Indian Journal of Biochemistry & Biophysics
Vol. 59, March 2022, pp. 268-275

Blood formed elements of the women with uterine tumors as one of the criterion for assessment of severity of the pathology

Nanuli Kotrikadze[1]*, Tamar Tuphinashvili[1], Manana Alibegashvili[1], Liana Ramishvili[1], Irina Nakashidze[2], Mukhamed Artmeladze[3], Manana Gordeziani[1], Maia Zibzibadze[1], Ana Khazaradze[1], Bela Sepiashvili[1] & Sarfraz Ahmad[4]*

[1]Department of Biology, Faculty of Exact and Natural Sciences, Ivane Javakhishvili Tbilisi State University, Tbilisi-0186, Georgia

[2]Department of Biology, Natural Scienceand Health Care, Batumi Shota Rustaveli State University, Batumi-6010, Georgia

[3]High Technology Hospital Medcenter, Batumi-6000, Georgia

[4]AdventHealth Cancer Institute, Orlando, FL-32804, USA

Received 16 March 2021; *revised 11 March 2022*

The indicators for structural analysis of blood formed elements are prominent in the assessment of pathologies, diagnostics and the degree. Therefore, we aimed to evaluate the ongoing alterations that reflect on the structural characteristics of blood formed elements based on the hormonal imbalance among menopausal women with uterine tumors. Blood samples from the women with benign (n=20), malignant (n=20) uterine tumors, and healthy menopausal women (control, n=20) were used. Enzyme-linked Immunosorbent assay (ELISA) kits were used for the quantitative determination of hormones. The blood formed elements ultrastructure observations were conducted using transmission electron microscope. Compared to control (33.8±0.7 pg/mL), estradiol level was higher in benign (45.7±0.9 pg/mL) and malignant (70.7±3.7 pg/mL) cases ($P< 0.001$). Similar pattern was noted in testosterone levels [control=0.38±0.03 ng/mL, benign=0.55±0.04 ng/mL ($P< 0.01$), malignant=1.56±0.14 ng/mL ($P< 0.001$)] was higher in malignant cases. In contrast, progesterone levels were decreased in the disease cases [control=0.93±0.05 ng/mL, benign=0.44±0.003 ng/mL, malignant=0.31±0.02 ng/ml ($P< 0.001$)]. Assessments of the morphologic structure of erythrocytes revealed pathological forms of erythrocytes (poikilocytosis) in case of benign, as well as in malignant tumors. particularly target cells (codocytes), hamlet cells, teardrop cells (dacrocytes), sickle cell (drepanocytes) erythrocytes. Using ELISA and transmission electron microscopy our results demonstrate that in case of malignant uterine tumor quantitative/structural changes occur in blood formed elements indicating ongoing alterations in hormonal imbalance. Assessing these changes in structural characteristics would be useful in examining uterine pathologies and subsequent treatment plans.

Keywords: Blood Formed Elements, Hormonal Imbalance, Menopausal Women, Morphological Characteristics, Pathological Assessments, Transmission Electron Microscopy, Uterine Cancer

Uterine (endometrial) cancer is one of the most common gynecologic malignancies in women. The structural analysis of blood formed elements are prominent in the assessment of the severity degree of the pathology, as well as in diagnostics. It is well known that the steroid hormones - estradiol and progesterone play prominent regulatory role in the development of women's tumors, among them are gynecological malignancies. It is well established that estrogen receptor (ER) and progesterone receptor (PR) are ligand-dependent transcription factors that belong to the nuclear receptor superfamily. The imbalances in the levels of sex steroid hormones can cause the hyperplasia of the glandular epithelial tissue, and eventually endometrial cancer, when there is lower progesterone or excessive estrogen action on the endometrial tissue[1]. It's also recognized that progesterone inhibits estrogen-induced endometrial growth. The imbalance between estrogens and anti-growth progestogens leads to the cancer formation. Animal model studies have indicated that high levels of estrogen unopposed by progesterone lead to endometrial hyperplasia or cancer[2-6]. Similar studies suggest that the lack of estrogen/progesterone balance can contribute to the early stages of endometrial cancer formation.

Experimental as well as epidemiological studies suggest that testosterone plays a crucial role in the pathogenesis of endometrial cancer, but the precise mechanism has not been fully understood until now. The elevated level of blood free testosterone may be associated with increased risk of endometrial cancer in postmenopausal women[7].

*Correspondence:
Phone: +995 551 94 01 09; +1-407-312-2766
Fax: +995 32 53 71 25; +1-407-303-2435
E-mail: nanuli.kotrikadze@tsu.ge (NK);
sarfraz.ahmad@adventhealth.com (SA)

Therefore, we aimed to evaluate the ongoing alterations that reflect or not on the structural characteristics of blood formed elements based on the hormonal imbalance among the menopausal women with uterine benign and malignant tumors. Taking into consideration that gynecological cancers belong to hormone-dependent cancers, we also investigated sex-steroid hormones: estradiol (E2), progesterone (P) and testosterone (T) in the blood of menopausal women with uterine tumors (benign, malignant).

