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The diagnostic algorithm in pre-invasive cervical lesions

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ABSTRACT

The screening for pre-invasive cervical lesions has significantly decreased the incidence of cervical neoplasm. It is recommended to be performed starting with the age of 21 with a frequency of 3-5 years and it consists of pap smear testing and HPV genotyping, and, if required, it can be continued with colposcopy or biopsy followed by pathological assessment. The importance of the early diagnosis of pre-invasive cervical lesions has led to several studies on this topic. The paper analyzed the modern literature published on the PubMed and Scopus databases. Reference studies have found that most intraepithelial lesions are caused by the presence of HPV. Other commonly associated factors are immunosuppression, multiparity and other viral infections. HPV infection can be prevented by vaccination. It is recommended for people between 11 and 26 years old and also over 27 years old if they associate risk factors. A meta-analysis performed on patients diagnosed with CIN2 revealed a lower recurrence rate in vaccinated women than in unvaccinated women. Other studies have shown the transient nature of HPV infection and spontaneous regression of pre-invasive lesions. The early diagnosis of pre-invasive lesions is necessary for the initiation of therapeutic and follow-up behavior as soon as possible, with the aim of reducing the incidence of cervical cancer. This is possible and easy to access through national health programs.

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Introduction

The early diagnosis of pre-invasive cervical lesions has been a challenge since the twentieth century. National screening programs have been developed to reduce the incidence of cervical cancer, but this pathology remains a public health problem. In the United States, the incidence of malignant tumor pathology of the cervix has decreased since the 1970s, after the evolution of the first national screening programs [1,2].

Intraepithelial cervical lesions (CIN) are premalignant lesions of non-keratinized multilayered epithelial covering tissue [3]. The cervix consists of endocervix (cylindrical epithelium) and exocervix (squamous epithelium). The screening for pre-invasive and invasive cervical lesions includes Pap smear and HPV genotyping. Depending on the results, these investigations can be completed with colposcopic examination and pathological assessment [4,5].

In order to be able to diagnose these lesions at an early stage, every woman older than 21 years old has to undergo screening tests: Papanicolau smear (Pap smear) once every three years, or HPV genotyping together with Pap smear every 5 years [6,7].

Pap smear examines cervical cellularity, thus being able to diagnose malignant intraepithelial lesions, inflammation, atrophy, or other physiological and pathological changes in the cervical epithelium [8,9]. On the referral note of the cytological examination, one must specify the date of the last menstruation, the presence of intrauterine devices and pregnancy [10]. HPV genotyping seeks to multiply the HPV genome to identify viral strains [11].

For the diagnosis of intraepithelial cervical lesions, the colposcopic examination is also used, which analyzes the modifications of the external layer of the cervix and of the squamous-columnar junction. The investigation is indicated in case of unfavorable results at Pap smear

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examination or HPV genotyping [12]. During the colposcopic examination, a tissue fragment can be collected for the histopathological examination. This procedure does not require the hospitalization of the patient and it has few contraindications: cervicitis, hemorrhagic diathesis, vaginal bleeding, pregnancy and immunosuppression [13,14].

The next step in the diagnosis of the lesions is the pathological evaluation. Conization together with the diathermic loop electro resection and bioptome biopsy are reliable methods that provide tissue for the histopathological examination. Uterine curettage is not a routine indication if conization is performed. It is indicated only if there are glandular cell abnormalities on Pap smear or if they have risk factors for endometrial hyperplasia or endometrial adenocarcinoma [15].

The literature search was conducted in PubMed and Scopus databases using the terms “Cervical intraepithelial lesions”, “excisional procedures” and “screening and diagnostic for cervical lesions”. No date restriction was applied. Language was restricted to English and French. Additional studies from the reference list of the articles were searched.

Discussions

CIN occurs most frequently in the squamous-columnar junction, being secondary to the HPV infection. Most intraepithelial lesions are thought to be caused by HPV, but not all HPV infections cause CIN [16,17]. Besides the HPV infection, other risk factors are immunosuppression, diet, multiparity, first delivery before the age of 17 years, multiple sexual partners, lack of condom use, smoking, herpes virus infection. A study conducted by Anttila revealed that Chlamydia trachomatis has an implication in the development of pre-invasive lesions [18-21]. A study conducted by Aldieh revealed how a person living with HIV is affected by the co-infection with HPV. If left untreated, the HIV infection decreases the number of CD4 positive lymphocytes. On the other hand, an HIV positive undetectable person has a pro-inflammatory status [22]. Another important exponent is smoking, which releases nicotine-derived ketones that lead to decreased local immunity [23].

