

UNIVERSITY OF MEDICINE AND PHARMACY CRAIOVA
DOCTORAL SCHOOL

DOCTORAL THESIS

**CLINICAL, COLPOSCOPIC, ULTRASONOGRAPHIC AND
IMMUNOHISTO HORMONAL IMPLICATIONS IN CONGENITAL
CERVICAL ECTROPION**

-ABSTRACT -

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State of Knowledge

1. Uterine cervix – reviews elements of embryology, anatomy, pathophysiology essential for understanding cervical malignant and premalignant pathology. Using data from recent literature, cervical organogenesis stages are systematically exposed. At the same time, updated definitions of the reserve cell and metaplasia support the premise of a modern vision on the diagnosis and follow-up regimen in patients with congenital ectropion.

2. Cervicitis. Implications of viral infections in the pathogenesis of cervical carcinoma - this chapter describes the pathophysiology and etiopathogenesis of cervicitis and the main physico-chemical or microbiological etiological factors involved in this pathology. The importance of this chapter resides from the evidence in both classical as well as latest literature, on the implications of this type of pathology in the genesis of preneoplastic and neoplastic lesions of the cervix.

3. Carcinoma of the cervix. Etiological theories. HPV, stem cell, the reserve cell - Chapter deepens into the pathophysiological mechanisms of carcinogenesis and make a foray into the most popular and debated etiological theories of cervical carcinoma (HPV-mediated carcinogenesis, the implications of stem cell, the reserve cell). Each of these theories is supported by recent evidence of international recognition in the literature.

4. Cervical intraepithelial neoplasia is the centerpiece of this chapter. It describes the general characteristics and factors involved in the occurrence of CIN, histogenesis, diagnostic methods (clinical examination and laboratory tests) and the role of biopsy in the management of patients with suspected CIN. Classification, evolution and prognosis of CIN conclude a chapter covering the main points needed to understand the complex mechanisms involved in the development of this pathology.

5 Colposcopy - one of the main tools for diagnosis of changes that occur in the neck, is the colposcopic examination. This chapter is an overview of the maneuver (guidelines for implementation) and the changes found in case of cervical ectropion and congenital cervical intraepithelial neoplasia.

Personal contribution

1 Doctoral Thesis Objectives - This paper aims to update the knowledge about cervical lesions with high oncogenic potential and tries to clarify the hormonal and infectious context and the need for creating a successful diagnosis and therapeutic algorithm, since the incidence of cervical cancer is the third leading cause worldwide of malignancy in women and 7th in both sexes, ranking in the top five diseases that cause mortality worldwide. It is known that cervical ectropion is the highest oncogenic risk cervical lesions, but studies worldwide, are still insufficient on solving these types of injuries. We decided to extend the current research to standardize lesion by creating degrees of ectropion correlate them with local and systemic hormonal interference. As hormonal interface we chose the luteal phase progesterone dosing, reference dosing for relative hyperestrogenism demonstration, valuable component to potentiate viral and "virus-like" locally. As novelty we established the study extrapolation to estrogen and progesterone receptors in the ectropion epithelium with the expectation of involvement in addressing situations of this kind and their correlation with other markers studied so far in cervical tissue lesions such as Ki 67 and p16. Complete cervical screening by volumetric and linear ultrasound measurements and uterine artery resistance indices are not fully studied therefore we extended the study to this segment imaging unexpected results.

2 Material and methods – We conducted a prospective study that included 126 patients lot who presented in OG outpatient setting between 2010 and 2014. During that period, patients were admitted into the study by signing an informed consent, and then were subjected to an investigation protocol with a prior established set of measurable, specific parameters, for case monitoring.

Patients were selected on the basis of local clinical examination (gynecologic exam) when presenting for routine ambulatory consultations, and were subsequently subjected to further investigation. At admission in the study group, several factors were considered: age, the area of origin, characteristics of the menstrual cycle (onset, frequency, flow, premenstrual syndrome), the presence or absence of comorbidities (thyroiditis), a heredo- collateral history.

Study definition and procedure

We chose to conduct a study in patients diagnosed with congenital ectropion, to investigate the importance of various risk and prognosis factors for cervical dysplasia development in this population. These patients were identified with congenital ectropion through clinical examination, and they were investigated by venous blood sampling and vaginal cultures in order to set the infectious context (serology for IgG, IgM and HPV DNA, HVS2). First intention diagnosis of Chlamydia, Mycoplasma, Ureaplasma, HPV, HVS2 infections set the patient into a viral etiology oncogenic risk group. For further classification of patients into risk groups we proceeded to venous blood sampling on day 21 for determining hormonal context for the evolution of ectropion.

