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PECULIARITIES OF THE ENDOCRINE STATUS OF WOMEN WITH PRECANCEROUS LESIONS OF THE VULVA

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Abstract

The purpose of the study was to determine the hormonal profile of peripheral blood in women with precancerous lesions of the vulva.**Material and methods.** 309 women with precancerous lesions of the vulva and 60 gynecologically healthy women aged 25 to 70 years were included in the study. Patients with precancerous lesions were divided into 4 groups depending on the nosological unit: 87 women with severe vulvar dysplasia dependent on HPV (VHSIL), 154 individuals with differentiated vulvar dysplasia independent of HPV (dVIN), 36 patients with extramammary Paget's disease of the vulva (VPD) grade Ia and 32 women with melanoma *in situ* of the skin vulva In the groups with VHSIL, with dVIN, with melanoma *in situ*, there were women of premenopausal and postmenopausal age, therefore, for the comparison of hormonal data in these groups, subgroups were selected according to age: subgroups with persons younger than 50 years, subgroups of women aged 50 years and

older. 60 conditionally healthy women of the control group also included 30 people under 50 years and 30 people 50 years and older. The levels of estradiol (E2), progesterone (P4), free testosterone (Tf), follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), anti-Müllerian hormone (AMH), insulin, thyroid-stimulating hormone (TSH), free triiodothyronine (T3f), free thyroxine (T4f), antibodies to thyroperoxidase (ATPO)in blood serumand HOMA indexwere determined. **The results.** There was no difference between serum LH and FSH levels in women with various precancerous diseases of the vulva under the age of 50 years. Individuals with dVIN were characterized by the highest level of PRL (24.44±1.55 ng/ml). Among women aged 50 years and older, the highest levels of LH, FSH and PRL were observed in persons with melanoma in situ - respectively 36.90±2.09 mIU/ml, 74.67±4.06 mIU/ml, 9.85±2.03 ng/ml; the level of FSH and PRL was higher, and the LH/FSH ratio was lower than in controls in all precancerous diseases of the vulva. No statistically significant difference was found between the basal levels of serum P4 and AMH in subgroups of women younger than 50 years and 50 years and older. Among both premenopausal and postmenopausal women, the highest average levels of E2 were recorded in women with melanoma in situ (104.61±10.22 pg/ml and 30.71±1.90 pg/ml), Tf and Tf index - in patients with dVIN (3.20±0.10 pg/ml and 1.58±0.05 pg/ml vs. 8.25±0.24% and 3.77±0.10%, respectively). Features of the thyroid status were the highest indicators of TSH and ATP in persons with dVIN both in premenopause (2.68±0.30 mIU/ml and 196.39±76.48 IU/ml) and in postmenopause (2.23±0 .09 mIU/ml and 123.46±16.07 IU/ml). The average level of 25(OH)D did not differ statistically significantly between women with various precancerous diseases of the vulva. The level of insulin and NOMA index among premenopausal women was the highest in women with melanoma in situ (14.33±1.55 mIU/ml), in postmenopausal women with VPD (14.90±1.02 mIU/ml), HOMA index – respectively, with dVIN (2.87±0.30) and with VPD (3.60±0.27). Insulin resistance was most common in premenopause among individuals with dVIN (53.33%), and in postmenopause - with VPD (72.22%).**Conclusions.** Features of the hormonal profile of people with precancerous diseases of the vulva under the age of 50 are the highest levels of PRL, TSH and ATPO in women with dVIN, the highest average levels of Tf and Tf index, insulin level and HOMA index – in patients with VPD, the highest average levels of E2– in women with melanoma *in situ*. Among postmenopausal patients, the highest levels of TSH and ATPO in women with dVIN, the highest average levels of Tf and Tf index, the level of insulin and NOMA index in patients with VPD, the highest average levels of LH, FSH, PRL and E2 in women with melanoma are characteristic features situ. The average level of 25(OH)D does not differ statistically significantly between women

with various precancerous diseases of the vulva. Insulin resistance is most common in premenopausal individuals with dVIN, and in postmenopausal individuals with VPD.

Key words: vulvar squamous intraepithelial neoplasia; high-grade intraepithelial neoplasia of the vulva; differentiated intraepithelial dysplasia of the vulva; Paget's disease of the vulva; melanoma *in situ* of the skin of the vulva; hormonal profile; thyroid status; insulin resistance.

Cancer of the external genital organs makes up almost 8.0% of the total incidence rate of malignant tumors of the female genitalia and ranks fourth after cancer of the cervix, uterine body, and ovaries [1-5]. It accounts for approximately 2–5% of all gynecological cancers and 1% of all cancers diagnosed in women [5]. The issues of pathogenesis, diagnosis and treatment of precancerous lesions of the vulva remain the least studied in modern gynecology and oncogynecology.

According to the consensus of the European Society of Gynecological Oncology (ESGO), the International Society for the Study of Vulvovaginal Diseases (ISSVD), the European College for the Study of Vulvar Diseases (ECSVD) and the European Federation of Colposcopy (EFC) in 2022, precancerous lesions of the vulva include the human papillomavirus (HPV)-associated squamous high-grade vulvar intraepithelial lesions (VHSIL) and HPV-independent vulvar intraepithelial lesions (differentiated vulvar intraepithelial neoplasia (dVIN)), differentiated exophytic vulvar intraepithelial lesion (DEVIL), vulvar acanthosis with altered differentiation (VAAD)). Non-squamous precancerous lesions of the vulva combine vulvar Paget's disease (VPD) and melanoma *in situ* [6]. The most common precancerous lesions of the vulva are VHSIL and dVIN.

Single works to date are devoted to the state of hormonal homeostasis in precancerous lesions of the vulva.

It is known that estrogens and/or progestins do not cause abnormal mitotic activity in vulva tissues [7]. Cancer of the vulva is rarely a hormone-dependent tumor [8].

The occurrence of lichen sclerosis (LS) and dVIN are among the risk factors for the development of vulvar cancer. LS often accompanies such a precancerous lesion of the vulva as dVIN [9].

