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DIAGNOSTICS OF UTERINE CORPUS CANCER AND THE IMPORTANCE OF ELECTRON MICROSCOPIC EXAMINATION IN ITS PROGNOSIS

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Applicant: **Samira Ilyas Safarova**

ABSTRACT
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The work was performed at the Oncology Department of Azerbaijan Medical University

Scientific supervisor: academican
Ahliman Tapdig Amiraslanov

Scientific consultant: doctor of medical sciences, professor
Habil Kamil Muradov

Official opponents: doctor of medical sciences, professor
Islam Sharif Mahalov

doctor of medical sciences
Abulfaz Agasoltan Soltanov

doctor of medical sciences
Mahmud Mir Vaqif Bagirzade

Dissertation council FD 1.02 at the National Center of Oncology of the Supreme Attestation Commission under the President of the Republic of Azerbaijan

Chairman of the Dissertation council:
academician
Jamil Aziz Aliyev

Secretary of the Dissertation council:
PhD in medicine
Rashad Salch Zeynalov

Azərbaycan Respublikası
Milli Onkologiya Mərkəzi
ELMI KATİB

Chairman of the scientific seminar:
doctor of medical sciences, professor
Fuad Alovsat Mardanli

GENERAL DESCRIPTION OF THE RESEARCH

The actuality of the subject. Endometrial cancer (EC) is the second most common tumor in women after breast cancer, accounting for 7.1% [1]. Each year new disease cases up to 200.000 and death cases up to 50.000 are registered in the world. According to Cancer-research.uk, Endometrial cancer cases have increased by 56% since 1990. By 2030, the incidence is expected to increase by 60%, which naturally requires a more in-depth study of the actuality of the problem, diagnostic, and prognostic factors. Uterine corpus cancer (UCC) relates to the main nosological forms in the structure of malignant tumors in women in the Republic of Azerbaijan. Its extensive indicators are 7.3%. The overall mortality rate from this pathology is 3.2% per 100,000 population across the city. Mortality rate is 19.7% [2].

EC belongs to the group of hormone-dependent cancers and acts as a target for sex steroid hormones. Functional and anatomical changes in the hypothalamic-pituitary-ovarian system, disruption of hormonal homeostasis lead to the proliferative process in the endometrium, and in the future - the development of hyperplastic processes. These hyperplastic processes provide the background for the development of malignant neoplasia[3][4].

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The most decisive and recent diagnostic method of EC is the histological method that allows determining the nature of morphological changes. The histological variant of the tumor is one of the most important factors in the prognosis of patients with UCC. Sometimes the method of pathohistological examination alone is not enough to select diagnostic and prognostic factors, to properly assess the process. The Transmission Electron Microscope (TEM) currently in use has a number of advantages over noemal light microscopes (LM). The electron microscopic (EM) method allows for differential diagnosis of endometrial carcinomas both between multiple histological variants and between benign and atypical hyperplasia [56].

The presence of diagnostic errors is due to the imperfection of the technology of morphological examination. Even though the fact that there is a small number of negative errors in modern histology, this problem has not been fully solved and is still relevant. In this regard, the application of the EM method eliminates the disadvantages of differential diagnosis. The use of EM examination helps to detect developing cancer factors, accurate and timely diagnosis, selecting optimal treatment tricks and taking preventive measures [789].

At the ultrastructure level, some signs of cellular atypism can be detected. Disorder and/or loss of intracellular contacts is one of the

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main symptoms of the neoplastic process. Although various cellular disorders are widely described in adenocarcinoma of the endometrium, EC-specific electron-microscopic image, quantitative and qualitative criteria have not yet been fully clarified and systematized.

The object and subject of the research. The research contingent consisted of patients diagnosed with uterine corpus cancer who were treated and kept under observation at the Oncology Clinic of the Azerbaijan Medical University in 2011-2018. The number of patients included in the research contingent is 167 people. 132 - main group, patients with uterine corpus cancer, 35 - control group: patients with Atypical glandular hyperplasia (AGH). These patients were examined by clinical, laboratory, instrumental, radiological, histological, electron microscopic methods. A computer-electronic database of the obtained results was created, a comparative algorithm of the results of electronic microscopic and pathohistological examination of the main and additional groups of patients was developed.

The purpose of the research: Determination of the importance of electron microscopic examination in the diagnosis, histogenesis, and prognosis of the uterine corpus cancer.

Objectives:

1. Clinical and morphological characterization of patients with endometrioid type of uterine corpus cancer in 2011-2018
2. Comparative analysis of the diagnostic, electron-microscopic image of atypical glandular hyperplasia and endometrial adenocarcinomas
3. Formation of electron microscopic, diagnostic algorithm of endometrial adenocarcinomas
4. Systematization of ultrastructural indicators of different histological variants of endometrial adenocarcinomas
5. Investigation of the prognostic significance of the identified ultrastructural parameters.

