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ABSTRACT

PRECANCEROUS LESIONS OF THE CERVIX:
EPIDEMIOLOGY, DIAGNOSIS, TREATMENT AND IMPLICATIONS
ON PATIENTS PERSONAL AND SOCIAL LIFE

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PART I – GENERAL ASPECTS

SPECIAL PART – PERSONAL CONTRIBUTIONS

CHAPTER I
ETIOPATHOGENY OF PRECANCEROUS LESIONS

Cervical cancer (CC) is one of the leading causes of cancer death. Thus, in 2008, the GLOBOCAN study **(1)** globally ranked cervical cancer as the third most common cancer in women, after breast cancer and colorectal cancer. In Romania, cervical cancer remains a social problem as the incidence of this disease is 10 times higher and the death rate 5 times higher than the EU average.

Although in the etiopathogenesis of precancerous lesions and of the cervical cancer several risk factors are involved it is not imperative that women who have such factors to develop cancer. The most important risk factor is the infection with human papillomavirus (HPV), most commonly involved being the HPV types 16, 18, 31, and 45 **(2, 3)**. Other etiologic factors are smoking, sexually transmitted diseases, birth control methods, diet and genetic factors **(4, 5, 6)**.

CHAPTER II
PRECANCEROUS LESIONS OF THE CERVIX

Cervical cancer is preceded by a series of cellular abnormalities characterized by cytological and histological changes considered precancerous lesions. These include precursor lesions for squamous cell carcinoma: cervical intraepithelial neoplasia (CIN) or squamous intraepithelial lesions (SIL) as well as for adenocarcinoma: glandular intraepithelial neoplasia (CIGN) or adenocarcinoma "in situ" **(7)**.

These lesions have different evolutionary character, with a tendency towards regression for LSIL lesions while HSIL lesions tend to progress. Progression from mild dysplasia to moderate dysplasia occurs in 9 to 13 years, from moderate to severe dysplasia in another 2-4 years, and progression from severe dysplasia to invasive carcinoma in about 10 to 20 years **(8)**.

CHAPTER III
DIAGNOSIS OF PRECANCEROUS LESIONS

Diagnosis of precancerous lesions of the cervix involves a clinical diagnosis and a complex laboratory tests (cytology, colposcopy with biopsy, detection of HPV infection). The clinical picture of precancerous lesions is poor and nonspecific, the dominant clinical sign being leucorrhea.

Cytological diagnosis is the main method of diagnosis **(9)**. In Romania, the screening method used for the diagnose of cervical cancer is the Pap test in the case of women aged over 25 years who do not have a confirmed diagnosis of CC. The testing can be stopped in the case of women over 64 years only if the last 3 smears were normal and the testing interval is 3 years.

Colposcopy is an important step in diagnosis, the most common indication being an abnormal Pap smear **(10)**. Correlated with biopsy it represents the "gold standard" for the diagnosis of precancerous lesions **(11)**. The histopathological examination confirms the presence and type of precancerous lesions.

Since 1990 the detection of HPV was introduced in the management of precancerous lesions, testing being indicated only in lesions with atypical squamous cells.

CHAPTER IV
TREATMENT OF PRECANCEROUS LESIONS

There are several methods available for the treatment of precancerous lesions. Surgery is the main therapeutic method and it may be conservative (ablation or conization) or radical (hysterectomy) and refers to CIN II, CIN III / Cis and Ais lesions. CIN I lesions are subject to regular monitoring and surveillance.

Chemotherapy and radiotherapy have limited indication being reserved for cases with contraindication for surgery. Prevention in precancerous lesions mainly focuses on: avoiding the risk of HPV infection, avoiding the risk factors involved in the CC etiopathogeny and HPV vaccination.

In the last 15 years a number of independent studies have assessed the efficacy of LLETZ as the most effective technique in the treatment of CIN lesions **(12)**.

All patients, regardless what precancerous lesions were treated against, require further careful monitoring, the methods used being cytology, colposcopy, endocervical curettage and ADN HPV testing.

CHAPTER V
IMPACT OF DIAGNOSIS AND TREATMENT ON PATIENTS' PERSONAL AND SOCIAL LIFE

Patients with precancerous lesions of the cervix experience complex problems - physical, psychological and sexual related to the consequences of diagnosis and treatment. Stress is another problem in the case of these patients, on one hand because it causes discomfort and on the other because it is one of the risk factors that can coact in the progression of CIN to invasive lesion **(13)**.