Despite the fact that LS affects women of any age, the peak of its prevalence occurs in two different age periods: prepubescent girls (1 in 900) and women in peri- or postmenopause (that is, after menopause) [10, 11]. These peaks support the idea that hypoestrogenia plays a role in the development of LS and most likely dVIN. It is expected that the first peak will be

7-15% of all cases [12]. Nevertheless, not all authors consider it consistent [13, 14]. The average age range at the time of diagnosis is from 52.6 to 60 years [13-17]; however, the length of time that symptoms may persist prior to this can be considerable (68 ±11.2 months) [9], raising doubts about the actual role of menopause in the development of the disease.

A possible explanation for the origin of LS is a decrease in the level of the enzyme 5α-reductase in the vulva. In a study of 30 women with untreated LS, serum hormone levels (estradiol (E2), free testosterone (Tf), dihydrotestosterone, androstenedione, and sex hormone-binding globulin) were measured. Untreated LS patients had significantly higher levels of free testosterone (Tf) and significantly lower serum levels of dihydrotestosterone and androstenedione compared to typical values for their age [18]. In addition, a number of studies have shown that LS have fewer nuclear androgen receptors (AR) and that well-developed LS have fewer ARs than early LS [19-21]. It is believed that the loss of ARexpression (downregulation) in women with LS may be secondary to a change in squamous cell phenotype rather than a hormonal etiology [19].

The transition of vaginal tissue to vulvar tissue in a healthy woman is characterized by an increase in AR and a decrease in estrogen (ER) and progesterone (PR) receptors. A subset of patients with LS has lower vulvar AR expression [19, 20]. Recent studies suggest that oral contraceptives, especially those with antiandrogenic properties, may disrupt androgen-dependent vulvar skin growth, thereby causing early onset of LS in a minority of susceptible young women [22, 23].

VPD is the most common extramammary Paget's disease and accounts for approximately 1–2% of all vulvar neoplasms [24]. Premalignant conditions include primary (cutaneous) VPD, which originates in the epidermis and can be classified as in situ or intraepithelial (conventional type). In a recent systematic review and meta-analysis, Angelico G. et al. (2020) of VPD patients, hormone receptor expression levels were: 12% (95% CI = 0.03–0.36) for ER, 9% (95% CI = 0.03–0.25) for PR, and 40% (95% CI = 0.34-0.47) for AR [25]. This contradicts the results of Garganese G. et al. (2021), who reported the following expression rates: 70% ER, 20% PR, and 75% AR positive among 41 patients with VPD [26].

Since the 1970s, many epidemiologic studies have focused on the possible association between female endocrine characteristics and melanoma, but there is considerable debate regarding the potential influence of hormones on melanoma risk [27]. Estrogens have been found to play an important role in female survival, which appears to be reversed in the postmenopausal period when estrogen levels decline [28-30]. A US study of 167,503 women found a strong association between exposure to ambient UV radiation and melanoma in

women who experienced early menarche and late menopause [31], further suggesting an important role of hormones in pathogenesis melanomas A recent cohort study of 684,696 Norwegian women found an increased risk of melanoma among postmenopausal women using estrogen-only hormone therapy (pill and vaginal forms). Whereas combined estrogen-progestogen hormone therapy was not associated with an increased risk. This finding seems to suggest that exogenous estrogen is a risk factor for melanoma. Additional studies are needed to confirm these findings [32]. In vitro, sex hormones and gonadotropins stimulate melanogenesis by directly affecting melanocytes. However, in vivo, their antagonists and agonists appear to be effective antitumor drugs [28].

Meta-analysis Sun Q. et al. (2020). ≥20 years may be associated with an increased risk of melanoma in women. The authors observed that the use of menopausal hormone therapy was specifically associated with an increased risk of superficial spreading melanoma but not nodular melanoma, and estrogens and estradiol may be the main active agents contributing to the increased risk of melanoma. Increasing the number of births may reduce the risk of melanoma in women. However, any use of oral contraceptives, years since last use, age at first use, menopausal status, and age at menarche were not associated with melanoma incidence in women, according to this meta-analysis [33].

Based on the fact that there are contradictory and isolated data on the endocrine status of women with precancerous lesions of the vulva, **the purpose** of the study was to determine the hormonal profile of peripheral blood in women with this pathology.

Material and methods

To solve the problem, a study of the hormonal profile of the peripheral blood of patients with precancerous lesions of the vulva and gynecologically healthy women who sought medical help at the National Cancer Institute (Kyiv, Ukraine) and the "Verum" clinic (Kyiv, Ukraine) in 2017–2024 was done.

309 women with precancerous lesions of the vulva and 60 gynecologically healthy women aged 25 to 70 years were included in the study. Patients with precancerous lesions were divided into 4 groups depending on the nosological unit: group G1 – 87 women with severe dysplasia of the vulva depending on HPV (VHSIL); group G2 - 154 individuals with differentiated dysplasia of the vulva independent of HPV (dVIN), group G3 - 36 patients with extramammary Paget's disease of the vulva (VPD) grade Ia, and group G4 - 32 women with melanoma *in situ* of the skin of the vulva.

In the groups with VHSIL, with dVIN, with melanoma in situ, there were women of premenopausal and postmenopausal age, i.e. before 50 and after 50 years, therefore, for the

comparison of hormonal data in these groups, subgroups a and b were selected according to age: subgroups G4a, G5a and G8a - younger than 50 years, subgroups G4b, G5b and G8b - 50 years and older.

60 conditionally healthy women of the CG control group included CG1 (30 people under 50 years) and CG2 (30 people 50 years and older) subgroups.

Venous blood sampling for the study was performed on an empty stomach in premenopausal women on the 2nd-5th day of MC in accordance with generally accepted recommendations, and in postmenopausal women on any day after consulting a doctor. The vacutainer tubes with the gel were left at room temperature (15-20°C) until complete clot formation for an average of 1-1.5 hours, and after the clot formation, the vacutainer tubes were centrifuged at a speed of 3000 revolutions/min for 5-10 min. with the help of a medical centrifuge LMC-3000. Laboratory studies were performed in the certified laboratories of the "Institute of Pediatrics, Obstetrics and Gynecology named after academician O. M. Lukyanova of the National Academy of Sciences of Ukraine", the National Cancer Institute of Ukraine and the "Dila" LLC (Kyiv).