Of great importance was the colposcopic examination of patients diagnosed with ectropion, in particular to determine the degree of ectropion, and to detect any preexisting

lesions. However, at the time of colposcopy, guided biopsy was performed from representative areas. The fragments collected were subjected to histopathological and immunohistochemical examination (Ki 67, p53, progesterone receptors and estrogen).

Dynamic ultrasound surveillance: we calculated volumes of internal genitalia anatomical segments for days 5-7 and established IR levels for the uterine artery; supervision of cervical volume change, for days 7, 14 and 21 after the first ultrasound, and simultaneously velocimetry changes; after determining the degree of ectropion, progesterone deficiency and infectious context prescription of hormonal, antiviral and anti-infectious case specific treatment followed every 3-6 months depending on the severity of each case.

3. Ultrasonographic study

Ultrasound examination was performed according to the protocol described in the previous chapter. Measurement of the size of the cervix was performed by endovaginal ultrasound. No statistically significant differences were found between the size of the cervix before and after therapy, however numerical differences were found. Cervical volumes were influenced by the presence or absence of coinfection studied and the number of progesterone receptors, however not by histopathological or virologic findings.

The evaluation of the variations of ultrasound volumetry of the uterine cervix showed no statistically significant differences when they were compared with the degrees of congenital ectropion (the number of crypts).

One of the surprises of this study was the evaluation of Doppler resistance indices of uterine artery. Of note is that these indices have suffered the most significant changes in periovulatory stage ultrasound and left uterine artery. Diagnostic and prognostic implications of this finding requires a follow-up study on a larger group of selected patients, patients who are selected through application of diagnosis and therapeutic protocol described in this thesis. Uterine artery resistance indices shows improvement in value after treatment in both arteries but only left uterine artery measurements had significant variations during statistical processing.

4.Colposcopic examination result-colposcopies were performed following the previously described protocol generating videocolposcopic images with the evolution of lesions, which had a major positive impact on patients. Colposcopic evaluation included comparative interpretation of images after application of acetic acid and Lugol solution.

5.Histopathological study

Histopathological study on HE staining, of the 126 cases revealed a number of changes in the area of ectropion. It was noted that in some cases were identified two or more of the microscopic changes: inflammatory changes - inflammation dominated by polymorphonuclear cells(11 cases), predominantly lymphocytic inflammation – diffuse (34 cases), nodular (6 cases), inflammation with predominant plasmocytes(14 cases), micropolipoid-like area of ectropion (73 cases), metaplastic changes of the columnar epithelium - squamous metaplasia (50 cases), tubal metaplasia (28 cases), epithelium hyperplasia changes of the metaplastic epithelium (8 cases), columnar epithelium reactive atypia (8 cases), dysplastic changes of the metaplastic epithelium – LSIL (36 cases) and HSIL (6 cases).

Immuno marking of Ki67 and anti-p16 antibodies was performed in order to correct classification of SIL lesions and squamous metaplasia occurring on areas of ectropion and accurate grading purposes of squamous intraepithelial lesions. Also, the p16 Immuno marking helped in SIL lesions (LSIL especially type), to the selection of cases that will have an unfavorable prognosis. The analysis carried out was compared with mature squamous epithelium of control batch.

Immuno Assay marking of ER and PR involved in the first instance positive hormone receptors cases detection. ER Immuno Assay marking was positive in the control cases in 15 patients (83.33%), at the site of squamous metaplasia in 14 patients (77.78%) and in the areas of the LSIL in 10 patients (76.93%). HSIL areas were negative in all cases. Statistical analysis revealed a statistically significant correlation between ER positivity in areas of LSIL and HSIL. Thus, patients with LSIL were statistically significantly more frequently positive than those with HSIL ER ($p = 0.0316$), ER positivity was significantly associated with LSIL. There was no correlation between the ER positivity in areas of squamous metaplasia compared with normal exocervix.

6. Combined therapy of congenital cervical ectropion

6.1. Conservative treatment with antibiotics, antivirals, local and systemic progesterone.

Targeted antibiotic bacterial "viral like" sterilization are chlamydia, ureaplasma mychoplasma detected in the first line of investigation. Out of 126 patients, 67 were negative, we identified chlamydia infection in 14 patients, 34 with ureaplasma, and 11 patients with mychoplasma.

Specific viral therapy has been started for the 2 types of infections identified, HPV and HVS2 as follows: -for HPV identified in 36 cases, Isoprinosine was introduced in the therapeutic scheme - for HVS2 identified in 17 cases, Valaciclovir was introduced.

Following luteal phase progesterone dosing we found in the initial group of 126 patients, that 38 patients had serum progesterone values below the lower limit of the reference range, 43 cases had serum progesterone in the lower 1/3 of the reference range, 37 patients were classified within the middle of the reference range and 8 cases in the upper 1/3 of the interval.

From the initial group of 126 patients, 48 were treated with topical treatment and in 78 cases we considered this type of therapy was not required, obtaining favorable results after initiation of antiviral therapy and progesterone.