The HPV infection is a sexually transmitted infection, and 75-80% of sexually active people have had the infection. Its natural evolution has several stages: latent infection (minimal clinical, colposcopic and histopathological changes) is the most common form of HPV infection. Active infection, without genomic integration (the virus replicates intensely, cell nuclei become large, multinucleated and hyperchromatic with a halo around the nucleus) corresponds to low-grade lesions (L-SIL) on the cytological evaluation. The evolution at this stage depends on the host's immune system. Antibodies directed against viral particles are produced by CD4 + T lymphocytes and macrophages. Most people produce these antibodies efficiently, so the evolution is favorable.

The last stage in the evolution of the infection is the genomic integration, which corresponds to the malignant tumor pathology [24-28].

Clinical presentation

Intraepithelial lesions do not have a characteristic symptomatology, most often we encounter abnormal vaginal bleeding, changes in bowel movement, changes in bladder function, pelvic pain syndrome, dyspareunia, macroscopic lesions on the exocervix [29-31].

Paraclinical evaluations

The diagnosis of pre-invasive cervical lesions uses multiple paraclinical evaluations.

Pap smear

Pap smear examines cervical cellularity for abnormal cells. It is a screening method that must be done every three years. The appearance of a lesion on a properly performed screening is very small: 10-> 66 / 10,000. It is not recommended to be performed annually, as it does not decrease the risk of death from cervical cancer. A study conducted by Sawaya et al. covering patients aged 21 to 29 years revealed that the risk of death from cervical cancer is identical, regardless of whether the cytological evaluation is done at one, two or three years [6,10,32].

The evaluation is not done during menstruation. It is important not to touch the exocervix when inserting the vaginal speculum, as dysplastic cells can attach to the speculum. Although during menstruation it is recommended not to perform the examination, it should not be delayed in case of cervicorrhagia, metrorrhagia or abnormal secretion because they can be symptoms of a malignant pathology of the cervix.

On the referral note of the cytological examination, it is important to mention the date of the last menstruation, because the appearance of the vaginal and cervical epithelium varies with the ovarian cycle. Another thing to mention is pregnancy, exogenous hormone therapy, metrorrhagia, a history of dysplastic lesions, menopause, the use of intrauterine devices (IUD). In the case of IUD, it is recommended to take cells from the anterior vaginal wall as well, as these patients have an increased risk of developing vaginal cancer [33]. The Bethesda 2014 classification is used to provide the cytological result. Intraerpithelial lesions are subdivided into several groups. Squamous cell atypia-like lesions (AUC): lesions of determined significance (AUC-US) or lesions that cannot rule out a high-grade lesion (AUC-H). Low-grade lesions L-SIL include CIN 1, HPV infection, mild dysplasia. High-grade lesions: H-SIL include severe, moderate dysplasia, CIN2, CIN3, carcinoma in situ and the invasive lesion represents squamous cell carcinoma of the cervix [34,35].

The HPV genotyping can be done alone or together with Pap smear once every 5 years. The most common strains 16, 18 are routinely tested on the cytological examination. It is useful to do it alone in patients who do not have access to screening services [11,34,36].

Colposcopy

Colposcopy is used to evaluate the cervix. It is indicated in case of macroscopically visible genital tract lesions, abnormal cytological evaluation, positive HPV evaluation, intrauterine exposure to diethylstilbestrol, unexplained genital tract bleeding [12,33]. The examination can confirm if one has vulgar warts, cervicitis, pre-invasive lesions of the cervix, vagina or vulva.

It has few risks; it allows the biopsy to be performed. Among the risks that arise after tissue sampling are: bleeding, infection and pelvic pain syndrome. Pre-procedural training includes: no sexual intercourse 24-48 hours before, no intravaginal tampons should be used 1-2 days before, no intravaginal medication and no menstruation [37,38]. The examinations that are performed: the native macroscopic evaluation of the cervix, the examination with 3-5% acetic acid, which stains the aceto-white lesions, the Lahm-Schiller test using lugol. Normal cells have estrogen receptors; thus, those cells have glycerol in their cytoplasm. On the other hand, dysplastic cells lack these receptors and they have a lower quantity of glycerol. The last evaluation on colposcopy finds dysplastic cells using Lugol (an iodine solution). If the cells have glycerol, iodine will impregnate them. The last stage is omitted in the case of patients allergic to iodine or contrast agents. The results that suggest a cervical lesion are: acetone-white epithelium, mosaic, punctures and leukoplakia.