The levels of estradiol (E2), progesterone (P4), free testosterone (Tf), follicle-stimulating hormone (FSH) and luteinizing hormone (LH), prolactin (PRL), anti-Müllerian hormone (AMH), insulin were determined in blood serum by an immunochemical method with electrochemiluminescence detection (ECLIA) (Cobas 6000 analyzer, Roche Diagnostics test systems, Switzerland). The thyroid profile was assessed by the content of thyroid-stimulating hormone (TSH), free triiodothyronine (T3f), free thyroxine (T4f), antibodies to thyroperoxidase (ATPO) on an automatic immunochemiluminescent analyzer MaglumiX8 (DiaSystem Scandinavia AB, Sweden).

The HOMA index (insulin resistance index) (Homeostasis Model Assessment) was calculated using the formula: NOMA = (fasting blood glucose (mmol/l) x fasting insulin).

Patients were included in the study after obtaining written informed consent in accordance with the principles of the Helsinki Declaration of Human Rights, the Council of Europe Convention on Human Rights and Biomedicine, and the relevant laws of Ukraine. The diagnosis was established on the basis of the medical history, complaints, clinical examination, vulvoscopy, dermoscopy and the results of the morphological examination of biopsies or resected tissues of pathologically changed tissues.

Statistical processing and data analysis were carried out using the software Statistica 7.0 for Windows and Microsoft Excel. The work uses standard methods of descriptive and comparative analysis. Mean value (M), standard deviation (SD) and odds ratio (OR) were

calculated. The reliability of parametric values was evaluated according to the Student's criterion. A value of $p<0.05$ was considered statistically significant.

Results and their discussion

The average age of women with VHSIL was 40.08±1.13 years, with dVIN – 58.30±0.82 years, with VPD – 53.11±0.26 years, with melanoma *in situ* – 55.50 years, control CG group - 48.80±1.28 years. The average age of the examined persons in the subgroups and the body mass index are presented, respectively, in fig. 1 and fig. 2.

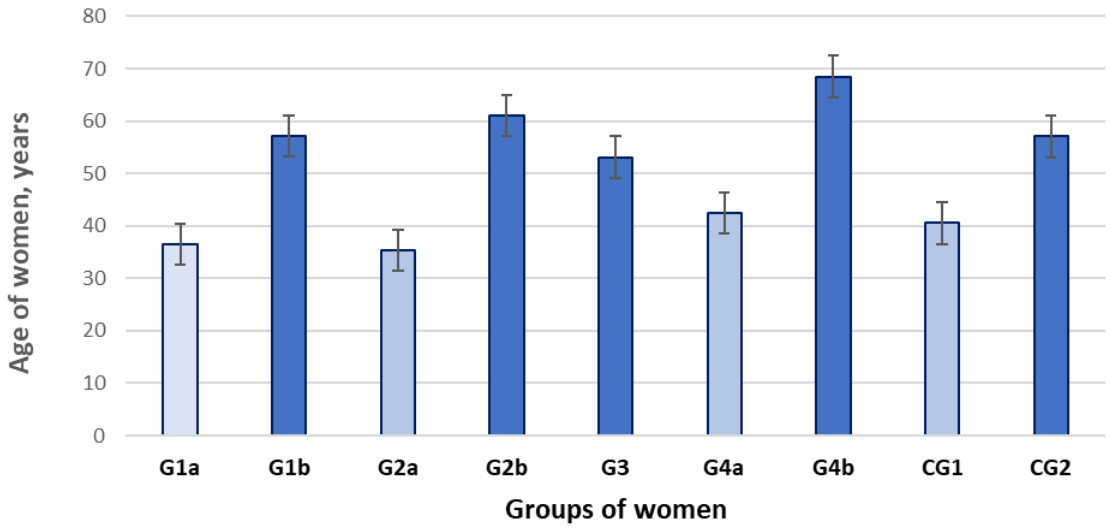

Figure 1 – Average age of persons of the studied groups.

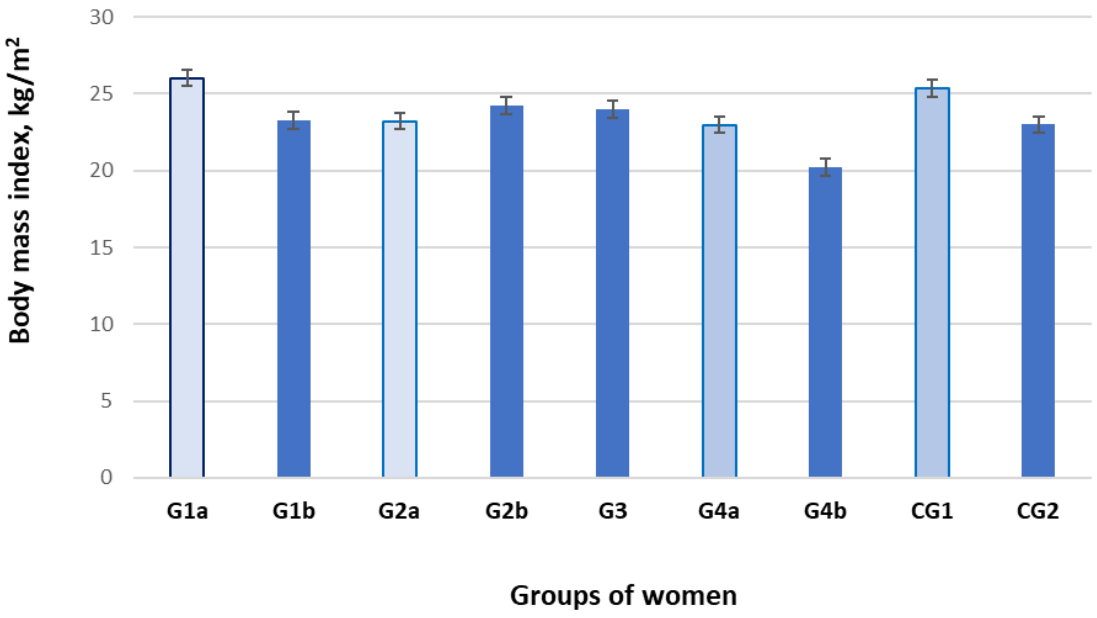

Figure 2 – Average body mass index of persons of the studied groups.

