GENERAL REVIEW

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Cervical, vaginal and vulvar intraepithelial neoplasms

Intraepitelijalne neoplazme grlića materice, vagine i vulve

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Intraepithelial neoplasms of the uterine cervix

Cervical intraepithelial neoplasms

The cervical intraepithelial neoplasms (CINs) are abnormalities of the squamous ectocervical epithelium. The first group consists of squamous intraepithelial lesions of low histological stage (LSIL) and includes flat condyloma and CIN I, while the second group consists of squamous intraepithelial lesions of high histological stage (HSIL) including CIN II and CIN III.

Epidemiological data

Epidemiological data on the frequency of the incidence and detection of these changes are scarce and varying, so they cannot be accepted with certainty. It is only confirmed that these changes are being increasingly detected and what is more, in the younger age groups. This is explained by new knowledge of the etiologic factors of these changes, which makes them classified in the group of sexually transmitted diseases, as well as by altered behavior regarding to sexual sphere [1–5].

Risk factors

Human papilloma viruses (HPV) play a significant role in the development of cervical neoplasms [2, 6, 7]. The most important issues are a promiscuous behavior of both women and men, early sexual activities, poor sexual hygiene with frequent infections of the genital organs, failure to use barrier methods of contraception, etc. [8]. There are other risk factors contributing to initiation and development of pathological event. They are designated as cocarcinogens and such as impair immunity, smoking, genital infections caused by other agents (genital herpes virus, HIV, cytomegalovirus, *chlamydia trachomatis*) as well as some medications (cytostatics, immunosuppressants) [6, 8–11].

Molecular biology examinations verified the presence of specific viral DNA sequences (E6 and E7) integrated into the genome of the atypical cervical epithelial cells. It is possible to document it in over 80% of intraepithelial neoplasms and in over 98% of cervical cancers [7]. Presently, it is known that less than 2% of cervical cancers are negative to HPV DNA, provided that they were caused by, at that time, undetected types of viruses, or HPV genetic material was lost in the process of oncogenesis [11]. Pathogenetic mechanism of malignant cell transformation has been elucidated, as well. Viral genome has circular shape and as such enters the host cell through the skin or mucosal membrane microabrasions; or, in case of the uterine cervix, through the cells of basal or parabasal layer within the transformation zone. There is a continuous mitotic activity in these layers, and these are the target cells for HPV infection of the cervix. Virus entry into the cell is the initial event, which may, upon a series of other intracellular events, result in development of neoplasia. Viral DNA, immediately upon the entry of viral particle in the host cell, is being freed from capsid and travelling to the cell nucleus. Herein, the circular viral genome will be placed episomally, not inducing any cytopathogenetic effect. It will be a latent infection and it may remain as such. It has been proved that about 10% of female population has latent HPV cervical infection, and no signs of disease. However, if so far undefined factors designated as cocarcinogens, caused breakage of the viral genome ring, its sequences would be freed and the process of mutagenesis would be initiated. The sequences E1 and E2 of the viral genome lose control over the sequences E6 and E7, which are then integrated into the host cell genome. These sequences bind to tumor suppressor

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genes of host cells p53 and pRB, thus breaking the normal control mechanisms of the cell growth. The result of these events is the alteration of the host cell structure, which acquires proliferative properties, characteristic for oncogenesis. The lesion developed in this way is, as a rule, monoclonal, meaning that it originated from a single cell in which the process originally started.

Infections with HPV 16 and 18 and other oncogenic types are more likely to persist than infections with low-risk HPV types. In women 15–25 years of age, ~80% of HPV infections are transient. In older women, cervical HPV infections are more likely to persist. Persistent oncogenic HPV infection is a precursor to invasive cervical cancer. The risk starts from sexual debut and continues through out life. Incident infection of oncogenic types is estimated to be 5.3% (range: 5%–10%) in women 25–55 years of age. Immune function declines with aging resulting in a decreased capacity to respond to both new and previously encountered infections. Up to 80% of sexually active women are infected with HPV at some point of their lifetime. Prior HPV infection may not always induce sufficient immunity to prevent subsequent infection. Knowing this, vaccination against HPV 16/18 is very important [11–13].

