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КЛІНІЧНА ЕНДОКРИНОЛОГІЯ

REDOX STATUS AND CELL MEMBRANE ALTERATIONS OF CIRCULATING LEUKOCYTES AND ERYTHROCYTES IN ABNORMAL UTERINE BLEEDING*

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Abnormal uterine bleeding (AUB) is defined as an irregularity of the menstrual cycle manifesting as a heavy blood discharge [1, 2]. Its prevalence depends on age can reach up to 40% in women aged from 40 to 49 years [3]. Thus, AUB is a quite common and frequent condition, which significantly affects the quality of life influencing physical and emotional state of women [4]. According to the International Federation of Obstetrics and Gynecology (FIGO), the causes of AUB can be summarized in the PALM-COEIN (polyp, adenomyosis, leiomyoma, malignancy, coagulopathy, ovulatory disorders, endometrial, iatrogenic and not otherwise classified) acronym [1]. Analysis of the mechanisms and factors that underlie AUB has revealed that reduced endometrial vasoconstriction, abnormal angiogenesis, fragile vascular wall, insufficient blood clotting, local inflammation, and poor tissue repair may contribute to AUB occurrence [5, 6]. There is accumulating evidence that hormonal imbalance can be associated with a higher prevalence of AUB [7, 8]. It has been reported that thyroid disorders, especially hypothyroidism, is common in females with AUB [9–11].

It is important to note that thyroid hormones are known to interact with cell membranes affecting their phospholipid composition, lipid order, fluidity, and microviscosity [12, 13]. The maintenance of normal cell membrane lipid composition and physic-chemical properties is crucial for controlling cell functions and cell membrane alterations result in instability and malfunction of cell membranes [14]. The possible impact on cell membrane abnormalities along with the role of erythrocytes and leukocytes in AUB and AUB accompanied by hypothyroidism is poorly investigated. Cell membrane abnormalities may reduce cell viability and promote cell death [15]. Anemia due to blood loss is very common among

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females with AUB [16]. One of the factors that can worsen the anemia is excessive eryptosis, which is a programmed cell death of erythrocytes similar to apoptosis of other cells [17, 18]. The contribution of eryptosis to AUG-associated anemia has not been covered.

Thus, **the aim** of this study was to assess eryptosis degree, the state of cell membranes and redox status of circulating red blood cells and leukocytes in women with abnormal uterine bleeding alone and in combination with thyroid disorders.

MATERIALS AND METHODS

Patients and groups

Patients, 74 women aged 18 to 49 years, were examined, which were divided into 3 groups:

- group I – women with abnormal uterine bleeding (AUB) (24 patients);
- group II — with AUB and thyroid pathology (30 patients, of whom 18 women had primary hypothyroidism and 2 — secondary hypothyroidism);
- group III — control group (20 healthy women, who had never had menstrual irregularities).

The average age of patients (n = 74) was 38.5 ± 7.7 years. The average age by subgroups was 39.5 ± 7.5 years, 35.4 ± 5.1 years, 38.7 ± 3.1 respectively. 65% of all women with AUB and thyroid gland lesions had heavy and prolonged menstruation, which led to secondary anemia. In patients of group 1, this percentage was 42%. 37% of women in group 1 and 49% of women in group 2 had pronounced pain syndrome on the eve and during menstruation. Bleeding in the intermenstrual period worried 7 (29.1%) women of group 1 and 11 (36.6%) women of group 2.

Analyzing the etiological factors of AUB according to ultrasound, it was determined that the frequency of cases of endometrial hyperplasia in women with concomitant thyroid pathology prevailed by 12%. the frequency of uterine leiomyomas in women with concomitant thyroid pathology was comparable to the corresponding indicator of group 1 (22.2% and 21.4%). The incidence of cervical canal polyps was three times higher in women with AUB mono-run than in women with concomitant thyroid involvement: 19.6% and 5.6% respectively.

In the structure of thyroid lesions, 33.3% of cases of euthyroidism were identified. The distribution by stages of autoimmune thyroiditis showed a reliable ($\chi 2 = 10.889$, $p = 0.012$) prevalence of the frequency of autoimmune thyroiditis stage 1 (55.6%). Postoperative hypothyroidism was diagnosed in 33.3%.

When analyzing complaints, it was found that primary infertility occurred in women with thyroid pathology much more often, and amounted to 18%, while in women of group 1, this percentage was 4%. Among somatic pathology in 15% of patients of group II and in 10% of women of group 1, obesity of grade II was detected, body weight index 30–34.9 kg/m^2. Hypertension occurred in 10% of patients of group 1 and 13% of patients of group 2.

Eryptosis indices

Eryptosis was estimated using flow cytometry by analyzing externalized phosphatidylserine (PS) level in erythrocyte cell membranes via fluorochrome-labelled annexin V binding and evaluating intracellular reactive oxygen species (ROS) levels by 2′,7′-dichlorodihydrofluorescein diacetate (H2DCFDA) staining. Erythrocyte suspensions obtained as described above were stained with annexin V-FITC (BD Pharmingen™ FITC-Annexin V, BD Biosciences, San Jose, CA, USA) and H2DCFDA [19, 20].