One thing that is easily neglected is the patients point of view is the fact that they consider the diagnosis and treatment procedures very stressful. There is an intensification of the sense of fear from the moment when the diagnosis is communicated **(12, 14, 15, 16)**.

CHAPTER VI
MOTIVATION, PURPOSE AND OBJECTIVES OF THE RESEARCH

MOTIVATION: The incidence of cervical cancer is increasingly higher that why it is necessary to come up with solutions that lead to its decline. Motivation is supported by the findings documented by multiple studies which have pointed out that after the introduction of screening methods for cervical cancer the incidence of this disease in these countries fell by 80%.

AIM: the evolution in well defined stages of precancerous lesions under the influence of certain risk factors within a rather

long interval involves on one hand developing methods of early diagnosis of precancerous lesions and on the other hand establishing a rigorous treatment protocol for confirmed disease.

The OBJECTIVES of this work were:

- identification of risk factors involved in the etiopathogenesis of precancerous lesions of the cervix;
- assessment of methods for screening and early diagnosis of cervical cancer;
- establishment of modern management criteria of precancerous lesions;
- analysis of the impact of diagnosis and treatment on patients personal and social life;

CHAPTER VII
MATERIAL AND METHOD

In our study there were included 500 patients who solicited gynaecological consultation for various diseases or for prescription of contraceptives in the Gynaecology Clinic of Craiova Municipal Hospital and at a private clinic of gynaecology in Craiova within 5 years, between 2006 and 2010. Of these, 233 patients had histologically confirmed precancerous lesions.

The biological material used was the product of vaginal discharge for microbiological examination, cells collected from the superficial layer of the cervix for cytopathological examination and tissue fragments collected through targeted biopsy during the colposcopy examination for histological examination and HPV genotyping.

M**icrobiological examination** consisted of making smears with specific stain for an identification of microorganisms such as Candida albicans, Trichomonas vaginalis, Gardnerella, Streptococcus, Staphylococcus, Chlamydia trachomatis, Mycocoplasma, Ureaplasma urealyticum, E coli, Klebsiella and growing in growth media for the identification of germs and for the antibiogram determination.

Cytological examination or the PAP test is the analysis of normal or abnormal exfoliated cells in the cervix, cells showing morphological changes similar to the tissues of origin.

Colposcopic examination was performed in all patients with abnormal inflammatory smear in accordance with the general recommendations for colposcopy examination – examination in successive stages after local application of saline solution, acetic acid and Lugol solution **(17)**. To classify the lesions and analyse the clinical data we used the Reid colposcopic index (RCI) which is based on four colposcopic characteristics **(18, 19)**: border of the lesion, acetowhite staining, type of vascular pattern and staining with Lugol solution.

Histological examination was performed on the biological material obtained through biopsy in all cases examined colposcopically. Interpretation of results was done by dividing the lesions of the cervix in squamous epithelial lesions (CIN) or glandular epithelial lesions (CIGNA) **(12)**.

HPV determination was performed using the PCR method on tissues harvested during biopsy and fixed in paraffin which were already sent to the laboratory for histological examination, the specificity of the reaction was not influenced by the method of fixation used prior to introduction in paraffin **(20, 21)**.

In order to assess the **implication of diagnosis and treatment on patients personal and social life** we conducted a study using the RAND SF – 36 analysis system (version 1.0), a universally accepted system used for determining the quality of life. The system quantifies 8 parameters of general health and thus of the quality of life: physical functioning (PF), role limitations in daily activities due to physical health problems (RP), bodily pain (BP), general health (GH) perceptions, vitality (VT), social functioning (SF), role limitations in daily activities due to personal or emotional problems (RE) and general mental health (MH). These 8 parameters help to calculate the physical health - PCS (PCS - 36) and mental health - MCS (MCS - 36) **(22)**.

As we are monitoring both the impact of diagnosis and treatment on the quality of life we decided to carry out the test one month after the treatment, restricting thus the number of candidates that could be included in the study to 184 (78.9%).

