for Ki-67 and p16, and 101 cases were immunostained for pRb and p53.
Results: The odds of LSIL persistence and progression were significantly higher in women 30–39, 40–49 and 50+ years old, as compared to women 20–29 years old (OR 1.89, 2.52 and 2.39, respectively). The odds of persistence and progression were higher in women infected with HPV16, 18, 33 and 52 (OR 3.5, 3.1, 3.5 and 2.9, respectively). There were no significant differences in expression of immunomarkers (p16, p53, pRB and Ki-67) between the lesions that regressed versus the lesions that persisted or progressed.
Conclusions: Patients 30 years of age and older have a higher risk of LSIL progression or persistence as compared to 20–29 year olds. In addition, HPV genotyping, but not the cell cycle markers, may aid in prognosis of LSIL course.

1. Introduction

Human papillomavirus (HPV) infection is now recognized as the main pathogenetic factor of cervical carcinoma. Eighteen mucosal HPV types have been detected in cancers of genital and oropharyngeal mucosa and those types are identified as "high oncogenic risk." HPV16 alone accounts for 50–60% of cervical cancers cases [1].

Invasive cervical carcinoma is preceded by a long period of cervical intraepithelial neoplasia (CIN), also termed squamous intraepithelial lesion (SIL). SIL is a heterogeneous group of lesions graded as either low grade (LSIL) or high grade squamous intraepithelial lesions (HSIL), depending on the severity of their histological abnormalities. LSIL reflects a productive viral infection and it may follow various clinical courses. Most LSIL (60%) are transient and regress spontaneously within 12–24 months, but 30% of LSIL persist, and approximately 10% of LSIL progress to pre-malignant HSIL within 2-year follow-up [2–4]. Since only a small fraction of patients with LSIL are expected to develop HSIL, optimally such patients should be prospectively identified and triaged for excisional treatment, while all other patients with LSIL could be safely followed with repeat Pap tests and colposcopic examinations until the resolution of the lesion. In some medical centers patients with LSIL are offered a loop electrosurgical excision procedure (LEEP) in order to prevent progression to HSIL. Such an approach results in over-treatment of patients who have only a transient HPV infection, contributing to unnecessary high costs of medical care and occasional iatrogenic complications.

Understanding the factors involved in progression from benign, productive HPV infection to premalignant HSIL and invasive carcinoma could be utilized to identify patients who are truly at risk for carcinoma. Such markers may help in developing efficient individualized therapy and cost-effective triage protocols for patients with diagnosis of LSIL. The aim of this study was to

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http://dx.doi.org/10.1016/j.ejogrb.2013.07.012

identify prognostic markers of the course of LSIL in order to predict which patients with LSIL may require excisional treatment.

This study examined the relationship between LSIL regression, persistence and progression and patients' age, HPV genotype and immunohistochemical expression of four main cell cycle regulating proteins – p53, pRb, p16, and Ki-67.

2. Materials and methods

2.1. Case selection

The Institutional Review Board's permission was obtained for the study. The surgical pathology files of the Department of Pathology at Weill Medical College of Cornell University were searched from 1995 to 2005 to identify consecutive cervical punch biopsies with the diagnosis of LSIL (''index biopsy''). Only LSIL cases with available 2-year follow-up information (cervical cytology or biopsy) were selected from the computer-database. Regression of LSIL was defined as normal cervical cytology or cervical biopsy at the end of 2-year follow up. Persistence was defined as LSIL or atypical cells of undetermined significance (ASCUS) on cervical cytology or biopsy at the end of 2-year follow up. Progression was defined as diagnosis of HSIL any time within the 2 years of follow-up on cervical cytology, punch biopsy or cone biopsy. If LSIL and HSIL lesions were detected simultaneously in the initial ''index'' cervical biopsy, such a case was also classified as progression.

All slides were re-reviewed to confirm the diagnosis. Of the 584 LSIL cases with clinical follow up, 328 (57%) cases had enough remaining lesional tissue available for both HPV detection and immunohistochemical studies.

2.2. HPV DNA amplification and genotyping

HPV testing was performed on all available 328 cases of LSIL. Tissue digestion and DNA release were performed using standard methods. Briefly, 250 μL of the proteinase K solution was added to the 1.5 mL tubes containing tissue sections and incubated for 18 h at 70 °C. Proteinase K was subsequently inactivated at 95 °C for 10 min. 10 μL of the supernatant was used for PCR reaction.