It is also known that the process of oncogenesis is relatively slow and develops principally as a biological continuum, from the initial intraepithelial neoplasm to the invasive cancer. It usually lasts several years, but there have been some described cases with a 6-month period of genesis. A progressive course of the disease is mostly caused by high oncogenic potential viral infection, although it may be significantly affected by cocarcinogens, particularly the immune status of a patient. According to different and numerous results of studies, a significant number of HPV infections regresses spontaneously or persists in a latent stage, while lesser number leads to cervical neoplasms. Already developed cervical intraepithelial neoplasm may also regress spontaneously, but the possibility for this to happen would be lesser if the neoplasm stage was higher. For instance, the cancer *in situ* will progress into invasive cancer in about 70% of cases [14–17].

Symptomatology

Symptoms of CINs are practically absent, so they are referred to as asymptomatic stage of disease.

Diagnostics

The diagnosis of CIN is based on the methods of HPV testing, cytology, colposcopy and histopathological examination of the bioptic tissue specimen. Due to its simplicity and cost-effectiveness, cytology is the most often used diagnostic method, representing the basis of screening program in many countries worldwide. It must be pointed out that its sensitivity in discovering the intraepithelial stages of disease is limited. In histologically verified CIN, the percentage of false negative results of cytodiagnostics is considerable. This percentage is higher if the stage of the intraepithelial neoplasm is lower, therefore, the normal cytological finding may be seen in the majority of the initial and even mid-severe intraepithelial lesions [7]. Other than cytodiagnostics, colposcopy is another basic method for detection of the cervical neoplasms, characterized by considerably higher sensitivity of detecting the lowest stages of disease [18].

The baseline of colposcopy is in recognition of pathological changes of the cervical epithelium pathognomonic for CIN, which are based on significant protein increase in dysplastic cells and considerable loss of glycogen, as well as on the changes of the stromal vascular net regarding the number, appearance and capillary arrangement. Upon the application of 3% acetic acid and Lugol's solution on the cervix, these changes are, under colposcope, presented with specific colposcopic images.

CIN undergoing a colposcopic examination are manifested as characteristic pathological pictures of acetowhite epithelium, mosaic, punctations, leukoplaques, negative epithelium iodine or atypical blood vessels. Qualitative extent of a change and their associated manifestations are proportional to stage of the intraepithelial neoplasm. They are a sign of the pathological events in the epithelium and need to be histologically explained [19].

Besides classical colposcopy, recently the method of microcolpohysteroscopy has been used for CIN diagnostics.

For verification and determination of a pathological change extent detected by HPV testing, colposcopy and/or cytological examination, it would be necessary to perform biopsy of the exocervix and endocervical curettage if the change extended to cervical channel. The biopsy is targeted, colposcopy-assisted, and a specimen obtained from the most evident epithelial changes. It is not rare that multiple biopsies are required, so the pathologist will be supplied with most representative bioptic tissue specimens for histological analysis. A resulting finding will make the final diagnosis of a pathological change and represent the basic parameter for decision-making on the future treatment according to current protocols [19].

Cervical glandular intraepithelial neoplasm

Intraepithelial neoplasm in the cervical channel originates from cylindric epithelium. In recent decades, it has been detected more frequently, probably due to better diagnostic procedures, including the endocervical smear, HPV testing and the utilization of microcolpohysteroscopy. In comparison to CIN, cervical glandular intraepithelial neoplasm (CGIN) is being detected relatively late, not uncommonly in the invasive disease stage. HPV is a major etiological factor.