Materials and Methods

Samples

The blood samples of participants were collected and investigations have been performed during the 2018-2019 years. The study participants were enrolled from the High-Tech Hospital Med Center, Batumi, Georgia. All participants belonged to the European (Caucasian) race. Blood specimens from women with benign (n=20), malignant (n=20) uterine tumors, and healthy menopausal women (control, n=20) were used. The age range of the patients was 45-65 years.

The study was approved by the Independent Ethic Commission of High-Tech Hospital Med Center Ltd, Batumi, Georgia (*Identification Number: 445466870*). Written informed consent was obtained from all subjects.

Hormonal assay

For quantitative evaluation of hormones, liquid biopsy specimens (venous blood samples) were collected on day 20th of the regular menstrual cycle and serum specimens utilized for the test. The serum samples were stored at 2-8°C for 24 h and frozen at −20°C or lower until further use. Enzyme-linked immunosorbent assay (ELISA) was performed for the quantitative determination of hormones using the kits (Monobind, Statistical methods. Inc., Lake Forest, CA, USA). We calculated the mean absorbance value (A_{450}) for each set of reference standards, controls, and the test samples.

Electron microscope

The blood formed elements ultrastructure observation was conducted by the method of transmission electronic microscopy (TEM)[8]. We used 5 mL blood, fixed (twin fixed) in 2-4% glutaraldehyde buffer solution, then performed repetitive fixation with osmium fixator (osmium tetroxide buffering solution). After the fixation, we prepared the experimental materials for slicing. Accordingly, during the first stage, we performed dehydration of material (ethanol, 70%-100%), then embed epon (epon, Araldite) and completed the process of the material: material was sliced on ultramicrotome (OmU2, Austria), and obtained thin slices were placed on the pattern and performed their contrast, and investigated by transmission electron microscope (BS-500, Tesla, The Czech Republic) by using boost voltage.

Statistical analysis

Experimental data processing was performed using the variation statistical methods, Graphpad prism computer program (and $P< 0.05$ was considered statistically significant).

Results

In the present study, we determined sex-steroid hormones -estradiol (E2), progesterone (P), and testosterone (T) in the blood of menopausal women with uterine tumors (benign, malignant). We observed that the level of E2 was increased in both benign (1.3-fold, $P< 0.0001$) and malignant (0.09-fold, $P< 0.0001$) tumor cases (Table 1), compared to the control group. As for the antagonist hormone – anti-estrogenic P, the levels of P were decreased in the following order: control group → benign tumor → malignant tumor (2.11-fold and 3-fold, respectively, $P< 0.0001$) (Table 1). Based on our investigation, T levels were higher in case of tumors in menopausal women. Particularly, the level of T was elevated by 1.44-fold in case of benign tumor ($P= 0.0038$), and by 4-fold in cancer group patients ($P< 0.0001$, respectively) (Table 1).

In this study, we also evaluated the changes in the morpho-structural indices of erythrocytes, leukocytes, and thrombocytes by using transmission electron microscope. The study of the morphologic structure of erythrocytes demonstrated numerous pathological forms of erythrocytes (Poikilocytosis) as benign, also in case of malignant tumors in the menopausal women (Fig. 1A &

Table 1 — The quantitative assessments of alteration of steroid hormones in the blood of menopausal women with uterine tumors

Object	Estradiol (E2) pg/mL	Progesterone (P) ng/mL	Testosterone (T) ng/mL
Control Group	33.80±0.72	0.93±0,038	0.38±0.03
Benign Tumor	45.65±0,85 $P< 0.0001$	0.44±0,002 $P< 0.0001$	0.55±0.04 $P= 0.0038$
Malignant Tumor (*Endometrial Cancer*)	70.67±3.68 $P< 0.0001$	0.31±0,02 $P< 0.0001$	1.56±0.14 $P= 0.0001$

n=20, number of patients in each study group; $P< 0.05$; The age range of the patients 45-65 years

B). In particular, target cell (codocytes) (Fig. 1.1), hamlet cell (Fig. 1.2), teardrop cells (dacrocytes) (Fig. 1.3), Sickle cells (Drepanocytes) erythrocytes (Fig. 1.4). Presumably, appearance of the above noted pathological forms of erythrocytes is the result of abnormal and irregular distribution of hemoglobin[9]. Based on these evaluations, the appearance of schistocytes was also noticed (fragmented part of red blood cells) (Fig. 1.5 & Fig. 1E).

