Clinical Management and Follow-up of Squamous Intraepithelial Cervical Lesions during Pregnancy and Postpartum

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Abstract. *Background: The incidence of cervical cancer in pregnancy is estimated to be 1-10/10000 pregnancies. Approximately 3% of cervical cancers are diagnosed during pregnancy. The incidence of abnormal Pap smears has been reported to be 5%-8%. Data on the spontaneous evolution of an intraepithelial neoplasia during pregnancy are quite diverse. Of dysplasia cases diagnosed during pregnancy, 10%-70% regress and sometimes even disappear postpartum, while persistence in the severity of cervical neoplasia is reported in 25%-47% and progression occurs in 3%-30%. However, adequate follow-up and definitive management in the postpartum period is important. The objective of the study was to assess proper management of squamous intraepithelial lesion (SIL) during and after pregnancy, to assess regression, persistence and risk of progression and the predictive role of HPV tests. Materials and Methods: Thirty-one out of 721 pregnant women with a diagnosis of low- and high-grade SIL were observed. All patients were triaged using standard colposcopy. The histological diagnosis was assessed by colposcopic direct biopsies. In patients affected by high-SIL with colposcopic findings of suspected micro-invasive lesions, a loop electrosurgical excisional procedure (LEEP) was carried out in pregnancy. High risk HPV tests were performed using PCR. The patients were followed up with cytology and colposcopy every 6-8 weeks during gestation and nine weeks postpartum. They were re-evaluated using cytology, colposcopy and histology for a final diagnosis and, when necessary, submitted to treatment. The patients were followed up for a minimum of 5 years. The HPV test was performed once at 6-8 weeks during gestation and annually during the follow-up. Results: Of the 31 patients with abnormal cytology, histological analysis revealed 10 cervical intraepithelial neoplasia (CIN) 1, 5 CIN 2 and 16 CIN 3. The HPV test at diagnosis was positive for HPV 16 type in 22 cases and negative in 9. Five patients with CIN 2 and 11 with CIN 3 were followed up; 5 patients with CIN 3 with colposcopic findings of suspected microinvasive lesions were submitted to an excisional procedure with LEEP before the 16th week of pregnancy. Conclusion: Performing high-risk HPV tests may improve the follow-up of patients with SIL in pregnancy and postpartum in addition to cytology and colposcopy to indicate persistence/progression of the lesions. Proper management and adequate follow-up could be proposed in pregnancy and postpartum.*

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The majority of pregnancies occur between the ages of 18 and 35 years, corresponding to the age range associated with the greatest incidence of cervical intraepithelial neoplasia (CIN)(1). The incidence of cervical cancer in pregnancy is estimated to be 1-10/10000 pregnancies, depending on the inclusion of carcinoma *in situ* and postpartum patients (2, 3). Approximately 30% of women diagnosed with cervical cancer are in their reproductive years (2), and 3% of cervical cancer cases are diagnosed during pregnancy (2).

The incidence of abnormal Pap smears and human papillomavirus has been reported as 5-8% in pregnant and non pregnant women (2-7). As in the non-pregnant population, the pathological subtype is squamous in most cases (>80%) and the remainder are adenocarcinomas (9, 10). Data on the spontaneous evolution of intraepithelial neoplasias during pregnancy are quite diverse.

Studies report that 10%-70% of dysplasia cases diagnosed during pregnancy regress and sometimes even disappear postpartum (3, 6, 7, 10-13), while persistence in the severity of cervical neoplasia is reported in 25%-47% of cases (3, 6, 11) and progression in 3%-30% of cases (3, 10-12).

Suspect microinvasive lesions should be treated by an excisional procedure, until the 20th-24th week of pregnancy (14). However, adequate follow-up and definitive management should be provided in the postpartum period.

The objective of this study was to assess the management of squamous intraepithelial lesion (SIL) during and after pregnancy, to evaluate regression, persistence and risk of progression, and the predictive role of HPV tests.