Research methods. Ambulatory cards, disease histories, results of the clinical, ultrasound, CT scan, MRI and immunoenzymatic examinations of the patients with uterine corpus cancer were analyzed. Electron microscopic examination was performed in the scraping, and tissue materials of patients diagnosed with pre-uterine

corpus cancer and uterine corpus cancer. Initially, the obtained materials were examined histologically, and after reviews' being clarified, examination materials of the patients with uterine corpus cancer were investigated by EME. Ultrastructural investigations were conducted with the help of electron microscope JEM-1400 (Japan). A computer database of collected materials was created, the information obtained was processed using biostatistical methods.

Main points presented to the defense of the dissertation:

1. There are different specific ultrastructural structures of uterine corpus adenocarcinomas

2. Electron-microscopic indicators of endometrial cells have differential-diagnostic value

3. The electron-microscopic structure of various variants of uterine corpus adenocarcinomas depends on the histological differentiation of the tumor.

4. Ultrastructural indicators of endometrial adenocarcinomas are one of the most important prognostic criteria.

Scientific novelty of the research. Either morphological or electron-microscopic image of uterine corpus adenocarcinomas was analyzed in detail. Important information was obtained on the disorder of the cell and tissue architecture during malignant changes of the endometrium at the ultrastructural level, reorganization of cell contacts and formation of neovascularization with the help of electron microscopic method. Moreover, the quantitative and qualitative changes in the components of organoid of cells that make up the endometrium adenocarcinomas at the expense of the mentioned examination were investigated. The obtained indicators were also investigated during atypical glandular hyperplasia, the mentioned tissue and cytospecific images of the pathology were described, and a comparative diagnostic algorithm was established. This algorithm facilitates the differential diagnosis between atypical glandular hyperplasia and endometrial adenocarcinomas. At the same time, differential diagnostic criteria were established by making electron-microscopic characterization of well, moderately and poorly differentiation variants (G1, G2, G3) of endometrial adenocarcinomas.

Prognostic significance of the ultrastructural indicators of vari-

ous types of uterine corpus adenocarcinomas was evaluated. The impact of these criteria on clinical situation and disease prognosis depending on the histological type of tumor was studied.

Theoretical and practical significance of the dissertation. The results of the dissertation help to improve the effectiveness of treatment in addition to facilitating differential diagnosis and prognosis of this pathology in patients with uterine corpus cancer. Ultrastructural changes of endometrial adenocarcinoma cells allow for reliable prognostic evaluation during different tumor differentiation variants.

The results of the dissertation are important in the daily practice of oncogynecologists, surgeons, and morphologists and are of practical importance.

Approbation and application of the dissertation. The fragments of the dissertation were discussed at the following scientific conferences: Scientific-practical conference dedicated to the anniversary of National leader H.A.Aliyev (Baku, 2017);

Speech at the seminar of ANAS dedicated to Science Week (on biology and medicine) on March 11-17, 2019 (Baku, 2019); International scientific-practical conference "Actual problems of medicine 2019" dedicated to the 100th anniversary of the establishment of the medical faculty under Baku State University (Baku, 2019) poster and speech; The eye of the medicine. International scientific-practical conference dedicated to topical problems of radiation diagnostics (Baku, 2019); The main provisions of the dissertation were initially discussed at the joint meeting of the Oncology, Histology, Embryology and Cytology Departments of the Azerbaijan Medical University, Oncology Clinic on 01.04.19 (Baku, 2019) and discussed at the scientific seminar of the Dissertation Council FD 1.02 operating under the National Center of Oncology on protocol № 1, 06.04.2021 (Baku, 2021)

Publications. 23 articles involved the main provisions of the dissertation, 10 scientific articles, 2 of them without co-authors were published in local publications, 2 of them without co-authors were published in foreign publications, 13 theses, 4 of them without co-author were published in foreign, 2 theses without co-authors were

published in local publications

Application of the obtained results. The results of the research are being applied at the Oncology Clinic of Azerbaijan Medical University and the Department of Histology, Cytology, and Embryology. The dissertation work won the Grant Competition №EIF/GAM-2-2013-2 (8) -25/19/3 of the Science Development Foundation under the President of the Republic of Azerbaijan.

Name of the organization where the dissertation was performed. The dissertation work was performed at the base of the Oncology Clinic of Azerbaijan Medical University and electron-microscopic laboratory of the Research Center.

Total volume and structure of the dissertation. The dissertation was written in Azerbaijani language, total volume consists of 240,000 characters with an introduction (13.200 characters), I chapter – review (60,000 characters), II chapter- materials and methods (21,000 characters), 3 chapters dedicated to the personal research (30.400 + 77.350 + 7.850 characters), conclusions, results, practical recommendations (30,200 characters) and list of references. The dissertation is visualized with 16 tables, 45 figures and graphs. The list of references covers 159 sources.

