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Noncancerous vulvar disorders – own experience

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SUMMARY

Vulvar intraepithelial neoplasia (VIN) is one of the rarest vulvar pathologies and its diagnosis is a challenge for gynecologists. Since lesions of the VIN type are believed to be precursor lesions of vulvar squamous cell carcinoma, their early detection and treatment are significant. Currently, 2 types of VIN are distinguished: uVIN, the occurrence of which is associated with human papilloma virus (HPV) infection, and dVIN, which is independent of infection.

The paper presents a detailed description of the latest VIN classification, evolution of nomenclature, basic aspects of epidemiology, etiology as well as characteristics of macro- and microscopic lesions.

Key words: vulvar intraepithelial neoplasia; VIN, dVIN, uVIN, human papilloma virus

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INTRODUCTION

In light of contemporary studies, there are two basic types of vulvar intraepithelial neoplasia, which correspond to two distinct pathogenetic pathways to vulvar squamous cell carcinoma (VSCC). The incidence of and mortality due to vulvar cancer is stable in the population. However, the incidence of VIN is increasing. Vulvar intraepithelial neoplasia of the uVIN type (usual type) is caused by HPV infection, affects younger women and is multifocal. dVIN (differentiated type), on the other hand, is not associated with HPV and develops in postmenopausal women, frequently due to chronic vulvar dermatoses, such as lichen sclerosus. Despite the fact that dVIN is diagnosed more rarely than uVIN, it carries a higher risk of malignant transformation and progresses over a shorter period of time. The microscopic diagnosis of uVIN is not usually problematic due to the similarity of cellular architecture, which is altered by human papilloma virus in a typical way, to HPV lesions in other tissues. The histological diagnosis of dVIN is more difficult and poorly reproducible; the lesions are more subtle and must be differentiated from a vast spectrum of other vulvar pathologies.

TERMINOLOGY

For years, many terms and classifications for epithelial precancerous vulvar lesions have been used. These lesions were first described in 1912 by an American dermatologist, J.T. Bowen (Bowen's disease), who distinguished the following typical microscopic features: enhanced epithelial cell proliferation, granular layer atrophy, increased mitosis and cell nucleus clusters. He differentiated these lesions from vulvar carcinoma (no stromal invasion), but suggested their precancerous nature.

Towards the end of the 1950s, Hildebrant and Woodruff introduced the term carcinoma in situ (CIS), but further investigation revealed that some lesions referred to as CIS regressed

spontaneously, particularly in young pregnant women or women with multifocal disease. In order to distinguish them from lesions that progress to invasive carcinoma, the term bowenoid papulosis was used. In 1976, the International Society for the Study of Vulvar Disease (ISSVD) approved two terms: squamous cell carcinoma in situ and hyperplastic dystrophy, which was divided based on the level of cellular atypia into mild, moderate and severe.

The term vulvar intraepithelial neoplasia (VIN) was proposed in 1967 by Richart, and later, at the beginning of the 1980s by Crum in order to standardize the terminology describing epithelial vulvar lesions. In 1986, the International Society for the Study of Vulvar Disease (ISSVD) accepted this term as replacing previous diagnoses, such as leukoplakia, erythroplasia of Queyrat, bowenoid dysplasia, Bowen's disease or bowenoid papulosis. This classification distinguishes three stages of advancement based on the histological image of the vulvar stratified squamous epithelium (VIN I/II/III), analogously to lesions in the cervical epithelium (CIN), where VIN I denotes lesions limited to 1/3 of the lower epithelial thickness, VIN II signifies lesions encompassing 2/3 of this layer and VIN III refers to lesions occupying more than 2/3 of this layer.

Since then, lots of evidence has appeared confirming that VIN I, II and III are not a continuum on the way to the development of VSCC. In 2004, ISSVD revised the aforementioned classification taking into account different etiology, morphology and oncogenic potential of these lesions, and proposed new terms: u-VIN (usual VIN) referring to lesions that develop due to human papilloma virus infection and d-VIN (differentiated VIN) denoting lesions developing irrespective of HPV infection and occurring in the course of chronic vulvar dermatoses, usually vulvar lichen sclerosus.