Materials and Methods

From 1993 to 2000, 31 out of 721 pregnant women attended the Department of Gynaecology, Perinatology and Child Health, University "La Sapienza", and the Department of Obstetrics and Gynaecology, Catholic University, with a diagnosis of low and high grade SIL. Cytological diagnosis was formulated in agreement with the Bethesda System (15). A structured questionnaire was administered regarding sociodemographic, gynaecological, obstetric and sexual behavioural characteristics.

All patients were triaged with standard OM50 Zeiss colposcopy (Carl Zeiss, Oberkochen Germany) using a 5% acetic acid solution followed by a Lugol test. The colposcopic findings were interpreted according to the International Nomenclature (16).

The histological diagnosis was assessed with colposcopic direct biopsies in the areas revealing the greatest degree of abnormality. In patients affected by high-SIL with colposcopic findings of suspect microinvasive lesions, a loop electrosurgical excisional procedure (LEEP) was carried out during gestation.

High risk HPV tests (type 16 and 18) were performed using a polymerase chain reaction (PCR). Cytological samples were collected in sterile polypropylene tubes and resuspended in 100 μl of digestion buffer with proteinase K, incubated overnight at 37˚C, and boiled for 5 min. Aliquots (10 μl) of each were used for PCR amplification. Each cytological sample was analyzed using PCR for HPV open reading frame sequences using the following primers: HPV-16: 5'-ACC gAA ACC ggT Tag TATAAAAgC-3' and 3'-gAT CAT TTg TCT CTg gTT gCA AAT-5'; HPV-18: 5'-CAC ACC ACA ATA CTA Tgg CgCgCT-3' and 3'-CTg CTg gAT TCA ACg gTT TCT ggC-5'. Every amplification experiment included one negative and one positive control for each viral type. A portion of exon 15 of the human APC gene was routinely amplified as a positive control using the following primers: APC: 5'-gTCCTTCACAgAAt gAAAgATg-3' and 3'-CTg CTT gAA gAA gAC ATA TgTTCg-5'. The size of the amplified fragments were 576, 360 and 520 bp, respectively. Amplification reactions were carried out in 100 μl of reaction buffer containing 50 mM KCl, 2 mM $MgCl_2$, 10 mM Tris (pH 8.3), 200 μM each deoxynucleotide triphosphate, 2.5 units of Taq DNA polymerase (Perkin-Elmer-Cetus, Norwalk, CT, USA), 100 pmol of each primer, and 10 μl of proteinase K-digested sample. Samples were denatured at 95˚C for 5 min, followed by 40 cycles of amplification (denaturation at 94˚C for 1.5 min, annealing at 55˚C for 2 min, except APC, where annealing was at 40˚C and 57˚C, respectively, with extension at 72˚C for 2 min; the final extension was prolonged to 7 min).

Amplified products (15 μl) were electrophoresed through 1.6% agarose gels. The gels were analyzed using UV after staining with ethidium bromide (17).

The patients were followed up using cytology and colposcopy every 6-8 weeks during gestation and nine weeks postpartum. The patients were re-evaluated with cytology, colposcopy and histology for a final diagnosis and, when necessary, submitted to treatment and followed up for a minimum of 5 years.

An HPV test was performed once at 6-8 weeks of gestation and then annually during the follow-up.

Table I. *Cytology, histology and HPV test results.*

Initial cytology	Histology	HPV test	
Number of patients (%)		Positive	Negative
Low SIL 10 (33%)	CIN 1 10 (33%)	1	9
	CIN 2 5 (16%)	5	-
High SIL 21 (67%)	CIN 3 16 (51%)	16	-
Total: 31		22	9

SIL=squamous intraepithelial lesion; CIN=cervical intraepithelial lesion.

Results

Of the 31 patients with abnormal cervical cytology, in 10 cytology revealed low-grade SIL and high-grade SIL in 21.The mean gestational age at diagnosis was 16 weeks (5-27).

