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Significance of the Blood Serum Epidermal Growth Factor and the Endometrial Epidermal Growth Factor Receptor in Endometrial Hyperplasia

Abstract of the dissertation for the PhD in Medicine

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The abstract has been distributed on 2015

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Description of the Study

Prevalence of the Problem

Endometrial hyperplasia is most frequently observed in pre-menopause period when normal estrogen-progesterone balance characteristic of a menstrual cycle is altered; endometrial hyperplasia may as well develop in young women and teenagers, who are often observed to have an anovulatory cycle (Chen, Zang, Feng et al., 2009; Robbins, Cotran, 2010). Endometrial hyperplasia is a nonphysiologic, noninvasive proliferation of the endometrium and is considered as a precancer condition for the development of endometrial carcinoma caused by excessive estrogen (Chen, Zang, Feng et al., 2009). Estrogen stimulates the synthesis of Epidermal Growth Factor (EGF) in the endometrium (Vlodavsky, Brown, Gospodarowich, 1978). The higher serum estrogen level, the more expressed is endometrial proliferation, with the activity of Epidermal Growth Factor and the expression of Epidermal Growth Factor Receptor (EGFR) being increased (Kurman, Kaminski, Norris, 1985). However, EGF can act independently to stimulate the epithelial cell growth (Atasoy, Bozdoğan, 2006). The majority of researchers think that EGF plays a prominent role in the development of hyperplastic processes in the endometrium and is the risk carrier of tumor occurrence (Wang, Konishi, Koshyama et al., 1993; Blaustein, Kurman, 2002; Wang, Pudney, Song et al., 2003; Ejskjaer, Soresen, Poulsen et al., 2007; Ejskjaer, Soresen, Poulsen et al., 2009; Santoro, 2010; A. Tavartkiladze, M. Kasradze, D. Kasradze, 2012). Besides, there is an assumption that EGF does not correlate with tumor malignancy degree, stage, and clinical outcome (Fuller, Seiden, Young, 2004). Regarding the blood serum EGF content, the mean index of EGF is higher in endometrial carcinomas than that in normal condition (Tomaszevski, Miturski, Kotarski, 1996).

Based on the above mentioned, the relationship between EGF and its receptor should be attached a particular importance in the development of hyperplastic endometrium into neoplastic endometrium.

The abundant literature data are focused on the expression of Epidermal Growth Factor Receptor (EGFR) in human hyperplastic endometrium. These findings are often controversial: according to some authors, EGFR is much more revealed in hyperplastic endometrium than in normal state (Leone, Constantini, Gallo et al., 1993; Amezcua, Zheng, Muderspach et al., 1999; Wang, Pudney,

Song et al., 2003; Citri, Yarden, 2006; Altieri, 2008; Koike, Sekine, Kaymiya et al., 2008; Margulis, Lotan, Shariat, 2008). However, other researchers report on equal levels of EGFR expression in almost all conditions (Gershtein, Bocharova, Ermilova et al., 2000). Again, some authors show that EGFR is more frequently expressed in endometrial carcinomas, than in hyperplasia (Nyholm, Nielsen, Ottesen,1993), while others state quite opposite: the EGFR does express in all cases of edometrial hyperplasia however in carcinomas it is found only in some cases (Niikura, Sasano, Matsunaga et al., 1995; Niikura, Sasano, Kaga et al., 1996). While some authors think that EGF expresion is higher in normal condition rather than in endometrial carcinomas (Niikura, Sasano, Matsunaga et al., 1995; Niikura, Sasano, Kaga et al., 1996; Miturski, Semeczuk, Postawski, Jakowicki, 2000; Fuller, Seiden, Young, 2004; Santoro, 2010). A number of studies show that high expression of EGFR is in correlation with histologically poorly differentiated tumors (Llorens, Bermejo, Salcedo et al., 1989; Brmelin, Zimmer, Sauerbrei et al., 1992; Scambia, Benedetti, Battaglia et al., 1992; Scambia, Benedetti, Ferrandina et al., 1994). Some of these authors think that EGFR expression is not associated with the malignancy degree, histological type and the extent of invasion (Nyholm, Nielsen, Ottesen, 1993; Scambia, Beneditti, Ferrandina et al., 1994; Miturski, Semczuk, Postawski, Jakowicki, 2000; Fuller, Seiden, Young, 2004).

Based on the literature, it is also known that in certain tumors with positive EGFR, the outcome is much worse than with negative EGFR (Santoro, 2010; A. Tavartkiladze, M. Kasradze, D. Kasradze, 2012). Finally, most researchers suggest that EGFR plays an important role in the development of hyperplastic processes in the endometrium and is the risk carrier of tumor occurrence (Wang, Konishi, Koshyama et al., 1993; Blaustein, Kurman, 2002; Wang, Pudney, Song et al., 2003; Ejskjaer, Soresen, Poulsen et al., 2007; Ejskjaer, Soresen, Poulsen et al., 2009; Santoro, 2010; A. Tavartkiladze , M. Kasradze, D. Kasradze, 2012).

It is noteworthy that there are scarce data concerning the analysis of EGF-EGFR interaction (in the cases of endometrial hyperplasia and neoplasia), particularly, we have seen only one study revealing both EGF and EGFR in the normal, hyperplastic and neoplastic endometrium tissue, which, however, does not involve an intercorrelation analysis. Also, no data are available regarding the measurement of blood serum EGF content (Niikura, Sasano, Kaga, et al.,1996); there is another study that describes the measurement of blood serum EGF level only in endometrial neoplasias with the conclusion made in the discussion that "not very high content of blood serum EGF and the scarcity of EGF receptor in the endometrial tissue are likely to indicate an increased risk of endometrial carcinogenesis" (Tomaszewski, Miturski, Kotarski, 1996.) Besides, we could not find studies concerning the interaction between the blood serum EGF content and endometrial EGFR expression in respect of antiproliferative or antineoplastic factors in endometrial

hyperplasia/neoplasia. We assume that this interaction is likely to have a particular significance in the evaluation of endometrial hyperplasia malignization. We also think that identification of the interaction between EGF and EGFR as risk factors and Melatonin, the universal hormone that has antiproliferative/antineoplastic effects, will acquire more importance in the assessment of estrogen-dependent endometrial carcinogenesis risk. With that, we think that the detection of the EGF-EGFR interaction in terms of their relationships with Melatonin both in endometrial hyperplasias and endometrial carcinomas will enable to more clearly determine the predictive importance of EGF and EGFR in the evaluation of uterine body precancer condition.

Objectives and Goals of the Study

The goal of our study was to identify the significance of blood serum EGF content as well as EGFR expression in the endometrium in simple and complex endomemetrial hyperplasias.

The objectives involve the following:

1. Identification of EGFR expression in endometrial and tumor tissues in patients with simple/complex endomemetrial hyperplasia, and endometrial carcinoma (excessive estrogen mediated endometrial carcinoma);

2. Identification of the defining features of blood serum EGF content in simple/complex endometrial hyperplasia and endometrial carcinoma (excessive estrogen mediated endometrial carcinoma);

3. Identification of the defining features of blood serum Melatonin concentration in simple/complex hyperplasia and endometrial carcinoma (excessive estrogen mediated endometrial carcinoma);

4. Evaluation of the complex study results, determination of their interaction and correlativity (statistical analysis);

5. Based on the complete analysis and discussion of the study results, determination of the importance of EGF and EGFR for the assessment of endometrial hyperplasia malignization risks.

Scientific Novelty of the Study

The following correlations were first revealed in simple and complex non-atypical endometrial hyperplasias: 1. Correlation between blood serum EGF content and EGFR expression in the

endometrium, 2. Correlation between blood serum Melatonin content and EGFR expression in the endometrium, 3.Correlation between blood serum EGF and Melatonin contents;

The following correlations were first revealed in well and moderately differentiated adenocarcinomas: 1. Correlation between blood serum EGF content and EGFR expression in tumor tissue, 2. Correlation between blood serum Melatonin content and EGFR expression in tumor tissue, 3. Correlation between blood serum EGF and Melatonin contents.

Based on the analysis of the correlations, new aspects of EGF and EGFR predictive significance in respect of endometrial hyperplasia malignization were first elucidated.

General theses of the dissertation

1. With endometrial hyperplasia, in parallel with the complication of the hyperplasia type, strong expression of EGFR more frequently, while weak expression of EGFR less frequently is observed in the endometrium; in well and moderately differentiated endometrial adenocarcinomas the EGFR expression (in a tumor tissue) is always strong;
2. The more complicated type of endometrial hyperplasia, the stronger the EGFR expresion in the endometrium and the larger the increase in blood serum EGF level. Positive correlation has been revealed between the *endometrial EGFR expression* and *blood serum EGF level*. In complex non-atypical hyperplasia a sharply increased level of blood serum EGF is observed both with strong and weak endometrial EGFR expression;
3. The more complicated type of endometrial hyperplasia, the stronger the EGFR expression in the endometrium and the larger the decrease in blood serum Melatonin content: at the background of strong EGFR expression in the endometrium the level of blood serum Melatonin is always reduced both in simple and complex hyperplasia. A strong negative correlation has been revealed between the *endometrial EGFR expression* and *blood serum Melatonin content*, wherein *blood serum EGF level* is in a certain negative correlation with *blood serum Melatonin content*;
4. With well and moderately differentiated endometrial adenocarcinomas positive correlation between the *EGFR expression in tumor tissue* and *blood serum EGF level* is more pronounced than *endometrial EGFR expression* and *blood serum EGF level* in endometrial hyperplasia;
5. With well and moderately differentiated endometrial adenocarcinomas negative correlation between the *EGFR expression in tumor tissue* and *blood serum Melatonin content* is more pronounced than between *endometrial EGFR expression* and *blood serum Melatonin content* in endometrial hyperplasia;

6. With well and moderately differentiated endometrial adenocarcinomas negative correlation between *blood serum EGF* and *Melatonin levels* is more pronounced than that in endometrial hyperplasia;
7. In various clinical cases, under mostly identical conditions, where there is a strong expression of endometrial EGFR, the risk carrier of tumor development in the endometrium, as well as a sharp decrease in the blood serum content of Melatonin, the antiproliferative/antineoplastic hormone, it is the high level of blood serum EGF that, due to its proliferative activity, is very likely to be indicative of the disease aggravation and overall unfavorable situation.

Practical significance of the study

The results of the present study are supposed to have a sizable importance for gynecologists and oncologists. Considering and practical use of the methods (measurement of endometrial EGFR expression and blood serum EGF and Melatonin levels as additional diagnostic testing) will be helpful in terms of an objective assessment of the clinical course and therapeutic efficacy in endometrial hyperplasia. Additionally, it will enable to provide an ultimately objective assessment of the risk of endometrial carcinoma development at the background of endometrial hyperplasia and (with subsequent adequate treatment) to ensure the prevention of uterine body cancer.

Based on the above, these results may form the basis for uterine body cancer screening programs, taking into account that these programs are being worked over in many clinics on the world.

Publications

The main body of the research has been published in the form of 11 scientific articles.