Besides Poikilocytosis, in the women's blood with the malignant uterine tumor the examples of anisocytosis were also observed: erythrocytes with different sizes, with great outline, carriers of dense matrix, particularly large amount of macrocytes (>8 μM) (Fig. 1C) and a few megalocytes (>9.5 μM) (Fig. 1D).

In regards to leukocytes, it is well known that leucocytes (also known as blood white cells), their structure are divided into two broad groups: granulocytes (neutrophils, eosinophils, basophils) and agranulocytes (lymphocytes, monocytes)[10]. Accordingly, among the leukocytes, the neutrophils are presentin majority that can fight infections[11].

Structural indices of the neutrophils did not exceed physiological norms in the blood of the women with benign tumor (degranulation, dislocation of granules, perinuclear cisternae) (Fig. 2A). In our study, the large sized neutrophils were observed among women with uterine cancer (Fig. 2B & C). It should be noted that degenerative structural changes have been observed in the nucleus, as wells as in the cytoplasm of the neutrophils. In particular, the degenerative changes were noted by the nuclear segmentation and pyknosis of the nucleus (wrinkling, getting smaller) (Fig.2D). As for the degenerative changes inside the cytoplasm, the latter has been revealed by vacuolization, presence of numerous granules that indicates the intoxication. Notably, the integrity of the cell membrane (plasmalemma) is also impaired and/or altered (Fig. 2E).

Next, we studied the morphological picture of lymphocytes. It is known that the ability of an organism to react to any antigen is provided by different group of leukocytes[11]. Based on our results, in the blood samples of the patients with benign uterine tumor large sized lymphocytes were observed (Fig. 3A) with the dense matrix, twisted nuclear membrane, numerous mitochondria with the rinsed matrix and small sized hamulus at the plasma membrane. However, the countless normal lymphocytes with simply outlined plasma membrane and small size mitochondria were also found (Fig. 3B).

Among the uterine malignant tumors, large size lymphocytes with large nuclei were fixed in the

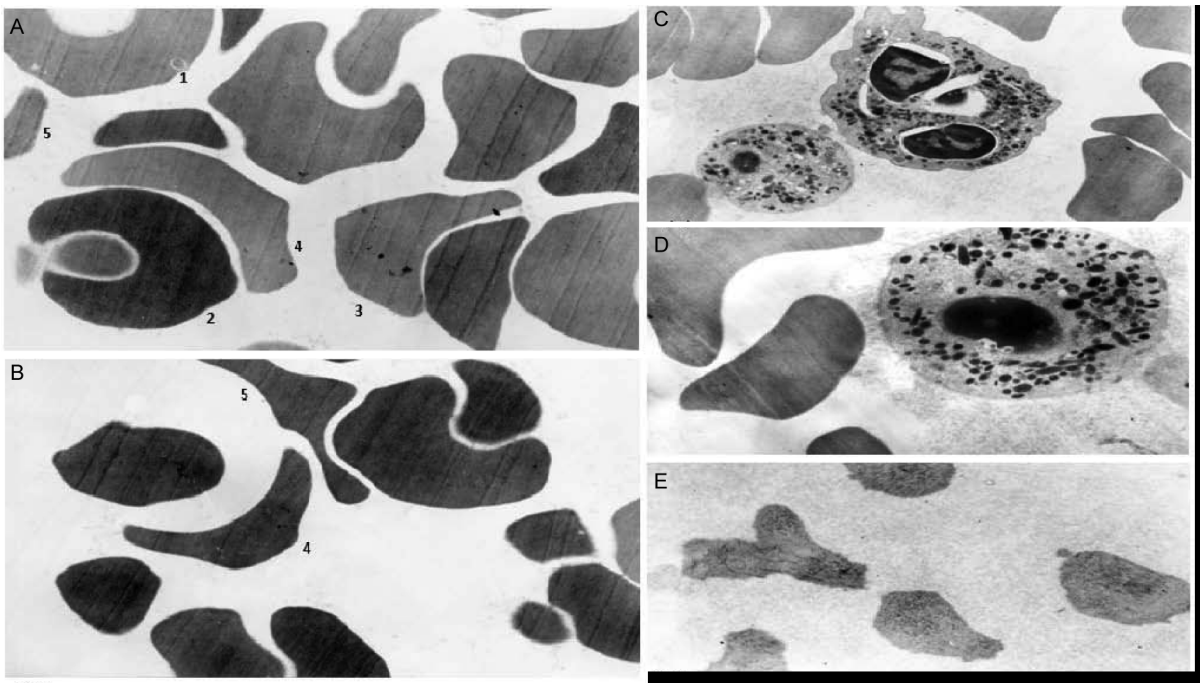

Fig. 1 — (A and B) The erythrocytes of the patients with uterine tumors (benign, malignant) at menopausal age (45-65 years) (pathological forms - Poikilocytosis). 1-Target cell (codocytes); 2-Hamlet cell; 3-Teardrop cells (dacrocytes); 4- Sickle cells (Drepanocytes); 5-Schistocytes; The erythrocytes of the patient with uterine malignant tumor, Erythrocytes – (C) Macrocyte; (D) Megalocyte; and (E) Schistocytes menopausal age (45-65 years), (4000×2.2)

cytoplasm, with dense lysosomes and mitochondria, numerous vacuoles, small size hamulus at the plasma membrane (Fig. 3C & D).