The use of the term VIN I was also discouraged due to low oncogenic potential of such lesions whose occurrence is usually associated with exposure to irritants or low-risk HPV infection (type 6 and 11). In 2005, Medeiros et al. proposed a classification based on the Bethesda system, used for describing cervical intraepithelial lesions, with two categories: LG-VIL (low grade vulvar intraepithelial lesion) and HG-VIL (high grade vulvar intraepithelial lesion; encompassing dVIN and uVIN).

The latest ISSVD classification of vulvar lesions (2015) includes:

- low-grade squamous intraepithelial lesions (LSIL) – lesions caused by HPV, including condyloma;
- high-grade squamous intraepithelial lesions (vulvar HSIL) – according to the nomenclature from 2004, these are lesions of uVIN 2 and 3 character;
- vulvar intraepithelial neoplasia, differentiated type (dVIN) in which no grading is used;
- vulvar intraepithelial neoplasia of unspecified type [1,2].

Despite constantly changing classifications of vulvar epithelial lesions, the terms VIN I, VIN II and VIN III are commonly used in the clinical practice.

EPIDEMIOLOGY

In the past three decades, the incidence of both usual and differentiated VIN has increased significantly with only slightly increased incidence of vulvar carcinoma. In the United States of America, the incidence of VIN increased by 411% in 1973–2000 with a 20% increase in the occurrence of vulvar cancer [3]. In 2013, there were 490 cases of vulvar cancer in Poland, which constitutes approximately 1% of newly diagnosed malignancies [4]. In the past decade, a slight decline has been observed in the incidence among older women, whereas it has remained stable in younger and middle-aged ones [4]. Vulvar carcinoma accounts for merely 2.5–5% of all female genital cancers, and the risk of the disease increases with age (particularly over the age of 50) without an evident peak. The incidence of VIN, however, is the highest in women aged 40–44 years (uVIN) and older than 55 years of age (dVIN).

These trends might reflect both greater detectability of precancerous lesions and greater treatment efficacy, which results in longer time needed for progression to VSCC. Due to anti-HPV vaccination programs, which are popular in many countries, as well as a predicted decline in the number of HPV-driven diseases, ageing population and greater awareness concerning the relevance of dVIN, the number of newly diagnosed dVIN cases is projected to increase while the number of uVIN cases – to drop.

ETIOLOGY

A strong correlation between uVIN and HPV infection has been confirmed; the virus is detected in >80% of women. In a study conducted in 2296 women with vulvar carcinoma

(1709) and VIN (587), De Sanjose et al. found that HPV was carried by 86.7% of patients with intraepithelial lesions and in 28.6% of patients with invasive cancer [5]. HPV 16 was detected most often (72.5%), followed by HPV 33 (6.5%) and HPV 18 (4.6%).

The pathogenesis of neoplasia induced by chronic HPV infection has been well researched. Viral protein E6 inactivates suppressor protein p53, thereby leading to cell cycle deregulation. Furthermore, protein E7 inactivates RB protein and releases E2F transcription factors, thus inducing cellular hyperproliferation. The role of RB protein consists in blocking transcription of kinase inhibitors p14 and p16. That is why p16 is typically overexpressed and p53 activity is detected only in trace amounts or is absent in HPV-driven cancers.

The fact that HPV is detected significantly less frequently in VSCC than in uVIN (in 15–79% of cases according to the literature) has prompted researchers to search for another cause of vulvar carcinoma and classify dVIN as a separate disease entity. In 134 cases of dVIN, HPV was detected in only 2 samples (1.5%).