Broad-spectrum HPV DNA amplification and genotyping was performed using HPV SPF10 PCR-DEIA-LiPA25, version 1 (Labo Biomedical Products, Rijswijk, The Netherlands). The PCR-products were analyzed on a 3% agarose gel. All HPV gel-positive samples were further genotyped using HPV LiPA25, version 1 kit (Labo Biomedical Products, Rijswijk, the Netherlands). This system is a reverse hybridization method that can identify 15 high risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66 and 68/73, and 10 low risk HPV types 6, 11, 34, 40, 42, 43, 44, 54, 70 and 74 [5].

2.3. Immunohistochemistry

Immunostaining was performed on 3-μm tissue sections from randomly computer-selected cases; 238 LSIL cases were immunostained for Ki-67 and p16, and 101 cases were stained for pRb and p53.

The sections were subjected to heat-induced antigen retrieval and incubated in an automated stainer with p16 antibody (Dako, Glostrup, Denmark) at a dilution of 1:25; p53 antibody (Dako, Glostrup, Denmark) at a dilution of 1:200; pRB antibody (Dako, Glostrup, Denmark) at a dilution of 1:250; and Ki-67 antibody (Zymed, San Francisco, CA) at a dilution of 1:50. For p16 the staining was graded as either ''no overexpression'' (completely negative or weak, focal nuclear or cytoplasmic blush), or ''overexpression'' (moderate to strong intensity nuclear and cytoplasmic staining with diffuse or patchy distribution). For p53 and pRB the staining was graded in the basal and parabasal epithelial layer as either ''retained staining'' (strong nuclear staining in >50% of basal and parabasal cells) or ''loss of staining'' (no staining or only spotty staining in the basal and parabasal cells). For Ki-67 the staining was assessed semi-quantitatively in three epithelial strata – lower, middle and upper 1/3 of the epithelial thickness. Presence of strongly staining nuclei was expressed as a percentage of all nuclei, and rounded up to the closest tenth percentage.

2.4. Statistical analyses

The two-sample *t*-test was used to compare age at diagnosis between groups and the Pearson's chi-square test or Fisher's exact was used, as appropriate, to assess relationships between categorical variables. All *p*-values are two-sided with statistical significance evaluated at the 0.05 alpha level. All analyses were performed in SPSS Version 21.0 (SPSS Inc., Chicago, IL).

3. Results

A total of 584 cervical biopsies with the diagnosis of LSIL and 2-year follow-up were identified, including 336 (57.5%) LSIL that regressed, 116 (19.8%) persistent LSIL and 132 (22.6%) LSIL that progressed to HSIL within 2 years. Age analysis was performed on all 584 cases, and out of these cases, 328 had available tissue material for HPV testing and immunohistochemical analysis.

3.1. Age analysis

The mean age of patients at index LSIL diagnosis was 29.8 years in the regression group and 33.2 years in the persistence/progression group. The difference was statistically significant (*t*-test, $p = 0.001$).

In the age group of 10–19 years, 61.4% of LSIL lesions regressed, compared to 66.8%, 51.6%, 44.4% and 45.7% in the age groups of

Table 1
Regression, persistence and progression of LSIL in different age groups.

Age group	*n*	LSIL regression % (*n*)	LSIL persistence % (*n*)	LSIL progression % (*n*)	OR of persistence (95% CI)	OR of progression (95% CI)	OR of persistence or progression (95% CI)	*p*[*]
10–19	44	61.4% (27)	15.9% (7)	22.7% (10)	1.19 (0.48–2.94)	1.33 (0.60–2.94)	1.27 (0.65–2.46)	0.483
20–29	247	66.8% (165)	14.6% (36)	18.6% (46)	1.00	1.00	1.00	[**]
30–39	186	51.6% (96)	23.1% (43)	25.3% (47)	2.05 (1.23–3.42)	1.76 (1.09–2.83)	1.89 (1.28–2.79)	0.001
40–49	72	44.4% (32)	30.6% (22)	25.0% (18)	3.15 (1.64–6.05)	2.02 (1.04–3.92)	2.52 (1.47–4.30)	0.0005
50+	35	45.7% (16)	22.9% (8)	31.4% (11)	2.29 (0.91–5.76)	2.47 (1.07–5.68)	2.39 (1.17–4.89)	0.014
All age groups	584	57.5% (336)	19.8% (116)	22.6% (132)	–	–	–	

OR = odds ratio, CI = confidence intervals.
[*] χ^2 test of regression versus persistence or progression.
[**] Reference group.