Washed erythrocytes were transferred to 1x BD Pharmingen™ Annexin V Binding Buffer produced by Becton Dickinson (USA) and stained with annexin V labeled with FITC. The mixtures were incubated for 15 minutes protected from sunlight.

H2DCFDA staining included loading of erythrocytes in suspensions of cells in PBS using a 10 mM stock solution in dimethyl sulfoxide (DMSO, Sigma-Aldrich, USA) to reach a 5 μM dye working solutions. The samples were incubated for 30 min in the dark. After staining, washing with PBS and resuspension of cell pellets in PBS (500 μL), fluorescence signal was determined by flow cytofluorometric method.

Both PS exposure and fluorescence of ROS-sensitive probe were detected in different samples by a BD FACSCanto™ II cell counter (BD Biosciences, USA) with an excitation at 488 nm and an emission at a wavelength of 525 nm.

Redox status of leukocytes

Leukocyte suspensions in PBS were stained with a H2DCFDA 10 mM stock solution in DMSO (10 μM). In addition, prior to incubation during 30 min under protection from light, 100 μL of solutions were stained with 5 μL BD Pharmingen™ APC-Cy™7 Mouse Anti-human CD45 (BD Biosciences, USA) and 10 μL 7-aminoactinomycin D (7-AAD, BD Pharmingen™ 7-AAD, BD Biosciences, USA) to identify viable leukocytes. Fluorescence detection was performed by BD FACSCanto™ II with an excitation at 488, 488, 633 nm and an emission at a wavelength of 525, 670, 780 nm for dichlorofluorescein, 7-AAD and labeled antibodies to leukocyte CD45 marker, respectively.

Fluorescent probes O1O and PH7

The cells were stained with the fluorescent probes by the same procedure: an aliquot of the probe stock solution in acetonitrile was added to the cell suspensions, thus, a final probe concentration of the probe was $5–10^{-6}$ mol/L and lipid-to-probe molar ratio was 200:1. Before the measurements of the probe fluorescence, the cell suspensions were incubated with the probes at room temperature during 1 hour. The fluorescence measurements were conducted on a fluorescence spectrometer «PerkinElmer FL8500». The parameters of the fluorescence measurements: the excitation wavelength was 330 nm; the fluorescence was detected in the range of 340–550 nm, with an increment of 0.1 nm; the emission and excitation slits were 5 nm; the emission scan speed was 240 nm/min.

Fluorescent probes O1O (2-(2¢-hydroxyphenyl)-5-phenyl-1,3-oxazole) and PH7 (2-(2¢-hydroxy-phenyl)-phenanthro[9,10-*d*]-1,3-oxazole) were used in this study, because their fluorescence characteristics depend on *proton-donor ability* and the polarity of the probe environment [21, 22], and, hence, depend upon the hydration of the microenvironment [23]. Because the changes in membrane hydration are connected with the changes of the membrane lipid order [24], the probes can indicate them.

The location of the probes in lipid membrane: probe O1O in the region of glycerol backbones of phospholipids closer to the center of the lipid bilayer, in the region of carbonyl groups of phospholipids and in the region of hydrocarbon chains of phospholipids near the region of the carbonyl groups of phospholipids; probe PH7 in the region of hydrocarbon chains of phospholipids closer to the center of the lipid bilayer [22].

When the probes O1O and PH7 are in the excited electronic state, they undergo excited state intramolecular proton transfer [21, 36]. During this process, the initial (or so-called «normal») form (N*) converts into phototautomer form (T*), which emits at significantly longer wavelengths in comparison with the initial form [21, 22]. The degree of the conversion of the normal form (N*) into the photoproduct (T*) depends on the probe microenvironment [22].

Two-band fluorescence of the probes enables us to perform ratiometric measurements, i.e. to use the phototautomer fluorescence intensity-to-the initial form fluorescence intensity ratio (I_{T*}/I_{N*}) as a parameter to estimate the changes in chemical and physical properties of the microenvironment: e.g., the ratio I_{T*}/I_{N*} decreases with the increase in hydration of the media [22].

Statistical analysis

Fluorescent probe and flow cytometric data processing was performed using the Kruskal-Wallis test followed by Dunn's post hoc test for multiple comparisons. Data are given as the median [interquartile range (the 25th and 75th percentile)]. GraphPad Prism 5 (GraphPad Software Inc., La Jolly, CA, USA) was used to process data. P values not above 0.05 indicated the statistical significance.

RESULTS AND THEIR DISCUSSION

Annexin V staining of erythrocytes was used to analyze the cell membrane scrambling in erythrocytes of the females with abnormal uterine bleeding alone and in combination with insufficiency of thyroid hormones.

The results are summarized in Table 1 and Figure 1.