MATERIALS AND METHODS OF THE RESEARCH

The research was conducted at the Oncology Clinic of Azerbaijan Medical University (AMU), electron-microscopic laboratory of the Research Center, Department of Histology, cytology and embryology of AMU by using of operation and archive data of patients diagnosed with endometrial adenocarcinoma, atypical glandular hyperplasia və simple hyperplasia. The outpatient card and disease history of these patients were reviewed. The research was conducted both retrospectively and prospectively. The research did not include the patients with non-resectable tumors, receiving neoadjuvant chemotherapy or radiation therapy; with distant metastasis; first applied with relapse; also patients with ovarian, vulvar and other genital organs' cancers, as well joint extragenital tumors. The basis of this research is 132 patients (main group) diagnosed with UCC and

35 patients with AGH of the control group.

Anamnesthetic data of patients were studied in detail, clinical, laboratory and instrumental investigations (examination, vaginal and ultrasound scan, scraping of the uterus) were performed. The results of the clinical, ultrasound, morphological and electron-microscopic examinations were systematized and analyzed.

The age limit of the patients changes from 39 to 76, with an average age of 49.6 ± 2.7 years. Complaints were non-specific for both groups of patients, with bloody or whitish-gray excretory in the uterus, pain in the lower abdomen.

Data on both near and distant treatment results were obtained by collecting detailed anamnesis of the patient's condition.

Samples from operation materials obtained from patients with uterine corpus cancer and pre-cancer for the purpose of studying light and electron microscopic examination (1.5-cm-sized tissue pieces consisting of all layers of uterus, taken from pathological derivative of uterine corpus, surround derivative and taken from the unchanged areas) were submitted to the electron microscopy laboratory by fixing in 2.5% paraformaldehyde, 4% sucrose, 0.1% picric acid solution prepared in 1.0 M phosphate buffer (pH = 7.4). Semithin (1mkm) sections were prepared using Leica EM UC7 ultramicrotome by separating fully prepared blocks from molds.

Ultrathin sections were investigated on a JEM-1400 transmission electron microscope (JOEL-Japan) under 80-120KW voltage and electronograms were recorded via lower and side chambers (Veleta).

The analysis of morphometric parameters (length, diameter, surface area, form factor, etc.) of the tissues and cells was performed by the computer program (The TEM imaging platform) developed by the German company “Olympus Soft Imaging Solution Gmbh”using microphotos and electrograms obtained in TIF format by the semi-automatic method. As the result of the research, 116 blocks, 720 semithin and 560 ultrathin sections were prepared from these blocks, 760 photo and 1227 electronograms were taken.

The number of indicators obtained during the research were implemented by applying methods of variation (U-Mann-Whitney), discriminant (Pearson Chi-Square) and regression (Kaplan-Meier cri-

teria with Log Rank (Mantel-Cox) model). All calculations were made in the electron table EXCEL-2016 and in the package program SPSS-22.

RESULTS AND DISCUSSIONS OF THE RESEARCH

3 histological variants of endometrial adenocarcinoma- well G1(53 patients, 40.2%), moderately G2 (40 patients, 30.3%), and poorly differentiated G3 (39 patients, 40.2%), as well clinical and ultrastructural features of 35 cases of atypical glandular hyperplasia were extensively studied. The obtained results help the formation of specific ultrastructural view, the diagnostic significance of some substructures, and show differential diagnostic capabilities and prognostic significance of a number of cell elements. Thus, the diagnostic algorithm and prognostic criteria were defined depending on the histological variant of endometrial adenocarcinomas.

The majority of disease cases for both groups (AGH and EC) were between 50 and 69 years, 71.43% (25 patients) and 72.0% (95 patients), respectively. While the diagnose of AGH did not occur in the age group over 70 years 11.4% of patients diagnosed with EC were registered. Frequently occuring of UCC and AGH in the menopausal period shows a crucial role of endocrine-metabolic disorders (estrogen-gestagen imbalance, metabolic syndrome, diabetes, obesity, etc.) in the pathogenesis of the aforementioned processes. Patients in both groups had similar clinical manifestations and were more likely to be diagnosed by the active examination of women during the reproductive age (40-49 years) (AGH – 60%, EC – 59.1%), whereas diagnosis in menopausal age is followed by the occurrence of specific complaints (AGH 88.6% and EC 93.2%) ($\chi 2=0.819$; $p = 0.365$)

Along with the personal anamnestic data of patients, family history was also investigated. It was found that 79.04% of patients diagnosed with EC had long-term treatment for infertility, in the AGH group, this rate was 20.96% ($p < 0.01$). In our view, this result is closely linked to the application of hormonal therapeutic methods for infertility; As a result, there is a severe proliferation of endometrium

and irreversible dysplasia and malignancy occurs in a hyperestrogenic state.