Prognosis

Considering the prognosis of these pathological changes, it is worthwhile mentioning that their biological behavior is unpredictable. The presence of HPV oncogenic types in the cells of intraepithelial change multiplies the possibility of its progressive development to invasive disease. The principle of preventive actions should be respected, not allowing for already diagnosed intraepithelial change to develop into invasive cancer [20].

Treatment

In LSIL pathological changes, only cytological and HPV typing will be sufficient. If the change persisted, it would be treated by some of destructive techniques, with previous endocervical curettage. If the oncogenic HPV types were detected in a LSIL pathological change, the typing would be repeated in 6 months, and if the infection was still persistent, the change would be treated by some of destructive techniques. If histological examination of a bioptic specimen reveals pathological HSIL change, the treatment will include excision techniques. Histological examination of excised tissue confirms the excision effect and depending upon the outcome, regular follow-up or additional excision treatment will be the options [12].

Treatment of LSIL presently involves several techniques, which, on one hand, should enable a complete cure, and on the other, preserve, to the largest extent possible, the function of the uterine cervix for future conception and birth since very young women are often in question. The techniques currently available for their treatment are described as destructive techniques, such as: laser vaporization, cryotherapy, cold coagulation and electrocauterization. They may be carried out in hospitals or on outpatient basis, under general or local anesthesia [21].

Laser vaporization

Laser vaporization is performed by means of CO_2 laser, which emits a beam of 10.6 μm wave length. The output temperature at the laser beam and tissue contact is over 100 ºC, resulting in vaporization of the extra- and intracellular fluid and carbonization of the cell structures and intracellularly located virus particles. Postoperative complications as infection or hemorrhage are extremely uncommon, and the effect of cure after just one treatment is very high, accounting for 96%–98% [20, 22].

This method is advantageous over other techniques by extremely good postoperative regeneration of vaporized tissue, what significantly contributes to rapid restoration of anatomic and functional integrity of the cervix.

Cryotherapy

The method of cryotherapy is based on cervical tissue freezing by liquid nitrogen, which pressurized passes through special tubes leaning against the cervical tissue. The best destructive effect of pathological change is achieved by "freezing-defrosting-freezing" technique [22].

Cold coagulation

A special Semm coagulator is used for performance of this technique. It achieves tissue destruction by heat, which is transferred via a thermic tube to the uterine cervix. Upon positioning a tube on the cervix, the device is being activated and the temperature of 120 ºC on the top of the tube will be reached in 15–20 seconds. For destruction of pathological change in the uterine cervix, it is enough to keep on leaning the tube against the cervical tissue for two minutes [22].

Electrocauterization

This method is designated as electrodiametry as well, and based on the effect of electrical power on pathological cervical tissue. The power of 40W–50 W is used, which is transferred to the tissue by means of special tubes in the shape of a needle or small ball. The procedure is painful, so it is performed in general anesthesia, which together with some other limitations, reduces its broad utilization.

Cervical HSIL changes, involving CINs of stages II and III (CIN II and CIN III), call for treatment using these methods, which will enable a partial cervical removal or extirpation of the whole uterus and its postoperative histological analysis. These are so-called excision methods, including the following: scalpel conization, laser conization, "loop" excision and hysterectomy [22].

Scalpel conization – It is a classical excision technique, where a part of the cervix is excised by scalpel in the shape of cone or cylinder, depending upon the localization of a pathological change. Surgery is carried out in general anesthesia, with different modes of hemostasis, while the suture technique is completed by Sturmdorf. The effectiveness of surgery, i.e. elimination of the pathological HSIL as a whole, varies, while the percentage of the incomplete removal has been reported in 1% to 13% what may be verified by additional histopathological examination of the removed cone [22].

Laser conization – Comparing to classical techniques, it differs in that cervical incision is made by a 30 W–60 W laser beam. As in scalpel excision, the cervical tissue is excised in the shape of the cone or cylinder, depending upon the localization of a pathological change.