Patients included in the study were selected from patients presented for a routine checkup or contact bleeding and menstrual cycle linked hidroreea or for procreation reasons. Treatment of patients was carried out over a period of 5 years, sequential or combined treatment regimens.

6.2 Interventional therapy

Cervical biopsy was done with the biotom, which is the most used tool for this maneuver, allowing painless outpatient sampling without significant bleeding and intact lesion margins and maintaining optimal histopathological evaluation of the piece. Fragmentation biopsies targeted

colposcopic findings with the highest degree of severity of lesions visible on the cervix. In 76 cases sequential biopsies led to regression of the lesion using the biotom and the electric cautery in case of bleeding.

7. Conclusions

- Between this thesis novelties, we consider as primary the immunohistochemical study of estrogen and progesterone receptors in the transformation zone of congenital cervical ectropion, which is discussed only in mice and in this context of absolute hyperestrogenism by administration of estrogen and transformation zone growth. The administration of progesterone is innovative in conjunction with the transformation zone restriction supervised by progesterone receptors and demonstrating this phenomenon by colposcopy.

- "Viral-like" infections and viral infection negativity as early as possible by widespread informing of an infectious screening at sexual debut lowers the risk of subsequently developing a degree of dysplasia and thus blocking oncogenesis.

- There were statistically significant differences noted in the cervical displacement measurement in day 7 and day 21 before and after the treatment of patients infected with Chlamydia regarding cervical volume after treatment.

- Immuno marking of Ki-67 and p16 showed that cell cycle of cells in the epithelium of immature squamous preinvasive lesions deviates from normal, is markedly amplified compared to normal cervix.

- Progressive dysfunction of cervical epithelial cell proliferative activity correlates with the preinvasive grade, Ki-67 expression suggesting poor prognosis independent of high grade squamous lesions of the cervix.

- Ki67 as a marker of cell proliferation presents particular utility in assessing the fairness of preinvasive squamous lesions classification and differentiation of non-neoplastic lesions and other similar aspects in common stains (inflammatory atrophy, squamous metaplasia), and may guide the differential diagnosis of lesions of low grade (LSIL) and high grade (HSIL).

- The association between p16 protein and Ki67 in cases with dysplastic lesions occurring on areas of ectropion realize their correct classification as low-grade lesions and high-grade lesions and clearly separated these lesions that mimic the usual staining.

- Immune marking of the p16 plays a special role in selecting patients with congenital ectropion and associated LSIL lesions that will progress to high-grade lesions and thus allows proper management.

- p16 positivity correlates significantly with the high-grade squamous intraepithelial lesions (HSIL) ($p < 0.05$) and permits the selection of patients at risk for rapid progression to invasive carcinoma.

- Positivity in both hormone receptor (ER and PR) in patients with congenital ectropion was higher in areas of squamous metaplasia compared with adjacent normal cervix without statistically significant differences.

- Loss of ER and PR hormone receptors during development of squamous intraepithelial lesions LSIL to HSIL increase the risk of invasive lesions in patients with congenital ectropion, this phenomenon is not present in the initial stages of cervical carcinogenesis (hormone receptors are present in excess in case of squamous metaplasia compared to normal exocervix).

- The presence of hormone receptors in cases of HSIL patients with congenital ectropion can inhibit the invasive potential of these lesions and cervical squamous carcinomas appearance. Hormonal therapy according to the values of the ectropion serological luteal phase of progesterone and the progesterone receptor number. Also combined antiviro-hormonal therapy is another major new hormonal composition derived from the ectropion overlapped by genital viral potentially dysplastic infections and therefore oncogenic importance. Inducing early hormone replacement therapy may decrease the risk of ectropion evolution towards dysplasia.

- Cervical volumetric measurements were statistically significant for day 7 for patients with positive Chlamydia and Ureaplasma, before and after treatment.

- Cervical volumetric measurements for day 14 of menstrual cycle were statistically significant for patients HPVHR + and absent Chlamydia, in correlation with serological values of progesterone in the lower 1/3 of the range and surprisingly the presence of the marker Ki67. Also entered in the same range with high statistical significance patients had more frequent menstrual cycles (21-25 days) and to a lesser extent in patients with nonspecific inflammatory histopathological results.

- Requiring a study with a much larger number of patients, we can launch the hypothesis but not the explanation of left uterine artery IR sensitivity to progestin therapy in patients with relatively hyperestrogenism phenomena (lack of progesterone circulating influences can affect the digestive tract, causing swelling and distension colic, which can be one of the possible explanations for the post-therapeutic surprising answer in our study).