In control samples only a small percentage of cells had abnormal cell membrane scrambling, i.e. PS externalization. In women with

Table 1

Parameters that characterize phosphatidylserine externalization in erythrocytes of patients with abnormal uterine bleeding and abnormal uterine bleeding combined with hypothyroidism (Me [IQR])

Group of patients / Index	Control subjects (n = 12)	Abnormal uterine bleeding (n = 12)	Abnormal uterine bleeding and hypothyroidism (n = 12)
Percentage of annexin V-positive eryptotic cells, %	0.80 [0.60; 1.28]	1.20 [0.93; 1.48], $p_1 > 0.05$	1.35 [1.10; 2.38], $p_1 = 0.0027$ $p_2 > 0.05$
The mean fluorescence intensity of annexin V-FITC, a.u.	260 [226; 293]	290 [248; 318], $p_1 > 0.05$	294 [281; 325], $p_1 = 0.0262$ $p_2 > 0.05$

Note:
p_1 indicates the difference with the control group, while p_2 shows the statistical significance compared with the patients with abnormal uterine bleeding.

abnormal uterine bleeding this parameter was statistically insignificantly higher ($p_1 > 0.05$), while if the disease developed against the background of hypothyroidism the percentage of PS-exposing erythrocytes was statistically significantly higher ($p_1 = 0.0027$) compared to the healthy volunteers and did not differ ($p_2 > 0.05$) from the patients with just abnormal uterine bleeding. This tendency was also maintained when analyzing the MFI values of annexin V-FITC, which allowed characterizing quantitatively PS externalization in all red blood cells.

An increase in MFI values in the patients with abnormal uterine bleeding was statistically insignificant ($p_1 > 0.05$). However, our analysis revealed that a combination of abnormal uterine bleeding and hypothyroidism resulted in statistically significant elevation of MFI values of annexin V-FITC ($p_1 = 0.0262$) in comparison with the control group. Meanwhile, the difference in this eryptosis index was insignificant ($p_2 > 0.05$) between the women with the isolated abnormal uterine bleeding and the females with the combined pathology.

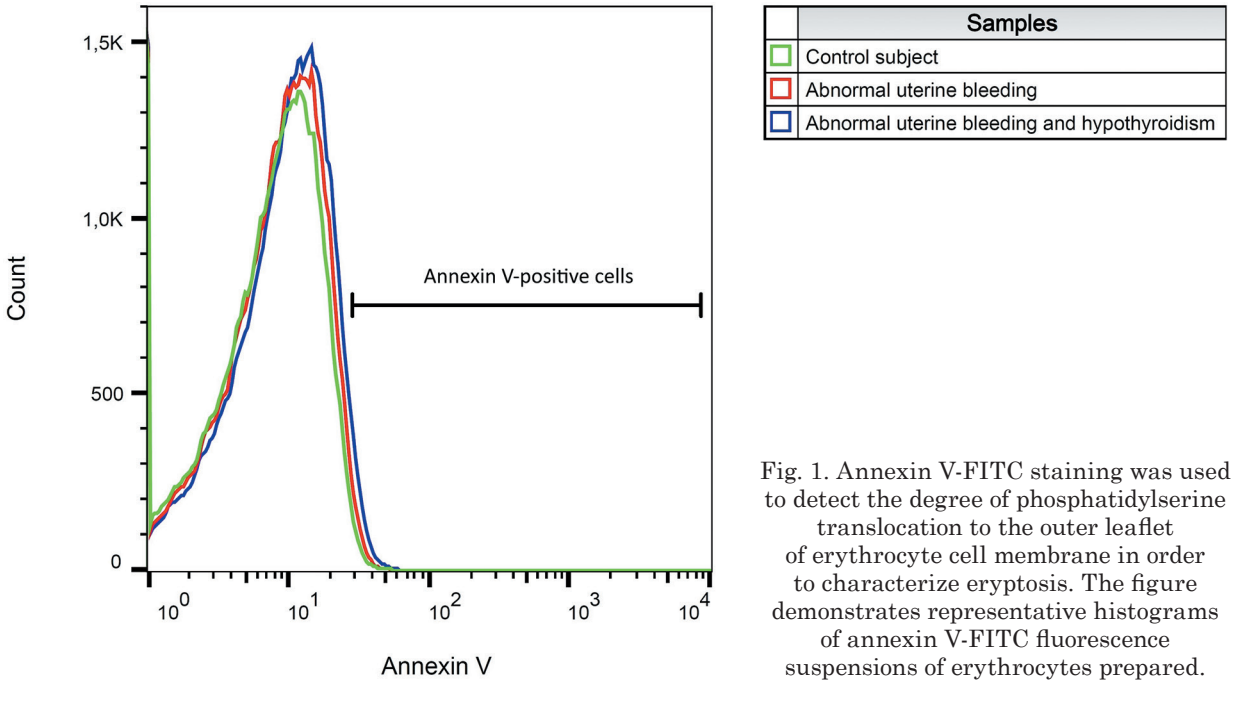

Fig. 1. Annexin V-FITC staining was used to detect the degree of phosphatidylserine translocation to the outer leaflet of erythrocyte cell membrane in order to characterize eryptosis. The figure demonstrates representative histograms of annexin V-FITC fluorescence suspensions of erythrocytes prepared.