Presence of natural killer (NK) cells in the blood of women with uterine cancer was also observed (Fig. 4A). NK cells play prominent role in the non-specific cytotoxic reactions and provide a first-line defence against intracellular pathogens and tumors. The NK cell effect or mechanisms are cytotoxicity and secretion of inflammatory cytokines and chemokines[12]. Dense

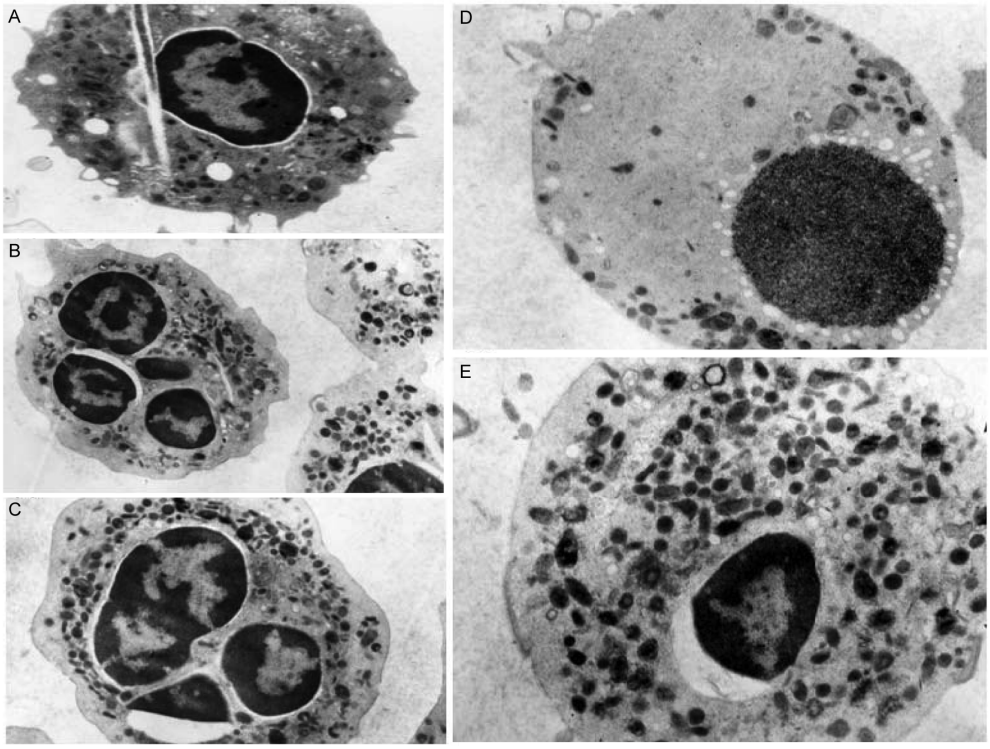

Fig. 2 — The neutrophils of the patients with uterine (A) benign; (B and C) malignant; and (D and E) tumors. The neutrophils of the patients with uterine malignant tumor, menopausal age (45-65 years), (8000×2.2)