Approbation of the dissertation

The basic theses of the dissertation were reported at: the International Congress ”Topical Issues of Women’s Health” (Batumi, 2012); XXI International Scientific-Practical Conference (arranged by Georgian Association of Pediatric Cardiologists, Tbilisi, 2013); IV Joint Multi-field Research Conference “International Standards of Clinical Practice” (Bakuriani, 2014). 2nd International Students and Young Scientists Conference (Tbilisi, 2014), The Joint Session of Academic/Scientific/Practitioner Personnel of Georgian Cancer and Internal Medicine Research Center, Louis Pasteur Laboratory, New Vision University Clinic, Maternity House “Hera”, David Tvildiani Medical University (Tbilisi, 2015).

The volume and the structure of the dissertation

The dissertation contains 148 pages and consists of the following parts: introduction, literature review, materials and methods, study results, discussion and analysis of the results, conclusions, recommendations, bibliography (list of references). The dissertation contains 8 tables, 14 diagrams and 8 figures (microphotography). The list of references contains 195 titles (including 2 Georgian, 1 Russian and 192 English language sources) and 6 Internet websites.

Materials and Methods of the Study

The study involved 66 patients.

45 patients were examined clinically and morphologically, including 27 women of reproductive age (27-45 years), with menstrual disorders manifested as menometrorrhagia, 8 patients of preclimacteric age with irregular menstrual cycle and dysfunctional uterine bleeding (endometrial hyperplasia with manifested metrorrhagia documented by clinical and ultrasonographic investigation), and 10 patients with postmenopausal bleeding. In 45 cases the morphological material was obtained from the endometrial scrape (curettage). During the morphological study the material was fixed in 4% neutral buffered formalin for 24 hs; then the material was embedded in paraffin. 4 mkm.- thick sections were affixed to poly-L-lysine covered glass, stained by haematoxylin and eosin (H&E) to obtain a histological preparations. Assessment of the type of hyperplasia was performed based on the histological preparation; clinical and morphological variants of endometrial hyperplasia were determined: out of 35 in 19 cases there was simple non-atypical hyperplasia, 15 cases – complex non-atypical hyperplasia, 1 case – complex atypical hyperplasia. Endometrial carcinoma was detected in 10 cases in patients of postclimacteric age. In addition, the object of our study was the material obtained at surgery – removed from 21 patients (reproductive age – 3, preclimacteric age – 3 and postclimacteric age – 15 patients). In each case the histopathological diagnosis of endometrial carcinoma was preliminarily documented and known to us: 8 cases – well differentiated endometrial adenocarcinoma, 13 cases – moderately differentiated endometrial adenocarcinoma.

In all 66 cases we carried out immunochistochemical study of the morphological material (the sections removed from the paraffin blocs) /*anti-EGFR was used as a primary antibody (Novocastra; Leica Biosystems Newcastle Ltd.,UK). The paraffin sections were dewaxed and treated with 3% Hydrogen Peroxide (10 min) for endogenous peroxidase blocking. The antigen restoration was performed in 0.01M citrate buffer and cooled for 20 min. Then it was washed with triphosphate buffer (Tbs) for 5 min and incubated in Protein Block for 5 min. Then it was washed with Tbs (2x5*

min), incubated in anti-EGFR 1:50 dilution for 60 min at 25°C and washed with Tbs (2x5 min). It was incubated in the Post Primary Block for 30 min, then washed with Tbs (2x5 min). Then it was incubated in Novo Link Polimer for 30 min, then washed with Tbs (2x5 min). Peroxidase activation was carried out using a working solution of Diaminobenzidine (DAB) for 5 min, then we washed it with water and stained the nuclei with haematoxylin (5 min)/. The immunohistochemical study revealed the staining intensity and spread of Epidermal Growth Factor Receptor. The staining intensity was evaluated as foloows: (++) - strong intensity, (+) - moderate intensity, (+/_) - weak intensity (by Niikura, Sasano, Kaga et al., 1996). The quantitative results are expressed in percents. EGFR spread was determined by the presence/absence of positive EGFR immune reactivity in glandular epithelium and stromal cells – in each case under study we investigated 3 visual fields (each containing 500-700 cells) and expressed the positive results obtained in percents (by Miturski, Semczuk, Postawski, Jakowicki, 2000). For comparison, norm standards were used (by Niikura, Sasano, Kaga et al., 1996): in a normal endometrium the EGFR expression is weak and is revealed in 58.3%; at the same time, regarding the spread, the EGFR immune reactivity is positive in the glandular epithelium and the stromal cells. In order to measure blood serum EGF and Melatonin content blood samples were collected from 35 patients with endometrial hyperplasia and 21 patients with endometrial adenocarcinoma (who had undergone the surgery). The blood serum EGF levels were measured using the method of HPLC *(high-performance liquid chromatography),* the results being expressed in units of ng/ml (normal serum EGF content is < 0.35 ng/ml). Based on the literature data (Tomaszewski, Miturski, Kotarski, 1996; Brzezinski, Lewinski, 1998), we evaluated the changes (elevation) in blood serum EGF content as follows: *slightly, insignificantly increased or actually borderline values (0.35 ng/ml - 0.40 ng/ml), moderately increased (over 0.40 ng/ml to 0.60 ng/ml), significantly increased (0.60 ng/ml - 0.85 ng/ml), markedly increased (over 0.85 ng/mol to 1.85 ng/ml), sharply increased (1.85 ng/ml to 3.5 ng/ml), very sharply increased (3.5 ng/ml to 5.0 ng/ml), extremely increased (5.0 ng/ml or more).* Blood serum Melatonin content was measured using the ELISA (*enzyme-linked immunosorbent assay*) method, the results being expressed in units of pg/ml (taking into account the circadian rhythms of Melatonin secretion 12 pm and/or 3 am is considered the adequate time for clinical evaluation of blood serum Melatonin level *(A.Tavartkiladze, 2008).* In our studies melatonin concentrations were measured at 12 pm when the blood serum Melatonin level is normal > 20 pg/ml). Based on the literature (Bartsch, Blask, Cardinali et al., 2001; В.М.Ковальзон, 2004; ა. თავართქილაძე, 2008; Montagness, Middleton, Mani et al., 2010; Nogueira, Sampson, Chu et al., 2013), the changes (decrease) in blood serum Melatonin content was evaluated as follows: *borderline values (20 pg/ml to 19 pg/ml), moderately decreased (19 pg/ml to 15 pg/ml), significantly decreased (15 pg/ml to 11 pg/ml), sharply decreased (11 pg/ml to 8 pg/ml), highly decreased (8 pg/ml to 5 pg/ml), extremely*

reduced (5 pg/ml or less). Eventually, the quantitative results were statistically processed using the program SPSS-12-ANOVA. The statistical processing program IBM SPSS, version 20 was used as well.

35 patients with endometrial hyperplasia were followed up clinically for 2 years; within 6-month intervals each patients underwent Transvaginal Ustrasonography. Accordingly, cases of recurrence were registered.

Results of the Study and their Analysis

In simple non-atypical hyperplasia EGFR is expressed in the endometrium with a different degree of intensity (table), i.e. 11 patients out of 19 patients (57.9%; $p< 0.05$) showed weak expression of EGFR , in 8 patients (42.1%; $p< 0.1$) the EGFR expression was strong (diagram 1).

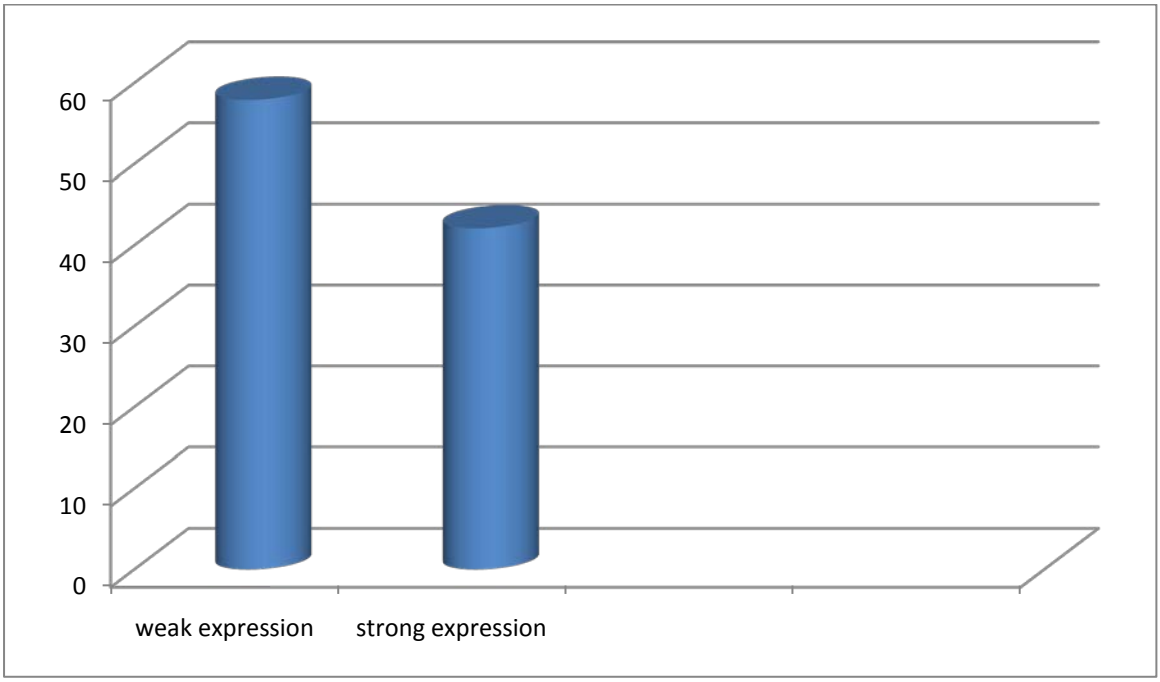
Table 1.

EGFR expression in the endometrium,
blood serum EGF and Melatonin levels in patients with simple and complex hyperplasia

Cases of simple endometrial hyperplasia	Cases of complex endometrial hyperplasia	EGFR expression in patients with simple endometrial hyperplasia EGF indices in blood serum in simple hyperplasia (normal level of blood serum EGF is < 0.35 ng/ml) Blood serum Melatonin indices in simple hyperplasia (normal level of blood serum Melatonin is > 20.0 pg/ml)			EGFR expression in patients with complex endometrial hyperplasia EGF indices in blood serum in complex hyperplasia (normal level of blood serum EGF is < 0.35 ng/ml) Blood serum Melatonin indices in complex hyperplasia (normal level of blood serum Melatonin is > 20.0 pg/ml)		
No 1	No1	Strong	0.55	17.3	Moderate	1.30	14.2
No 2	No 2	Weak	0.78	20.5	Strong	0.93	10.4
No 3	No3	Weak	0.22	23.8	Weak	1.70	17.9
No 4	No 4	Strong	0.45	15.7	Weak	0.85	23.1
No 5	No5	Weak	0.76	29.2	Strong	1.18	11.9
No 6	No 6	Weak	0.37	26.3	Weak	2.70	23.1
No 7	No7	Strong	0.56	17.4	Strong	1.90	12.7
No 8	No 8	Weak	0.81	28.7	Strong	2.20	9.2
No 9	No 9	Weak	0.97	26.9	Weak	0.77	16.0
No 10	No 10	Strong	0.65	11.2	Weak	3.20	19.3
No 11	No 11	Weak	0.88	20.1	Strong	2.60	12.1
No 12	No 12	Weak	0.38	29.2	Strong	1.15	10.7
No 13	No 13	Strong	0.30	10.3	Strong	2.05	13.2
No 14	No 14	Strong	1.02	17.8	Weak	1.18	17.1
No 15	No 15	Weak	0.51	25.8	Strong	2.60	10.1
No 16	No 16	Strong	0.70	8.2	Strong	1.19	8.5
No 17		Weak	0.23	32.1			
No 18		Strong	0.59	15.7			
No19		Weak	0,38	19.2			

Diagram 1.