The pathogenesis of vulvar squamous cell carcinoma that develops independently of HPV infection has not been fully explained. It has been proven that TP53 mutation is a relatively early phenomenon in dVIN and keratinizing VSCC development. Pinto et al. sequenced exons 2–11 of Tp53 from samples collected from 11 patients with dVIN (including 6 with VSCC) and found at least one mutation in 6 per 10 cases. Moreover, in 2 cases, they found identical mutations in dVIN and adjacent VSCC foci, thus supporting their monoclonal relationship [6]. In the study of Trietscha et al., TP53 mutations were found in 3% of HPV-related VIN and in 21% of lesions independent of the virus. Therefore, there must exist certain currently unexplored oncogenetic pathways to vulvar cancer arising in the background of dVIN. It is being studied whether mutations proven to occur in VSCC, such as mutations of CDKN2A, HRAS and PIK3CA, are also present in VIN and whether there is a potential correlation between them.

dVIN (DIFFERENTIATED VIN)

Clinical features

Differentiated vulvar intraepithelial neoplasia is a significantly rarer histological diagnosis than uVIN. It accounts for only approximately 2–10% of all VIN cases (some authors report 29%). In 164 patients with VIN followed by Scurry et al., 82.3% were diagnosed with uVIN and 18.7% with dVIN. Only one patient presented the coexistence of these two forms [7]. The disease is usually diagnosed in post-menopausal patients between the 6th and 9th decades of life (on average at the age of 68 years), with only occasional occurrence in younger women [8]. Al-Ghamdi et al. reported that amongst 21 patients younger than 40 years of age with VSCC, dVIN was concomitant in only 3 cases [9]. The main symptoms reported by patients include pruritus and pain in the vulvar region (60% of cases) [10]. The disease is asymptomatic in many patients, and morphological lesions are non-specific.

The morphology of lesions

Most lesions of the dVIN type are diagnosed in women with chronic vulvar dermatoses (usually lichen sclerosus, lichen simplex chronicus and lichen planus) [11,12], but these changes can also be isolated [13]. The physical examination usually reveals local thickened depigmentation regions (white–gray) with concomitant ulcerations, ill-defined raised plaques or papules or red hyperkeratotic lesions; in most patients these lesions are unifocal.

Course of the disease

Despite the fact that differentiated vulvar intraepithelial neoplasia accounts for few VIN cases, there is evidence suggesting that it progresses to invasive vulvar carcinoma significantly more frequently than uVIN. VSCC is estimated to develop in 32–33% of patients with dVIN [14,15]. Scurry et al. identified dVIN in 73% of patients with the history of, concomitant or secondary VSCC [7]. The occurrence of dVIN contiguous to vulvar carcinoma is estimated in the literature at 17–76%. Chiesa-Vottero et al. analyzed histological samples from 44 patients with vulvar carcinoma. In 38 samples described as keratinizing vulvar carcinoma, dVIN lesions coexisted in approximately 45% of cases [16]. Based on the foregoing, it can be concluded that the prevalence of dVIN is underestimated. It has also been shown that the time of transformation to invasive disease is shorter in dVIN compared with uVIN (22.8 months vs 41.4 months) [14].

Histology

The diagnosis of dVIN in paraffin sections is challenging even for experienced pathologists

[17], which frequently leads to wrong diagnoses (usually lichen sclerosus or squamous epithelial proliferation) [8]. These lesions are frequently seen in direct neighborhood of squamous cell carcinoma foci (mostly of the keratinizing type). They are characterized by thickened epithelium with numerous anastomoses between elongated rete ridges with occasional keratin pearls. Marked cellular atypia is limited to the basal and parabasal layers. Pathological keratinization (dyskeratosis or parakeratosis) and prominent intercellular bridges are usually observed. Basal layer keratinocytes are large, multiform cells filled with abundant eosinophilic cytoplasm. Nuclear chromatin is vesicular, and nuclei contain prominent nucleoli (particularly in the basal layer) [18].

The interobserver reproducibility of microscopic dVIN diagnosis is low. Van den Einden et al. [17] determined 5 typical features that facilitate dVIN identification: 1) atypical mitosis in the basal layer, 2) basal cellular atypia, 3) dyskeratosis, 4) prominent nucleoli, and 5) elongation and anastomosis of rete ridges. The histological picture of dVIN lesions is presented in Fig. 1, 2.

