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ORIGINAL RESEARCH PAPER

Endometrial hyperplasia-related inflammation: its role in the development and progression of endometrial hyperplasia

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Abstract

Background Endometrial hyperplasia (EH) is one of the most common gynecologic diseases in the world. Different statistical categories implicate an imbalance of estrogens and progestogens in the etiology of this disease. We propose that inflammation also plays a key role in the progression of endometrial hyperplasia.

Objective The aim of this study is to evaluate the role of inflammation in the transformation and progression of endometrial hyperplasia, using local inflammatory cytokines and nonspecific protease levels, CD 45^+ expression, and histological examination.

Design The study included 107 patients (ages 29–49 years) with different forms of endometrial hyperplasia. The enrolled patients were randomized into one of the four groups: normal endometrium ($n = 18$) as the control group, simple hyperplasia ($n = 41$), complex hyperplasia without atypia ($n = 36$), complex atypical hyperplasia or endometrioid adenocarcinoma ($n = 12$).

Methods The following were evaluated for patients with different forms of EH: steroid hormone levels in blood serum and uterine flushings, immunohistochemical estrogen and progesterone receptor expression patterns in the endometrial tissue, CD 45^+ (common leukocyte antigen) expression, the levels of the cytokines IL-1β, IL-6, and TNF-α, and nonspecific proteases and their inhibitors.

Results The level of estradiol in blood serum and especially in uterine flushings was elevated dramatically in simple EH as compared to that of controls, but there was no significant difference between estradiol levels among the different forms of EH. The estimation of CD 45^+, the levels of the cytokines IL-1β, IL-6, and TNF-α, and the activity of proteases (elastase-like and trypsin-like activities) and their inhibitors showed that levels of nonspecific inflammatory markers increase with EH progression.

Conclusions We suggest that the initial responsibility for the development of simple endometrial hyperplasia belongs to systemic hyperestrogenemia and, in particular, local hyperestrogenia, but that the role of inflammatory processes increases in complex and atypical EH. Development of inflammatory changes in endometrial hyperplasia may be considered as a factor in the promotion and progression of pathology, as well as an attributed risk factor for malignancy in endometrial hyperplasia. In this study, we have established a role for CD 45^+ expression cells, non-specific proteases, and the inflammatory cytokines IL-1β, IL-6, and TNF-α in endometrial hyperplasia-related inflammation.

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Keywords Endometrial hyperplasia · Inflammation · Cytokines · Nonspecific proteases · Carcinogenesis

Introduction

Endometrial hyperplasia (EH), the result of an abnormal proliferation of endometrial cells, has also been associated with a high risk of carcinogenesis in the endometrial mucous membrane in persistent long-term cases [1, 2]. Classification according to the architectural crowding and nuclear atypia has identified several EH types: simple hyperplasia, characterized by minimal endometrial glandular crowding; complex hyperplasia, characterized by greater endometrial glandular crowding; and atypical hyperplasia, comprising endometrium with complex glandular crowding, cytologic atypia, and the high risk of endometrial carcinoma progression [1]. Clinical observations show that simple hyperplasia can spontaneously regress and rarely progresses to endometrial malignant neoplastic process [3]. According to different statistical categories, complex hyperplasia and atypical hyperplasia, in particular, are more likely to progress to cancer and are, therefore, commonly treated with a progestin or hysterectomy [4, 5]. In that context, the causes and mechanisms of the progression of endometrial hyperplasia are problems whose solutions are of the most immediate interest.

It is considered that the key factor in the development of hyperplasic processes in the endometrium is hyperestrogenemia [4]. Estrogens stimulate cell division in the endometrial glandular epithelium, whereas progesterone inhibits the effects of estrogen. The balance between estrogen and progesterone levels during the menstrual cycle must be precisely maintained, and a shift in either direction—a gain in estrogen or a loss in the antagonistic activity of progesterone—stimulates abnormal proliferation [6, 7].

However, regulation of cell proliferation activity in the endometrium is not controlled solely by estrogens and progesterone. The endometrial proliferation activity depends on a complex set of factors, which involves interactions among different cell types, cytokines, adhesion molecules, and growth factors that provide signaling for intercellular cooperation [8]. Some of these factors are produced during endometrial inflammation [9]. Inflammation can result in disorders in the regulation of cell division, leading to excessive mitosis, decreased apoptosis, mutations, and thus the initiation and promotion of neoplastic transformation [10]. Among the factors that reflect the severity of the inflammatory changes in the endometrium, recent studies have focused on the role of cytokines and matrix metalloproteinases [11]. It is known that in endometrial hyperplasia and endometrial cancer, there is an increase in the production of pro-inflammatory cytokines, which exist in a state of imbalance. However, most of the investigations were based on studies of pro-inflammatory mediators' levels in blood serum, which did not completely characterize the processes that develop in the uterine cavity.