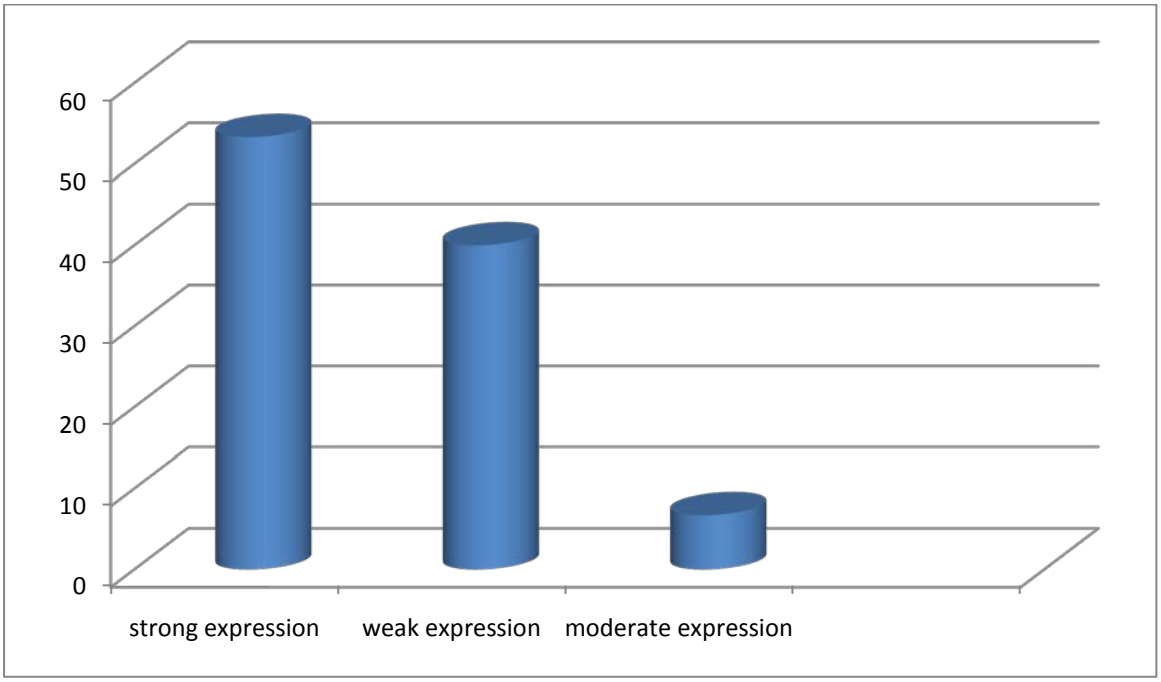
Endometrial EGFR expression in the in patients with simple endometrial hyperplasia



In complex non-atypical endometrial hyperplasia EGFR in the endometrium is revealed both by strong and weak expression; 8 patients out of 15 (53.3%; $p<0.05$) showed a strong EGFR expression, 6 patients (40.0%; $p< 0.1$) showed weak EGFR expression and in 1 patient (6.7%; $p< 0.1$) EGFR expression was moderate (diagram 2). In complex atypical endometrial hyperplasia (although there was only one case) EGFR is expressed with strong intensity.

Diagram 2.

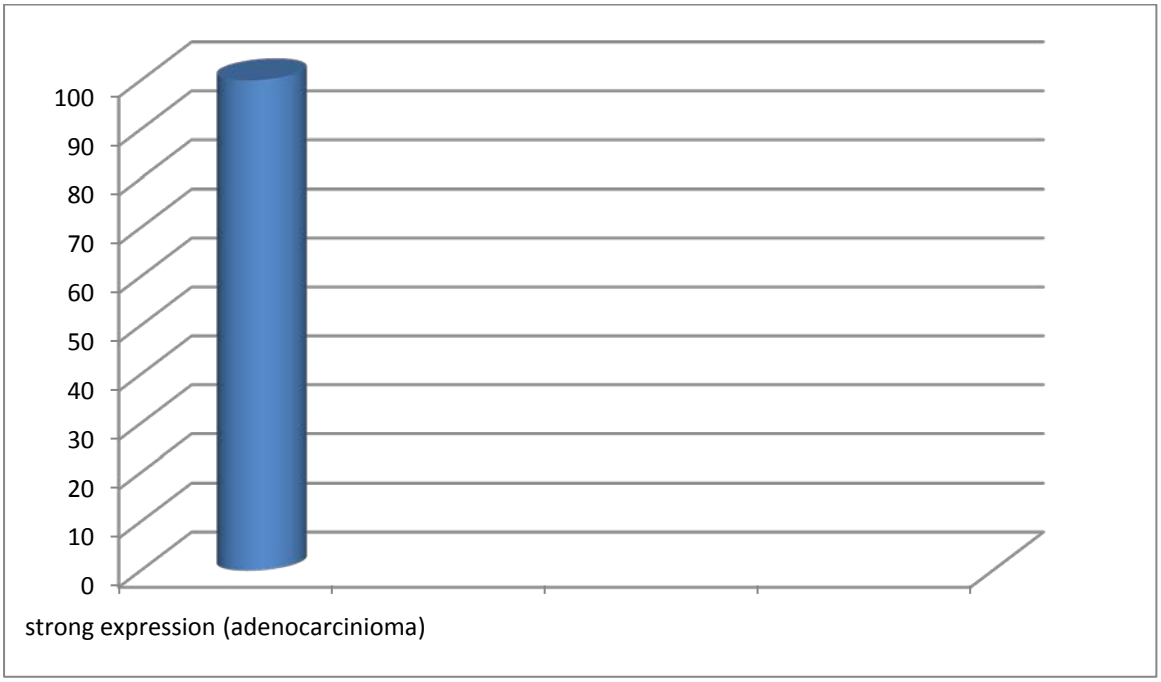
Endometrial EGFR expression in patients with complex endometrial hyperplasia



The analysis of EGFR expression frequency using Pearson χ^2 criterion showed no reliable difference between the two groups (patients with simple endometrial hyperplasia and patients with complex endometrial hyperplasia): the meaning of the criterion is 0.300; however some difference is possible which is confirmed by so called risk assessment [odds ratio (simple endometrial hyperplasia/complex endometrial hyperplasia) is 2.063]. In all ten cases of endometrial carcinoma (the morphological material being collected by curettage), EGFR expression in the tumor tissue was strong. In the morphological material (endometrial tissue from 21 patients – after surgery) where a preliminary histopathological diagnosis documented endometrial adenocarcinoma, EGFR expression was strong in all the cases (100%) (Diagram 3).

Diagram 3.

EGFR expression in the tumor tissue in patients with endometrial adenocarcinoma



The analysis of EGFR expression frequency in simple endometrial non-atypical hyperplasia and endometrial adenocarcinoma using Pearson χ^2 criterion showed a drastic difference (the criterion value – 0.00004). However this is evident without any analysis because in all cases of adenocarcinoma EGFR expression is strong. This refers to the risk assessment as well [odds ratio (adenocarcinoma/simple non-atypical hyperplasia) is 2.375]. In complex non-atypical endometrial hyperplasia and endometrial adenocarcinoma, the analysis of EGFR expression frequency using Pearson χ^2 criterion showed a drastic difference (the criterion value – 0.001). However this is evident without any analysis because in all cases of adenocarcinoma EGFR expression is strong. This refers to the risk assessment as well [odds ratio (adenocarcinoma/complex non-atypical hyperplasia) is 1.667)].

Thus, based on our investigation, in simple non-atypical endometrial hyperplasia EGFR expression in the endometrium is mostly weak, while in complex non-atypical endometrial hyperplasia strong EGFR expression is prevalent. With that, in well/moderately differentiated endometrial adenocarcinoma EGFR is revealed by a strong expression (100%) in the tumor tissue.

Our results, to a certain extent, coincide with those of other authors that in hyperplastic endometrium EGFR is revealed in 100% (Niikura, Sasano, Matsunaga et al., 1995; Niikura, Sasano, Kaga et al., 1996). They are also in tune with other studies reporting on a high EGFR expression in endometrial carcinomas (Berchuck, Soisson, Olt et al., 1989; Prentice, Thomas, Weddell et al., 1992; Nyholm, Nielsen, Ottesen, 1993; Zarcone, Bellini, Cardone et al.,1995; Niikura, Sasano, Kaga et al., 1996; Oza, Eisenhauer, Elit et al., 2008; Pierpaoli, Regelson, Colman, 2011).

In patients with simple non-atypical endometrial hyperplasia the average content of blood serum EGF (0.56 ng/ml) is by 66.29 % less ($p<0.01$) than that in patients with complex non-atypical endometrial hyperplasia (1.71 ng/ml) (table 1, diagram 4, diagram 5); according to the additional statistical study the average content of blood serum EGF in simple non-atypical endometrial hyperplasia is considerably lower than that in complex non-atypical endometrial hyperplasia [the T-criterion (p(T) value is 0.0000225, while the Mann-Whitney nonparametric criterion (p(U) value is 0.0000026).

Diagram 4.

Characteristics of EGF concentration in human blood serum in simple/complex endometrial hyperplasia (on the diagram the upper broken line represents the blood serum EGF level in complex endometrial hyperplasia while the lower one shows the same index in simple endometrial hyperplasia

Noteworthy, each point on the broken lines on diagrams 4, 6, 12, 13 corresponds to a proper case number given in the tables, while each height (apex) represents the absolute number, i.e. the absolute index of blood serum EGF/Melatonin levels which is identical to the numbers given in the vertical column