When clarifying the presence of simple glandular hyperplasia in anamnesis, we found that approximately $^{2}/_{3}$ of patients in the UCC had this episode (75.76%), but this was 34.43% in patients with AGH, $p < 0.001$. Obtained figures show that endometrium can be perceived as an indirect indicator of the potential of proliferation and malignancy during simple glandular hyperplasia.

When analyzing family medical history, special attention was paid to the presence of ovarian cancer and uterine corpus cancer in the family. Thus, these cases were diagnosed 29.55% in the EC group and 5.71% in women in the AGH group, $p < 0.05$. This fact proves once more that, there is a certain role of genetic factors in the development of the EC, as seen from literature review.

During the research, the frequency of occurrence of diabetes that manifestation of metabolic syndrome, arterial hypertension, and obesity were also studied. The diseases mentioned above were found in 37.12%, 34.85%, 59.85% of the patients during EC ($p<0.05$). In AGH, obesity was 28.57% ($p < 0.001$). Diagnostic procedures are done almost in the same size, the most important of these are ultrasound scan, MRI and diagnostic scraping of the uterus. Ultrasound scan mainly focuses on parameters such as endometrial thickness, tissue size, and neovascularization. Endometrial thickness was defined in 88.6% of cases in AGH and 82.6% of cases in UCC ($p>0.05$). Intensive bleeding of the tissues was defined 53.8% of cases in the EC group, 22.8% of cases in the AGH ($p<0.05$) with the help of a Doppler ultrasound.

The distribution of patients on clinical stages shows that the vast majority of patients were 73.48% in Phase I. When examining the disease stage with age, it becomes clear that Phase I prevails in all age groups, but as age increases, the rate of II and III phases also increase. Thus, in women of reproductive age, Phase II and III occur in 26.52% of cases and 30.0% of cases in patients over 50 years, $p<0.05$.

As it is known, the clinical condition and prognosis of the disease depend on the spreading locally of the tumor, metastases in the

lymph nodes, the morphological structure of the tumor. This approach is true in all types of tumors, but these indicators are extremely important in endometrial adenocarcinomas. In our research, we aim to select the correct diagnostic and prognostic criteria by carrying out a mutual evaluation of clinical and morphological (histological and ultrastructural) signs of uterine adenocarcinomas.

Well-differentiated adenocarcinomas were found in 40.15%, moderately differentiated adenocarcinomas in 30.3% and poorly differentiated adenocarcinomas in 29.55% of 132 patients diagnosed with EC. If we look at the incidence rate of the differential rate of adenocarcinomas in the different age groups, we see that there are certain features. Anterior gradient adenocarcinomas occur in 72.7 % of women in reproductive age, moderately and poorly differentiated tumors occur in 27.3% of cases, $p<0.05$. Generally, G3 tumors were found in only one patient (4.6%) in this category of patients. In the premenopausal period, the course of the disease was slightly different: well-differentiated carcinomas were found in 33.64% of cases, moderately and poorly differentiated carcinomas were found in 66.36% cases, $p<0.05$. The general tendency is manifested as an increasing share of poorly-differentiated tumors as age increases. In our view, it is due to the occurrence of cell atypism by combining mutation changes made as a result of the effect of Hyperestrogenism for a long time. When we investigated the distribution of differentiated tumors of G1, G2, and G3 at different stages, some points attracted our attention. Thus, in the IA stage, well-differentiated tumors predominate - 71.7%, G3 tumors - 28.21%. The information obtained is consistent with clinical facts: IA stage is characterized by the fact that miometrial invasion of tumor is less than ½ of its thickness, and at the same time, the spread potential of well-differentiated tumors is also low.

Poorly-differentiated adenocarcinoma was found in 15 patients (38.46%) in IIIC stage, at this stage, the G1 tumor was confirmed in only 1.89% of cases. The predominance of poorly differentiated tumors in stage IIIC can be explained as follows ($\chi 2 = 35.001$; $p <0.001$). At this stage, regional lymph node damage is observed, and poorly-differentiated adenocarcinomas have a high potential for ma-

lignancy, which is manifested by regional metastases.