With intracervical application of vasoconstrictors, the surgery is performed without bleeding, what excludes the need for hemostatic sutures. This is the core of the absence of subsequent scarred deformities of the cervical remnant and preservation of its functional integrity. It is especially valuable in operations of young women, whose fertile ability should be spared. Apart from the above-mentioned, laser conization has other advantages over classical methods. It may be carried out on outpatient basis, under local anesthesia, and the proportion of the intraoperative and postoperative complications is lesser as compared to other techniques [22].

"Loop" excision – This technique requires a generator, a power source and a series of thin wire loops, circular or rectangular in shape. By its holder, the loop is passed through the tissue of the uterine cervix, while the electric power running through the loop is being warmed up, thus accomplishing the effect of tissue cutting. The hemorrhage is stopped by means of the ball electrode.

The method is simple to perform, and its application is restricted by pathological process localized high up in the cervical channel, as well as by immensely spread ectocervical changes, which cannot be excised by a single cut.

In so far described excision techniques of management of cervical HSIL, it is necessary to point out the need for curettage of the cervical channel residue and histological verification of the respective bioptic tissue specimen [22].

Postoperative controls

In addition, it is important to emphasize the need for postoperative controls, due to low but always present risk of residual or recurrent disease.

If the excised cervical tissue histological examination show that a pathological change had been removed *in toto*, it is be followed by a cytologic 6-month follow-up and HPV testing subsequently, once a year. If the lesion has not been removed completely, that is, it involves the resection margin of the cone as well, it is possibile to opt for cytological and colposcopic (histological) monitoring for 4–6 month, with HPV-typing in 6 months. Moreover, repeated conization would be another options, and if not feasible or HSIL change recurred, the hysterectomy should be done [12, 20–27].

Vaginal intraepithelial neoplasms

Intraepithelial neoplasms (VAINs) of the vagina are less frequent than those of the cervix, accounting for 0.4%–0.5% of all intraepithelial neoplasms of the female lower genital tract. In relation to epithelial involvement by atypical cells, they are classified in a similar way as CIN, i.e. to VAIN I – with changes in the lower third of the epithelium, VAIN II – pathological change involves lower two-thirds of the epithelium and VAIN III – atypical cells involved the full epithelial thickness. The risk and etiological factors of the VAIN etiology are the same as for CIN, with predominant role of the human papilloma virus infection. For this reason, VAIN is most commonly discovered in patients treated for CIN or cervical cancer. The association with cervical neoplasms is the reason for its presentation in the upper third of the vagina. In further course, VAIN may tend to regress spontaneously, persist in the same stage for a long period of time or progress into cancer. Such progression is less frequent than in CIN, and sometimes it may manifest as spread of neoplastic process from cervix to vaginal fornix. The diagnostics of VAIN, which is typically asymptomatic disease, is based on cytological examination, colposcopy and histological verification of a bioptic tissue specimen. The treatment of VAIN may be completed by the laser technique, cryotherapy or electrocauterization. Given that VAIN frequently localized in the vaginal fornices is very difficult to access, laser technique, which appeared to yield good results, is preferential. If VAIN was present together with cervical neoplasm, its treatment would include partial vaginectomy, in the same surgical act applied for cervical malignancy. Therapeutical treatment of VAIN will be followed by regular controls in 6 months as it was in CIN case [28, 29].

Vulvar intraepithelial neoplasms

Vulvar neoplasms (VINs) may appear in the squamous epithelium designated as VIN as well as intraepithelial neoplasms not originating from squamous epithelium.

This disorder is classified into two main groups: 1) VIN, usual type, which encompasses the former subcategories of VIN, warty type; VIN, basaloid type, and VIN, mixed / warty, basaloid / type; 2) VIN, differentiated type, which encompasses the former category simplex type.

Rare cases that do not fit into these categories are termed "unclassified type".

The term VIN is limited to histologically high-grade squamous lesions for which treatment is indicated to prevent progression to cancer.