Table 2

Redox status of circulating erythrocytes and leukocytes collected from the patients with abnormal uterine bleeding and abnormal uterine bleeding combined with hypothyroidism (Me [IQR])

Group of patients / Index	Control subjects (n = 12)	Abnormal uterine bleeding (n = 12)	Abnormal uterine bleeding and hypothyroidism (n = 12)
The mean fluorescence intensity of dichlorofluorescein in erythrocytes, a.u.	270 [247; 290]	287 [258; 305], $p_1 > 0.05$	298 [273; 335], $p_1 = 0.0176$ $p_2 > 0.05$
The mean fluorescence intensity of dichlorofluorescein in leukocytes, a.u.	4706 [3971; 5291]	4752 [4183; 5311], $p_1 > 0.05$	4548 [4251; 5140], $p_1 > 0.05$ $p_2 > 0.05$

Note:
p_1 indicates the difference with the control group, while p_2 shows the statistical significance compared with the patients with abnormal uterine bleeding.

Thus, annexin V staining shows that eryptosis is activated in the patients with a combination of abnormal uterine bleeding and hypothyroidism.

The redox status of blood cells was assessed by H2DCFDA staining, which allows revealing ROS levels inside the cells. In particular, MFI values of DCF in the women with abnormal uterine bleeding did not show any difference with the control blood samples ($p_1 > 0.05$). On the other hand, the combined pathology led to ROS overgeneration, evidenced by a statistically significant increase in DCF fluorescence ($p_1 = 0.0176$). The difference ($p_2 > 0.05$) in ROS production in erythrocytes between two pathologies was insignificant (Table 2 and Figure 2).

The fluorescence of DCF in viable leukocytes (CD45-positive, 7-AAD-negative cells) is presented. Our findings indicate that no changes in the DCF fluorescence were observed in circulating leukocytes extracted from the individuals with abnormal uterine bleeding and abnormal uterine bleeding combined with hy-

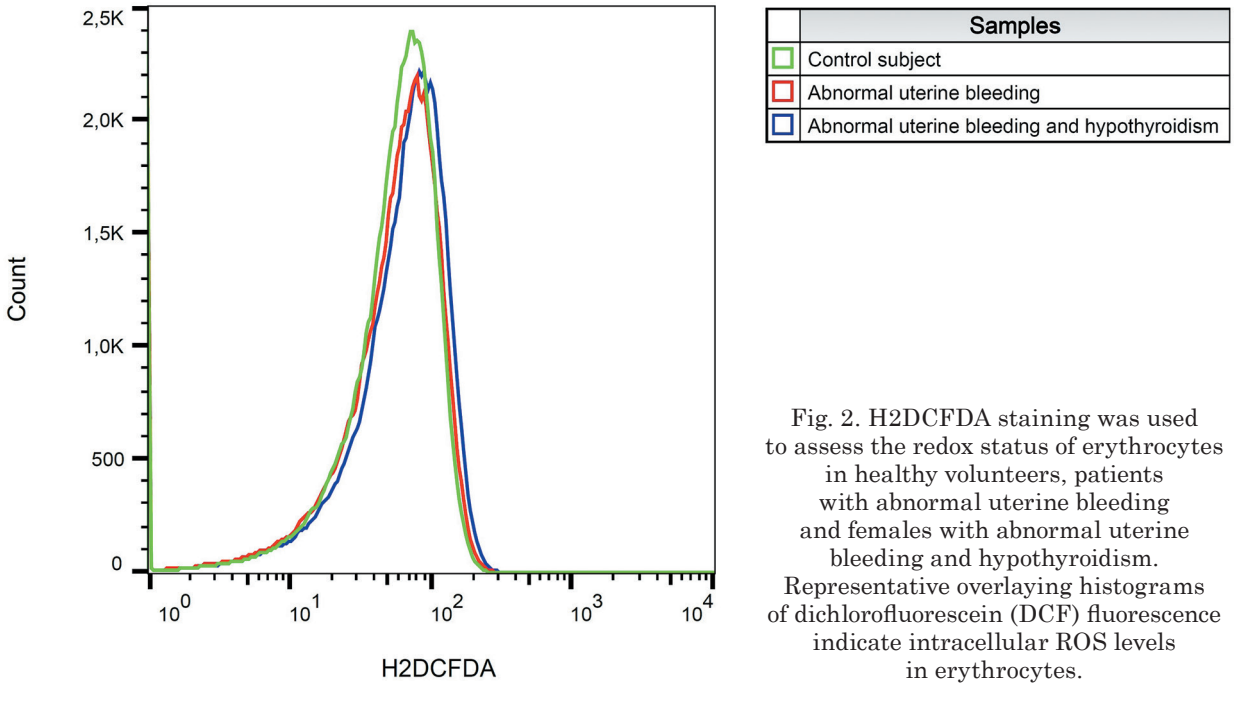

Fig. 2. H2DCFDA staining was used to assess the redox status of erythrocytes in healthy volunteers, patients with abnormal uterine bleeding and females with abnormal uterine bleeding and hypothyroidism. Representative overlaying histograms of dichlorofluorescein (DCF) fluorescence indicate intracellular ROS levels in erythrocytes.