Genetic and molecular changes

Expression of p53 was analyzed immunohistochemically in 10 of 12 (83%) dVIN samples by Yang and Hart. They obtained positive results in the basal and parabasal layers (with a positive reaction of >90% of basal cells). By contrast, in normal epithelium contiguous to the lesions, the staining was irregular and concerned <10% of basal cells without involvement of the cells in the upper layers [19]. In further studies, the authors detected p53 expression in 66–100% of dVIN cases. The usefulness of this test is, however, debatable due to a considerable percentage of positive staining observed in other vulvar pathologies, particularly LS (up to 80% of results).

Another useful marker that differentiates dVIN from epithelial hyperplasia and normal epithelium is Ki-67. In uVIN and dVIN, this parameter is strongly positive in the basal and upper layers, but negative in the normal epithelium for the basal layer. In uVIN, basal cells and a thin layer of cells located above them are Ki-67-positive. uVIN is characterized by positive full thickness staining [21–22]. For comparison, in sections of epithelium with LS, Ki-67-positive cells are located only in the basal layer [23].

uVIN (USUAL VIN)

Clinical features

uVIN is identified in young patients, usually between the 3rd and 5th decade of life [24]. Risk factors include: smoking, multiple sexual partners, early sexual initiation and immunodeficiency (usually HIV infection). Coinfection with herpes simplex virus is detected in as many as 30% of cases. The main symptoms reported by women are pruritus and burning sensation in the region of the vulva as well as dysuria. Some patients remain asymptomatic (approximately 20–40% of cases), and lesions are detected during a routine pelvic examination or when features of HPV infection in other genital regions (usually in the cervix uteri) are identified [25].

The morphology of lesions

On physical examination, the lesions usually present themselves as multifocal, well-defined white or red spots, or flat papules with a tendency to merge and form papillary structures, sometimes with accompanying hyperpigmentation (approximately 10% of lesions).

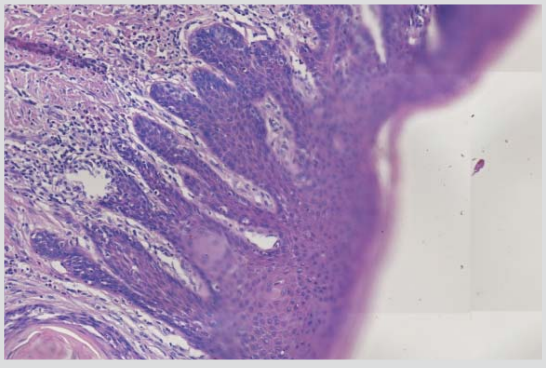

Fig. 1. Characteristic histological features of dVIN lesions

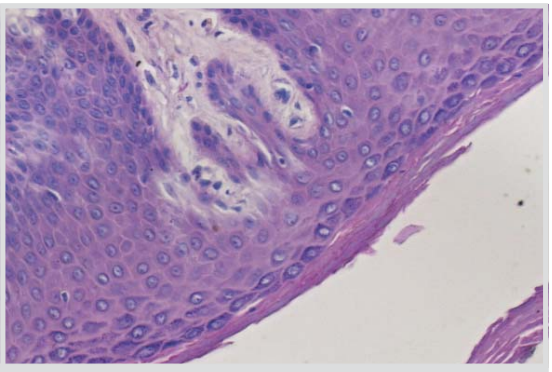

Fig. 2. Characteristic histological features of dVIN lesions

Course

Approximately 41% of patients with uVIN have a history of, concomitant or future HPV lesions (this refers to 3% of patients with dVIN). Moreover, patients with previously diagnosed uVIN more frequently present with cervical HSIL lesions (35% compared with 2% of patients with dVIN); in both locations, the same high-risk HPV was identified. Van de Nieuwenhof et al. found that the risk of uVIN progression to VSCC increases and the time in which vulvar carcinoma develops decreases with age [14,18]. Apart from advanced age, other risk factors of invasive disease include the state post radiotherapy and compromised immunity [26,27]. Invasive vulvar carcinoma is estimated to develop in 4–6% of uVIN cases [14]. In an analysis encompassing over 3000 patients, van Seters et al. found that only 9% of untreated and 3.3% of treated patients with uVIN developed squamous cell carcinoma [28]. The risk of uVIN relapse is estimated at 13–36%. It remains uncertain, however, whether these are new lesions or whether they result from the primary chronic HPV infection. The disease regresses spontaneously in a small percentage of women (1.2%). This process is more likely in patients <35 years of age and during pregnancy.