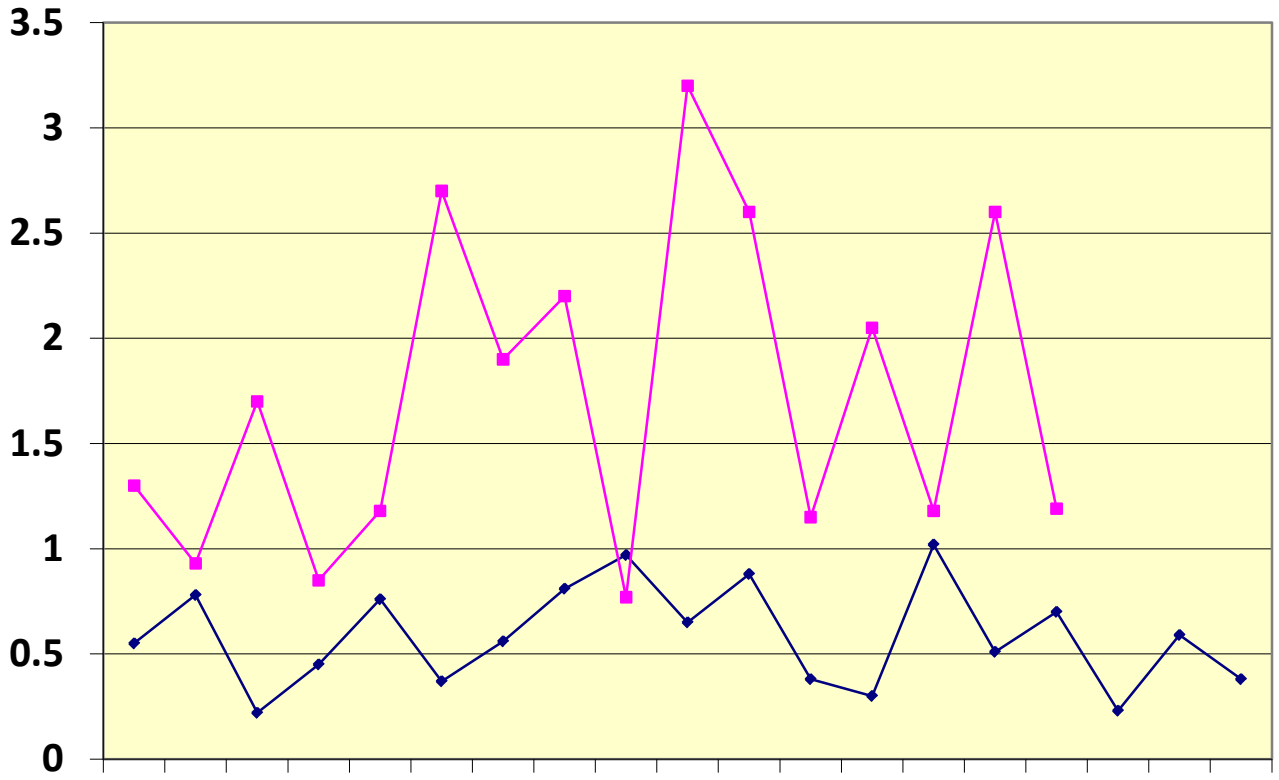
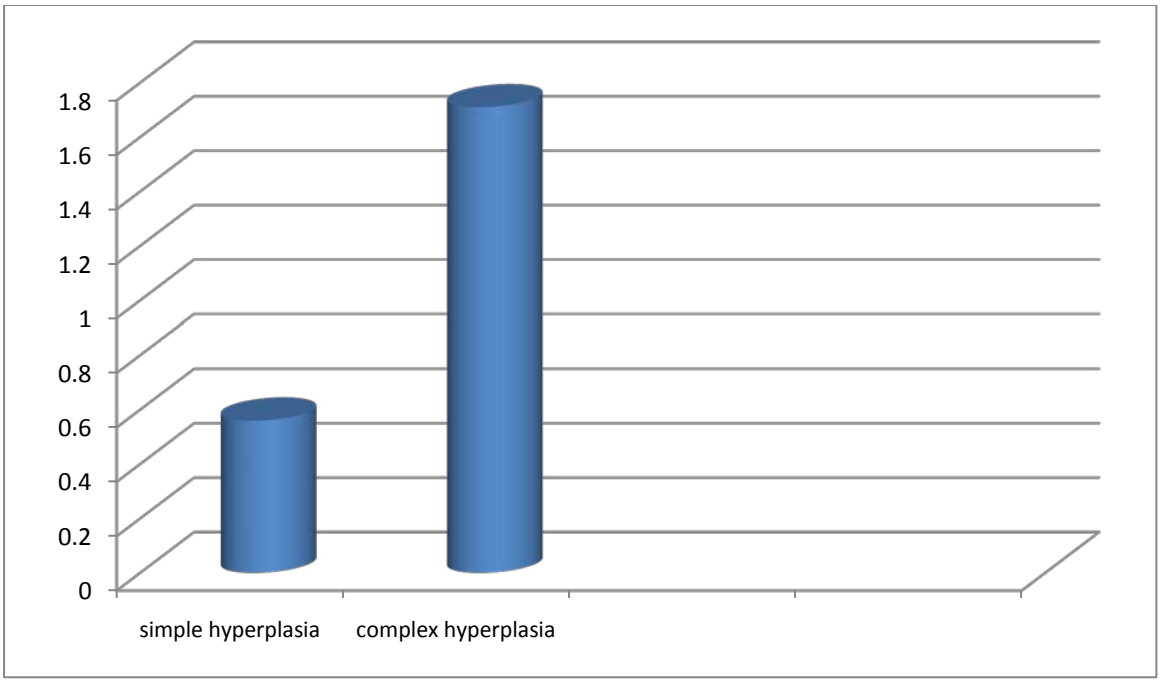


Diagram 5.

Average index of blood serum EGF in patients with simple/complex endometrial hyperplasia



In patients with simple non-atypical endometrial hyperplasia the average level of blood serum Melatonin (20.8 pg/ml) is by 29.33% more ($p<0.01$) than that (14.7 pg/ml) in complex non-atypical endometrial hyperplasia (table 1, diagram 6, diagram 7); according to the additional statistical study the average level of Melatonin in simple non-atypical hyperplasia is *considerably higher* than that in complex non-atypical endometrial hyperplasia. [T-criterion (pT) value is 0.003, while the Mann-Whitney nonparametric criterion (p(U) value is 0.007).

Diagram 6.

Characteristics of Melatonin concentration in human blood serum in simple and complex endometrial hyperplasia (on the diagram the upper broken line represents the blood serum

Melatonin level in simple endometrial hyperplasia while the lower one shows the same index in complex endometrial hyperplasia)

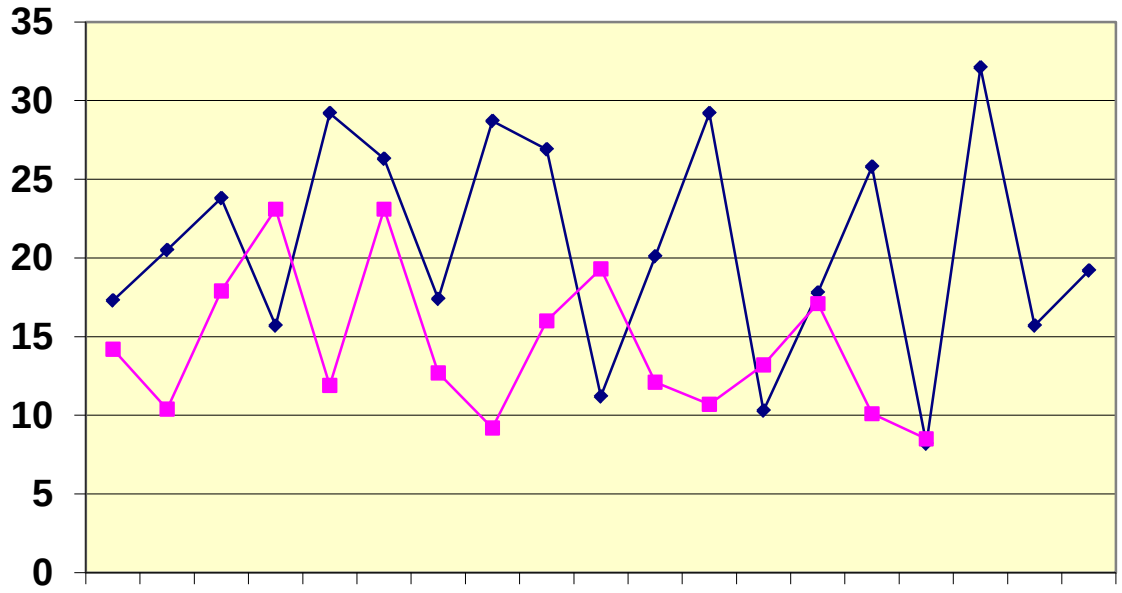
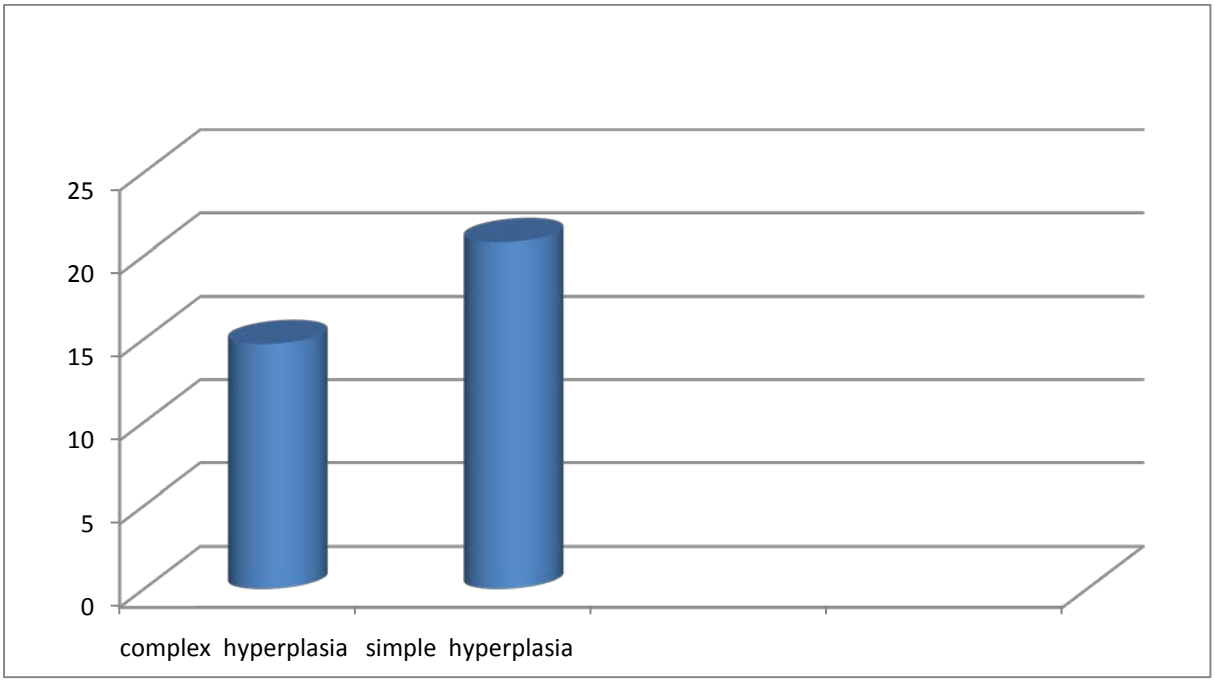


Diagram 7.

Average index of blood serum Melatonin content in patients with simple/complex endometrial hyperplasia



As mentioned above, in simple non-atypical endometrial hyperplasia weak expression of endometrial EGFR is revealed in 57.9% (11 patients out of 19), while blood serum EGF level equals to 0.22-0.97 ng/ml (table 1). Accordingly, the index was: normal (2 cases); slightly increased, virtually showing the borderline values (3cases); moderately increased (1 case); significantly

increased (3 cases); markedly increased (2 cases). In all 11 cases the content of Melatonin was practically within normal limits (one case showing borderline values).

It turns out that according to the cases reviewed (i.e. where endometrial EGFR expression was weak) in respect of Melatonin, blood serum EGF level “should be of relatively less importance “ – whether normal or slightly/moderately/significantly/markedly increased (though the “influence” of blood serum EGF content on Melatonin cannot be completely excluded). Apparently, a weak expression of EGFR is “much more important” within this context. In these circumstances the index of blood serum melatonin mostly remains normal.