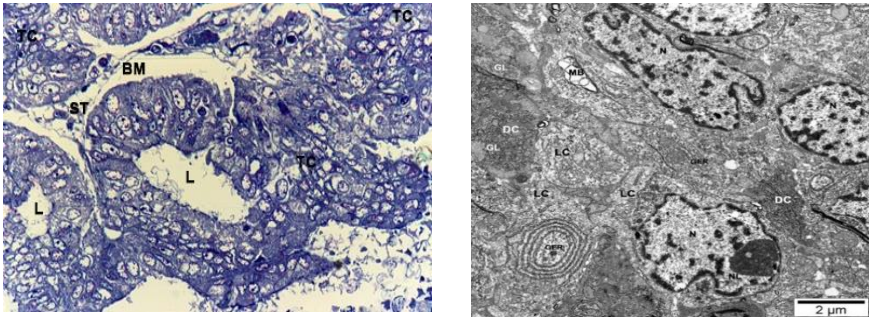
In general, the study investigated the metastatic abilities of various differentiated adenocarcinomas, some of the ultrasonic transformational indicators obtained are associated with the degradation of the malignant process, allows to think about the clinical course of the tumor and the probability of spread, as well as they have diagnostic and prognostic value.

Cytochorectomy of the endometrium during the electron-microscopic examination, changes in the shape, size and structure of gllandulocytes, number and structure of organelles, intercellular contacts, also the modifications of active secretory cells that provide hemostasis of the inner layer of the uterine wall during the malignant process were investigated and systematized. Structural changes described by us include atypical glandular hyperplasia and adenocarcinomas of the endometrium.

In order to make a comparative assessment of cell and tissue structure disorders at the electron-microscopic level, some indicators have been based, and changes in atypical and neoplastic processes have been identified. This includes plasma membrane, dark and light cells, intercellular contacts, cell nuclei, mitochondria, Golgi complex, as well as amount of these cells, and features of neovascularization.

The main point in the course of **AGH** is the swelling of the basement membrane in endometrial cells and to produce invaginations of different sizes. These types of invaginations, which are noticeable as ultrasturcture, were not encountered during gland polyps and simple hyperplasia. Ultrastructurally, the endoplasmic reticulum is recorded as a network of narrow, parallel cisterns. Weak osmophilic structures of cells with elongated cisterns anastomosed with each other are also found. Mitochondria are closely associated with ergocytoplasm and are swollen. Their size has increased, the color of their matrixes has become clearer, and the crystals are bulging. The above mentioned pathological changes act as a sign of impaired synthetic-transport function of cells during atypical glandular hyperplasia. The plumpness of interstitial stromal capillaries one of the symptoms of AGH. The main characteristic features of AGH are produc-

ing invaginations of the basement membrane, mitochondrial swelling, rough crystals and plumpness of interstitial stromal capillaries. Mitochondrial swelling causes the disorder of reactions in membranes. Functional changes in the mitochondria indicate more energy consumption of cells during the pathological process. Mitochondria are mainly found in the basal part of the plasma membrane. Golgi complex is usually positioned on the nuclear surface. Generally, the plumpness of interstitial stromal capillaries is one of the symptoms of AGH. It is important to note that cells acquire atypical symptoms and maintain tissue specificity, while damage to the basal membrane is not detected. (fig. 1).



a) **b)**

Figure 1. Atypical glandular hyperplasia.

a) Dye: hematoxylin-eosin, Zoom:ok12.5,ob.40. L-lumen. BM-basal membrane ST- stroma. Half thin section.

b) Electronogram. Light cells (LC) and dark cells (DC), cytoplasmic granules, granular endoplasmic reticulum (GER), granular endoplasmic reticulum in the form of concentric circles indicating high protein levels in the light cell. In dark cells, granular endoplasmic reticulum (GER) is enlarged, the accumulation of cisterns and glycogen granules is seen.

Dye: uranyl-citrate and lead citrate.

The main characteristic features of AGH are producing invaginations of basement membrane, mitochondrial swelling, rough crys-

tals and plumpness of interstitial stromal capillaries. It should be noted that the cell maintains tissue cytospecificity by acquiring atypical symptoms. Basal membrane damage is not detected.

Well differentiated adenocarcinomas of the endometrium (WDA) was met in patients of 40.2%, the 5-year survival rate was 84.9%. The granular endoplasmic reticulum is in the shape of enlarged figures, with thin fibrous structures in the center. Largely fragmented crystal mitochondria are found. The contours of the nuclei are irregular, characterized by deep invaginations of the nuclear membrane, twisting and narrowing of the inner channels of the nucleus, and excessive expansion in the area around the nucleus. Despite the basal membrane is fragmented, complete destruction is not detected. Although the mitochondria have a dense matrix, the crystals are swollen, edematous, and the collapse of some of the crystals is visualized. Numerous dense osmiophilic bodies are revealed between the inner and outer membranes of mitochondria. pathological center. Desmosomes and covering plates providing intracellular contacts are found, but in some areas, structural disorders of the desmosomes are revealed.