We excluded patients who refused participation in the study - 74 (40.2%), those who did not understand how to conduct the tests and have completed it wrongly and those who have completed it intentionally wrongly - 31 patients (16, 8%), thus remaining with a total of 79 patients (42.9%) who met the required profile and have consented to participate in the test.

CHAPTER VIII
RESULTS

We mention that the study group is represented by the 233 cases with histologically confirmed precancerous lesions, the rest of the cases being excluded from the study. Of these, 226 were squamous lesions (CIN I - 26 cases, CIN II - 55 cases, CIN III / Cis - 145 cases) and 7 were glandular lesions – Ais.

A) ***Epidemiology*** of precancerous lesions:

- ***incidence*** of lesions and distribution of cases by calendar years:

For the time interval studied we found a slight increase in the number of cases diagnosed annually. Thus, if in 2006 there were 40 patients diagnosed with precancerous lesions (17.1%) in 2010 there were diagnosed 51 patients (21.8%).

At the same time there is a relatively uniform distribution of cases throughout the period considered, so CIN I lesions vary between 1.2% and 2.5%, CIN II between 3.4% and 5.5% and CIN lesions III / Cis between 9.8% and 13.7%. Only in the case of CIN III lesions we can notice a significant growth of incidence over time.

- age distribution of cases***:***

Analyzing the age distribution of squamous precancerous cervical lesions - 226 cases (96.9%), it can be seen that LSIL lesions predominate up to 30 years - 21 cases (9%), while HSIL lesions reach a peak between 20 to 40 years - 179 cases (76.8%).

Incidence decrease after 40 years is explained by the fact that most HPV- induced cellular changes spontaneously regress within a variable period of time **(56, 121, 122)** and only about 20% of women infected with HPV develop CIN lesions **(48)**.

- *origin* distribution of cases:

Analyzing the distribution of the 233 cases with precancerous lesions according to area of origin is noted that predominant are patients from the urban areas - 145 cases (62.2%) comparative to those from the rural areas - 88 cases (37.7%).

Although we have noticed the predominance of patients from urban areas (62.2%) over the patients from rural areas (37.7%), involvement of this factor in the etiopathogenesis of the disease is not conclusive; the only explanation could be the educational and socio-economic level that could influence the patients participating in screening tests.

B) Risk factors for precancerous lesions:

- ***smoking***:

From data obtained from the 233 patients with precancerous lesions we identified a total of 162 patients (69.5%) smokers and found that both squamous precancerous cervical lesions - 14 cases (5.3%) LSIL and 143 cases (54.3 %) HSIL and glandular precancerous lesions - 5 cases (1.9%) are associated with smoking patients. It also obvious that in the case of LSIL lesions the proportion of cases is approximately equal, but in the case of HSIL lesions the proportion is much higher for patients who smoke.

- ***use of contraceptives***:

In the group examined the majority of patients did not use contraceptives - 167 cases (71.6%). Only 66 patients (28.3%) used this birth control method.

The use of contraceptives is also a risk factor provided that the duration of their use exceeds 10 years. Our study revealed the use of contraceptives in a number of 66 patients (28.3%), but only in 11 cases (4.7%) the use of contraceptives exceeded 10 years, so we can not consider contraception as a proven risk factor.

- ***HPV infection***:

Considered by the specialized literature as the main etiologic factor, the HPV infection, the most common sexually

transmitted infection in the world, with an incidence of approximately 75-90% of sexually active women **(11)**, was one of the main objectives of our study.

The diagnosis of HPV infection was made by PCR technique from samples sent for histopathological examination in all the 233 cases with histologically confirmed precancerous lesions, being detected in 97.4% of cases.

It is noted that while high-risk viral types were identified in 90.9% of cases, subtypes 16 and 18 were the most frequently encountered and identified mainly in HSIL lesions (CIN II and CIN III / CIS).

Our results confirm the data mentioned by the specialized literature that the HPV infection is present in the majority of precancerous lesions, and when it is present subtype 16 is the most commonly involved (19).

C) Clinical diagnosis

The clinic of precancerous lesions is poor and non-specific, the dominant clinical sign that usually brings patients to the gynaecological examination being leucorrhea, present in 77 patients (33%). Other clinical signs were: vulvovaginal discomfort, burning, pain, itching, bleeding after intercourse, dyspareunia and 65 cases (27.8%) were asymptomatic, the cervical lesion being occasionally discovered after a routine gynaecological examination and in 9 cases (3.8%) the lesion was identified during an abortion.