Endometrial hyperplasia predisposes the uterus to carcinoma, and its presenting clinical symptoms, menorrhagia and menometrorrhagia, often lead to emergency and outpatient evaluations [1–4]. In addition, patients and the health care system bear the cost and burden of diagnostic evaluations and surgical and medical treatment (including biopsy, curettage, hysteroscopy, etc.). Despite the fact that endometrial hyperplasia is the one of the most common gynecologic pathologies in the world, very little is known about the role of inflammation in the pathogenesis and progression of EH. This aspect has stimulated the search for biomarkers to improve the diagnostic tools available to evaluate premalignant lesions in patients with EH, and led to more studies of the progression of hyperplasia. Thus, the aim of this study is to evaluate the role of inflammation in the transformation and progression of endometrial hyperplasia using the levels of local inflammatory cytokines and nonspecific proteases, CD 45^{+} expression, and histological examination. Identifying targets for treatment and potentially improving systemic therapy strategies is also important.

Materials and methods

Study design

This study included 107 patients (ages 29–49 years) with different forms of endometrial hyperplasia who underwent curettage and/or hysteroscopy. Patients were recruited from among those seen at the Department of Obstetrics and Gynecology of Simferopol Hospital in association with the Department of Gynecology at our university for a study period of 1.5 years (August 19, 2013–February 27, 2015). The ethical committees of Crimea State Medical University approved the study protocol, which was in accordance with the 1964 Helsinki Declaration, and informed consent was obtained from all individual participants included in the study.

All unequivocally diagnosed cases of EH reported from the specimens obtained from endometrial curettage and/or hysteroscopy performed under general anesthesia at the Department of Obstetrics and Gynecology of Simferopol Hospital, and received in the Department of Pathological Anatomy for histopathological examination during the study period, were included in the study after conventional tissue processing, standard staining with hematoxylin and eosin, and examination using light microscopy. Inadequate

specimens, improperly processed specimens, and cases with insufficient clinical data and more than one differential diagnosis were excluded from the study. Clinical data regarding the age, menstrual history, presenting complaints, and radiological findings were obtained for each patient whose tissue was used in the study.

The enrolled patients were randomized into one of the four groups: normal endometrium (NE, $n = 18$) as the control group, simple hyperplasia (simple EH, $n = 41$), complex hyperplasia without atypia (complex EH, $n = 36$), or complex atypical hyperplasia or endometrioid adenocarcinoma (complex atypical EH, $n = 12$). All controls reported menstrual cycles with regular intervals (25–35 days) and no clinical evidence of EH.

EH histological scoring

Histological typing of endometrial hyperplasia was done according to the Standard International Society of Gynecological Pathologists and WHO criteria [12]. Uterine tissue for this study was collected from the specimens of endometrial curettage and/or hysteroscopy. The study required paraffin-embedded endometrial tissue from the patients. All of the accumulated data were analyzed for descriptive statistics. Subdivision of endometrial hyperplasia cases was based on the degree of glandular complexity and crowding. Thus, a proliferative lesion displaying no evidence of cytologic atypia and minimal–moderate glandular crowding was termed simple hyperplasia, whereas one with marked glandular crowding and complex glandular architecture was termed complex hyperplasia. An endometrial proliferation displaying cytologic atypia accompanied by marked crowding and complexity was designated complex atypical hyperplasia.

Blood and uterine flushing fluid samples

Venous blood samples were collected by venipuncture from an antecubital vein before any other manipulation. Blood samples were collected into serum separation tubes or vacutainers containing ethylenediaminetetraacetic acid (EDTA) and were immediately centrifuged at 1000*g* for 10 min. Serum and plasma were then removed and stored at −20 °C for future analysis.

Uterine flushing fluid samples were collected by the method described previously [11]. Briefly, an 8 Fr Foley catheter was first inserted into the uterine cavity through the cervix and then connected to a syringe. Subsequently, ≤5 mL saline solution was injected into the cavity and aspirated immediately without contamination by the vaginal and cervical fluids. Uterine flushings were centrifuged (1000*g*, 10 min, 4 °C), the supernatants collected, and uterine flushings free from debris, blood corpuscles, large-sized protein molecules, and other impurities were stored at −20 °C until further analysis.