Histology

The microscopic image of uVIN resembles lesions detected in other areas of mucous membranes affected by HPV [29] (CIN, VaIN, AIN) and represents the integration of genetic material of human papilloma virus with the host cell genome. Typical features include thickened epithelium with superficial hyperkeratosis and/or parakeratosis, acanthosis and presence of rod-shaped rete ridges with intact basal membrane. Next to dysplastic cells of the squamous epithelium with a slight amount of cytoplasm and hyperchromatic nucleus, other findings include dyskaryotic cells with eosinophilic cytoplasm. Nuclear pleomorphism, loss of cell maturation features and increased mitotic activity (abnormal mitotic figures) are other features seen in a histological examination. In one third of cases, uVIN is detected within hair follicles and sebaceous glands.

uVIN is divided into two subtypes: warty and basaloid types. Also, a mixed time, with structures typical of both groups, has been distinguished. Coilocytosis, dyskaryosis and multinucleated cells are typical of the warty type. Basaloid type, however, which clinically presents with flat lesions, is characterized by the presence of basal cells along the whole epithelial thickness. Studies on whether any of these two subtypes is more likely to become malignant are underway. The histological picture of uVIN lesions is presented in Fig. 3, 4.

Genetic and molecular changes

Positive immunohistochemical staining for p16 correlates with high-risk HPV infection in over 90% of cases and is commonly used for its detection. The pattern obtained during this examination should be dispersed, clear and encompass basal cells and its adjacent upper epithelial layers (at least 1/3 of its thickness). In almost 100% of HSIL cases, the result is strongly positive whereas in LSIL, it is less prominent and heterogeneous. The result is positive (weakly positive) in only approximately 0–17% of dVIN cases. In diseases such as lichen sclerosus or epithelial hyperplasia, this reaction is always negative.

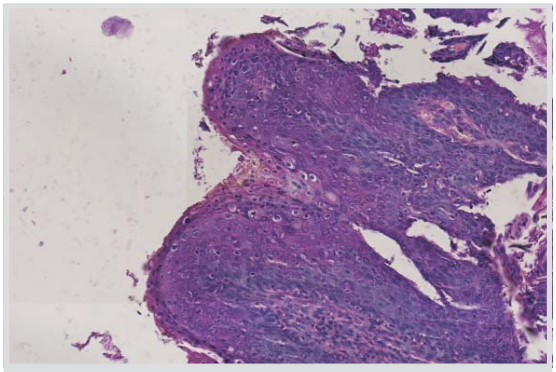

Fig. 3. Characteristic histological features of uVIN lesions

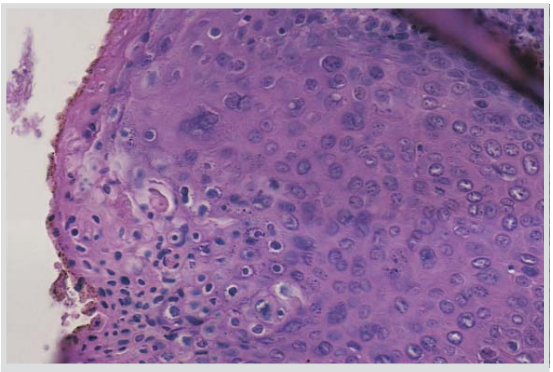

Fig. 4. Characteristic histological features of uVIN lesions

INCIDENCE OF VULVAR PATHOLOGIES

Incidence of vulvar pathologies in patients diagnosed in the department and clinical unit of gynecology, obstetrics and gynecologic oncology in Bytom.