D) Cytological diagnosis

The 500 patients have undergone in the first stage a Pap test and the results of this test were: 49 cases (9.8%) with normal smear and 451 cases (90.2%) with abnormal smear of which 161 cases (32, 2%) with inflammatory smear and 290 cases (58%) with abnormal cytology. The distribution of the 290 cases with abnormal cytology based on cytological aspects identified was: 31 cases (10.6%) with ASCUS cytology, 73 cases (25.1%) with LSIL cytology, 167 cases (57.5%) with HSIL cytology and 19 cases (6.5%) with AGC cytology.

The cytological appearance of LSIL lesions smears is characterized by isolated cells or cells arranged as groups and

nuclei volume increasing of hyperchromes. In HSIL lesions the form anomalies are more intensive and more pronounced than in LSIL.

E) **Microbiological diagnosis**

Of the 161 cases with inflammatory smear studied, 29 cases (18%) were sterile after seeding cervicovaginal secretions in growth media and 24 cases (14.9%) had polyetiology infection. In the remaining 108 cases, after sowing, in order of frequency, the following bacterial load resulted: Candida albicans - 35 cases (21.7%), Trichomonas vaginalis - 33 cases (20.4%), Chlamydia - 9 cases (5, 5%), Gardnerella - 8 cases (4.9%), Streptococcus and Proteus in 7 cases (4.3%), E coli - 5 cases (3.1%) and Staphylococcus - 4 cases (2.4%).

F) Colposcopic diagnosis

Colposcopic examination was performed in all cases with abnormal smear - 290 (58%). After the initial colposcopic examination, 27 cases (6 cases with polyps, 10 cases with condylomas and 11 cases with leukoplakia - benign lesions) were excluded from the study, for further analysis remaining only 263 cases.

Following the colposcopic examination we noticed in the first place the net predominance of squamous lesions - 245 cases (93.1%) compared with glandular lesions - 18 cases (6.8%). Most squamous lesions were - 154 cases (58.5%) CIN III / Cis followed in order by CIN II - 50 cases (19%) and CIN I - 28 cases (10.6%), results that confirmed that colposcopy is more accurate in differentiating high grade lesions from low grade lesions, data which are similar to the specialised literature data **(70, 132, 133)**.

F) Histological diagnosis

Histological analysis was done on biopsy samples taken during colposcopy.Histological appearance of CIN I lesions is characterized by abnormal proliferation of parabasal cells with nuclear atypia and architectural disorganization in the basal 1/3 of the epithelium. In combination with HPV infection koilocytosis appears.

CIN II and CIN III lesions are histologically characterized by altering the proliferation and maturation of parabasal cells and

cytoarchitectural disorganization in the middle 1/3 in CIN II or in the uppermost 1/3 in CIN III. In combination with HPV infection the number of koilocytes is reduced and limited to the superficial layer.

The hystopathological examination confirms the presence of precancerous lesions and their type. It was performed in all cases that were examined colposcopic and showed squamous lesions in 245 cases (93.1%) and glandular lesions in 18 cases (6.8%). In squamous lesions were identified the following types of injuries: 55 cases (20.9%) CIN II, 145 cases (55.1%) CIN III / Cis, 26 cases (9.8%) CIN I and 6 cases (2.2%) were normal.

G) Study of therapeutic methods used

In the group studied, from the total of cases with precancerous lesions there have been treated 160 patients (77.2%), while 47 patients (22.7%) refused treatment.

The conservative therapy was performed in 138 cases (86.2%) for lesions type CIN II - 38 cases (23.7%) and CIN III / Cis - 100 cases (62.5%). Of these, LLETZ was performed in 90 cases (56.2) and conization in 48 cases (30%).

The reexamination confirmed residual disease in 9 cases (5.6%) of which: 3 cases (1.8%) with CIN II and 6 cases (3.7%) with CIN III. The persistent disease was identified after conservative surgery in 3 cases (1.8%) of which 1 case (0.6%) with CIN II and 2 cases (1.2%) with CIN III in patients with positive margins after excision.