The study conducted by Sophia S. analyzed the incidence of malignant tumor subtypes located in the cervix, and as a result of the screening programs, the incidence of squamous cell carcinoma of the cervix is decreasing, but the lack of experience in endocervical cell evaluation has led to an increase of endocervical adenocarcinomas [12,33].

Conization

There are also invasive diagnostic methods: conization with the cold scalpel, conization with the electrocautery or excision with the diathermic loop.

Conization with the cold scalpel offers a good piece for histopathological evaluation, it is simple, it requires general or local anesthesia, but it has a higher complication rate than other methods [39]. The patient is placed in a lithotomy position, the vaginal speculum is mounted, the colposcopic evaluation or Lugol can be used to observe the areas with lesions. Two sutures can be made at 3 and 9 o'clock to limit bleeding. Sometimes it is useful to inject vasopressors which lead to a decrease in the intraoperative bleeding, in the absence of contraindications (hypertension). A circumferential incision is made that goes as deep as possible, it is excised and the neck is restored with Strumdorf suture [33].

Conization with the electrocautery offers a good specimen, good hemostasis and it has a lower complication rate, but it is more difficult, it can cause thermal damage in the adjacent structures, it is difficult to make a small radius cone [39-41].

The loop electrosurgical excision procedure is easy, fast, has few complications and provides a good piece for histopathological examination, but it can cause thermal damage to adjacent tissues and it is difficult to perform for a large area of tissue. Lidocaine can be used for local anesthetic purposes, but also to limit bleeding. It penetrates the cervix with a loop and in a single movement a fragment of the cervix is excised and sent for the histopathological examination [33,39,42].

A study conducted by Hu X looked at the rate of HPV infection among medical staff practicing electrocautery or diathermic excision. Procedures that lead to smoke generation are dangerous for doctors and nurses because they expose them to HPV that will be found to the nasal epithelium (the incidence is 9-10% among doctors who treat people positive for HPV, compared to 2- 3%). If medical personnel use KN95 masks, the infection was 0%. [43].

All these methods provide enough tissue for the pathological evaluation. The histopathological result is classified into CIN1, 2 and 3. CIN1 is also called L-SIL: mild, moderate dysplasia, located in the basal third, HPV infection, with an increased regression rate. High-grade intraepithelial lesions (H-SIL) are subdivided into CIN2 and CIN3. CIN2: moderate dysplasia in two basal thirds, CIN3: severe dysplasia in two basal thirds, but can affect the entire epithelium, a situation called carcinoma in situ. [44].

A study led by Bansal, which included 680 patients, analyzed the evolution of CIN1 lesions. At 6 months after diagnosis: 49% regressed spontaneously, 35% had CIN1 lesions, and 7% had a negative outcome. Out of the patients with negative lesions at 6 months, at the one-year reassessment: 80% had no lesions, 16% had low-grade intraepithelial lesions, and 4% had an unfavorable evolution. In the case of patients with positive lesions at 6 months, at the one-year reassessment: 50% did not present lesions, 46% CIN1, and 4% evolved negatively [45,46].

The study conducted by Tainio K revealed that CIN2 lesions in half of the cases regress without treatment. This meta-analysis covering 36 studies and including 3,160 patients showed that at the two-year evaluation: 50% underwent regression of the lesion, 32% had persistent lesions, and 18% progressed to CIN3, carcinoma in situ, invasive cervical carcinoma [47].

CIN3 lesions may regress spontaneously in 32-47% of cases or may progress to invasive carcinoma in 12-40% of cases [48]. A study conducted by McCredie evaluated patients at 10 years and 30 years, comparing the patients' evolutions according to the treatment undergone. In the case of patients who opted for the expectant attitude, 20% developed invasive carcinoma at 10 years, and 31% developed malignant tumor pathology at 30 years. The patients who opted for the surgical treatment had an incidence of 0.3% at 10 years and 0.7% at 30 years [49].