In the first three quarters of 2016, a total of 63 patients, aged 26–80 years, were diagnosed in the Department and Clinical Unit of Gynecology, Obstetrics and Gynecologic Oncology due to vulvar pruritus.

VIN lesions were found in three patients (4.8%), including 2 women with dVIN and one, a 39-year-old patient, with uVIN. The most common cause of vulvar pruritus was inflammation (vulvitis chronica), which was found in 24 patients (38%). Lichen sclerosus atrophicus was found in 14 patients (22%), and lichen planus in 4 women (6.3%). Incidentally, G1 squamous cell carcinoma, basal-cell carcinoma, leukoplakia and condyloma were diagnosed.

The occurrence of VIN lesions in 5% of patients indicates that this pathology is one of the rare female diseases. However, its effective treatment suggests that they should be included in standard daily gynecological practice.

REFERENCES

1. **Horn LC, Brambs CE, Handzel R et al.** Nomenclature of squamous cell precursor lesions of the lower female genital tract: Current aspects, *Pathology* 2016;37(6):526-533.
2. **Hoang LN, Park KJ, Soslow RA, Murali R**. Squamous precursor lesions of the vulva: current classification and diagnostic challenges. *Pathology* 2016;48(4):291-302.
3. **Akerman G, Dussour C, Haddad B et al.** Epidemiology of vulvar intra-epithelial neoplasias, *Gynecol Obstet Fertil* 2007; 35(12):1251–1256.
4. **Wojciechowska U, Didkowska J**. Zachorowania i zgony na nowotwory złośliwe w Polsce. Krajowy Rejestr Nowotworów, Centrum Onkologii - Instytut im. Marii Skłodowskiej-Curie. <http://onkologia.org.pl/raporty/#tabela_wojewodztwa
5. **de Sanjosé S, Alemany L, Ordi J et al.** Worldwide human papillomavirus genotype attribution in over 2000 cases of intraepithelial and invasive lesions of the vulva. *Eur J of Cancer* 2013;49(16):3450-3461.
6. **Pinto AP, Miron A, Yassin Y et al**. Differentiated vulvar intraepithelial neoplasia contains Tp53 mutations and is genetically linked to vulvar squamous cell carcinoma. *Mod Pathol* 2010;23(3):404–12.
7. **Scurry J, Campion M, Scurry B et al**. Pathologic audit of 164 consecutive cases of vulvar intraepithelial neoplasia. *Int J Gynecol Pathol* 2006;25(2):176-181.
8. **Mulvany NJ, Allen DG**. Differentiated intraepithelial neoplasia of the vulva. *Int J Gynecol Pathol* 2008; 27(1):125–35.
9. **Al-Ghamdi A, Freedman D, Miller D et al**. Vulvar Squamous Cell Carcinoma in Young Women: A Clinicopathologic Study of 21 Cases, *Gynecol Oncol* 2002;84,1:94 – 101.
10. **McNally OM, Mulvany NJ, Pagano R et al**. VIN 3: a clinicopathologic review. *Int J Gynecol Cancer* 2002;12: 490–5.
11. **Carlson JA, Ambros R, Malfetano J et al**. Vulvar lichen sclerosus and squamous cell carcinoma: a cohort, case control, and investigational study with historical perspective; implications for chronic inflammation and sclerosis in the development of neoplasia. *Hum Pathol* 1998;29(9): 932–48.
12. **Del Pino M, Rodriguez-Caruncio L, Ordi J et al**. Pathways of vulvar intraepithelial neoplasia and squamous cell carcinoma. *Histopathology* 2013;62(1):161–75.
13. **Eva LJ, Ganesan R, Chan K et al.** Differentiated-type vulval intraepithelial neoplasia has a high-risk association with vulval squamous cell carcinoma. *Int J Gynecol Cancer* 2009;19(4):741-744.
14. **van de Nieuwenhof HP, van Kempen L, Massuger LF et al**. Differentiated-type vulval intraepithelial neoplasia has a high-risk association with vulval squamous cell carcinoma. *Int J Gynecol Cancer* 2010;20(1):194.