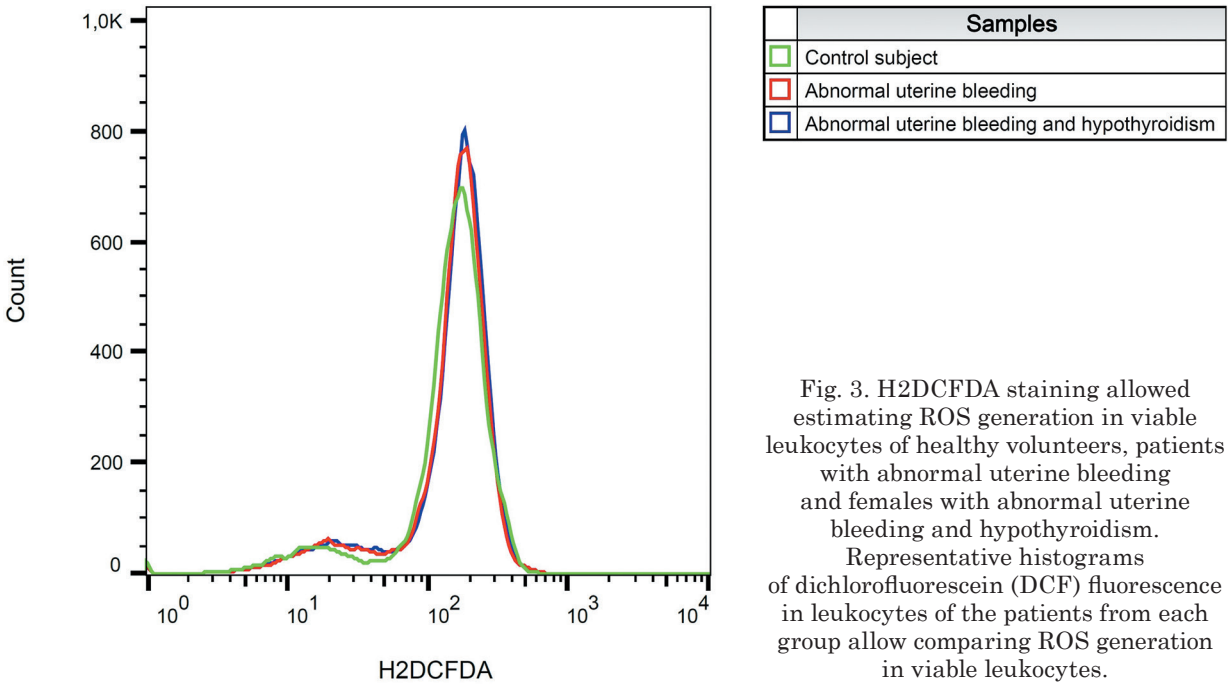

Fig. 3. H2DCFDA staining allowed estimating ROS generation in viable leukocytes of healthy volunteers, patients with abnormal uterine bleeding and females with abnormal uterine bleeding and hypothyroidism. Representative histograms of dichlorofluorescein (DCF) fluorescence in leukocytes of the patients from each group allow comparing ROS generation in viable leukocytes.

Table 3

The I_{T*}/I_{N*} fluorescence intensity ratio of probes 2-(2¢-hydroxy-phenyl)-5-phenyl-1,3-oxazole and 2-(2¢-hydroxy-phenyl)-phenanthro[9,10-*d*]-1,3-oxazole in circulating erythrocytes and leukocytes of the patients with abnormal uterine bleeding and abnormal uterine bleeding combined with hypothyroidism (Me [IQR])

Group of patients / Parameter	Control subjects (n = 12)	Abnormal uterine bleeding (n = 12)	Abnormal uterine bleeding and hypothyroidism (n = 12)
The I_{T*}/I_{N*} fluorescence intensity ratio of 2-(2¢-hydroxy-phenyl)-5-phenyl-1,3-oxazole in erythrocytes, a.u.	1.25 [1.19; 1.34]	1.34 [1.30; 1.40], $p_1 = 0.0003$	1.38 [1.34; 1.42], $p_1 < 0.0001$ $p_2 > 0.05$
The I_{T*}/I_{N*} fluorescence intensity ratio of 2-(2¢-hydroxy-phenyl)-phenanthro[9,10-*d*]-1,3-oxazole in erythrocytes, a.u.	1.58 [1.48; 1.69]	1.61 [1.54; 1.75], $p_1 > 0.05$	1.56 [1.47; 1.67], $p_1 > 0.05$ $p_2 > 0.05$
The I_{T*}/I_{N*} fluorescence intensity ratio of 2-(2¢-hydroxy-phenyl)-5-phenyl-1,3-oxazole in leukocytes, a.u.	2.72 [2.59; 2.95]	2.69 [2.55; 2.84], $p_1 > 0.05$	2.71 [2.54; 3.00], $p_1 > 0.05$ $p_2 > 0.05$
The I_{T*}/I_{N*} fluorescence intensity ratio of 2-(2¢-hydroxy-phenyl)-phenanthro[9,10-*d*]-1,3-oxazole in leukocytes, a.u.	1.68 [1.61; 1.78]	1.63 [1.60; 1.70], $p_1 > 0.05$	1.66 [1.57; 1.72], $p_1 > 0.05$ $p_2 > 0.05$

Note:
p_1 indicates the difference with the control group, while p_2 shows the statistical significance compared with the patients with abnormal uterine bleeding.

pothyroidism compared with healthy subjects (Figure 3).