HPV infection can be prevented by vaccination. It is recommended for all people between 11 and 26 years old and, in carefully selected cases, for people over 27 years old. It is recommended for women with a history of dysplasia and patients with vulgar warts. Vaccination has no therapeutic effects, but a decrease in the risk of recurrence has been observed. A meta-analysis performed by de Villiers EM et al. revealed that the recurrence in vaccinated patients is 1.9% compared to 5.9% in non-vaccinated patients with CIN2 [50].

Conclusions

Most pre-invasive cervical lesions are caused by the HPV infection, but not every infection causes the lesions, so it is not advisable to do it routinely among the young HPV genotypic population.

The diagnosis of pre-invasive cervical lesions is eminently made through paraclinical investigations.

Pap smear is an effective method of diagnosis and screening that must be performed every three years, performing it earlier does not reduce the risk of mortality from cervical cancer.

If there is an abnormal Pap smear result, colposcopy is indicated for a thorough assessment of the squamous-column transit area. Pathological lesions with pathological appearance are leukoplakia, mosaicism, punctures, aceto-white epithelium.

Invasive examinations provide the basis for the histopathological analysis, thus providing a careful assessment of the endocervix.

Conflict of interest disclosure

There are no known conflicts of interest in the publication of this article. The manuscript was read and approved by all authors.

Compliance with ethical standards

Any aspect of the work covered in this manuscript has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

References

1. Nieminen P, Kallio M, Hakama M. The effect of mass screening on incidence and mortality of squamous and adenocarcinoma of cervix uteri. *Obstet Gynecol*. 1995; 85(6):1017-21. doi: 10.1016/0029-7844(95)00063-W
2. Iancu IV, Anton G, Botezatu A, Huica I, Nastase A, Socolov DG, Stanescu AD, Dima SO, Bacalbasa N, Plesa A. LINC01101 and LINC00277 expression levels as novel factors in HPV-induced cervical neoplasia. *J Cell Mol Med*. 2017 Dec;21(12):3787-3794. doi: 10.1111/jcmm.13288
3. Montz FJ. Management of high-grade cervical intraepithelial neoplasia and low-grade squamous intraepithelial lesion and potential complications. *Clin Obstet Gynecol*. 2000 Jun;43(2):394-409. doi: 10.1097/00003081-200006000-00018
4. Solomon D, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M, Raab S, Sherman M, Wilbur D, Wright T Jr, Young N; Forum Group Members; Bethesda 2001 Workshop. The 2001 Bethesda System: terminology
for reporting results of cervical cytology. *JAMA*. 2002;287(16):2114-9. doi: 10.1001/jama.287.16.2114
5. Luff RD. The Bethesda System for reporting cervical/vaginal cytologic diagnoses. Report of the 1991 Bethesda workshop. *Am J Clin Pathol*. 1992 Aug;98(2):152-4. doi: 10.1093/ajcp/98.2.152
6. Sawaya GF, Kerlikowske K, Lee NC, Gildengorin G, Washington AE. Frequency of cervical smear abnormalities within 3 years of normal cytology. *Obstet Gynecol*. 2000 Aug;96(2):219-23. doi: 10.1016/s0029-7844(00)00882-6
7. Feldman S, Haas JS. How the Coronavirus Disease-2019 May Improve Care: Rethinking Cervical Cancer Prevention. *J Natl Cancer Inst*. 2021 Jun 1;113(6):662-664. doi: 10.1093/jnci/djaa089
8. Bacalbasa N, Balescu I, Dimitriu M, Balalau C, Vilcu M, Brezean I. Does sentinel lymph node detection play a role in patients with vaginal cancer? *J Clin Invest Surg*. 2019;4(1):1-4. doi: 10.25083/2559.5555/4.1/1.4
9. Kulasingam SL, Havrilesky L, Ghebre R, Myers ER. Screening for Cervical Cancer: A Decision Analysis for the U.S. Preventive Services Task Force [Internet]. Rockville (MD): Agency for Healthcare Research and Quality (US); 2011 May. Report No.: 11-05157-EF-1
10. Screening for squamous cervical cancer: duration of low risk after negative results of cervical cytology and its implication for screening policies. IARC Working Group on evaluation of cervical cancer screening programmes. *Br Med J (Clin Res Ed).* 1986;293(6548): 659-64. doi: 10.1136/bmj.293.6548.659
11. Winer RL, Lin J, Tiro JA, Miglioretti DL, Beatty T, Gao H, Kimbel K, Thayer C, Buist DSM. Effect of Mailed Human Papillomavirus Test Kits vs Usual Care Reminders on Cervical Cancer Screening Uptake, Precancer Detection, and Treatment: A Randomized Clinical Trial. *JAMA Netw Open*. 2019;2(11): e1914729. doi: 10.1001/jamanetworkopen.2019.14729