As was mentioned, in 40% of patients (8 out of 19 patients) with simple non-atypical endometrial hyperplasia EGFR is strongly expressed, while blood serum EGF level is 0.3-1.02 ng/ml (table 1). Accordingly the index was: normal – 1 case (blood serum Melatonin content is sharply decreased, respectively), moderately increased – 4 cases (blood serum Melatonin content is moderately decreased, respectively), significantly increased – 2 cases (blood serum Melatonin content is significantly/sharply decreased, respectively), markedly increased – 1 case (blood serum Melatonin content is moderately decreased, respectively). Of all these cases in the first case (despite the normal blood serum EGF content) the situation is likely to be dangerous because the sharp expression of endometrial EGFR, the carcinogenesis risk carrier, as well as the sharp decrease of blood serum Melatonin, that has antiproliferative/antineoplastic effects. In the next 4 and 2 cases, blood serum EGF level shows a certain negative correlation with blood serum Melatonin content, which was confirmed by the correlative analysis (there is a *certain negative correlation* between blood serum EGF level and Melatonin content: Spearman nonparametric correlation coefficient is -0.372; Pearson correlation coefficient is -0.331). The last of above mentioned cases, wherein blood serum EGF level is markedly increased requires more attention to make an appropriate assessment.

It should be emphasized that any increase in blood serum EGF level, especially significant or marked, is dangerous at the background of strong endometrial EGFR expression, because blood serum Melatonin content tends to decrease. In short, Melatonin is particularly “responsive” to EGFR expression and with weak expression of the receptor in simple non-atypical endometrial hyperplasia its level is usually within normal or, rarely, borderline limits, while in complex non-atypical endometrial hyperplasia the content of Melatonin is normal/borderline/moderately decreased (regardless of blood serum EGF level). With strong expression of endometrial EGFR, the content of blood serum Melatonin is always decreased, even when the level of EGF is normal (as in one case mentioned above).

Though there is a *certain negative correlation* between blood serum EGF and melatonin levels, which is basically revealed in the cases with strong endometrial EGFR expression, there also might be a rare exception (as in the 2 cases mentioned above). Therefore, the decrease in blood serum melatonin level seems to be impending in endometrial hyperplasia (even with simple non-atypical endometrial hyperplasia).

As was mentioned, the strong expression of EGFR in the endometrium is revealed in 53.3% of complex non-atypical endometrial hyperplasia cases (8 out of 15 patients) with markedly/sharply increased blood serum EGF level (0.93-2.60 pg/ml) and significantly/sharply decreased blood serum melatonin (from 13.2 pg/ml to 9.2 pg/ml). Accordingly, the same picture was observed in one case where endometrial EGFR expression was moderate (blood serum EGF level was markedly increased, while blood serum melatonin content was significantly decreased). In complex atypical endometrial hyperplasia (1 case) at the background of strong endometrial EGFR expression the level of blood serum melatonin is very low (8.5 pg/ml), however blood serum EGF level is markedly (not sharply) increased.

In short, in complex non-atypical endometrial hyperplasia (with strong EGFR expression in the endometrium), if blood serum EGF level is markedly or sharply increased then the level of blood serum melatonin is significantly or sharply decreased (the correlation analysis performed showed that there is a *weak positive correlation* between endometrial EGFR expression and blood serum EGF level: Spearman nonparametric correlation coefficient is 0.195; Pearson correlation coefficient is 0.147. Also, the correlation analysis performed showed that there is a *strong negative correlation* between endometrial EGFR expression and blood serum melatonin level: Spearman nonparametric correlation coefficient is -0.830; Pearson correlation coefficient is -0.805). In this case, a particular attention should be paid to blood serum EGF content, i.e. excessive increase of EGF is indicative of an unfavorable condition. A better situation in non-atypical endometrial hyperplasia is when EGF values are not very increased at the background of the normal/borderline level of blood serum melatonin.

As was mentioned, weak expression of endometrial EGFR is revealed in 40% of complex non-atypical endometrial hyperplasia cases (6 patients out of 15). Blood serum EGF level was 0.77-3.20 ng/ml. With that, blood serum EGF index was: significantly increased *(blood serum melatonin level - normal or moderately decreased)* – 2 cases; markedly increased *(blood serum melatonin level - moderately decreased)* – 2 cases; sharply increased *(blood serum melatonin level - normal or borderline)* – 2 cases.

Interestingly, in same cases of complex non-atypical endometrial hyperplasia, the serum index of melatonin is normal, however, this requires the background of weak expression of endometrial EGFR is necessary (despite the sharply increased blood serum EGF index in certain cases). In all cases of complex non-atypical endometrial hyperplasia, blood serum melatonin index is higher with weak rather than strong endometrial EGFR expression, not to mention simple endometrial hyperplasia, where blood serum melatonin content is constantly normal with weak endometrial EGFR expression, regardless of blood serum EGF level.

As it has been revealed, in complex non-atypical endometrial hyperplasia blood serum EGF level is always significantly/markedly/sharply increased at the background of weak, moderate or strong EGFR expression in the endometrium. Eventually, one can probably assume that in complex non-atypical endometrial hyperplasia (regardless of the difference in blood serum EGF levels) blood serum melatonin content is always normal/borderline or moderately decreased with weak endometrial EGFR expression, which is not indicative of poor condition, and vice versa, with strong endometrial EGFR expression blood serum melatonin content is decreased (even when blood serum EGF level is normal) and any elevation, especially significant or marked, in the levels of blood serum EGF is dangerous.

Finally, melatonin is much more “responsive” to endometrial EGFR expression than to the changes in blood serum EGF levels which directly indicates that in the given situation it is endometrial EGFR that indicates “carcinogenicity” rather then blood serum EGF. That is, the variant where only blood serum EGF content is elevated and endometrial EGFR expression is weak, implies a better condition than, say, the one with strong endometrial EGFR expression and moderately increased blood serum EGF level.

Regarding endometrial carcinoma (table 2), the correlation analysis showed the following:

Table 2.

EGFR expression in tumor tissue and blood serum EGF and Melatonin indices; malignancy degree (G), illness stage (T), invasion into lymphatic nodes (N) and distant organs (M) in patients with endometrial adenocarcinoma

Cases of endometrial adenocarcinoma Tumor malignancy degree (G), stage of disease (T), invasion in the lymphatic nodes(N), and distant organs (M)					EGFR expression in the tumor tissue of patients with endometrial adenocarcinoma Blood serum EGF level in endometrial adenocarcinoma (normal level of EGF is <0.35 ng/ml) Blood serum Melatonin content in endometrial adenocarcinoma (normal level of melatonin is <20.0 pg/ml)		
No 1	G2	T3	N2	M0	Strong	3.4	11.1
No 2	G2	T2	N2	M1	Strong	1.2	2.5
No 3	G2	T3	N2	M1	Strong	5.5	1.2
No 4	G2	T1	N0	M0	Strong	7.0	4.09
No 5	G2	T3	N0	M0	Strong	1.0	7.3
No 6	G2	T3	N2	M0	Strong	1.3	8.1
No 7	G2	T2	N0	M0	Strong	0.5	3.2
No 8	G2	T1	N1	M0	Strong	7.7	2.1
No 9	G2	T3	N0	M0	Strong	0.8	1.9
No 10	G2	T3	N2	M1	Strong	2.0	5.3
No 11	G1	T3	N0	M0	Strong	1.9	12.1
No 12	G1	T2	N0	M0	Strong	3.4	7.8
No 13	G2	T3	N1	M0	Strong	7.0	2.8
No 14	G 1	T3	N2	M0	Strong	5.5	0.24
No 15	G1	T1	N0	M0	Strong	4.0	2.6
No 16	G2	T3	N0	M0	Strong	0.9	7.1
No 17	G 1	T3	N0	M0	Strong	1.2	11.8
No 18	G1	T2	N0	M0	Strong	5.0	3.2
No 19	G1	T1	N0	M0	Strong	0.2	4.25
No 20	G2	T3	N2	M1	Strong	5.3	2.17
No 21	G1	T2	N0	M0	Strong	0.4	7,7

In endometrial adenocarcinoma there is a *relatively weak positive correlation* between endometrial EGFR expression in the tumor tissue and blood serum EGF level: Pearson correlation coefficient is 0.307, and Spearman nonparametric correlation coefficient is 0.308.

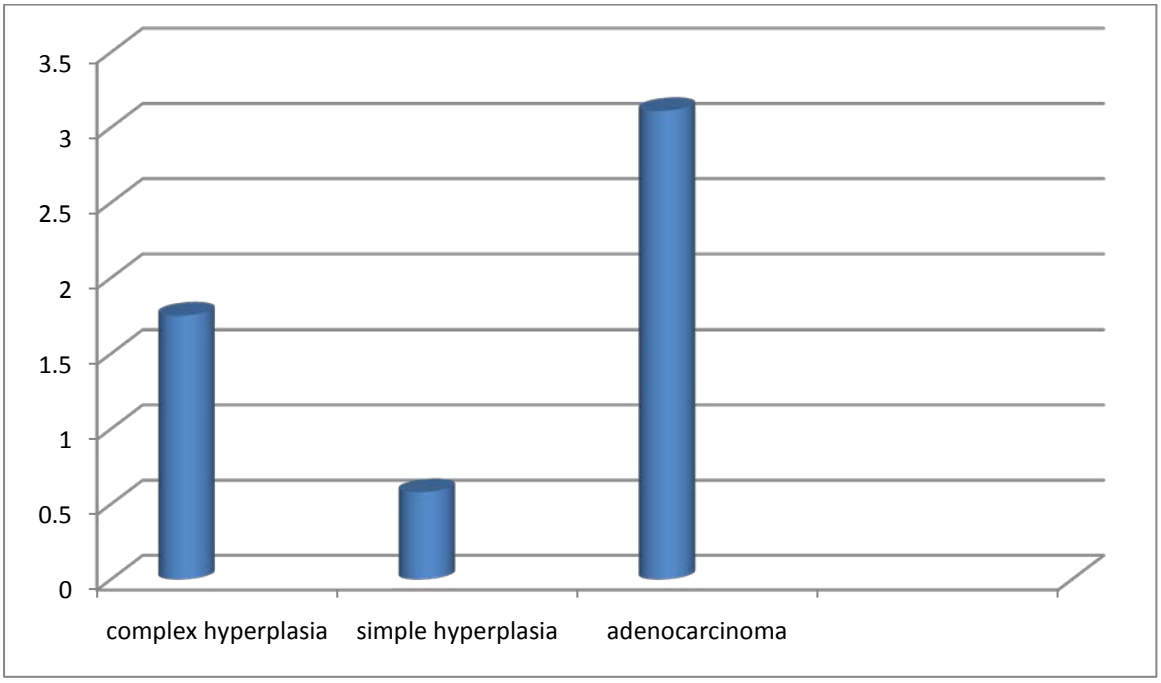
With endometrial adenocarcinoma we also observed a *strong negative correlation* between EGFR expression in the tumor tissue and blood serum Melatonin content: Pearson correlation coefficient is -0.818, and Spearman nonparametric correlation coefficient is -0.790.

Patients with endometrial adenocarcinoma show a *negative correlation* between blood serum EGF and Melatonin levels: Pearson correlation coefficient is -0.533, and Spearman nonparametric correlation coefficient is -0.473. These results demonstrate that the correlative parameters are more pronounced in endometrial carcinoma than those in endometrial hyperplasia (see above).