Thus, abnormal uterine bleeding in a combination with hypothyroidism is accompanied by altered redox homeostasis in erythrocytes with the unaffected redox status of leukocytes.

Outcomes of fluorescence measurements of the spectra of fluorescent probes O1O and

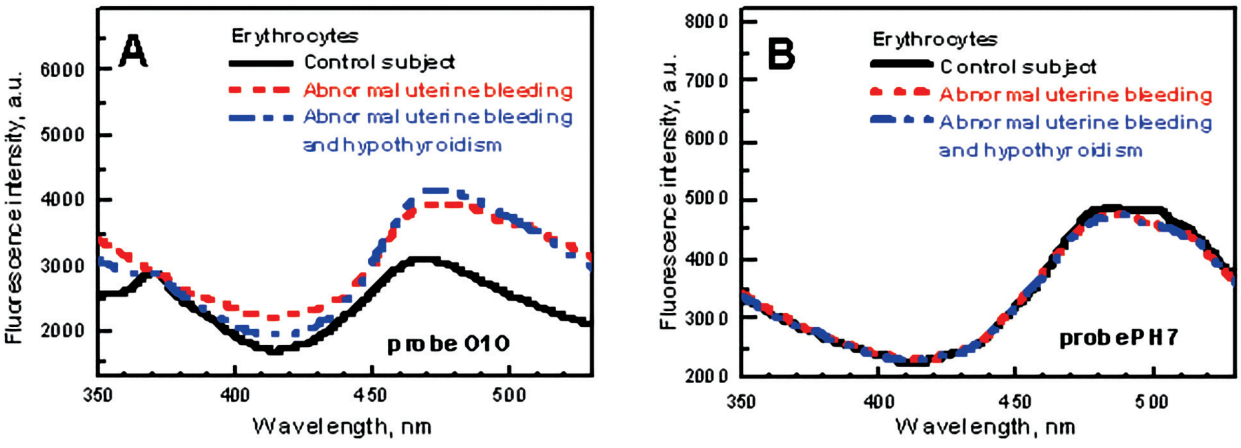

Fig. 4. Representative fluorescence spectra of probes O1O (panel A) and PH7 (panel B) in erythrocyte suspensions: (a) the control subject (black solid line), (b) abnormal uterine bleeding (red dashed line), (c) abnormal uterine bleeding and hypothyroidism (blue dash-dot-dot line).

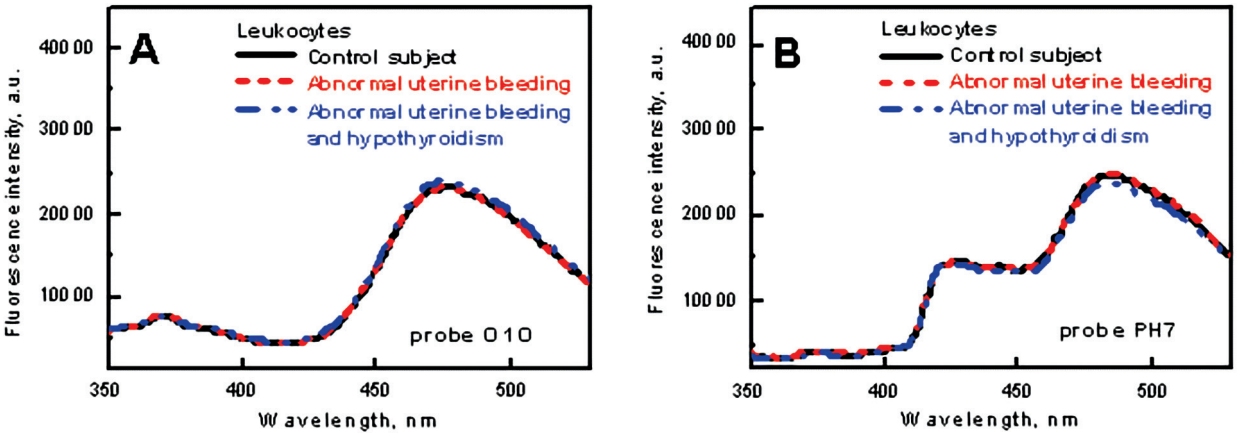

Fig. 5. Representative fluorescence spectra of probes O1O (panel A) and PH7 (panel B) in leukocyte suspensions: (a) the control subject (black solid line), (b) abnormal uterine bleeding (red dashed line), (c) abnormal uterine bleeding and hypothyroidism (blue dash-dot-dot line).