12. Moyer VA; U.S. Preventive Services Task Force. Screening for cervical cancer: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med*. 2012 Jun 19;156(12):880-91, W312. doi: 10.7326/0003-4819-156-12-201206190-00424
13. Kulkarni A, Policarpio M, Strub S, Jembere N, Kupets R. Performance Indicators for Colposcopy in Ontario. *J Obstet Gynaecol Can*. 2020 Feb;42(2):144-149.e1. doi: 10.1016/j.jogc.2019.07.002
14. Pană M, Sima RM, Bălălău OD, Stănescu AD, Pleş L, Poenaru MO. The quality of sexual life after vaginal surgical interventions. *J Mind Med Sci*. 2020;7(2):201-205. doi: 10.22543/7674.72.P201205
15. Rubin SC, Battistini M. Endometrial curettage at the time of cervical conization. *Obstet Gynecol*. 1986 May;67(5):663-4. doi: 10.1097/00006250-198605000-00011
16. Aitken CA, Siebers AG, Matthijsse SM, Jansen EEL, Bekkers RLM, Becker JH, Ter Harmsel B, Roovers JWR, van Kemenade FJ, de Kok IMCM. Management and treatment of cervical intraepithelial neoplasia in the Netherlands after referral for colposcopy. *Acta Obstet Gynecol Scand*. 2019 Jun;98(6):737-746. doi: 10.1111/aogs.13547
17. Mitra A, Tzafetas M, Lyons D, Fotopoulou C, Paraskevaidis E, Kyrgiou M. Cervical intraepithelial neoplasia: screening and management. *Br J Hosp Med (Lond)*. 2016 Aug 2;77(8):C118-23. doi: 10.12968/hmed.2016.77.8.C118
18. Heard I. Prevention of cervical cancer in women with HIV. *Curr Opin HIV AIDS*. 2009 Jan;4(1):68-73. doi: 10.1097/COH.0b013e328319bcbe
19. Anttila T, Saikku P, Koskela P, Bloigu A, Dillner J, Ikäheimo I, Jellum E, Lehtinen M, Lenner P, Hakulinen T, Närvänen A, Pukkala E, Thoresen S, Youngman L, Paavonen J. Serotypes of Chlamydia trachomatis and risk for development of cervical squamous cell carcinoma. *JAMA*. 2001 Jan 3;285(1):47-51. doi: 10.1001/jama.285.1.47
20. Puca BM, Braila AD, Obleaga CV, Braila M, Saad H, Lungulescu C, Deca M. Conservative surgical treatment in cervical dysplastic lesions associated with cystorectocele. *J Mind Med Sci*. 2019;6(2):340-345. doi: 10.22543/7674.62.P340345
21. Herrington CS. Human papillomaviruses and cervical neoplasia. II. Interaction of HPV with other factors. *J Clin Pathol*. 1995 Jan;48(1):1-6. doi: 10.1136/jcp.48.1.1
22. Ahdieh L, Muñoz A, Vlahov D, Trimble CL, Timpson LA, Shah K. Cervical neoplasia and repeated positivity of human papillomavirus infection in human immunodeficiency virus-seropositive and -seronegative women. *Am J Epidemiol*. 2000;151(12): 1148-57. doi: 10.1093/oxfordjournals.aje.a010165