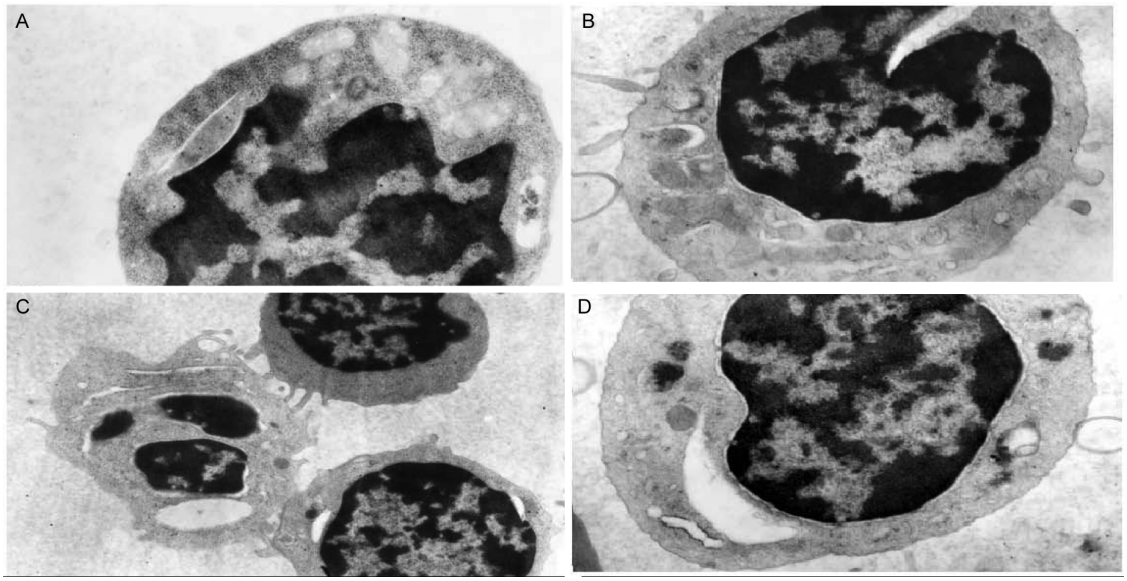

Fig. 3 — (A) Lymphocytes of the patients with uterine benign tumor (menopausal age, 45-65 years), (14000×2.2); (B) The lymphocytes of the patients with benign uterine tumor (menopausal age,45-65 years), (6000 × 2.2); and (C and D) Lymphocytes of the patients with uterine malignant tumor, menopausal age (45-65 years), (8000×2.2)

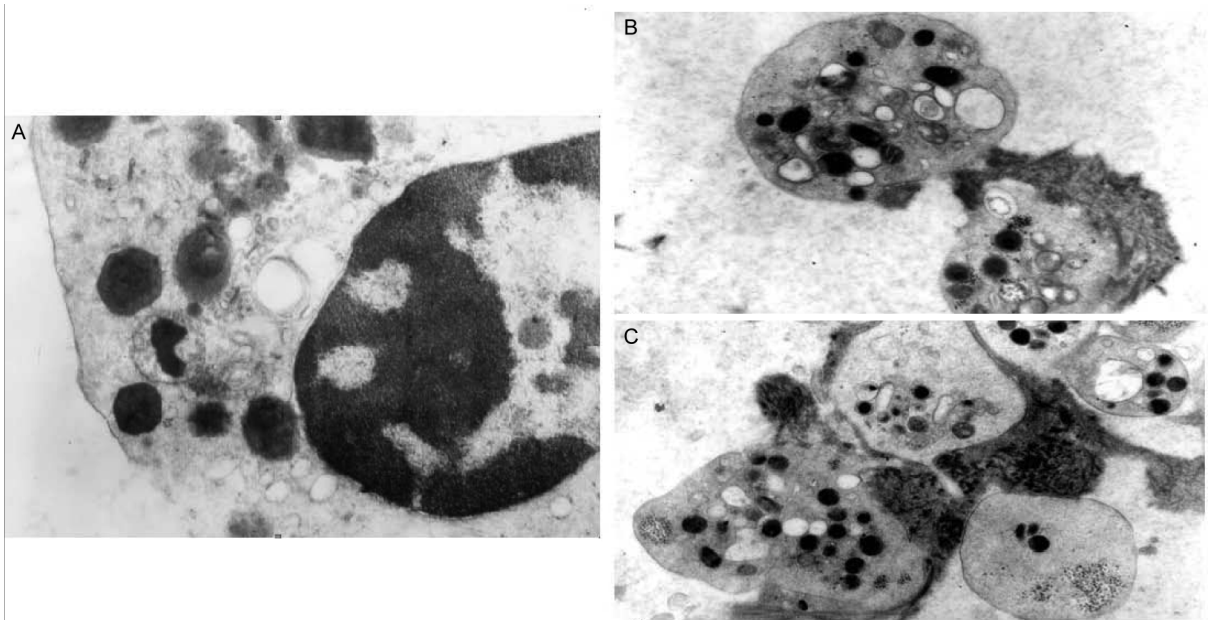

Fig 4 — (A) Natural killer cells of the patients with uterine malignant tumor (menopausal age, 45-65 years); (14,000×2.2); (B and C) Thrombocytes of the patients with uterine malignant tumor, menopausal age (45-65 years), (10,000×2.2)

granules were observed on the morphological picture of NK cells, which occupied the place near the nucleus. The contour of some granule's membrane is obscure; some of them contain only remains of the matrix. Between the granules, a large number of vacuoles and numerous narrow canals of the endoplasmic reticulum are also found. The abovementioned group of lymphocytes did not reveal any immunological specificity that have the ability to destroy tumor cells[13].

The observation on the morphologic picture of blood formed elements in the malignant tumor demonstrated numerous, much bigger size blood formed platelets, thrombocytes, and rinsed out granules (Fig. 4B & C).