Analysis of the expression of pituitary hormones in subgroups of women with precancerous diseases of the vulva aged less than 50 years showed no difference between serum levels of LH and FSH, while the mean basal level of PRL was the highest in the subgroup with dVIN - 24.44±1.55 ng/ml versus 17,16±0.55 ng/ml in the subgroup with VHSIL ($p<0.01$), 17.25±1.63 ng/ml in the subgroup with melanoma in situ ($p<0.01$) and 16.70±0 .63 ng/ml in the CG1 group ($p<0.01$) (Table 1).

Table 1 – Average levels of pituitary hormones in women of the studied subgroups, M±SEM

Group	LH, mIU/ml	FSH, mIU/ml	LH/FSH	PRL, ng/ml
Subgroups of women under 50 years of age				
G1a, VHSIL, n=72	7.52±0.19	5.59±0.11	1.34±0.02	17.16±0.55[2]
G2a, dVIN, n=15	7.41±0.30	5.81±0.15	1.27±0.04 [k1]	24.44±1.55[1,4,k1]
G4a, melanoma*in situ*, n=16	7.64±0.36	5.80±0.28	1.34±0.08	17.25±1.63 [2]
CG1, n=30	7.58±0.20	5.45±0.13	1.41±0.05	16.70±0.63
Subgroups of women aged 50 years and older				
G1b, VHSIL, n=15	33.00±0.74	66.00±4.98 [k2]	0.53±0.03 [2,3,k2]	7.50±1.78 [k2]
G2b, dVIN, n=139	32.86±0.22 [3.4]	73.96±0.80 [k2]	0.45±0.01 [1,4,k2]	9.39±0.56 [k2]
G3, VPD, n=36	33.57±0.16 [2]	73.54±0.93 [k2]	0.46±0.01 [4,k2]	7.89±1.12 [4,k2]
G4b, melanoma*in situ*, n=16	36.90±2.09 [2]	74.67±4.06 [k2]	0.51±0.02 [2,k2]	9.85±2.03 [3,k2]
CG2, n=30	34.56±0.93	57.01±1.41	0.61±0.01	5.18±0.28
Note. [1, 2, 3, 4, k1, k2] - a statistically significant difference with the indicators of groups G1, G2, G3, G4, CG1, CG2 ($p<0.05$).				

Among women aged 50 years and older, the highest levels of LH, FSH and PRL were observed in persons with melanoma in situ - respectively 36.90±2.09 mIU/ml, 74.67±4.06 mIU/ml, 9.85±2 .03 ng/ml, while the LH/FSH ratio was the highest in patients with VHSIL (0.53±0.03). Subgroups of postmenopausal women with precancerous diseases of the vulva by age had statistically significantly higher serum levels of FSH and PRL, a lower ratio of LH/FSH than women of the CG2 group.

No statistically significant difference was found between the levels of serum P4 and AMH in the subgroups of women younger than 50 years and 50 years and older. In the subgroups of people younger than 50 years, the highest average levels of E2 were recorded in

women with melanoma in situ (104.61±10.22 pg/ml), Tf and Tf index in patients with dVIN (3.20±0.10 pg/ml and 8.25±0.24%, respectively). Levels of E2 were statistically significantly higher than the similar indicator of control in persons with melanoma *in situ*, Tf and index Tf - in patients with dVIN (Table 2).

Table 2 – Average levels of sex steroid hormones and AMH in women of the studied subgroups, M±SEM

Group	E2, pg/ml	P4, ng/ml	Tf, pg/ml	TV index, %	AMH, ng/ml
Subgroups of women under 50 years of age					
G1a, VHSIL, n=72	75,85±2,71 4	1,50±0,04	2,76±0,10 2	7,42±0,28 2	1,61±0,07
G2a, dVIN, n=15	79,13±5,31 4	1,39±0,06	3,20±0,10 1,4,k1	8,25±0,24 1,к1	1,70±0,14
G4a, melanoma *in situ*, n=16	104,61±10,22 1,2,k1	1,40±0,06	2,60±0,14 2	7,29±0,40	1,33±0,16
CG1, n=30	79,88±2,96	1,51±0,05	2,70±0,06	7,16±0,17	1,60±0,10
Subgroups of women aged 50 years and older					
G1b, VHSIL, n=15	21,17±1,03 4	0,54±0,03	1,27±0,08 2	3,44±0,13 2	0,06±0,01
G2b, dVIN, n=139	22,49±0,42 4	0,54±0,01	1,58±0,05 3,4,k2	3,77±0,10 1	0,08±0,01 k2
G3, VPD, n=36	23,57±2,60 4	0,54±0,03	1,31±0,10 2	3,52±0,09	0,09±0,03
G4b, melanoma*in situ*, n=16	30,71±1,90 1,2,3,k2	0,52±0,04	1,08±0,09 2,k2	3,20±0,30	0,10±0,07
CG2, n=30	21,01±0,65	0,48±0,03	1,32±0,04	3,60±0,06	0,05±0,00
Note. [1, 2, 3, 4, k1, k2] - a statistically significant difference with the indicators of groups G1, G2, G3, G4, CG1, CG2 ($p<0.05$).					

Similar changes in the secretion of sex steroids were observed in women aged 50 years and older. The highest average levels of E2 were recorded in women with melanoma in situ (30.71±1.90 pg/ml), Tv and Tf index - in patients with dVIN (1.58±0.05 pg/ml and 3.77±0.10%, respectively). Levels of E2 in patients aged 50 years and older exceeded similar indicators of controls in persons with melanoma *in situ*, Tf and AMH in women with dVIN. Tf levels in the G8 group were lower than the similar indicators in the CG2 group - 1.08±0.09 pg/ml vs. 1.32±0.04 pg/ml ($p<0.01$).

Analysis of thyroid diseases among the studied groups revealed their highest specific weight among individuals with dVIN and melanoma *in situ* in both subgroups of premenopausal and postmenopausal women (Fig. 3).