The study results have revealed that in patients with endometrial adenocarcinoma blood serum level of EGF makes up 3.11 ng/ml on average. The statistical study also showed that in simple non-atypical endometrial hyperplasia the average level of blood serum EGF (0.58 ng/ml) is 81% less ($p<0.01$) than that in endometrial adenocarcinoma cases. In complex non-atypical endometrial hyperplasia the average level of blood serum EGF (1.75 ng/ml) is 43% less ($p<0.01$) than that in endometrial adenocarcioma (diagram 8).

Diagram 8.

Average indices of blood serum EGF level in patients with simple/complex endometrial hyperplasia and endometrial adenocarcinoma

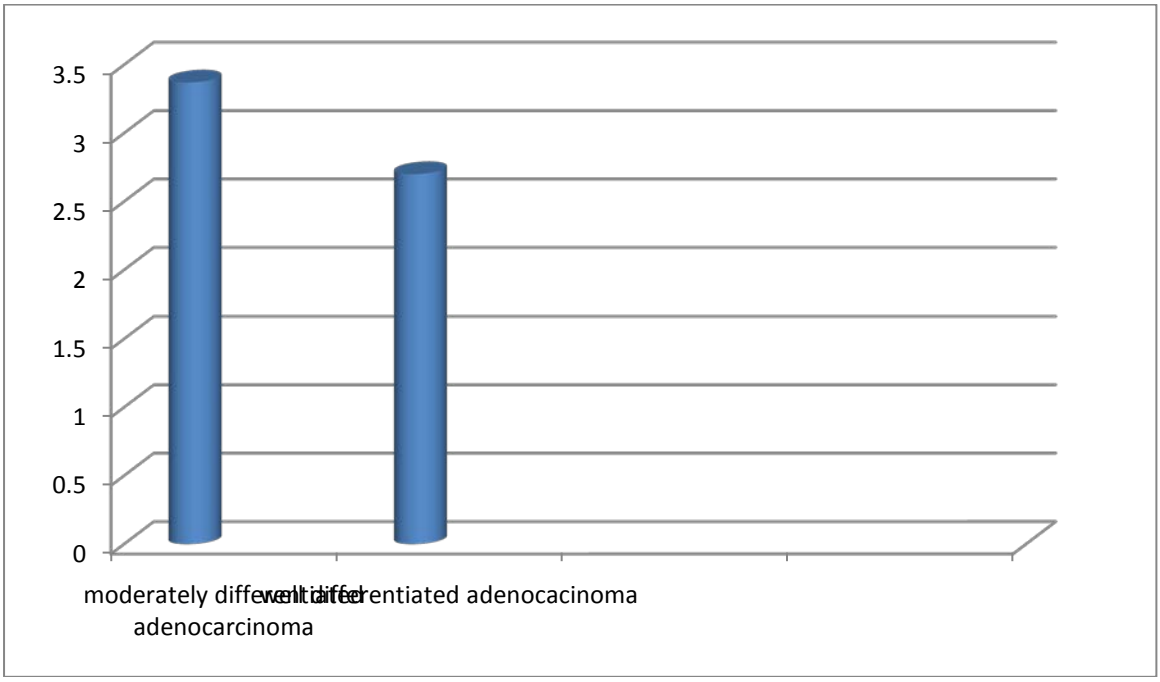


Additional statistical analysis has shown: in patients with simple non-atypical endometrial hyperplasia the average level of blood serum EGF is *significantly lower* than that in patients with endometrial adenocarcinoma [T-criterion (p(T) value is 0.00014; Man-Whitney (p(U) nonparametric criterion value is 0.00005]. In patients with complex non-atypical endometrial hyperplasia the average level of blood serum EGF is *somewhat lower* than that in patients with endometrial adenocarcinoma [T-criterion (p(T) value is 0.018; Man-Whitney (p(U) nonparametric criterion value is 0.141]. It was also established, based on the study results, that in moderately differentiated

endometrial adenocarcinoma the average level of blood serum EGF was 3.37 ng/ml, while in well differentiated endometrial adenocarcinoma the average level of blood serum EGF made up 2.70 ng/ml (diagram 9); the latter index was by 19.38% less ($p<0.05$) than the former. As is shown, our data are consistent with the results of some authors who reported that in endometrial carcinomas the average blood serum EGF level exceeds normal values (Tomaszewski, Miturski, Kotarski, 1996). The same authors also reported on the highest average level of blood serum EGF in moderately differentiated endometrial carcinomas.

Diagram 9.

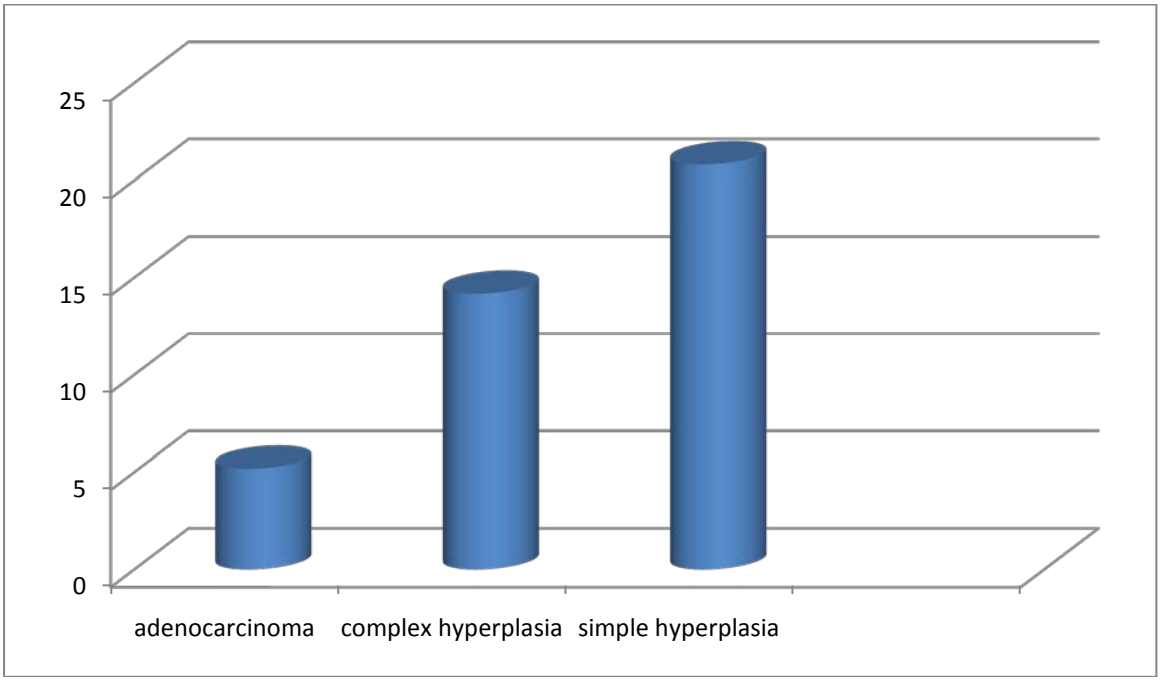
Average indices of blood serum EGF level in patients with well/moderately differentiated endometrial adenocarcinoma



The results of the study have demonstrated: in patients with endometrial adenocarcinoma the level of blood serum Melatonin is 5.17 pg/ml on average. According to the statistical analysis, in endometrial adenocarcinoma the average level of blood serum Melatonin is by 75.16% less ($p<0.01$) than that in simple non-atypical endometrial hyperplasia (20.81 pg/ml), and by 64.94% less ($p<0.01$) than that in complex non-atypical endometrial hyperplasia (14.73 pg/ml) (diagram 10).

Diagram 10.

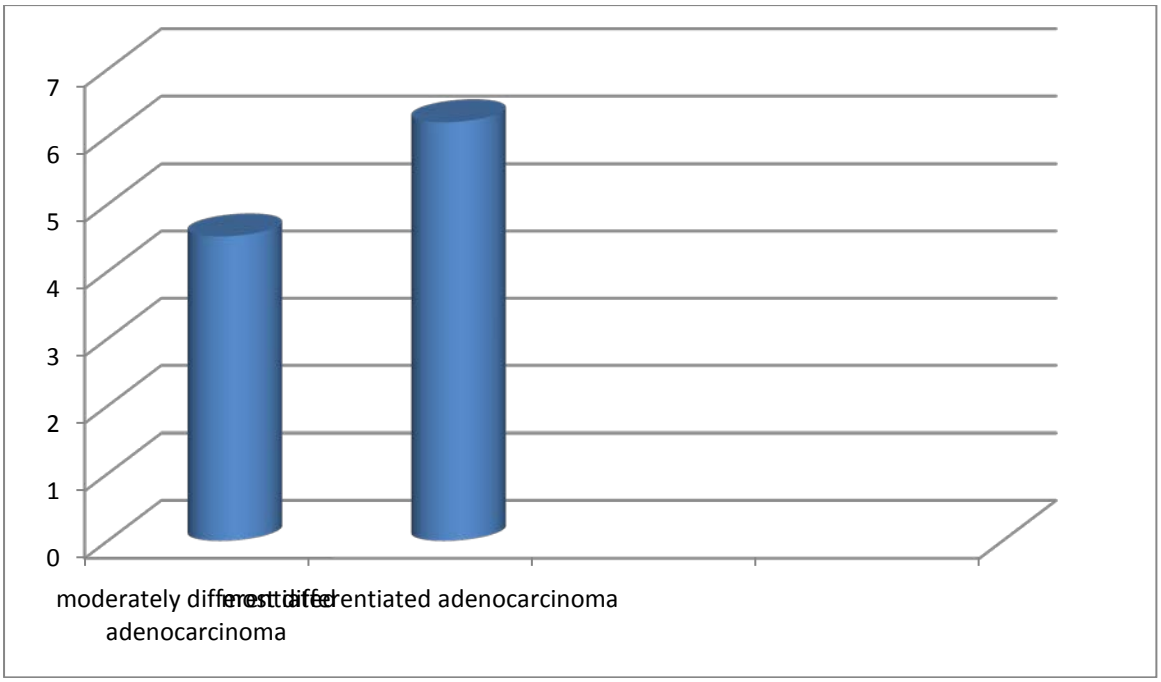
Average indices of blood serum Melatonin content in patients with simple/complex endometrial hyperplasia and endometrial adenocarcinoma



Additional statistical analysis has shown: in patients with simple non-atypical endometrial hyperplasia the average level of blood serum Melatonin is *significantly higher* than that in patients with endometrial adenocarcinoma [T-criterion (p(T) value is 0.00000003; Man-Whitney (p(U) nonparametric criterion value is 0.0000002]. In patients with complex non-atypical endometrial hyperplasia the average level of blood serum Melatonin is also *significantly higher* than that in patients with endometrial adenocarcinoma [T-criterion (p(T) value is 0.00000007; Man-Whitney (p(U) nonparametric criterion value is 0.0000034]. Based on the study results it has been established: in moderately differentiated endometrial adenocarcinoma the average level of blood serum Melatonin is 4.52 pg/ml, while in well differentiated endometrial adenocarcinoma it makes up 6.21 pg/ml (diagram 11); the latter index is by 27.21% higher ($p<0.01$) than the former. It seems interesting to know whether the authors have obtained any data regarding the role and importance of Melatonin in neoplastic processes of the endometrium as well as the relationship between the average blood serum levels of Melatonin and tumor differentiation. However there are no definite findings in this respect.

Diagram 11.