15. **van de Nieuwenhof HP, Massuger LF, van der Avoort IA et al.** Vulvar squamous cell carcinoma development after diagnosis of VIN increases with age. *Eur J Cancer* 2009;45(5):851-856.
16. **Chiesa-Vottero A, Dvoretsky PM, Hart WR et al.** Histopathologic study of thin vulvar squamous cell carcinomas and associated cutaneous lesions: a correlative study of 48 tumors in 44 patients with analysis of adjacent vulvar intraepithelial neoplasia types and lichen sclerosus *Am J Surg Pathol* 2006;30(3):310-318.
17. **van den Einden LC de Hullu JA, Massuger LF et al.** Interobserver variability and the effect of education in the histopathological diagnosis of differentiated vulvar intraepithelial neoplasia. *Mod Pathol* 2013;26(6):874–80.
18. **van de Nieuwenhof HP, Bulten J, Hollema H et al**. Differentiated vulvar intraepithelial neoplasia is often found in lesions, previously diagnosed as lichen sclerosus, which have progressed to vulvar squamous cell carcinoma. *Mod Pathol* 2011;24(2):297-305.
19. **Yang B, Hart WR.** Vulvar intraepithelial neoplasia of the simplex (differentiated) type: a clinicopathologic study including analysis of HPV and p53 expression. *Am J Surg Pathol* 2000;24(3):429-441.
20. **Stewart CJR, Crook ML**. Fascin and cyclin D1 immunoreactivity in non-neoplastic vulvar squamous epithelium, vulvar intraepithelial neoplasia and invasive squamous carcinoma: correlation with Ki67 and p16 protein expression, *J Clin Pathol* 2014;67(4):319–25.
21. **van der Avoort IA, Shirango H, Hoevenaars BM et al**. Vulva squamous cell carcinoma is a multifactorial disease following two separate and independent pathways. *Int J Gynecol Pathol* 2006;25(1):22–9.
22. **Hoevenaars BM, van der Avoort IA, de Wilde PC et al**. A panel of p16(INK4A), MIB1 and p53 proteins can distinguish between the 2pathways leading to vulvar squamous cell carcinoma. *Int J Cancer* 2008;123(12):2767–73.
23. **Scurry J, Beshay V, Cohen C et al.** Ki67 expression in lichen sclerosus of vulva in patients with and without associated squamous cell carcinoma, *Histopathology* 1998;32(5):399–404.
24. **Beurden M, Ten Kate FJW, Smits HL et al.** Multifocal vulvar intraepithelial neoplasia grade III and multicentric lower genital tract neoplasia is associated with transcriptionally active human papillomavirus. *Cancer* 1995;75(12): 2879–2884.

25. **de Bie RP, van de Nieuwenhof HP, Bekkers RLM et al.** Patients with usual vulvar intraepithelial neoplasia-related vulvar cancer have an increased risk of cervical abnormalities, *B J Cance*r 2009;101(1):27-31
26. **Jones RW, Rowan DM, Stewart AW et al.** Vulvar intraepithelial neoplasia: aspects of the natural history and outcome in 405 women, *Obstet Gynecol* 2005(6);106: 1319–26.
27. **Chafe W, Richards A, Morgan L et al**. Unrecognized invasive carcinoma in vulvar intraepithelial neoplasia (VIN), *Gynecol Oncol* 1988;31(1):154–65.
28. **van Seters M, van Beurden M, de Craen AJ et al**. Is the assumed natural history of vulvar intraepithelial neoplasia III based on enough evidence?, A systematic review of 3322 published patients Gynecol Oncol 2005;97(2):645 – 651.
29. **Reyes MC, Cooper K.** An update on vulvar intraepithelial neoplasia: terminology and a practical approach to diagnosis *J of Clin Pathol* 2014;67(4):290-294.