- The volumetric ultrasound is important according to the antiviral therapy administered especially on day 14 of the menstrual cycle where important statistical significance was demonstrated by Isoprinosine. Left uterine artery resistance index values are particularly sensitive in patients who have not received antibiotic and antiviral. It can be argued that progestin therapy is more effective as the ectropion is "clean" and the bacteriological and viral hyperestrogenism influence and improve resistance index of right uterine artery in the 21st day of the menstrual cycle. So hyperestrogenism renders ultrasonographic effects and patients require extensive supervision.

8.Selective Bibliography

1. ACOG Practice Bulletin: Clinical Management Guidelines for Obstetrician-Gynecologists. No 61, April 2005. Human Papillomavirus. . Obstet Gynecol 2005; 105:905.
2. Anton AC, Peltecu G. Leziunile precursorale ale cancerului de col uterin. In: Irinel Popescu, editor. Tratat de chirurgie, vol. V A, editor Peltecu G, editura Academiei Române, București 2008;

3. Belfiore P, Costa E, De Cantis S, et al. Effectiveness and persistence of a topical treatment for cervical ectropion with deoxyribonucleic acid. *Minerva Ginecol* 2005; 57:461.
4. Benacerraf BR, Shipp TD, Bromley B. Improving the efficiency of gynecologic sonography with 3-dimensional volumes. *J Ultrasound Med* 2006; 25:165.
5. Berek JS. *Berek & Novak's Gynecology, Thirteenth Ed. Benign Diseases of the female reproductive tract.* Pag 438, Lippincott, Philadelphia, 2002
6. Bergonzini, V., Salata, C., Calistri, A., Parolin, C. & Palu, G. 2010. View and review on viral oncology research. *Infectious Agents and Cancer, Vol.5*
7. Castellsague X, Diaz M et al. Worldwide HPV etiology of cervical adenocarcinoma and its cofactors: implication for screening and prevention. *J Natl Cancer Inst* 2006; 98:303.
8. Clifford G, Franceschi S. *Int J Cancer* 2008; 122(7): 1684-5;
9. *Colposcopy Principles and Practice- Second Edition*, Barbara S.Apgar, Gregory L. Brotzman, Mark Spitzer, zona de transformare pag.149, index Reid's 175, 2008.
10. Cuzick J, Clavel C et al. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. *Int J Cancer* 2006; 119:1095
11. Dallenbach-Hellweg G, Knebel Doeberitz MV, Trunk MJ, Normal histology, regeneration and repair, in *Color atlas of histopathology of the cervix uteri*, 13-29, second edition, Springer Verlag Berlin, 2006
12. Himes KP, Simhan HN: Time from cervical conization to pregnancy and preterm birth. *Obstet Gynecol* 2007; 109(2 pt 1):314-319;
13. International Collaboration of Epidemiological Studies of Cervical Cancer, Appleby P, Beral V, et al. Cervical cancer and hormonal contraceptives: collaborative reanalysis of individual data for 16,573 women with cervical cancer and 35,509 women without cervical cancer from 24 epidemiological studies. *Lancet* 2007; 370:1609.
14. Jeronimo J, Schiffman M. Colposcopy at a crossroads. *Am J Obstet Gynecol* 2006;195:349Y53.
15. Junior, J. E., Giraldo, P. C., Gonçalves, A. K. S., do Amaral, R. L. G. and Linhares, I. M. 2014, Uterine cervical ectopy during reproductive age: Cytological and microbiological findings. *Diagn. Cytopathol.*, 42: 401–404. doi: 10.1002/dc.23053
16. Keating JT, Cviko A, Riethdorf S, et al Ki-67, cyclin E, and p16INK4 are complimentary surrogate biomarkers for human papilloma virus-related cervical neoplasia. *Am J Surg Pathol.* 2001 Jul;25(7):884-91.
17. Khalbuss, Walid E. et al. "Cytomorphology of Unusual Infectious Entities in the Pap Test." *CytoJournal* 9 (2012): 15. PMC. Web. 3 Dec. 2014.

18. McIver, Christopher J. et al. "Multiplex PCR Testing Detection of Higher-Than-Expected Rates of Cervical Mycoplasma, Ureaplasma, and Trichomonas and Viral Agent Infections in Sexually Active Australian Women ." *Journal of Clinical Microbiology* 47.5 (2009): 1358–1363. PMC. Web. 3 Dec. 2014.
19. Mehlhorn G, Hautmann SK, Koch MC, Strehl JD, Hartmann A, Hilfrich R, Beckmann MW, Griesser H. HPV16-L1-specific Antibody Response Is Associated with Clinical Remission of High-risk HPV-positive Early Dysplastic Lesions. *Anticancer Research* September 2014 vol. 34no. 9 5127-5132
20. Montz FJ. Management of high-grade cervical intraepithelial neoplasia and low-grade squamous intraepithelial lesion and potential complications. *Clin Obstet Gynecol* 2000; 43:394.