Moderately differentiated adenocarcinomas of the endometrium (MDA) have been found in 30.3% of patients, the five-year survival rate is 85%. Small mitochondria with the dense matrix are found between the membranes of the Ergocytoplasm. Mitochondrial matrix contains osmiophilic granules and few crystals. The nuclei are characterized by a rough surface. Mitochondria are located close to the plasma membrane of cells. The color change of the matrix characterizes the anastomosis of the crystals, which are located in numerous perpendiculars. On the contact surface of cells, interdegradations and complex contacts are selected. The number of desmosomes and covering plates has been reduced, with the weakening of intercellular contacts seen as the detection of tumor cells between the stromal elements in areas around the pathological center (Fig.2). The basal areas of cancer cells have been undergone fragmentation. Endoplasmatic network has been undergone degranulation. The folds of the inner nuclear channels are widened in the areas close to the nuclear membrane and narrowed in the area around the nucleus. Thus, ultrastruc-

tural change in the moderately differentiated adenocarcinoma is characterized by a disorder of intracellular metabolism.

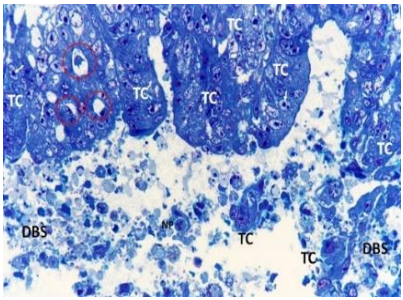


Figure 2. MDA.
Die: hematoxylin-eosin. Zoom: ok 12.5, ob 24.0
NL-nucleolus, TC-tumor cells, Dark cells (DC), and light cells (LC).
Interaepithelial lymphocytes are marked with a red circle. Debric (DBC)

Poorly differentiated adenocarcinomas of endometrium (PDA) have been found in 29.55% of patients, the 5-year survival rate is 64.1%. Electron microscopic studies have revealed that the most notable process in the cytoplasm of atypical cells is multivesicular bodies, consisting of endoplasmic reticulum elements. (fig. 3).

The cytoplasm of cells is characterized by light-stained areas. The endoplasmic reticulum is fragmented, partially vacuolated. There is a sharp increase in the size of the nucleus, irregularly shaped membrane, a severe change in the shape of the nucleus due to numerous deep invaginations, complete destruction in intranuclear unique channels, necrosis, fragmentation, decomposition. Invagination is found in the plasmalemma. In some areas of destruction, intercellular communication is disrupted, and epithelial cells become more flattened. Golgi complex is fragmented in 50-60% of observations. Mitochondria are completely decomposed, the crystals are fragmented. Intercellular communication is completely disrupted in more than 50% of cases.

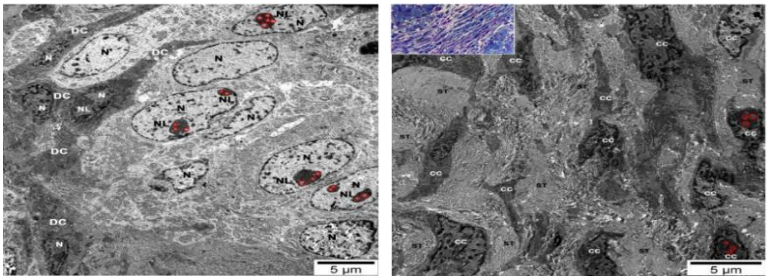


Figure 3. Poorly differentiated adenocarcinoma
Metastatic chain development of dark tumor cells from the collagen fibers. Invasion occurs in connective tissue stroma (ST) due to the extensive development of dark cells. Cancer cells (CC). Compared to light cells, outlines of the dark cell nuclei are distorted, the cell and nuclear polymorphism are clearly visible. Large fibrillar centers are in a red circle.
Dye: uranyl-citrate and lead citrate. Zoom - 1: 5000.

Thus, the data obtained indicate that electron microscopic research is important not only in the differential diagnosis of endometrial adenocarcinomas but even in the assessment of prognosis. In addition to complementing the existing histological, histochemical and other methods, these indicators are fundamentally new approach that allows studying the metastatic potential of tumor cells by studying intracellular structures. As a result of the research, we have found that with increased levels of malignancy there is a sharp decline in intracellular contacts. Firstly, it concerns desmosomes. Thus, if the structure of the desmosome is maintained during atypical glandular hyperplasia, deformation of the structure of desmosome in some of the cells in well differentiated adenocarcinoma, decrease in the number of desmosomes in the G2 tumors, and the complete disintegration of the small number of desmosomes in G3 are observed.

One of the important points here is that these symptoms coincide with histological research and clinical indicators. In the low gradient endometrial adenocarcinomas, the distribution of large numbers of tumor cells in the stroma is determined, the loss of intracellular

contacts in this type of tumor is, in our opinion, an indicator of the growth rate of cancer and high metastatic potential. The number and structure of desmosomes are of great prognostic significance.