Hormone assays

All subjects underwent thorough medical evaluations, including a screen for hormones including estradiol, progesterone, and prolactin in the serum and uterine flushings. The concentration of estradiol was assessed using an enzyme immunoassay method (ELISA) (DRG Diagnostics, Germany) per the manufacturer's instructions. Progesterone and prolactin—were assayed using a commercial ELISA kit (Alcor Bio, Russia).

Immunohistochemistry

Immunohistochemical estrogen and progesterone receptor expression patterns in the endometrial tissue were evaluated. For immunohistochemistry the following antibodies were used: estrogen receptor (Thermo Fisher Scientific Inc, USA, clone SP1, concentration 1:50) and progesterone receptor (clone PgR 636, concentration 53.8 mg/L). For the detection of bound primary antibody, a DAKO Real Detection Multilink System was used, with anti-rabbit and anti-mouse antibodies, respectively. A series of studies using positive (endometrial tissue) and negative (brain tissue, as a benchmark) samples was performed.

The quantitative analysis of CD 45^+ (common leukocyte antigen) expression employing a scoring method based on the immunohistochemical staining frequency was performed as previously described [13]. Briefly, paraffin-embedded sections from clinical endometrial tissue samples were subjected to immunostaining for CD 45^+ using an anti-human monoclonal antibody to common leukocyte antigen CD 45^+ (Clone 2B11 + PD7/26) (DAKO) and EnVision™ FLEX+, High pH (Dako Autostainer/Autostainer Plus) Code K8024 visualization system. Each tissue sample was examined under an Olympus microscope CX41 and photographed using an OLYMPUS C 5050Z camera (Olympus, Tokyo, Japan).

Pro-inflammatory mediators

The levels of the cytokines IL-1β, IL-6, and TNF-α in uterine flushings were investigated using a standard Enzyme-Linked Immunosorbent Assay (ELISA) in accordance with manufacturer's specifications (Biomedica, Vienna, Austria).

The trypsin-like (TLA) and elastase-like (ELA) activities and levels of acid-nonstable antitrypsin activity (ATA) and acid-stable inhibitors (ASI) in uterine flushings were measured using enzyme methods with specific synthetic substrates [14]. All results were recalculated to 1 mg of

intrauterine flushing protein. Briefly, trypsin-like activity, based on a standard curve generated with 0.1–0.6 μg of active enzyme, was determined spectrophotometrically using *N*α-benzoyl-L-arginine ethyl ester hydrochloride (BAEE) (Sigma). A preincubation medium was prepared by adding trypsin. The medium was then brought up to 1 mL using phosphate buffer with a pH of 7.6, followed by incubation at 37 °C for 30 min. After incubation, the reaction was initiated by adding 950 μL BAEE (0.25 mM) to the phosphate buffer. The absorbance of the *N*α-benzoyl-L-arginine from hydrolysis of the BAEE was then monitored at 253 nm every 30 s for 5 min. One (1) unit of enzyme is defined as enzyme activity sufficient for the breakdown of 1 nM of substrate/mg/min.

Measurement of elastase-like activity (ELA) in uterine flushings was carried out by detection of hydrolysis of the synthetic substrate *N-t*-BOC-L-Alanine *p*-Nitrophenyl Ester (BANPE) (Sigma). For this purpose, the following were mixed in a spectrophotometer cuvette maintained at 25 °C: a 0.1–1.0 mL uterine flushing sample and the amount of 0.05 M Na phosphate buffer (pH 6.5) needed to bring the contents of the cuvette to a final volume of 2.9 mL. After 15 min, 0.1 mL of 0.01 M BANPE solution in acetonitrile was added to the sample. The increase in optical density at 347.5 nm was measured and expressed in nM of substrate/mg/min.

ATA in the in uterine flushings was analyzed using an enzymatic assay for trypsin inhibitory capacity (Sigma-Aldrich Co.). The principle is: trypsin hydrolyses a trypsin substrate *N*α-benzoyl-L-arginine ethyl ester hydrochloride to *N*α-benzoyl-L-arginine and trypsin inhibitors inhibit this reaction. The activity is expressed in mIU/mg. To determine the ASI, acid labile proteins were previously precipitated with 0.1 mL 0.05 M Na-acetate buffer (pH 4.1). The activity of acid-stable inhibitors in uterine flushings was then determined according to the method for ATA. Uterine flushing total protein quantification was performed using a modified Lowry assay procedure [14].