23. Sasson IM, Haley NJ, Hoffmann D, Wynder EL, Hellberg D, Nilsson S. Cigarette smoking and neoplasia of the uterine cervix: smoke constituents in cervical mucus. *N Engl J Med*. 1985 Jan 31;312(5):315-6. doi: 10.1056/nejm198501313120516
24. Nucci MR, Crum CP. Redefining early cervical neoplasia: recent progress. *Adv Anat Pathol*. 2007 Jan;14(1):1-10. doi: 10.1097/PAP.0b013e31802e0de7
25. Wang R, Pan W, Jin L, Huang W, Li Y, Wu D, Gao C, Ma D, Liao S. Human papillomavirus vaccine against cervical cancer: Opportunity and challenge. *Cancer Lett*. 2020;471:88-102. doi: 10.1016/j.canlet.2019.11.039
26. Carter JJ, Koutsky LA, Hughes JP, Lee SK, Kuypers J, Kiviat N, Galloway DA. Comparison of human papillomavirus types 16, 18, and 6 capsid antibody responses following incident infection. *J Infect Dis*. 2000 Jun;181(6):1911-9. doi: 10.1086/315498
27. Motofei IG, Rowland DL, Georgescu SR, Tampa M, Paunica S, Constantin VD, Balalau C, Manea M, Baleanu BC, Sinescu I. Post-Finasteride Adverse Effects in Male Androgenic Alopecia: A Case Report of Vitiligo. *Skin Pharmacol Physiol*. 2017;30(1):42-45. doi: 10.1159/000455972
28. Bontkes HJ, de Gruijl TD, Walboomers JM, Schiller JT, Dillner J, Helmerhorst TJ, Verheijen RH, Scheper RJ, Meijer CJ. Immune responses against human papillomavirus (HPV) type 16 virus-like particles in a cohort study of women with cervical intraepithelial neoplasia. II. Systemic but not local IgA responses correlate with clearance of HPV-16. *J Gen Virol*. 1999 Feb;80 (Pt 2):409-417. doi: 10.1099/0022-1317-80-2-409
29. Sghaier S, Ghalleb M, Bouaziz H, Chemlali M, Hechiche M, Slimane M, Rahal K. Sentinel lymphnode for edometrial cancer: where are we? *J Clin Invest Surg*. 2020;5(1):1-8. doi: 10.25083/2559.5555/5.1/1.8
30. Arany I, Tyring SK. Activation of local cell-mediated immunity in interferon-responsive patients with human papillomavirus-associated lesions. *J Interferon Cytokine Res*. 1996 Jun;16(6):453-60. doi: 10.1089/jir.1996.16.453
31. La Russa M, Jeyarajah AR. Invasive cervical cancer in pregnancy. *Best Pract Res Clin Obstet Gynaecol*. 2016 May;33:44-57. doi: 10.1016/j.bpobgyn.2015.10.002
32. Sawaya GF, McConnell KJ, Kulasingam SL, Lawson HW, Kerlikowske K, Melnikow J, Lee NC, Gildengorin G, Myers ER, Washington AE. Risk of cervical cancer associated with extending the interval between cervical-cancer screenings. *N Engl J Med*. 2003;349(16):1501-9. doi: 10.1056/NEJMoa035419
33. Tsikouras P, Zervoudis S, Manav B, Tomara E, Iatrakis G, Romanidis C, Bothou A, Galazios G. Cervical cancer: screening, diagnosis and staging. J BUON. 2016 Mar-Apr;21(2):320-5.