The mean age of the patients was 31 years (22-38), the mean parity 1.05 (0-4), mean age for first sexual intercourse was 17 years (14-28), mean number of sexual partners was 3 (1-8) and 47% reported tobacco use.

In each of the 31 patients, colposcopy visualized an entirely detectable squamous columnar junction (SCJ) and an ectocervical abnormal transformation zone (ANTZ). Ten (33%) ANTZ were grade I of abnormality and 21 (67%) were grade II.

Histological analysis of colposcopy-directed biopsies revealed 10 (33%) cases of CIN 1, 5 (16%) of CIN 2 and 16 (51%) of CIN 3.

The HPV test at diagnosis was positive for HPV 16 in 22 cases (71%): 1 CIN 1, 5 CIN 2 and 16 CIN 3, and HPV-negative in 9 (29%) CIN 1 (Table I).

All patients affected by CIN 1 were followed up during the pregnancy and postpartum for a minimum of 5 years.

A further 5 patients with CIN 2 and 11 with CIN 3 were followed up; 5 cases of CIN 3 associated with cytological and colposcopic abnormalities suggestive of microinvasive lesions were submitted to an excisional procedure with LEEP before the 16th week of pregnancy.

In 4 patients the final histological analysis of the excised specimens confirmed the degree of the lesions that had been characterized at the previous biopsy. In one case, the final histological analysis revealed a microinvasive carcinoma (stage IA1, invasion <1 mm).

The excisional procedure did not modify the duration of pregnancy, its outcome or delivery. The first follow-up check was performed 9 weeks after delivery and provided negative cytological and colposcopic findings. Only the case affected by a microcarcinoma was submitted, at 9 weeks postpartum, to cold-knife conization. The final histological diagnosis

revealed a residual CIN 3 lesion. At five years follow-up the check showed normal cytology and colposcopy findings.

On the first and second follow-up check during pregnancy: CIN 1 lesions (10 pts) whether HPV-test negative or HPV-test positive were confirmed with grade I colposcopic findings and abnormal cytology; CIN 2 (5 pts) and CIN 3 lesions (11 pts) were confirmed with grade 2 colposcopic findings and abnormal cytology.

Postpartum at the first follow-up check, a CIN 1 lesion, HPV test-positive, showed a colposcopic grade 2, with abnormal cytology and histology determined by subsequent colposcopy-directed biopsy revealing a CIN 2 lesion. The patient was submitted to LEEP and analysis of the excised specimens confirmed the degree of the lesion. Nine patients affected by CIN 1 lesions at the same time revealed negative cytological and colposcopic findings.

Two CIN 2 lesions had regressed at the first postpartum follow-up check; 3 CIN 2 lesions were confirmed with abnormal cytological and colposcopic findings and excised specimens using LEEP confirmed the degree of the lesion.

Eight out of 11 CIN 3 at the first postpartum check revealed negative cytological and colposcopic findings and 3 out of 11 at the same check were confirmed with abnormal cytological and colposcopic findings and were submitted to an excisional procedure using LEEP; the histological specimen confirmed the degree of the lesion.

The 5-year follow-up check revealed negative cytological and colposcopic findings in all patients.

Discussion

An increasing incidence of high-grade squamous intraepithelial lesion (HSIL) has been observed among young women. Consequently, an increased number of cases are being discovered during pregnancy. In fact, pregnancy is an opportune occasion to submit patients who have never had Pap smears in the past to cytological cervico-vaginal examination.

Controversies exist on the effect of pregnancy on human papillomavirus (HPV) infection. Some studies suggest the possibility that the physiological process of pregnancy modifies certain characteristics of the mother, increasing the risk of both infection and persistence of infection with HPV. Whether pregnancy has an influence on the course of the neoplasia remains unsolved.

Recently some authors refuted the hypothesis that the cervical trauma associated with delivery may induce regression in the CIN lesion, since they found no correlation between route of delivery and persistence of disease (7).