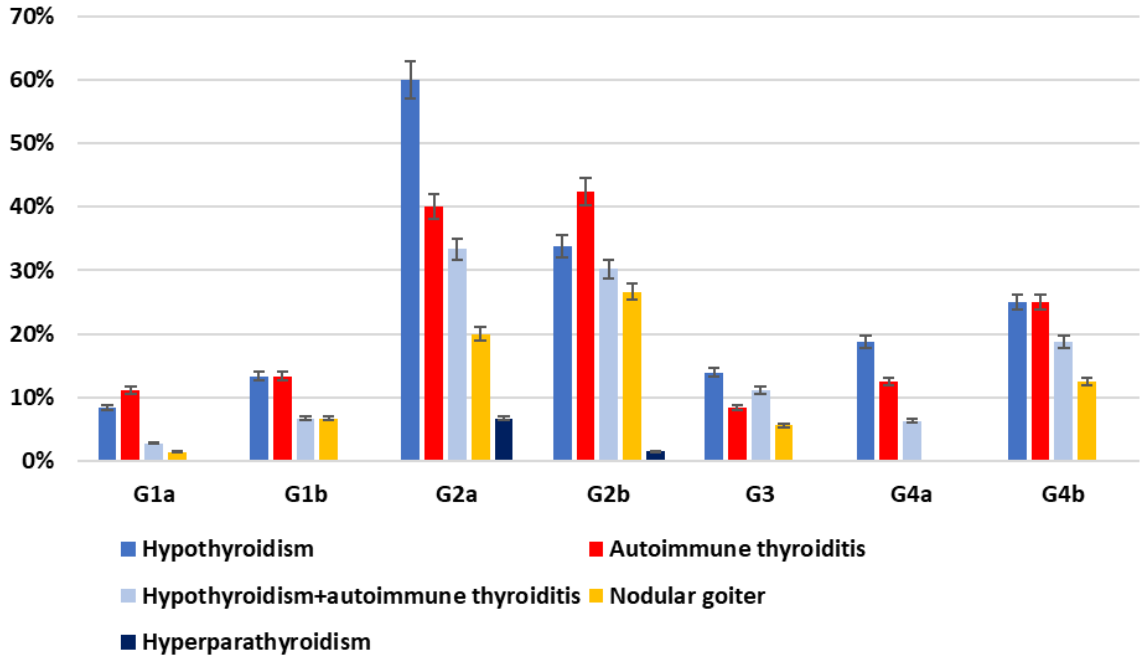

Figure 3 – Structure of thyroid diseases in women of the studied groups.

Among premenopausal women, the highest levels of TSH and ATPO were observed in persons with dVIN - 2.68±0.30 mIU/ml and 196.39±76.48 IU/ml, respectively, the lowest levels of T3f (2.62±0.15 ng /ml) and T4f (1.06±0.06 ng/dL). In the postmenopausal age group, the highest TSH and ATPO indicators were also observed in persons with dVIN – 2.23±0.09 mIU/ml and 123.46±16 .07 IU/ml, respectively the lowest levels of T3f (2.38±0.04 ng/ml) and T4f (1.09±0.04 ng/dL), TSH levels probably exceeded similar control indicators among women 50 years and older in the subgroups with dVIN, VPD and melanoma *in situ*, ATPO - in the subgroups with dVIN and melanoma in situ, the levels of T3f in the subgroups with dVIN and VPD, T4f - in the subgroups with dVIN and melanoma *in situ* were lower than these indicators in the control (table 3).

According to the level of 25(OH)D, the studied subgroups of premenopausal and postmenopausal age were homogeneous. The average level of 25(OH)D in subgroup G5a (22.44±2.33 ng/ml) was statistically significantly lower than that in group CG1 (28.81±1.42 ng/ml) ($p<0.02$), in the G5b subgroup (21.40±0.66 ng/ml) was lower than that in the CG2 group (24.79±1.25 ng/ml) ($p<0.02$) (Fig. 4).

Table 3 – Average levels of indicators of the thyroid system in women of the studied subgroups, M±SEM

Group	TSH, mIU/ml	ATPO, MO/ml	T3f, ng/ml	T4v, ng/dL
Subgroups of women under 50 years of age				
G1a, VHSIL, n=72	2.01±0.24	34.65±12.48 5,k1	3.00±0.04 5,k1	1.25±0.06 5
G2a, dVIN, n=15	2.68±0.30 8,k1	196.39±76.48 4,k1	2.62±0.15 4	1.06±0.06 4,k1
G4a, melanoma*in situ*, n=16	1.80±0.22 5	73.17±51.72	2.94±0.10 4	1.19±0.05
CG1, n=30	1.95±0.05	0.39±0.05	2.83±0.10	1.34±0.06
Subgroups of women aged 50 years and older				
G1b, VHSIL, n=15	2.01±0.24	37.95±27.23 5	2.94±0.05 5,6	1.23±0.11
G2b, dVIN, n=139	2.23±0.09 6,k2	123.46±16.07 4,6,8,k2	2.38±0.04 4,6,8,k2	1.09±0.04 6,k2
G3, VPD, n=36	1.90±0.12 5,k2	6.60±5.60 4,5	2.49±0.06 4,5,8,k2	1.22±0.04 5
G4b, melanoma*in situ*, n=16	2.07±0.19 к2	0.93±0.09 5,к2	2.83±0.10 5,6	1.11±0.06 k2
CG2, n=30	1.52±0.07	0.56±0.08	2.78±0.09	1.24±0.03
Note. 1, 2, 3, 4, k1, k2 - a statistically significant difference with the indicators of groups G1, G2, G3, G4, CG1, CG2 (p<0.05).				