Average indices of blood serum Melatonin content in patients with well/moderately differentiated endometrial adenocarcinoma



As is seen (table 2), in 11 patients out of 21 (52.4%; $p<0.05$) with endometrial adenocarcinoma blood serum EGF level is within the range common for endometrial hyperplasia (simple, complex), which was demonstrated in our study, and makes up 0.2-2.2 ng/ml (diagram 12); however, in 9 cases out of 11 mentioned blood serum Melatonin level is decreased as compared with normal values, making up 1.9-8.1 pg/ml; such a reduction was not observed with endometrial hyperplasia (diagram 13). This suggests that the strong expression of EGFR in the endometrium and simultaneous sharp/high/extreme decrease in blood serum Melatonin content are more “carcinogenic” than the increase in blood serum EGF level.

Diagram 12.

Characteristics of blood serum EGF concentration in simple/complex endometrial hyperplasia and endometrial adenocarcinoma (upper broken line depicts blood serum EGF level in endometrial

adenocarcinoma, middle broken line – the same index in complex endometrial hyperplasia, lower broken line – the same index in simple endometrial hyperplasia)

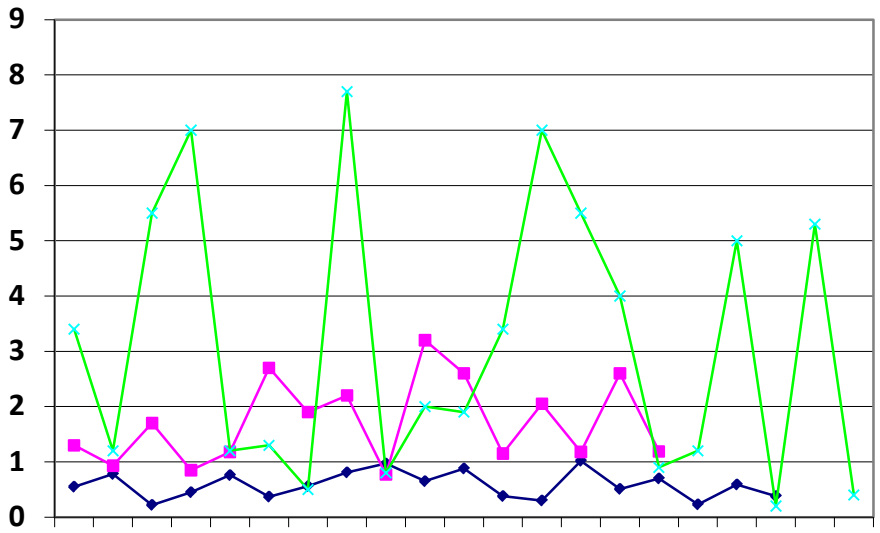
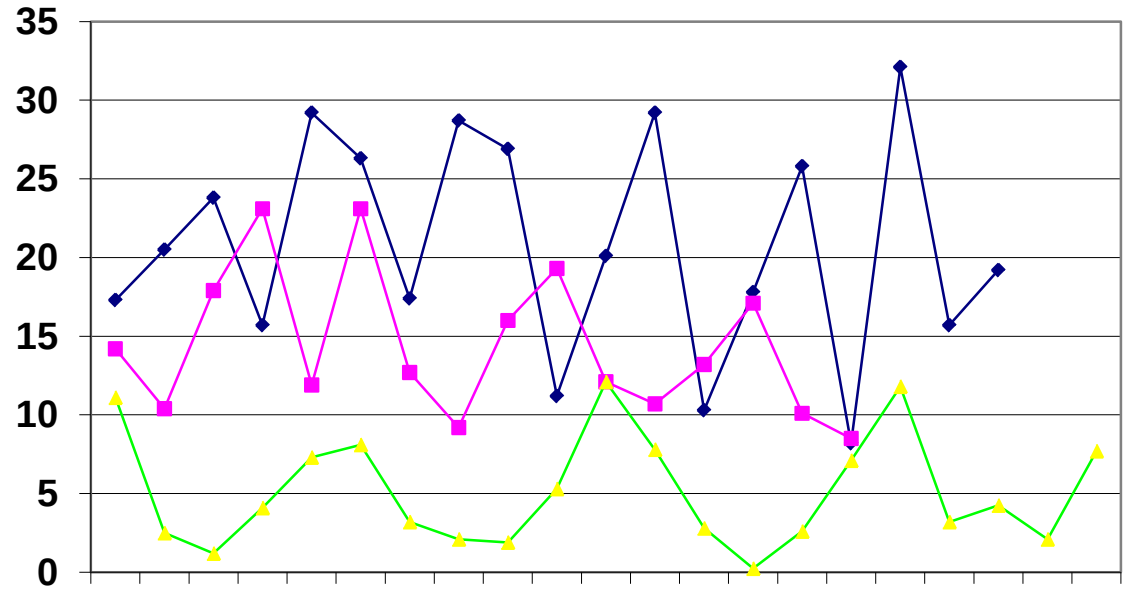


Diagram 13.

Characteristics of blood serum Melatonin concentration in simple/complex endometrial hyperplasia and endometrial adenocarcinoma (upper broken line depicts blood serum Melatonin level in simple endometrial hyperplasia, middle broken line – the same index in complex endometrial hyperplasia, lower broken line – the same index in endometrial adenocarcinoma)



Attention should also be paid to the 2 cases out of 11 (table 2) where blood serum EGF level was normal (0.2 ng/ml)

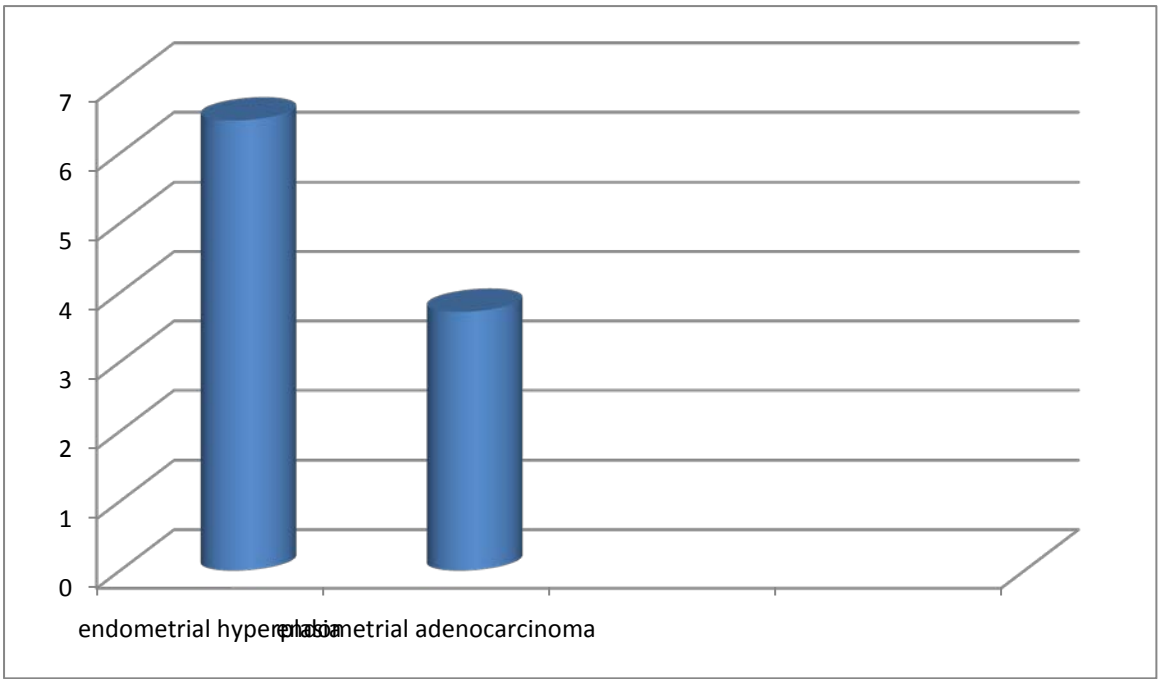
or bordline (0.4 ng/ml), while blood serum melatonin level was 4 pg/ml and 7.7 pg/ml, respectively (i.e. blood serum Melatonin content was within the typical of endometrial adenocarcinoma range, which was not seen in endometrial hyperplasias /diagram 13/). The question is why the EGF content in the blood serum is so close to the norm. Perhaps it was increased and has dropped by the moment. Why? The researchers – Tomaszewski, Miturski, Kotarski (1996) reported that blood serum EGF level is the highest in moderately differentiated endometrial carcinoma; they also showed that blood serum EGF lewel was low in well differentiated endometrial carcinoma. Is this the case in those two patients? Did the tumor differentiation change? Or, perhaps, "EGF is not correlation with the degree of tumor malignancy, stage and life expectancy'? (Fuller, Seiden, Young, 2004).It should be underlined that in these 2 cases the situation is different: in both cases adenocarcinoma is well differentiated; stage I is observed in the first case, and stage II is observed in the second case; neither of the cases show proximate or distant metastases (table 2). Therefore we assume that blood serum EGF level has not elevated enough, so far, to stimulate tumor "aggression".

As is seen (table 2, diagrams 12-13), in the remaining 10 cases out of 21 (47.6%; $p<0.05$) blood serum EGF level is high and makes up 3.4-7.7 ng/ml, while blood serum Melatonin content is 0.24-11.1 pg/ml (Melatonin level is extremely decreased in 9 cases and no similar values were found in endometrial hyperplasia, in which the highest value equals 7.8 pg/ml). In 7 out of 10 cases mentioned, blood serum EGF content is very high and makes up 3.4-5.5 ng/ml, while Melatonin content is 0.24-11.1 pg/ml; in the 7 cases mentioned the mean value of blood serum Melatonin level is 4.0444 pg/ml. In 3 out of 10 cases blood serum EGF content is extremely high and makes up 7.0-7.7 ng/ml, while Melatonin content is 2.1-4.09 pg/ml; in the 3 cases mentioned the mean value of blood serum Melatonin level is 2.997 pg/ml, which means that the latter is by 25.98% less ($p<0.01$) than the former. Based on the given data it can be concluded that blood serum EGF and Melatonin levels are in inverse proportion, which is confirmed by the statistical analysis.

In 11 patients out of 21 studied, who showed blood serum EGF levels consistent with the range typical of endometrial hyperplasia, the average level of blood serum Melatonin is 6.47 pg/ml; however, when blood serum EGF level (10 cases out of 21) fits within the range found merely in endometrial adenocarcinoma, blood serum Melatonin level makes up 3.72 pg/ml, being by 42.35% less ($p<0.01$) as compared with the former index (diagram 14).

Diagram 14.

Average level of blood serum Melatonin content in patients with endometrial adenocarcinoma: patients showing blood serum EGF level range typical of endometrial hyperplasias only; patients showing blood serum EGF level range typical of endometrial adenocarcinoma only



This diagram once again confirms that the elevation in blood serum EGF level as well as the decrease in blood serum Melatonin content are of predictive umportance in endometrial adenocarcinoma. We do not agree with the authors who suggest that "EGF is not in correlation with the degree of tumor malignancy and stage as well as with life expectancy" (Fuller, Seiden, Young, 2004). On the contrary, our results reveal that in endometrial adenocarcinoma (well/moderately differentiated adenocarcinoma) with a drastic increase in the average level of blood serum EGF the average level of blood serum Melatonin extremely decreases. If we compare the oncologic characteristics of above mentioned 11 and 10 patients (G, T, M, N), we will not see any significant difference (table 2). The fact is that in 10 patients whose range of blood serum EGF level is typically only of adenocarcinoma (in contrast to the EGF index range of the 11 patients, that fits also within that of endometrial hyperplasia) the health status will probably worsen more easily, because under equal conditions (strong expression of endometrial EGFR expression and extreme decrease of antiproliferative Melatonin content in blood serum) it is the high levels of blood serum EGF (due to the proliferative effect) that signal poor condition.