The second major point is the structure of the nuclear and mini nuclear. Nuclear size growth, generating deep and sharply expressed invaginations of nuclear layer, a large form of the nuclear, in short, sharply expressed nuclear polymorphism are indicators of the malignancy. Thus, in our study, this symptom was clearly expressed in G3 gradient tumors, with mini nuclei underwent hypertrophy, forming large fibrillary centers with complex configuration. On the other hand, we have observed high mitotic activity in tumors that sharply expresses nuclear polymorphism, which is one of the ultrastructural parameters of cancer progression.

A third important electron-microscopic finding is related to the structure of mitochondria. We did not observe any significant structural changes in mitochondria during AGH. However, as the degree of differentiation in the adenocarcinomas diminishes, these organelles become more swollen, and the crystals are destructed starting from partial fragmentation. These changes can be attributed to increased hypoxia during tumor progression and cells' rapid undergoing aerobic glycolysis.

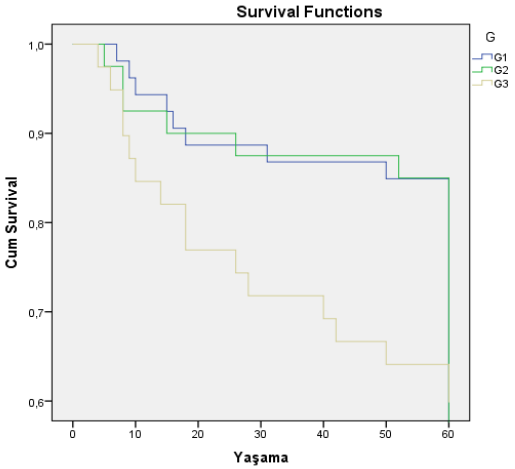
Another image revealed at the ultrastructural level is dark cells with the cytoplasm. We think that the number of these cells is closely related to malignancy; mentioned elements are rarely found in ultrathin cuts when atypical glandular hyperplasia occurs, their number increases in well differentiated tumors, and they are revealed more than 60% of area in poorly differentiated tumors. Even in G3 tumors, separate layers of these cells are found. In our view, dark cells have important differential diagnostic value. In addition, when investigating the features of stromal invasion, it was found that dark cells are one of the main components of the infection in poorly differentiated adenocarcinomas. We think that the number of these cells can be taken as prognostic factors (algorithm).

Algorithm of a set of electron microscopic features in endometrial adenocarcinomas

Electron microscopic signs	Well differentiated adenocarcinoma	Moderately differentiated adenocarcinoma	Poorly differentiated adenocarcinoma	F; p
Plasma membrane	With folds 15%	Invaginations30%	Invaginations 35%	F = 5.922 p = 0.003
Desmosomes	disrupted in cells 20%	disrupted in cells 50%	Completely destructed 50%	F = 14.850 p < 0.001
Nuclear	The size has increased Severe folding in intranuclear channels 30%	The size has increased increase in folding, fragmentation in most channels 50%	The size has grown dramatically fragmentation in intranuclear channels,decomposition 80%	F = 38.128 p < 0.001
Nuclear membrane	thickened 20%	thickened, invaginations in some parts 30%	With deep and numerous invaginations 40%	F = 5.034 p = 0.007
Nucleolus	Did not change 0%	hypertrophied 30%	They form numerous, complex fibrillar centers hypertrophied 50%	F = 56.430 p < 0.001
Endoplasmic reticulum	Vesiculation 10%	In the case of blisters and vesicles 20%	Multivesucilar bodies30%	F = 6.750 p = 0.001
Mitochondria	Edematous, swollen20%	Partial fragmentation 40%	Full fragmentation 60%	F = 21.214 p < 0.001
Mitochondrial crystals	Edematous, swollen 20%	Partial destruction 40%	Full destruction 60%	F = 21.214 p < 0.001
Golgi complex	Hypertrophic 20%	Hypertrophic 20%	Fragmented 40%	F = 7.425 p < 0.001
Lipid droplets	low 10%	High 40%	High 50%	F = 26.088 p < 0.001
Lysosomes	High 30%	High 40%	High 50%	F = 4.368 p = 0.014
Light cell	20%	30%	40%	F = 5.034 p = 0.007
Dark cell	50%	60%	85%	F = 17.471 p < 0.001

Thus, as a result of electron microscopic examination of endometrial adenocarcinomas, we have concluded that there are specific ultrastructural changes characteristic of various differentiated tumors, the modification of which can be used in the differential diagnosis and prognosis of cancer. In addition to the data of morphological, histochemical, etc., these indicators visualize the reorganization that takes place at the structural and substructural level in the cell. It also plays an important role in estimating the potential for tumor spread and in setting up an individualized treatment program, including the selection of patients in need of adjuvant therapy.