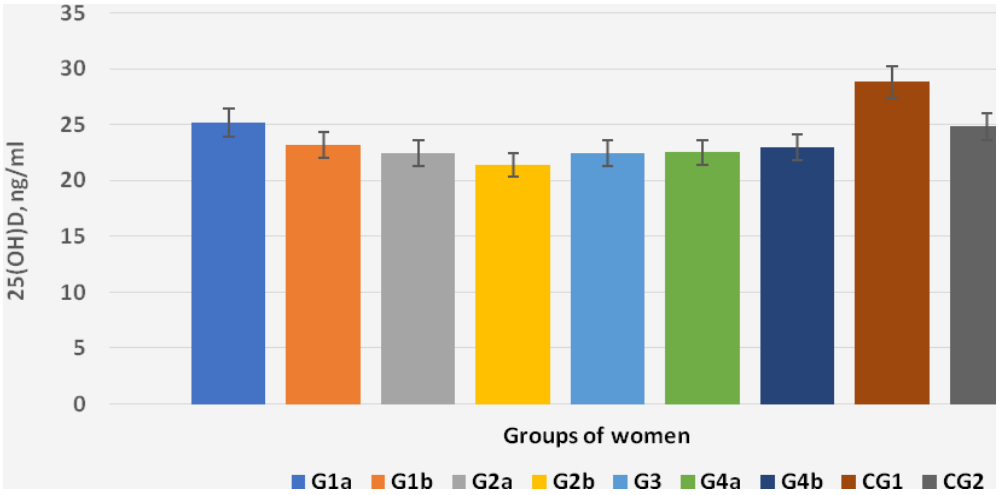

Figure 4 – Average levels of 25(OH)D in individuals of the studied groups.

Analysis of carbohydrate metabolism indicators showed that the level of insulin among women of premenopausal age was the highest in women with melanoma in situ (14.33±1.55 mIU/ml), it exceeded that of women with VHSIL (10.33±0.53 mIU/ ml, $p<0.02$), with dVIN (12.39±1.14 mIU/ml, $p>0.05$), CG1 group (8.71±0.46 mIU/ml, $p<0.01$). The level of insulin among postmenopausal women was the highest in women with VPD (14.90±1.02 mIU/ml), it exceeded that of women with VHSIL (11.83±1.13 mIU/ml, $p<0.05$), with dVIN (14.63±0.39 mIU/ml, $p>0.05$), individuals of group CG2 (8.49±0.34 mIU/ml, $p<0.01$). Average levels of insulin in all subgroups with precancerous diseases of the vulva exceeded similar indicators in the corresponding control groups.

Glucose levels were highest among premenopausal women in the dVIN group (5.15±0.12 mmol/L), and among postmenopausal women in the VPD group (5.40±0.10 mmol/L).

Accordingly, the HOMA index was the highest among premenopausal women in the group with dVIN (2.87±0.30), and among postmenopausal women in the group with VPD (3.60±0.27). The HOMA index among people under 50 years of age statistically significantly exceeded the similar indicator in the CG1 group (1.81±0.08) in people with VHSIL (2.18±0.12, $p<0.01$), with dVIN (2.87±0.30, $p<0.01$) and with melanoma *in situ* (2.74±0.32, $p<0.02$), and among persons 50 years and older it exceeded the similar one in the CG2 group (1.76±0 .08) among patients of subgroups with VHSIL (2.65±0.25, $p<0.01$), with dVIN (3.26±0.10, $p<0.01$), with VPD (3.60±0.27, $p<0.01$) and with melanoma *in situ* (3.44±0.37, $p<0.01$) (table 4).

Thus, the phenomena of insulin resistance were observed in the G4a subgroup in 18 (25.00%) people, in G5a - in 8 (53.33%), in G8a - in 6 (37.50%), while in the G4b subgroup in 7 (46.67%) of the examined, G5b – in 86 (61.87%), G6 – in 26 (72.22%), G8b – in 11 (68.75%) (Fig. 5).

Table 4 – Average levels of indicators of carbohydrate metabolism in women of the studied subgroups, M±SEM

Group	Insulin, mIU/ml	Glucose, mmol/l	HOMA index
Subgroups of women under 50 years of age			
G1a, VHSIL, n=72	10.33±0.53 ^{8,к1}	4.72±0.07 ^{5}	2.18±0.12 ^{5,к1}
G2a, dVIN, n=15	12.39±1.14 ^{к1}	5.15±0.12 ^{4,8,к1}	2.87±0.30 ^{4,к1}
G4a, melanoma*in situ*, n=16	14.33±1.55 ^{4,к1}	4.39±0.27 ^{5}	2.74±0.32 ^{к1}
CG1, n=30	8.41±0.46	4.85±0.10	1.81±0.08
Subgroups of women aged 50 years and older			
G1b, VHSIL, n=15	11.83±1.13 ^{5,6,к2}	5.07±0.10 ^{6}	2.65±0.25 ^{5,6,8,к2}
G2b, dVIN, n=139	14.63±0.39 ^{4,к2}	5.00±0.05 ^{6}	3.26±0.10 ^{4,к2}
G3, VPD, n=36	14.90±1.02 ^{4,к2}	5.40±0.10 ^{4,5,к2}	3.60±0.27 ^{4,к2}
G4b, melanoma*in situ*, n=16	14.84±1.55 ^{к2}	5.24±0.16	3.44±0.37 ^{4,к2}
CG2, n=30	8.29±0.34	4.93±0.09	1.78±0.06
Note. $^{1, 2, 3, 4, к1, к2}$ - a statistically significant difference with the indicators of groups G1, G2, G3, G4, CG1, CG2 (p<0.05).			

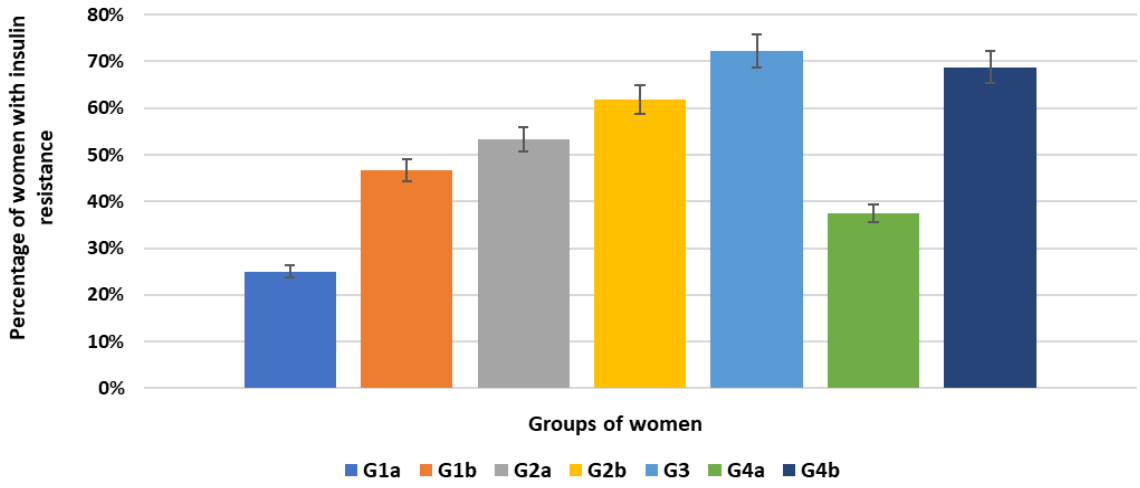

Figure 5 – Specific weight of patients in the studied groups with insulin resistance.