First intention radical surgery aimed Ais lesions (7 cases - 4.3%) and 15 cases with CIN III / Cis (9.3%) and consisted in total hysterectomy, surgery also practiced in 1 case (0.6%) of residual disease for lesion CIN III / Cis and 3 cases (1.8%) of recurrent disease of which 1 case (0.6%) for CIN II lesions and 2 cases (1.2%) for CIN III / Cis lesions.

H) Study of implications of the diagnosis and therapy on patients personal and social life

After calculating the parameters defining the quality of life: physical functioning (PF), role limitations in daily activities due to physical health problems, role limitations in daily activities due to personal or emotional problems, vitality, general mental health, social functioning, pain and general health perception and calculation average of the two indices for quantifying quality of life: physical health (PCS) and mental health (MCS) we obtained the following results:

- physical functioning: score of 100 with standard deviation 0
- role limitations in daily activities due to physical health problems: score of 80.37 with standard deviation $\pm$ 23.02
- role limitations in daily activities due to personal or emotional problems: score of 55.69 with standard deviation $\pm$ 17.67
- vitality (VT): score of 93.86 with standard deviation $\pm$ 38.49
- general mental health: score of 90.07 with standard deviation $\pm$ 37.06
- social functioning: score of 81.01 with standard deviation $\pm$ 34.94
- pain: score of 85.91 with standard deviation $\pm$ 33.11
- general health perception: score of 78.6 with a standard deviation of $\pm$ 31.58

Calculation of quality of life index revealed the following results:

- PCS = 83.36 with a standard deviation of $\pm$ 11.9
- MCS = 79.95 and a standard deviation of $\pm$29.02.

In interpreting the results we point out that the closer are the values to 100 the higher the quality of life. It thus appears that the diagnosis of precancerous lesions and appropriate treatment usually has a major impact on patients, which significantly affects patients quality of life at the personal, family or socio-professional level.

CHAPTER IX
CONCLUSIONS

1. Cervical cancer is a major public health problem, globally ranked as the third most common cancer in women, after breast cancer and colorectal cancer.

2. Cervical cancer is preceded by cellular abnormalities considered precancerous lesions including Cervical Intraepithelial Neoplasia (CIN) or Squamous Intraepithelial Lesions (SIL) for squamous cell carcinoma and glandular intraepithelial neoplasia (CIGN) or adenocarcinoma "in situ" for adenocarcinoma.

3. Maximum incidence of cervical cancer appears in the age group 40-50 years and precancerous lesions in the age group 20 to 30 years.

4. The main etiological factor is the HPV infection identified in our study in 97.4% of cases, subtypes 16 (38.6%) and 18 (31.3%) being the most common.

5. Diagnosis of precancerous lesions is a complex clinical and laboratory diagnosis including cytology, colposcopy with biopsy, and HPV identification.

6. The clinical picture is poor and nonspecific, the dominant clinical sign being leucorrhea (33% of cases).

7. Cytological diagnosis - the first stage of diagnosis of precancerous lesions can identify two types of abnormal smear: inflammation or abnormal cytology (ASCUS cytology - 10.6%, LSIL cytology - 25.1%, HSIL cytology - 57.5% or AGC cytology - 6.5%).

8. Colposcopy is mandatory in all patients with abnormal smears identifying precancerous lesion types: squamous (245 cases) or glandular (18 cases).

9. Histopathological examination performed on biological material collected through biopsy confirms the presence of precancerous lesions and identifies their type: 239 cases with squamous lesions (9.8% - CIN I, 20.9% - 55.1% CIN II - CIN III / Cis) and 16 cases with glandular lesions.

10. Treatment of precancerous lesions should be instituted immediately after diagnosis. It addresses lesions of the types CIN II, CIN III / Cis and Ais. CIN I lesions do not have initial therapeutic indication and they require regular monitoring and supervision.

11. Surgery is the main treatment method taking advantage of two treatment options: conservative (LLETZ - 56.2% and conization - 30%) or radical (hysterectomy - 16.8%) indicated in Ais lesions or recurrent / persistent disease.

12. Postoperative outcomes evaluated through monitoring and supervision were good, the rate of recurrent / persistent disease being 3.3%.

13. Identification of precancerous lesions and their treatment influences the patients' quality of life at the personal, family or socio-professional level.

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