It should be highlighted that in 18 cases out of 21 (85.71%;$p<0.01$) blood serum Melatonin level dropped from 8.1 to 0.24 pg/ml (table 2). This range of blood serum Melatonin content was not found in endometrial hyperplasia (diagram 13). There were only 3 cases, where blood serum

Melatonin content was 11.1-11.8-12.1, respectively; such indices of Melatonin level were also observed in endometrial hyperplasia. In 2 cases out of 3, increased index of blood serum EGF level was seen in endometrial hyperplasia as well (1.2-1.9 ng/ml). Presumably, significant decrease in blood serum Melatonin level at the background of strong EGFR expression is suggestive of a certain border between hyperplasia-neoplasia processes. In 1 out of 3 cases, where there is a sharp increase in blood serum EGF content (3.4 ng/ml), the drop in blood serum Melatonin levels should have been sharp, high or extreme. However, they did not decrease to that extent (11.1 pg/ml). Based on this, in endometrial hyperplasia, in the presence of strongly expressed EGFR in the endometrium and sharply increased level of EGF in blood serum, even a moderate decrease in blood serum Melatonin content demands attention.

Moreover, in 5 cases out of 6 (with recurrent hyperplasia of the uterine mucosa) (table 1: cases of simple endometrial hyperplasia – corresponding numbers 13, 16, 18; cases of complex endometrial hyperplasia – corresponding numbers 3, 8, 12) endometrial EGFR expression was strong (both in simple and complex non-atypical hyperplasia) and the decrease in blood serum Melatonin content was sharp in all cases and only in one patient it was moderate (table 1: case – corresponding number 18). Interestingly, in 1 out of 5 cases mentioned (with a sharp decrease in blood serum melatonin content) blood serum EGF level was normal (table 1: case – corresponding number 13).

Out of 6 cases mentioned, only in one case (table 1: case – corresponding number 3) endometrial EGFR expression was weak, with blood serum Melatonin being moderately reduced; however it is noticeable that in this case of complex endometrial non-atypical hyperplasia blood serum EGF level was markedly increased; in this respect it has to be emphasized that the treatment was belated.

To sum up:

- In simple non-atypical endometrial hyperplasia, weak EGFR expression prevails in the endometrium, while in complex non-atypical hyperplasia strong EGFR expression is more pronounced; with that, EGFR expression is revealed in all cases. In well/moderately differentiated endometrial adenocarcinoma EGFR is revealed by strong expression in the tumor tissue in 100%;
- There is a weak positive correlation between EGFR expression in hyperplastic endometrium and blood serum EGF levels , and a strong negative correlation between EGFR expression and blood serum Melatonin content; with that, in the patients' blood serum, there is a certain negative correlation between blood serum EGF and Melatonin levels. In endometrial adenocarcinoma, there is a relatively weak positive correlation between EGFR expression in the tumor tissue and blood serum EGF level and strong negative correlation between EGFR

expression and blood serum Melatonin content; in the patients' blood serum, there is a negative correlation between EGF and Melatonin levels. Correlation parameters in well/moderately differentiated endometrial adenocarcinoma are more pronounced than in endometrial hyperplasia;

- In complex non-atypical endometrial hyperplasia the average level of blood serum EGF is considerably higher than that typical of simple non-atypical endometrial hyperplasia; in patients with well/moderately differentiated endometrial adenocarcinoma the average level of blood serum EGF is somewhat higher than that in patients with complex non-atypical hyperplasia, however it is considerably higher in patients with simple non-atypical endometrial hyperplasia. In moderately differentiated endometrial adenocarcinoma the average level of blood serum EGF is much higher than that in e well differentiated endometrial adenocarcinoma. This indicates that EGF is involved in proliferative and neoplastic processes proceeding in the endometrium;
- Ocasionally (rarely), blood serum EGF content may fit within the normal range in simple non-atypical endometrial hyperplasia at the background of both strong and weak EGFR expression in the endometrium. However, in contrast to simple non-atypical hyperplasia, blood serum EGF level in complex non-atypical hyperplasia never reaches normal values. Moreover, in complex non-atypical hyperplasia with weak, moderate or strong EGFR expression in the endometrium, blood serum EGF level is always significantly, markedly or sharply increased;
- The average level of blood serum Melatonin in simple non-atypical endometrial hyperplasia is significantly higher than that typical of complex non-atypical endometrial hyperplasia. In patients with simple/complex non-atypical endometrial hyperplasia the average level of blood serum Melatonin is significantly higher than that in patients with endometrial adenocarcinoma. In well differentiated endometrial adenocarcinoma the average value of serum Melatonin is higher than that in moderately differentiated endometrial adenocarcinoma. All the above suggests that Melatonin is involved in antproliferative and antineoplastic processes proceeding in the endometrium;
- In simple/complex non-atypical endometrial hyperplasia, a sharply decreased level of blood serum Melatonin has been seen only at strong expression of endometrial EGFR and has not been found at weak EGFR expression;
- If blood serum EGF level values in endometrial adenocarcinoma may comply with the index range for endometrial hyperplasia, in endometrial adenocarcinoma blood serum Melatonin content is mostly low and does not fit within the range typical of endometrial hyperplasia.

CONCLUSIONS

1. In simple non-atypical endometrial hyperplasia, a significant/marked increase in blood serum EGF level (0.65-1.02 ng/ml) together with strong EGFR expression in the endometrium, may indicate that hyperplasia is likely to undergo some progression and complicate in the following direction: simple atypical hyperplasia/complex non-atypical hyperplasia/complex atypical hyperplasia/neoplasia, due to the downward tendency in blood serum Melatonin content (from 17.8 pg/ml to 8.2 pg/ml); a similar significant/marked elevation of blood serum EGF level (0.76-0.97 ng/ml) at the background of weak endometrial EGFR expression in simple non-atypical endometrial hyperplasia is probably not predictable of unfavorable condition, because in the mentioned situation blood serum Melatonin content is normal and does not tend to decrease (20.1-29.2 pg/ml);

2. In simple non-atypical endometrial hyperplasia, normal (0.22-0.23 ng/ml), borderline (0.37-0.38 ng/ml) or moderately increased (0.51 ng/ml) blood serum EGF level at weak EGFR expression in the endometrium, is likely to predict a favorable condition, because in the given situation blood serum Melatonin content is within normal (23.8-32.1 pg/ml) or borderline (19.2pg/ml) limits;

3. In complex non-atypical endometrial hyperplasia, a significant/marked increase (0.9-2.60 ng/ml) in blood serum EGF level at strong expression of EGFR in the endometrium, is not likely to be indicative of favorable situation, i.e. this condition may cause alteration of hyperplasia and subsequent complications (complex atypical hyperplasia/neoplasia because blood serum Melatonin content tends to decrease - significant or sharp reduction (from 13.2 pg/ml to 9.2 pg/ml); in complex non-atypical endometrial hyperplasia, a drastic increase in blood serum EGF level (2.70-03.20 ng/ml) at the background of weak EGFR expression in the endometrium, is probably not predictable of unfavorable condition if blood serum Melatonin content remains within normal (23.1 pg/ml) or borderline (19.3 pg/ml) limits;

4. Melatonin, the universal antiproliferative and antineoplastic hormone, is much more “responsive” to EGFR expression in the endometrium than to blood serum EGF level, which indicates that in the given situation (endometrial hyperplasias) endometrial EGFR, particularly, is more “carcinogenic” than serum EGF. In other words, if blood serum EGF level is significantly or extremely elevated (60 ng/ml and over), while EGFR expression

in the endometrium is weak, this condition is likely to be favorable in respect of clinical course and the outcome of the disease as compared to that at strong endometrial EGFR expression and moderately increased blood serum EGF levels (up to 60 ng/ml);

5. In simple non-atypical endometrial hyperplasia, a significant or sharp decrease in blood serum Melatonin content (11.2-10.3-8.2pg/ml) as well as strong EGFR expression in the endometrium, is not likely to be indicative of favorable condition that may result in hyperplasia progression and subsequent complications (simple atypical hyperplasia/complex non-atypical hyperplasia/complex atypical hyperplasia /neoplasia) not only with significantly elevated (0.65-0.70 ng/ml) but also with normal (0.30 ng/ml) blood serum EGF levels;

6. In complex non-atypical endometrial hyperplasia, normal or borderline (19.3-23.1 pg/ml) values of blood serum Melatonin content at weak EGFR expression in the endometrium is likely to indicate a favorable condition regardless of marked or sharp increase (0.85-3.20 ng/ml) in blood serum EGF level;

7. In simple non-atypical endometrial hyperplasia a moderate decrease in blood serum Melatonin content (from 17.4 pg/ml to 15.7 pg/ml), in the presence of strong EGFR expression in the endometrium, demands attention since the progression of hyperplasia (simple atypical hyperplasia/complex non-atypical hyperplasia/complex atypical hyperplasia/neoplasia) cannot be excluded, even if blood serum EGF level is, mostly, moderately increased (0.45-0.59 ng/ml). The index of the same range of blood serum Melatonin content (moderately decreased from 17.9 pg/ml to 16.0 pg/ml), at the background of weak EGFR expression in the endometrium, is to be paid attention in complex non-atypical endometrial hyperplasia as well because blood serum EGF level is significantly/markedly increased (0.77- 1.70 ng/ml).

PRACTICAL RECOMMENDATIONS

1. In endometrial hyperplasia, a gradual elevation of blood serum EGF that is directly proportional to endometrial hyperplasia type complications and estrogen-dependent

endometrial carcinogenesis, can probably be used as an additional marker in clinical gynecology and oncology for the monitoring of endometrial hyperplasia progression;

2. In complex non-atypical endometrial hyperplasia, high values of blood serum EGF level and, simultaneously, low index of blood serum Melatonin concentration can be used as a screening test for endometrial atypical hyperplasia or estrogen-dependent endometrial carcinoma;
3. In endometrial hyperplasia, a strong expression of EGFR in the endometrium can be used in clinical morphology as a factor for the prognosis of endometrial hyperplasia malignization;
4. A strong expression of endometrial EGFR associated with low concentrations of blood serum Melatonin can serve as a direct diagnostic test to detect a high malignancy potential in endometrial hyperplasia;
5. Scientific Implication:

   There are scare literature data on synchronous studies of blood serum EGF content and endometrial EGFR expression in endometrial hyperplasia or endometrial carcinoma. We did not find any findings on either synchronous or independent studies on blod serum Melatonin content in endometrial hyperplasia or endometrial carcinoma. Therefore, the results of our study may contributeto the activation of the organizations engaged in the investigation of endometrial hyperplasia progression (malignization) or estrogen-dependent endometrial carcinogenesis.

List ofthe dissertation-related publications

1. Growth Factor: Structure, biological effects, perspectives for future // Proceedings of the Georgian National Academy of Sciences, Biomedical Series. – 2011. – Vol.37, No. 3-4. – P. 221-231 (in Georgian).

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