Conclusions

In women with various precancerous diseases of the vulva under the age of 50, there is no difference between the serum levels of LH and FSH. Individuals with dVIN were

characterized by the highest level of PRL (24.44±1.55 ng/ml). Among women aged 50 years and older, the highest levels of LH, FSH and PRL are observed in persons with melanoma in situ - respectively 36.90±2.09 mIU/ml, 74.67±4.06 mIU/ml, 9.85±2.03 ng/ml; the level of FSH and PRL is higher, and the LH/FSH ratio is lower than in controls in all precancerous diseases of the vulva. No statistically significant difference was found between the basal levels of serum P4 and AMH in subgroups of women younger than 50 years and 50 years and older. Among both premenopausal and postmenopausal women, the highest average levels of E2 are recorded in women with melanoma *in situ* (104.61±10.22 pg/ml and 30.71±1.90 pg/ml), Tf and Tf index - in patients with dVIN (3.20±0.10 pg/ml and 1.58±0.05 pg/ml vs. 8.25±0.24% and 3.77±0.10%, respectively).

Peculiarities of the thyroid status are the highest indicators of TSH and ATPO in persons with dVIN both in premenopause (2.68±0.30 mIU/ml and 196.39±76.48 IU/ml) and in postmenopause (2.23±0.09 mIU/ml and 123.46±16.07 IU/ml). The average level of 25(OH)D does not differ statistically significantly between women with various precancerous diseases of the vulva. The level of insulin and HOMA index among women of premenopausal age is the highest in women with melanoma *in situ* (14.33±1.55 mIU/ml), in postmenopausal women with VPD (14.90±1.02 mIU/ml), the index HOMA – with dVIN (2.87±0.30) and with VPD (3.60±0.27), respectively. Insulin resistance is most common in premenopause among individuals with dVIN (53.33%), and in postmenopause - with VPD (72.22%).

References

1. Wilkinson EJ, Kneale B, Lynch PJ. Report of the ISSVD terminology committee. J Reprod Med Obstet Gynecol. 1986;31:973-4.

2. Sideri M, Jones RW, Wilkinson EJ, Preti M, Heller DS, Scurry J, et al. Squamous vulvar intraepithelial neoplasia: 2004 modified terminology, ISSVD Vulvar Oncology Subcommittee. J Reprod Med. 2005 Nov;50(11):807-10.

3. Darragh TM, Colgan TJ, Cox JT, Heller DS, Henry MR, Luff RD, et al. The Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology. J Low Genit Tract Dis. 2012 Jul;16(3):205-42. doi: 10.1097/LGT.0b013e31825c31dd.

4. World Health Organization, International classification of diseases for mortality and morbidity statistics (11th Revision), 2018. Available: https://icd.who.int/browse11/l-m/en#/http://id.who.int/icd/entity/ 913387730.

5. Bornstein J, Bogliatto F, Haefner HK, Stockdale CK, Preti M, Bohl TG, et al. Terminology of Vulvar Squamous Intraepithelial Lesions. J Low Genit Tract Dis. 2016 Jan;20(1):11-4. doi: 10.1097/LGT.0000000000000169.

6. Crum C, Herrington C, McCluggage W. Tumours of the vulva; epithelial tumors. In: WHO classification of tumours of female reproductive organs. Lyon: IARC Press, 2014.

7. Schindler AE, Hormone replacement therapy (HRT) in women after genital cancer. Maturitas. 2002 April;41:105-111. https://doi,org/10.1016/S0378-5122(02)00018-X.

8. Sherman K,J, Daling J,R, McKnight B, Chu J. Hormonal factors in vulvar cancer: A case-control study. J Reprod Med Obstet, Gynecol. 1994;39:857-86. doi: 10.1016/0378-5122(95)91220-9.

9. Vieira - Baptista P, Lima-Silva J, Cavaco - Gomes J, Beires J, Martinez – de - Oliveira J. What differentiates symptomatic from asymptomatic women with lichen sclerosus? Gynecol Obstet Invest. 2015;79(4):263-8. doi: 10,1159/000367788.

10. Powell J, Wojnarowska F. Childhood vulvar lichen sclerosus: an increasingly common problem. J Am Acad Dermatol. 2001 May;44(5):803-6. doi: 10.1067/mjd,2001.113474.

11. Neill SM, Tatnall FM, Cox NH; British Association of Dermatologists, Guidelines for the management of lichen sclerosus. Br J Dermatol. 2002 Oct;147(4):640-9. doi: 10.1046/j.1365-2133.2002.05012.x.

12. Powell JJ, Wojnarowska F, Lichen sclerosus. Lancet. 1999 May 22;353(9166):1777-83. doi: 10.1016/s0140-6736(98)08228-2.

13. Vieira - Baptista P, Lima - Silva J, Cavaco - Gomes J, Beires J, Martinez-de-Oliveira J. What differentiates symptomatic from asymptomatic women with lichen sclerosus? Gynecol Obstet Invest. 2015;79(4):263-8. doi: 10.1159/000367788.

14. Goldstein AT, Marinoff SC, Christopher K, Srodon M. Prevalence of vulvar lichen sclerosus in a general gynecology practice. J Reprod Med. 2005 Jul;50(7):477-80.