Discussion

The uterine endometrium represents one of the most sensitive organs towards hormones, among them are sex steroids. The abovementioned hormones contribute to several cellular processes (development, growth, *etc*.) and are responsible for the normal functioning of the women's reproductive system. Endometrial carcinogenesis is related to the overexposure of estrogen, as the increased levels of estrogen causes stimulation of the endometrium and rapid cell growth, then endometrial hyperplasia, leading to endometrial cancer[14]. Endogenous estrogen plays prominent role in the progression of the disease. The overexpression of the endogenous estrogen is connected with the enzymatic production in the adipose tissue.

Based on the study[15], nearly 40% of endometrial cancer incidence is caused by body weight. Investigation showed that obese women tend to convert the peripherally circulating androstenedione to estrone by the biochemical way[15].Lukanova and colleagues[16] reported a high tendency for endometrial cancer among women who have elevated estradiol and estrone levels. It has been suggesting that there is a positive correlation between circulating estrogen, testosterone levels, and endometrial cancer among the postmenopausal ages[16]. Allen *et al*[6] investigated that the elevated levels of free testosterone (and to a lesser extent, total testosterone) increases endometrial cancer risk among the postmenopausal ages. According to our previous study[17], a positive association between the high levels of estradiol and testosterone and uterine tumors (benign, malignant) was found at premenopausal, menopausal, and postmenopausal women.

In this study, large amount of macrocytes were observed (Fig. 1C) presumably due to the deficiency of the vitamin B12 that can be one of the reasons for increased amount of macrocytes. Notably, in case of uterine malignant tumor multivitamin metabolism is impaired. It should be noted that maybe it is caused by the impaired hematologic function of the liver[18], which is the accompanying process of the disease mentioned above[19]. We suppose, that the mechanism of erythrocytes formation (erythropoiesis) is impaired in case of uterine cancer[20].

The action of tumor on erythrocytes and erythropoiesis occurs directly through biologically active substances which are produced in tumor tissues to decrease the amount of erythropoietin, reduce the sensitivity of erythroblast towards erythropoietin, alter the exchange and absorption of iron and vitamin B, as well as by indirect way by through intermediate rings (*e.g*., neuroendocrine action). Presumably, these ongoing alterations in the hypothalamus cause inhibition of sympathetic nervous system activity, and therefore are involved in decreasing the processes of erythropoiesis. Also, it should be noted that hormones (particularly estrogens) affect not only the metabolism, but also erythrocytes and the bone marrow function (erythropoiesis)[11]. Our investigations have shown the high levels of estradiol in the cases of uterine benign and malignant tumors that may suppress the process of hemopoiesis (Table 1).

The investigation of the blood samples by the transmission electron microscopy (TEM) method have shown the changes in size and shape of erythrocytes, as well as alterations in the distribution of hemoglobin that occurs in tumors (benign, malignant). Taking into account all the mentioned changes create the point of view on the condition of erythropoiesis, which may play prominent role in the diagnostics and assessment of the severity/degree of pathologies.

Among peripheral blood lymphocytes, the natural killer (NK) cells make up a considerable proportion (5–20%)[13]. Two major subsets are well established: CD56bright NK cells are the dominant in the secondary lymphatic tissues and make up only 5-10% of the peripheral blood NK cells, while CD56dim NK cells are the mature and more cytotoxic cells and make up 90-95% of the peripheral blood NK cells[21]. Recent data revealed the presence of the natural killer cells in uterine tissue where they appear to regulate trophoblast invasion during pregnancy[22,23]. They are known as uterine natural killer cells and predominantly belong to CD56 bright NK cells. These type of NK cells reveal significantly lower cytotoxic activity compared to its counterparts in peripheral blood[22,24]. Exact function and physiological role of these NK cells in uterine tissue is not fully elucidated yet, though strong evidence supports that CD56bright NK cells still represent an immature phenotype that is able to differentiate into the *in vitro* and *in vivo* CD56dim NK cells. CD56dim NK cells are mature cells and their cytotoxic activity is significantly higher than that of CD56bright NK cells[25,26].

Besides the well-established anti-tumor effect, NK cells may support cancer (both by immunosuppression and by supporting tumor angiogenesis). The NK cell function is often altered during the tumor immune evasion, which allows tumor growth and tissue invasion[21]. It is supposed that cytokines and chemokines *e.g*., transforming growth factor (TGF)-β, interleukin (IL)-6, and human leukocyte antigen (HLA)-G actively produced by the tumor microenvironment have inhibitory effects on the NK cells function and activity[27,28]. It is established that neoplastic transformation significantly impacts on the NK cell phenotype and function[29,30]. Mamessier and coauthors have shown thatbreast cancer cells increase self-tolerance by modifying NK cell phenotype and these cells were unable to repress tumor growth[30]. Modification of NK cell structure as well as function in case of different tumor pathologies has also been described by several studies[21,27,28].