34. Loopik DL, Koenjer LM, Siebers AG, Melchers WJG, Bekkers RLM. Benefit and burden in the Dutch cytology-based vs high-risk human papillomavirus-based cervical cancer screening program. *Am J Obstet Gynecol*. 2021 Feb;224(2):200.e1-200.e9. doi: 10.1016/j.ajog.2020.08.026
35. Bălălău OD, Corbu AT, Bălălău C, Sima RM, Pleș L, Stănescu AD. Ultrasound signs in the diagnosis of placental anomalies: placenta accreta at the level of the uterine scar. *J Clin Invest Surg*. 2019;4(2):77-80. doi: 10.25083/2559.5555/4.2/77.80
36. Saslow D, Solomon D, Lawson HW, Killackey M, Kulasingam SL, Cain J, Garcia FA, Moriarty AT, Waxman AG, Wilbur DC, Wentzensen N, Downs LS Jr, Spitzer M, Moscicki AB, Franco EL, Stoler MH, Schiffman M, Castle PE, Myers ER; ACS-ASCCP-ASCP Cervical Cancer Guideline Committee. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. *CA Cancer J Clin*. 2012 May-Jun;62(3):147-72. doi: 10.3322/caac.21139
37. Phadnis SV, Atilade A, Young MP, Evans H, Walker PG. The volume perspective: a comparison of two excisional treatments for cervical intraepithelial neoplasia (laser versus LLETZ). *BJOG*. 2010;117(5): 615-9. doi: 10.1111/j.1471-0528.2010.02501.x
38. Motofei IG. A bihormonal model of normal sexual stimulation; the etiology of premature ejaculation. *Med Hypotheses*. 2001 Jul;57(1):93-5. doi: 10.1054/mehy.2001.1296
39. Bogani G, DI Donato V, Sopracordevole F, et al. Recurrence rate after loop electrosurgical excision procedure (LEEP) and laser Conization: A 5-year follow-up study. *Gynecol Oncol*. 2020 Dec; 159(3):636-641. doi: 10.1016/j.ygyno.2020.08.025
40. Frederiksen ME, Vázquez-Prada Baillet M, Jensen PT, Rygaard C, Hallas J, Lynge E. Conization and healthcare use: a population-based register study. *Eur J Cancer Prev*. 2019 Mar;28(2):124-130. doi: 10.1097/CEJ.0000000000000418
41. Greene SA, De Vuyst H, John-Stewart GC, Richardson BA, McGrath CJ, Marson KG, Trinh TT, Yatich N, Kiptinness C, Cagle A, Nyongesa-Malava E, Sakr SR, Mugo NR, Chung MH. Effect of Cryotherapy vs Loop Electrosurgical Excision Procedure on Cervical Disease Recurrence Among Women With HIV and High-Grade Cervical Lesions in Kenya: A Randomized Clinical Trial. *JAMA*. 2019 Oct 22;322(16):1570-1579. doi: 10.1001/jama.2019.14969
42. Hu X, Zhou Q, Yu J, Wang J, Tu Q, Zhu X. Prevalence of HPV infections in surgical smoke exposed gynecologists. *Int Arch Occup Environ Health*. 2021 Jan;94(1):107-115. doi: 10.1007/s00420-020-01568-9
43. Melnikow J, Nuovo J, Willan AR, Chan BK, Howell LP. Natural history of cervical squamous intraepithelial lesions: a meta-analysis. *Obstet Gynecol*. 1998 Oct;92(4 Pt 2):727-35. doi: 10.1016/s0029-7844(98)00245-2
44. Bansal N, Wright JD, Cohen CJ, Herzog TJ. Natural history of established low grade cervical intraepithelial (CIN 1) lesions. *Anticancer Res*. 2008 May-Jun;28(3B):1763-6.
45. Bohiltea R, Turcan N, Cavinder CM, Ducu I, Paunica I, Andronache LF, Cirstoiu MM. Risk factors, predictive markers and prevention strategies for intrauterine fetal death. An integrative review. *J Mind Med Sci.* 2020;7(1):52-60. doi: 10.22543/7674.71.P5260
46. Tainio K, Athanasiou A, Tikkinen KAO, Aaltonen R, Cárdenas J, Hernándes, Glazer-Livson S, Jakobsson M, Joronen K, Kiviharju M, Louvanto K, Oksjoki S, Tähtinen R, Virtanen S, Nieminen P, Kyrgiou M, Kalliala I. Clinical course of untreated cervical intraepithelial neoplasia grade 2 under active surveillance: systematic review and meta-analysis. *BMJ*. 2018 Feb 27;360:k499. doi: 10.1136/bmj.k499
47. Chan JK, Monk BJ, Brewer C, Keefe KA, Osann K, McMeekin S, Rose GS, Youssef M, Wilczynski SP, Meyskens FL, Berman ML. HPV infection and number of lifetime sexual partners are strong predictors for 'natural' regression of CIN 2 and 3. *Br J Cancer*. 2003 Sep 15;89(6):1062-6. doi: 10.1038/sj.bjc.6601196
48. McCredie MR, Sharples KJ, Paul C, Baranyai J, Medley G, Jones RW, Skegg DC. Natural history of cervical neoplasia and risk of invasive cancer in women with cervical intraepithelial neoplasia 3: a retrospective cohort study. *Lancet Oncol*. 2008 May;9(5):425-34. doi: 10.1016/S1470-2045(08)70103-7
49. de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H. Classification of papillomaviruses. *Virology*. 2004;324(1):17-27. doi: 10.1016/j.virol.2004.03.033
50. Baer A, Kiviat NB, Kulasingam S, Mao C, Kuypers J, Koutsky LA. Liquid-based Papanicolaou smears without a transformation zone component: should clinicians worry? *Obstet Gynecol*. 2002;99(6):1053-9. doi: 10.1016/s0029-7844(02)01998-1