The normal physiological alterations of the cervix make colposcopy in pregnancy difficult and it requires a high degree of expertise and experience on the part of the colposcopist. The most important purpose of colposcopy is to exclude invasive disease. On the one hand, pregnancy tends to exaggerate the colposcopic appearance of CIN, which might give rise to overdiagnosis (18). On the other hand several studies have shown that colposcopy alone without directed biopsy carries a significant risk of underestimating the severity of the lesion (7, 19, 20).

Cervical biopsies can be performed during pregnancy; if the biopsy shows normal histology, re-evaluation can be deferred until 6-12 weeks postpartum. However a conservative management is proposed and adequate follow-up and definitive management in the postpartum period should be provided (21). Suspect microinvasive lesions should be treated by an excisional procedure, no later than the 20th-24th week of pregnancy (14).

It is debatable whether the LEEP performed before 16 weeks influences pregnancy outcome. The percentage of abortions among those women who did not undergone treatment exceeds those who did (22, 23), however LEEP does not influence the outcome of successive pregnancies (24-26). Cervical conization during pregnancy is associated with a significant morbidity for both mother and fetus. Unfortunately, the introduction of loop excision of the cervix has not been able to decrease the morbidity of the traditional cold-knife conization in pregnancy. Recently Robinson (27) reported a complication rate of 25% following loop excision during pregnancy. As for cold-knife conization, the complications were due to transfusion-requiring haemorrhage, abortion and premature labor and delivery (27). In our series, 5 patients were treated using LEEP in early pregnancy. The procedure did not modify the duration of pregnancy, its outcome or delivery. The majority of LSILs will regress or remain stable during pregnancy. The HSILs will persist or remain stable during pregnancy and in postpartum and may even progress to invasive carcinoma.

Performing high-risk HPV tests may improve the follow-up of patients with SIL in pregnancy and postpartum in addition to cytology and colposcopy to indicate persistence/progression of the lesions.

Proper management and adequate follow-up should be proposed including cytology, colposcopy and HPV test in pregnancy and postpartum.

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References

1 Kaplan KJ, Dainty LA, Dolinsky B, Rose GS and Carlson J: Prognosis and recurrence risk for patients with cervical squamous intraepithelial lesions diagnosed during pregnancy. Cancer *25*: 228-232, 2004.