As for the amount of NK cells, it is noteworthy thatthese type of cells were observed only in peripheral blood of the women with uterine cancer. This fact should indicate on the increased number of NK cells in peripheral blood in case of uterine cancer. In our current study, presence of the NK cells was not observed in the blood of control group women, as well as in cases of benign uterine tumor. It is noteworthy, that increased amount of peripheral blood NK cells was also described by Mamessier and coauthors in blood of the women with breast cancer[31]. On the basis of gained results, we postulate that the increased level of NK cells in the blood of women with uterine malignant tumor can be caused by the increased proportion of immature and low cytotoxic CD56bright NK cells in the overall NK cell subsets in peripheral blood.

Conclusion

Our study utilizing transmission electron microscope have shown that in the uterine malignant tumor, structural changes of blood formed elements occurs, which indicates the ongoing alterations based on hormonal imbalance. In particular, in the blood samples of the women with uterine malignant tumor were found to be large sized young cells, the segmentation of nucleus, the pyknosis of the nucleus, the enormous amount of granules in the cell cytoplasm, abundant granulation, and projections at the membrane, which were not found in the benign and healthy women's blood. Thus, above noted changes of the structural characteristics may be useful

for assessing the degree of severity of uterine pathologies. Taken altogether, alterations of the morphological markers described above, can be used as a reliable prognostic and diagnostic test for the differentiation of endometrial tumors.

Acknowledgement

The authors are grateful for the research work supported by the Ivane Javakhishvili Tbilisi State University, Faculty of Exact and Natural Sciences, Tbilisi, Georgia.

Conflict of interest

All authors declare no conflicts of interest.

References

1 Kim JJ, Kurita T & Bulun SE, Progesterone action in endometrial cancer, endometriosis, uterine fibroids, and breast cancer. *Endocr Rev*, 34 **(**2013) 130.

2 Yang CH, Almomen A, Wee YS, Jarboe EA, Peterson CM & Janát-Amsbury MM, An estrogen-induced endometrial hyperplasia mouse model recapitulating human disease progression and genetic aberrations. *Cancer Med*, 4 *(*2015) 1039.

3 Cheng Y, Lv Q, Xie B, Yang B, Shan W, Ning Ch, Li B, Xie L, Gu C, Luo X, Chen X & Zhu Q, Estrogen and high-fat diet induced alterations in C57BL/6 mice endometrial transcriptome profile. *Endocr Connect*, 7 (2018) 36.

4 Brinton LA, Trabert B, Anderson GL, Falk RT, Felix AS, Fuhrman BJ, Gass ML, Kuller LH, Pfeiffer RM, Rohan TE, Strickler HD, Xu X & Wentzensen N, Serum estrogens and estrogen metabolites and endometrial cancer risk among postmenopausal women. *Cancer Epidemiol Biomarkers Prev*, 25 (2016) 1081.

5 Rodriguez AC, Blanchard Z, Maurer KA & Gertz J, Estrogen signaling in endometrial cancer: A key oncogenic pathway with several open questions. *Horm Cancer*, 10 (2019) 51.

6 Allen NE, Key TJ, Dossus L, Rinaldi S, Cust A, Lukanova A & Peeters PH, Endogenous sex hormones and endometrial cancer risk in women in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Endocr Relat Cancer*, 15 (2008) 485.

7 Lin SL, Yan LY, Liang XW, Wang ZB, Wang ZY, Qiao J, Schatten H & Sun QY, A novel variant of ER-alpha, ER-alpha36 mediates testosterone-stimulated ERK and Akt activation in endometrial cancer Hec1A cells. *Reprod Biol Endocrinol*, 7 (2009) 102.

8 Weakley BS, *Beginners' Handbook in Biological Electron Microscopy*. 2nd Ed. (Churchill Livingstone Publisher, Edinburgh, UK) 1981.

9 Dorgalaleh A, Mahmoodi M, Varmaghani B, Node FK, Kia OS, Alizadeh S, Tabibian S, Bamedi T, Momeni M, Abbasian S & Khatib ZK, Effect of thyroid dysfunctions on blood cell count and red blood cell indice. *Iran J Ped Hematol Oncol*, 3 (2013) 73.

10 Crutchlow EM, Dudac PJ, MacAvoy S & Madara BR, *Pathophysiology*, (Jones & Bartlett Learning, Sudbury, Massachusetts) 2002, 273.

11 Wagner DD & Frenette PS, The vessel wall and its interactions. *Blood*, 111 (2008) 5271.

12 Vivier E, Tomasello E, Baratin M, Walzer T & Ugolini S, Functions of natural killer cells. *Nat Immunol*, 9 (2008) 503.

13 Stabile H, Fionda C, Gismondi A & Santoni A, Role of distinct natural killer cell subsets in anticancer response. *Front Immunol*, 8 (2017) 293.