15. Bleeker MC, Visser PJ, Overbeek LI, van Beurden M, Berkhof J. Lichen Sclerosus: Incidence and Risk of Vulvar Squamous Cell Carcinoma. Cancer Epidemiol Biomarkers Prev. 2016 Aug;25(8):1224-30. doi: 10.1158/1055-9965.EPI-16-0019.

16. Micheletti L, Preti M, Radici G, Boveri S, Di Pumpo O, Privitera SS, et al. Vulvar Lichen Sclerosus and Neoplastic Transformation: A Retrospective Study of 976 Cases. J Low Genit Tract Dis. 2016 Apr;20(2):180-3. doi: 10,1097/LGT,0000000000000186.

17. Lee A, Bradford J, Fischer G. Long-term Management of Adult Vulvar Lichen Sclerosus: A Prospective Cohort Study of 507 Women. JAMA Dermatol. 2015 Oct;151(10):1061-7. doi: 10.1001/jamadermatol.2015.0643.

18. Friedrich EG Jr, Kalra PS. Serum levels of sex hormones in vulvar lichen sclerosus, and the effect of topical testosterone. N Engl J Med. 1984 Feb 23;310(8):488-91. doi: 10.1056/NEJM198402233100803.

19. Clifton MM, Garner IB, Kohler S, Smoller BR. Immunohistochemical evaluation of androgen receptors in genital and extragenital lichen sclerosus: evidence for loss of androgen receptors in lesional epidermis. J Am Acad Dermatol. 1999 Jul;41(1):43-6. doi: 10.1016/s0190-9622(99)70404-4. Erratum in: J Am Acad Dermatol 2000 Jan;42(1 Pt 1):148.

20. Hodgins MB, Spike RC, Mackie RM, MacLean AB. An immunohistochemical study of androgen, oestrogen and progesterone receptors in the vulva and vagina. Br J Obstet Gynaecol. 1998 Feb;105(2):216-22. doi: 10.1111/j.1471-0528.1998.tb10056.x.

21. Carlson JA, Murphy M. Androgen receptors and lichen sclerosus. J Am Acad Dermatol. 2000 Sep;43(3):559-60. doi: 10.1067/mjd,2000.109276.

22. Günthert AR, Faber M, Knappe G, Hellriegel S, Emons G. Early onset vulvar Lichen Sclerosus in premenopausal women and oral contraceptives. Eur J ObstetGynecolReprod Biol. 2008 Mar;137(1):56-60. doi: 10.1016/j.ejogrb.2007.10.005.

23. Murphy R. Lichen sclerosus, Dermatol Clin. 2010 Oct;28(4):707-15. doi: 10.1016/j.det.2010.07.006.

24. Caruso G, Barcellini A, Mazzeo R, Gallo R, Vitale MG, Passarelli A, et al. Vulvar Paget's Disease: A Systematic Review of the MITO Rare Cancer Group, Cancers (Basel). 2023 Mar 16;15(6):1803. doi: 10.3390/cancers15061803.

25. Angelico G, Santoro A, Inzani F, Straccia P, Arciuolo D, Mulè A, et al. Hormonal Environment and HER2 Status in Extra-Mammary Paget's Disease (eMPD): A Systematic Literature Review and Meta-Analysis with Clinical Considerations. Diagnostics (Basel). 2020 Dec 3;10(12):1040. doi: 10.3390/diagnostics10121040.

26. Garganese G, Inzani F, Fragomeni SM, Mantovani G, Della Corte L, Piermattei A, et al. The Vulvar Immunohistochemical Panel (VIP) Project: Molecular Profiles of Vulvar Squamous Cell Carcinoma, Cancers (Basel). 2021 Dec 19;13(24):6373. doi: 10.3390/cancers13246373.

27. Chiavarini M, Naldini G, Giacchetta I, Fabiani R. Effect of Exogenous Hormones and Reproductive Factors in Female Melanoma: A Meta-Analysis [Letter].Clin Epidemiol. 2022 Feb 22;14:211-212. doi: 10.2147/CLEP.S356205.

28. Dika E, Patrizi A, Lambertini M, Manuelpillai N, Fiorentino M, Altimari A, et al. Estrogen Receptors and Melanoma: A Review. Cells. 2019 Nov 19;8(11):1463. doi: 10.3390/cells8111463.

29. Kemeny MM, Busch E, Stewart AK, Menck HR. Superior survival of young women with malignant melanoma. Am J Surg. 1998 Jun;175(6):437-44; discussion 444-5. doi: 10.1016/s0002-9610(98)00070-1.

30. Mervic L, Leiter U, Meier F, Eigentler T, Forschner A, Metzler G, et al. Sex differences in survival of cutaneous melanoma are age dependent: an analysis of 7338 patients. Melanoma Res. 2011 Jun;21(3):244-52. doi: 10.1097/CMR.0b013e32834577c8.

31. Donley GM, Liu WT, Pfeiffer RM, McDonald EC, Peters KO, Tucker MA, et al. Reproductive factors, exogenous hormone use and incidence of melanoma among women in the United States. Br J Cancer. 2019 Apr;120(7):754-760. doi:10.1038/s41416-019-0411-z.

32. Beral V, Evans S, Shaw H, Milton G. Oral contraceptive use and malignant melanoma in Australia. Br J Cancer. 1984 Nov;50(5):681-5. doi: 10.1038/bjc.1984.236.

33. Sun Q, Sun H, Cong L, Zheng Y, Wu N, Cong X. Effects of Exogenous Hormones and Reproductive Factors on Female Melanoma: A Meta-Analysis. Clin Epidemiol. 2020 Oct 29;12:1183-1203. doi: 10.2147/CLEP.S273566.

Author's contribution

The work is solitary

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Conclusion of the commission on bioethics

A positive decision of the bioethics commission was received for conducting the research of the National Cancer Institute (Kyiv, protocol No. 2/21 dated 08.11.2021), the basic moral and ethical principles of the Helsinki Declaration of the World Medical Association for Biomedical Research are observed.

Statement of informed consent

Written informed consent for processing was obtained from the patient(s).
personal data and their further use.

Statement on data availability

All information is publicly available, data on a specific patient
can be obtained on request from the author.

Conflict of interest. The author declares no conflict of interest