In our study, we examined the 5-year survival rate of patients with uterine adenocarcinomas (graph 1).



G	Middle				Comparison	
	Indicator	Standard error	95% DI		Log Rank (Mantel-Cox)	
			Upper limit	Lower limit	χ^2	p
G1	53.887	2.138	49.697	58.077	7.369	0.025
G2	53.850	2.553	48.847	58.853		
G3	45.667	3.406	38.990	52.343		

Graph 1. Results of Log Rank (Mantel-Cox) Model with Kaplan-Meier criterion depending on tumor differentiation rate (χ2=7,369; p = 0,025).

The overall 5-year survival rate is 78.8%. When we look at different morphological variants, we see that the 5-year survival rates of G1 and G2 gradaded tumors are almost indistinguishable. It was 84.9% in G1 and 85% in G2. In the poorly-differentiatiated variant, it is lower than in the other groups since the first control year, and the 5-year results differ statistically significant - 64.1%, $p = 0.025$.

In general, the study investigated the properties of metastasis of various types of differentiated adenocarcinomas, some of the ultrastructural transformational indicators obtained are associated with the gradient of the malignancy, allowing us to advance an idea on the clinical course and potential for the tumor spread. They are of prognostic and diagnostic significance.

During the electron-microscopic examination, the cytoarchitectonics of the endometrium, the shape, size and structural changes of gllandulocytes, the number and structure of organelles, intercellular contacts, dark cells, as well as the modifications of secretory active cells that provide hemostasis of the inner layer of the uterus during the malignancy were studied and systematized.

Structural changes described by us include atypical glandular hyperplasia of the endometrium and adenocarcinomas.

RESULTS

1. The study found well differentiated adenocarcinomas in 40.2% of cases, (53 patients), moderately differentiated adenocarcinomas in 30.3% of cases (40 patients), poorly differentiated adenocarcinomas in 29.5% of cases (39 patients) [4,16]

2. Electron microscopically, nuclear polymorphism, degradation of the mitochondrial structure, and plasmatic membrane changes can be used in the differential diagnosis of AGH and EC [14, 15, 19].

3. The main diagnostic criteria for the degree of various differentiation of endometrial adenocarcinomas electronically microscopically: discompletion of the intranuclear channels, hypertrophy of the nuclei, deformation of the plasma membrane, dark cells proliferation, and destruction of the Golgi complex, disruption of the mitochondrial structure. [13, 14, 21].

4. During WDA, the destruction of visual field of desmosomes in 20% of cells ($p<0.001$), acute torsion in the intracellular canals was 30%. PDA desmosomes are completely disrupted in 50% ($p<0.001$) cells, acute nuclear polymorphism, abrupt increase in nuclear size, fragmentation in intranuclear channels, discompletion 80% ($p<0.007$), fibrillar centers in 50% of nuclei ($p<0.001$), dark cells are observed in 85% of the visual field ($p<0.001$) [8, 23].

5. Changes in the intracellular synthetic-energy apparatus, complete destruction of desmosomes in the cell membrane, nuclear polymorphism, unique intracellular channels, giant fibrillar centers within the nucleolus are of differential diagnostic and prognostic significance in endometrial adenocarcinomas. While the number of dark cells in the WDA is low, there is a significant increase in the number of dark cells in the PDA. Quantitative indicators of these ultrastructures can be considered as a prognostic factor ($p=0.025$) [3, 19, 22].

PRACTICAL RECOMMENDATIONS

1. The application of electron microscopic examination in different histological variants of endometrium adenocarcinomas is of differential diagnostic and prognostic significance.

2. Ultrastructural changes (intracellular contacts, nuclear polymorphism, mitochondrial structure, dark and light cells) can be used in the differential diagnosis of well, moderately and poorly differentiated adenocarcinomas, as well as in the diagnosis of AGH in complex cases.

3. Loss of intercellular contacts (desmosomes), the number of open and dark cells in the endometrium should be taken into account in the curation of patients, taking them as a negative prognostic factor.

4. Given that these indicators are more expressed sharply in poorly differentiated adenocarcinomas, full adjuvant treatment is considered appropriate in this group of tumors regardless of the disease stage.

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Abbrevations

AGH – Atypical glandular hperlasia
EC – Endometrial cancer
EM – Electron microscopy
EME – Electron microscopic examination
LM – Light microscope
G_1 – Well differentiated adenocarcinoma (WDA)
G_2 – Moderately differentiated adenocarcinoma (MDA)
G_3 – Poorly differentiated adenocarcinoma (PDA)
CT – Computer tomography scan
MRI – Magnetic resonance imaging
UCC – Uterine corpus cancer
US – Ultrasound

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Address: AZ 1022, Baku, H.Zardabi, 79b

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