VIN is most commonly seen in immunocompromised and postmenopausal women, but the incidence is increasing among healthy patients in younger age groups. Risk factors are similar to those observed for CIN. It is estimated that HPV infection is the major factor here, and it appears to be particularly often with the type 16. The commonest VIN localization is in the region of clitoris and lower third of the labia major and minor. It is often not manifested by symptoms, but some women may experience sense of discomfort, itching or burning sensation. Its evolution is very slow, and therefore, it is detected only a few years after the manifestation of symptoms. Clinically, i.e. vulvoscopically, VIN may be presented as a flat, elevated or papillary change, whitish, brown or red color with absent or present blood vessels, which may have atypical appearance. It is often multifocal or associated with VAIN or CIN. In addition, it may be associated with condylomatous growths, especially the bowenoid VIN type, which is more often presented in younger women. Basaloid VIN is more common in elderly women, has higher malignant potential and is not manifested concurrently with condylomata [17].

The change may vary, to a large extent, in surface, color and focality. Vulvoscopic examination are used to determine the exact location of the change for as adequate as possible biopsy. Histopathological examination of biopsy tissue is the only method to confirm the VIN diagnosis, considering the great similarity with many other skin diseases. Biopsies must often be multifocal and best performed by a special key excision instrument. The scalpel excision biopsy can also be done under local anesthesia.

The treatment of VIN involves different modalities including topical chemiotherapy, imiquimoid, CO_2 laser ablation, surgical exision, cryotherapy, loop electrosurgical excision procedure (LEEP), cavitron ultrasonic aspiration (CUSA) and interferon injections [30].

Imiquimod, an immune response modifier, has been successful in the treatment of external genital and perianal warts caused by low risk HPV, usually types 6 or 11. The drug antiviral and antitumor properties are thought to be due to its induction of cytokines, which stimulate a T-helper 1 or cell-mediated immune response. Recently, it has been shown that imiquimod may be potentially effective in the treatment of genital intraepithelial neoplasia caused by high-grade HPV [30].

Aldara Cream activates immune system to help body fight certain skin diseases, including actinic keratosis, superficial basal cell carcinoma and external warts. The most common side effects associated with using Aldara Cream involve skin reaction in the application area.

Given very long VIN evolution, lasting for years and even decades, as well as spontaneous regression of VIN has

been described (but not in high percentage – mainly seen in young women and is often related to pregnancy), give us opportunity to take the expectational standpoint and control the changes on regular basis only. In so far the signs of progression are noted during a follow-up (aneuploidy, presence of oncogenic HPV types), or in older patients, or in immunocompromised patients, radical methods of treatment should be considered. These include wide local scalpel or laser excision (for nonhairy vulvar regions), simple vulvectomy or vulvectomy with grafting, in case of changes found on a broad surface in younger patients. VIN management has also included some medicaments (5-FU, dinitro chloride-benzene, interferon), but without any encouraging results. Upon completion therapy, the regular control are necessary, not only of the vulva but also the entire lower genital tract, taking into account the identical features of the intraepithelial neoplasms which may manifest concurrently on these localizations. The patients with a previous history of cervical cancer also showed identical viral integration sites between their vaginal and/or vulvar lesions and their previous cervical tumors. Perhaps a large majority of high-grade lesions in the female genital tract emerge as monoclonal cell populations derived from the cervical transformation zone. Owing to this fact, we must consider vaccine efficacy in preventing cervical lesions in our estimate of how vaginal and vulvar epidemiology may change [23, 24, 27, 30, 31].

Out of VIN, not originating from the squamous epithelium, worth-mentioning are Paget's disease and melanoma *in situ*.

Conclusion

Great importance is ascribed to prevent and detect and well-timed treatment of these asymptomatic lesions because it is the most efficient way of fighting against the malignant diseases of the uterine cervix, vagina and vulva. It is best achievable by vaccination and systemic screening of female population.

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