14 Bender D, Buekers T & Leslie KK, Hormone receptors and endometrial cancer. *Proc Obstet Gynecol*, 2(2011) 25.

15 Bergstrom A, Pisani P & Tenet V, Overweight as an avoidable cause of cancer in Europe. *Int J Cancer*, 91 (2001) 421.

16 Lukanova A, Lundin E, Micheli A, Arslan A, Ferrari P, Rinaldi S, Krogh V, Lenner P, Shore RE, Biessy C, Muti P, Riboli E, Koening KL, Levitz M, Stattin P, Berrino F, Hallmans G, Kaaks R, Toniolo P & Zeleniuch-Jacquotte A, Circulating levels of sex steroid hormones and risk of endometrial cancer in postmenopausal women. *Int J Cancer*, 108 (2004) 425.

17 Nakashidze I, Diasamidze A, Baratashvili D, Nagervadze M, Alibegashvili M, Ramishvili L, Gordeziani M, Khazaradze A & Kotrikadze N, Alteration of sex and non-sex hormones and distribution features of blood ABO system groups among the women with uterine body tumors. *J Cancer Ther*, 5 (2014) 411.

18 Napoli VM, Nichols CW & Fleck S, Semi-quantitative estimate method for reporting abnormal RBC morphology. *Lab Med*, 11 (2016) 111.

19 Matsuo K, Gualtieri MR, Cahoon SS, Jung CE, Paulson RJ, Shoupe D, Muderspach LI, Wakatsuki A, Wright JD & Roman LD, Surgical menopause and the increased risk of non-alcoholic fatty liver disease in patients with endometrial hyperplasia and cancer.*Menopause*, 23 (2016) 189.

20 Yasuda Y, Fujita Y, Matsuo T, Koinuma S, Hara S, Tazaki A, Onozaki M, Hashimoto M, Musha T, Ogawa K, Fujita H, Nakamura Y, Shiozaki H & Utsumi H, Erythropoietin regulates tumour growth of human malignancies. *Carcinogenesis*, 24 (2003) 1021.

21 Bassani B, Baci D, Gallazzi M, Poggi A, Bruno A & Mortara L, Natural killer cells as key players of tumor progression and angiogenesis: Old and novel tools to divert their pro-tumor activities into potent anti-tumor effects. *Cancers* (*Basel*), 11 (2019) 461.

22 Moffett A, Regan L & Braude P, Natural killer cells, miscarriage, and infertility. *BMJ*, 329 (2004) 1283.

23 Blois SM, Klapp BF & Barrientos G, Decidualization and angiogenesis in early pregnancy: Unravelling the functions of DC and NK cells. *J Reprod Immunol*, 88 (2011) 86.

24 King K, Smith S, Chapman M & Sacks G, Detailed analysis of peripheral blood natural killer (NK) cells in women with recurrent miscarriage. *Human Reprod*, 25 (2010) 52.

25 Merkt W, Sturm P, Lasitschka F, Tretter T, Watzl C, Saure D, Hundemer M, Schwenger V, Blank N, Lorenz HM & Cerwenka A, Peripheral blood natural killer cell percentages in granulomatosis with polyangiitis correlate with disease inactivity and stage. *Arthritis Res Ther*, 17 (2015) 337.

26 Chan A, Hong DL, Atzberger A & Kollnberger S, CD56 bright human NK cells differentiate into CD56dim cells: Role of contact with peripheral fibroblasts. *J Immunol,* 179 (2007) 89.

27 Degos C, Heinemann M, Barrou J, Boucherit N, Lambaudie E, Savina A, Gorvel L & Olive D, Endometrial tumor

microenvironment alters human NK cell recruitment, and resident NK cell phenotype and function. *Front Immunol*, 10 (2019) 877.

28 Cifaldi L, Prencipe G, Caiello I, Bracaqlia C, Locatelli F, De Benedetti F & Strippoli R, Inhibition of natural killer cell cytotoxicity by interleukin-6: Implications for the pathogenesis of macrophage activation syndrome. *Arthritis Rheumatol*, 67 (2015) 3037.

29 Aktas ON, Ozturk AB, Erman B, Erus S & Tanju S, Role of natural killer cells in lung cancer. *J Cancer Res Clin Oncol*, 144 (2018) 997.

30 Mamessier E, Sylvain A, Thibult ML, Houvenaeghel G, Jacquemier J, Castellano R, Gonçalves A, André P, Romagné F, Thibault G, Viens P, Birnbaum D, Bertucci F, Moretta A & Olive D, Human breast cancer cells enhance self-tolerance by promoting evasion from NK cell antitumor immunity. *J Clin Invest*, 121 (2011) 3609.

31 Mamessier E, Pradel LC, Thibult ML, Drevet C, Zouine A, Jacquemier J, Houvenaeghel G, Bertucci F, Birnbaum D & Olive D, Peripheral blood NK cells from breast cancer patients are tumor-induced composite subsets. *J Immunol*, 190 (2013) 2424.