PH7 bound to the erythrocytes and to the leukocytes of the patients are presented in Table 3. The short-wavelength fluorescence bands, observed in the spectra of the probes belong to the normal forms of the probes, while the long-wavelength fluorescence bands are attributed to the fluorescence of the probe phototautomer forms.

The noticeable and statistically significant (p_1 = 0.0003 and p_1 < 0.0001) changes in the fluorescence spectra of probe O1O are detected in case of the erythrocytes extracted from the patients with abnormal uterine bleeding and a combination of abnormal uterine bleeding and hypothyroidism (Figure 4).

Hence, a considerable statistically valid increase in the fluorescence intensity ratio I_{T^*}/I_{N^*} was observed, which indicated a decrease in cell membrane hydration in erythrocytes. It should be noted that an additional evidence of the mentioned increase in the hydration of the region of probe O1O location is long-wavelength shift (~5 nm) of the fluorescence maximum of the phototautomer (T*).

On the other hand, the spectra of probe PH7 in erythrocyte membranes obtained from the female patients showed no significant difference in comparison with the control subjects: the changes in fluorescence intensity ratio I_{T^*}/I_{N^*} are found to be statistically negligible ($p_{1,2} > 0.05$). This result points to the absence of the changes in the membrane hydration, and, thus, in the lipid order in the region of probe PH7 location: i.e. in the less polar and more hydrophobic area of the lipid membrane.

In case of the leukocyte membranes obtained from the patients with abnormal uterine bleeding and the combined pathology, no changes in the fluorescence spectra of both probes were observed in comparison with the

corresponding spectra for the control subject (Figure 5), and, hence, no statistically significant changes ($p_{1,2} > 0.05$) in the ratio I_{T*}/I_{N*} were found for probes O1O and PH7 in this instance. This means that no changes in leukocyte lipid membrane hydration and, hence, in the membrane lipid order are observed.

AUB is known to be associated with anemia whose primary cause is blood loss [25, 26]. However, there is compelling evidence that eryptosis, which is referred to as erythrocyte apoptosis, can contribute to erythrocyte clearance from the bloodstream and, hence, reduction of red blood cell count [27]. Eryptosis is triggered in compromised or damaged erythrocytes via several mechanisms, including inadequate energy state, elevation of intracytosolic calcium ions, oxidative damage, osmolar dysregulation, ceramide accumulation, etc. [27]. At the cellular level, it manifests as cell membrane blebbling, cell shrinkage and PS externalization, which serves as a «destruction» signal for macrophages to engulf such erythrocyte and remove it from the bloodstream [28]. In this study, eryptosis is activated in patients with AUB combined with hypothyroidism. It is worth mentioning that ROS-dependent mechanisms are involved in eryptosis in these patients. However, eryptosis activation is not observed in women with AUB alone. Thyroid hormones are generally reported to be antiapoptotic [29] and mature erythrocytes possess receptors for thyroid hormones [30]. Conversely, there is some evidence that thyroid hormones can induce apoptosis of red blood cell progenitors [31]. Thus, our findings may be associated with the action of thyroid hormones. However, more studies are required to elucidate the proeryptotic action of thyroid hormones, especially in AUB.

ROS overgeneration is known to induce lipid peroxidation [32]. As a result of ROS-mediated lipid peroxidation, polyunsaturated fatty acids (PUFAs) are preferentially oxidized [33]. Oxidation of PUFAs by ROS has been reported to reduce the microfluidity of cell membranes [34]. The mentioned increase in I_{T*}/I_{N*} ratio indicates a decrease in membrane hydration [22] in the region of the probe location, and, hence, suggests the increase in membrane lipid order [24] of the rather polar area of the lipid membrane on occasion of the pathology. Such changes indicate exactly the reduction of microfluidity in cell membranes of erythrocytes of women with AUB and hypothyroidism. Such findings are consistent with data on ROS overproduction, since ROS mediate lipid peroxidation. It is important to note that changes in cell membranes are also observed in patients with AUB alone without the simultaneous oxidative stress.

In both AUB and AUB combined with thyroid disorders, the redox status of leukocytes remains undisturbed. Phospholipid bilayers of cell membranes in circulating leukocytes are unaffected as well. This suggests that erythrocytes are more vulnerable in these patients compared with leukocytes.

CONCLUSIONS

Abnormal uterine bleeding combined with hypothyroidism is associated with eryptosis activation, oxidative stress development in erythrocytes and changes in the physico-chemical properties of phospholipid bilayer of cell membranes in red blood cells.

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REDOX STATUS AND CELL MEMBRANE ALTERATIONS OF CIRCULATING LEUKOCYTES AND ERYTHROCYTES IN ABNORMAL UTERINE BLEEDING

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Aim. To analyze the eryptosis degree, the state of cell membranes and redox status of circulating red blood cells and leukocytes in patients with abnormal uterine bleeding and its combination with hypothyroidism.