2 Nguyen C, Montz FJ and Bristow RE: Management of stage I cervical cancer in pregnancy. Obstet gynecol Surv *55*: 633-643, 2000.
3 Palle C, Bangsboll S and Andreasson B: Cervical intraepithelial neoplasia in pregnancy. Acta Obste Gynecol Scand *79*: 306-310, 2000.
4 Campion MJ and Sedlacek TV: Colposcopy in pregnancy. Obstet Gynecol Clin North Am *20*: 153-163, 1993.
5 Kaminski PF, Lyon DS, Sorosky JL, Wheelock JB and Podzasky ES: Significance of atypical cervical cytology in pregnancy. Am J Perinatal *9*: 340-343, 1992.
6 Vlahos G, Rodolakis A, Diakomanolis E, Stefanidis K, Haidopoulos D, Abela K, Georgountzos V and Michalas S: Conservative management of cervical intraepithelial neoplasia (CIN (2-3)) in pregnant women. Gynecol Obstet Invest *54*: 78-81, 2002.
7 Coppola A, Sorosky J, Casper R, Anderson B and Buller RE: The clinical course of cervical carcinoma *in situ* diagnosed during pregnancy. Gynecol Oncol *67*: 162-165, 1997.
8 Jones WB, Shingleton HM, Russell A, Fremgen AM, Clive RE, Winchester DP and Chmiel JS: Cervical carcinoma and pregnancy. A national patterns of care study of the American College of Surgeons. Cancer *77*: 1479-1488, 1996.
9 Sood AK, Sorosky JI, Mayr N, Krogman S, Anderson B, Buller RE and Hussey DH: Radiotherapeutic management of cervical carcinoma that complicates pregnancy. Cancer *80*: 1073-1078, 1997.
10 Baldauf JJ, Dreyfus M, Ritter J and Philippe E: Colposcopy and directed biopsy reliability during pregnancy: a cohort study. Eur J Obstet Gynecol Reprod Biol *62*: 31-36, 1995.
11 Yost NP, Santoso JT, Mcintire DD and Iliya FA: Postpartum regression rates of antepartum cervical intraepithelial neoplasia II and III lesions. Obstet Gynecol *93*: 359-362, 1999.
12 Douvier S, Filipuzzi L and Sagot P: Management of cervical intra-epithelial neoplasm during pregnancy. Gynecol Obstet Fertil *31*: 851-855, 2003.
13 Siddiqui G, Kurzel RB, Lampley EC, Kangs HS and Blankstein J: Cervical dysplasia in pregnancy: progression *versus* regression post-partum. Int J Fertil Womens Med *46*: 278-280, 2001.
14 Robova H, Rob L, Pluta M, Kacirek J, Halaska M Jr, Strand P and Schlegerova D: Squamous intraepithelial lesion-microinvasive carcinoma of the cervix during pregnancy. Eur J Gynaecol Oncol *26*: 611-614, 2005.
15 The Revised Bethesda System for reporting cervical/vaginal cytologic diagnoses: report of the 1991 Bethesda workshop. J Reprod Med *37*: 383-386, 1992.
16 Stafi A and Wilbanks GD: An international terminology of colposcopy: report of the Nomenclature Committee of the International Federation of Cervical Pathology and Colposcopy. Obstet Gynecol *77*: 313-314, 1991.
17 Vecchione A, Zanesi N, Trombetta G, French D, Visca P, Pisani T, Botti C, Vecchione A, Croce CM, and Mancini R: Cervical displasia, ploidy, and human papillomavirus status correlate with loss of Fhit expression. Clin Cancer Res *7*: 1306-1312, 2001.
18 Ostergard DR and Nieberg RK: Evaluation of abnormal cervical cytology during pregnancy with colposcopy. Am J Obstet Gynecol *134*: 756-758, 1979.
19 Benedet JL, Selke PA and Nickerson KG: Colposcopic evaluation of abnormal Papanicolaou smear in pregnancy. Am J Obstet Gynecol *157*: 932-937, 1987.
20 Economos K, Veridiano NP, Delke I, Collado ML and Trancer Ml: Abnormal cervical cytology in pregnancy, a 17-year experience. Obstet Gynecol *81*: 915-918, 1993.
21 Murta EF, de Souza FH, de Souza MA and Adad SJ: High-grade cervical squamous intraepithelial lesion during pregnancy. Tumori *88*: 246-250, 2002.
22 Mitsuashi A, and Sekiya S: Loop eletrosurgical excision procedure (LEEP) during first trimester of pregnancy. Int J Gynecol Ostet *71*: 237-239, 2000.
23 Penna C, Fallani MG, Maggiorelli M, Zipoli E, Cardelli A and Marchionni M: High-grade cervical intraepithelial neoplasia (CIN) in pregnancy: clinicotherapeutic management. Tumori *84*: 567-570, 1998.
24 Crane JM: Pregnancy outcome after loop electrosurgical excision procedure: a systematic review. Obstet Gynecol *102*: 1058-1062, 2003.
25 Althuisius SM, Schornagel IJ, Dekker GA, van Geijn HP and Hummel P: Loop electrosurgical excision procedure of the cervix and time of delivery in subsequent pregnancy. Int J Gynaecol Obstet *72*: 31-34, 2001.
26 Paraskevaidis E, Koliopoulos G, Lolis E,Papanikou E, Malamou-Mitsi V and Agnantis NJ: Delivery outcomes following loop electrosurgical excision procedure for microinvasive (FIGO stage IA1) cervical cancer. Gynecol Oncol *86*: 10-13, 2002.
27 Robinson RR, Webb S, Tirpack J, Degefu S and O'Quinn AG: Management of cervical intraepithelial neoplasia during pregnancy with LOOP excision. Gynecol Oncol *64*: 153-155, 1997.

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