Materials and methods. Patients, 74 women aged 18 to 49 years, were examined, which were divided into 3 groups: group I — women with abnormal uterine bleeding (AUB) (24 patients); group II — with AUB and thyroid pathology (30 patients, of whom 18 women had primary hypothyroidism and 2 — secondary hypothyroidism); group III — control group (20 healthy women, who had never had menstrual irregularities).

Eryptosis of circulating erythrocytes was assessed by flow cytometry using annexin V staining and 2′,7′-dichlorodihydrofluorescein diacetate (H2DCFDA) staining. Fluorescent probes O1O (2-(2¢-hydroxy-phenyl)-5-phe-

nyl-1,3-oxazole) and PH7 (2-(2¢-hydroxy-phenyl)-phenanthro[9,10-*d*]-1,3-oxazole) were used to characterize changes in phospholipid bilayers of circulating erythrocytes and leukocytes. Lysed blood samples were stained with antibodies to CD45, 7-aminoactinomycin D and H2DCFDA to analyze the redox status of circulating viable leukocytes.

Results. Annexin V staining revealed eryptosis activation in females with abnormal uterine bleeding combined with hypothyroidism. In addition, in these patients, oxidative stress developed in red blood cells, evidenced by an increase in intracellular reactive oxygen species (ROS) levels. Oxidative stress was accompanied by changes in the physico-chemical properties of erythrocyte membranes, namely a decrease in membrane hydration and an increase in lipid order, which can indicate enhanced lipid peroxidation. These changes were observed in women with abnormal uterine bleeding alone, however, to a lesser extent. In this study, the redox state of leukocytes and phospholipid bilayers of their cell membranes were not affected in the patients from both groups.

Conclusions. Abnormal uterine bleeding combined with hypothyroidism is associated with eryptosis activation, oxidative stress development in erythrocytes and changes in the physico-chemical properties of phospholipid bilayer of cell membranes in red blood cells.

Keywords: eryptosis, flow cytometry, annexin V staining, reactive oxygen species, phospholipid bilayer.

ОКИСНО-ВІДНОВНИЙ СТАТУС ТА ЗМІНИ КЛІТИННОЇ МЕМБРАНИ ЦИРКУЛЮЮЧИХ ЛЕЙКОЦИТІВ ТА ЕРИТРОЦИТІВ ПРИ АНОМАЛЬНИХ МАТКОВИХ КРОВОТЕЧАХ

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Мета. Проаналізувати ступінь ериптозу, стан клітинних мембран і окисно-відновний статус циркулюючих еритроцитів і лейкоцитів у хворих на аномальну маткову кровотечу та її поєднання з гіпотиреозом.

Матеріал та методи. Обстежено 74 жінки віком від 18 до 49 років, яких було поділено на 3 групи: I група — 24 пацієнтки жінки з аномальними матковими кровотечами (АМК)); II група — 30 хворих з АМК та патологією щитовидної залози (з них 18 жінок мали первинний гіпотиреоз і 12 — вторинний гіпотиреоз); III група (контрольна) — 20 здорових жінок, без порушень менструального циклу.

Ериптоз циркулюючих еритроцитів оцінювали за допомогою проточної цитометрії з використанням фарбування аннексином V і 2',7'-дихлордигідрофлуоресцеїну діацетатом (H2DCFDA). Флуоресцентні зонди O1O (2-(2¢-гідрокси-феніл)-5-феніл-1,3-оксазол) і PH7 (2-(2¢-гідрокси-феніл)-фенантро[9,10-d]-1,3-оксазол) використовували для характеристики змін у фосфоліпідних подвійних шарах циркулюючих еритроцитів і лейкоцитів. Лізовані зразки крові фарбували антитілами до CD45, 7-аміноактиноміцину D і H2DCFDA для аналізу окисно-відновного статусу циркулюючих життєздатних лейкоцитів.

Результати. Фарбування аннексином V виявило активацію ериптозу у жінок з аномальними матковими кровотечами в поєднанні з гіпотиреозом. Крім того, у цих пацієнтів у еритроцитах розвинувся окислювальний стрес, про що свідчить підвищення рівня внутрішньоклітинних активних форм кисню. Окислювальний стрес супроводжувався зміною фізико-хімічних властивостей мембран еритроцитів, а саме – зниженням гідратації мембрани та підвищенням упорядкованості ліпідів, що може свідчити про посилення перекисного окислення ліпідів. Ці зміни спостерігалися також у жінок з аномальними матковими кровотечами окремо, однак меншою мірою. У цьому дослідженні у пацієнток обох груп не змінювався окислювально-відновний стан лейкоцитів і фосфоліпідних бішарів їх клітинних мембран.

Висновки. Аномальна маткова кровотеча в поєднанні з гіпотиреозом пов'язана з активацією ериптозу, розвитком оксидативного стресу в еритроцитах і зміною фізико-хімічних властивостей фосфоліпідного бішару клітинних мембран еритроцитів.

Ключові слова: ериптоз, проточна цитометрія, фарбування аннексином V, активні форми кисню, фосфоліпідний бішар.