Quantitative data were summarized as means and standard deviations and compared using Student's *t* test and Mann–Whitney *U* test. A probability value <0.05 was considered statistically significant. Calculations were made using the software package Statistica 6.0 by StatSoft (USA).

Results

Systemic and local hormonal status in endometrial hyperplasia

At the first stage of investigation, women were divided into two groups depending on the phase of their menstrual cycles; levels of sex hormones in blood serum and uterine flushings were measured. The level of estradiol in blood serum was significantly increased in the group with simple EH only during the first phase of the menstrual cycle as compared with the levels in the control group. In complex hyperplasia, the level of estradiol was increased in both phases as compared with those in the control group. However, there was no significant difference between simple and complex EH in the first phase. In complex atypical EH, the level of estradiol in both phases was notably elevated as compared with the level in the control group. There was no significant difference in the concentration of estradiol between complex EH and complex atypical EH.

Levels of progesterone in the blood serum of women with simple and complex EH did not change for the first phase of the menstrual cycle and were decreased during the second one as compared to those of controls.

Changes in the levels of sex hormones in uterine flushings were more substantial than those in blood serum. The level of estrogens in simple EH was threefold higher than those of first phase controls, and fivefold higher than those of the control level during the second phase ($p < 0.001$). In complex EH, there was no significant difference between the levels seen during the first and second phases, but complex EH and complex atypical EH both had levels 3.5- to 4-fold higher than the levels in the control group. In simple EH, the progesterone level in the first phase was twofold higher than the control level, but changes in the levels of progesterone for both complex and complex atypical EH were not statistically significant difference from those of simple EH.

The processes of steroidogenesis depend on pituitary regulation, which plays an important role with respect to local and systemic levels of prolactin. The study demonstrated a slight increase in prolactin levels in blood serum: 26 % ($p < 0.05$) in simple EH, 36 % ($p < 0.05$) in complex EH, and 42 % ($p < 0.05$) in complex atypical EH, as compared with the level in the control group. However, almost all levels of prolactin observed did not exceed the limits of the average rate of the reference values.

The endocrine study of uterine flushings showed that the prolactin levels did not depend on the phase of the menstrual cycle. Moreover, prolactin was almost absent from the uterine flushings of the control group. In contrast, the level of prolactin was increased to 17.9 ± 1.5 mIU/mg in women with simple EH, and to 27.3 ± 2.8 mIU/mg in women with complex atypical EH.

Analysis of the steroid hormone receptors in endometrial tissue showed that the most significant changes in the receptors for estrogen and progesterone, as well as in the levels of intra-uterine hormones, were seen in simple EH. Since the differences seen in complex and complex atypical EH depend on the phase of the cycle, the relative

change in local estrogen and progesterone receptor expression in the endometrial tissue disappears.

Markers of inflammation in endometrial hyperplasia

The second stage of our investigation was devoted to the study of the dynamics of nonspecific markers of inflammation in the development and transformation of EH. The results of histological examination of endometrial tissue with eosin and hematoxylin detected morphological features of chronic inflammatory processes in the endometrium in 67 % of patients ($p < 0.05$). Morphologically, chronic inflammation in the endometrium was mostly manifested by the presence of perivascular inflammatory infiltrates in the basal and functional layers of the endometrium, consisting of lymphocytes, macrophages, and plasma cells (Fig. 1).

The histological examination does not evaluate the activity of chronic inflammation and its dependence on the type of hyperplastic process. In this context, immunohistochemical analysis with the detection of common leukocyte antigen CD 45^+ expression was carried out for determining the severity of inflammatory process in different forms of EH (Fig. 2).

The immunohistochemical analysis revealed that the number of the cells that express CD 45^+ increased during the transition from simple to complex hyperplasia and complex atypical hyperplasia. Thus, in normal endometrium, only 4.2 ± 1.2 % of cells expressing CD 45^+ were detected; in simple hyperplasia, the percentage of CD 45^+ increased to 19.9 ± 0.9 % ($p < 0.001$), while in complex EH, the level of expression of CD 45^+ reached 31.2 ± 2.1 % ($p < 0.001$). Furthermore, the maximum level of CD 45^+ expression was observed in endometrioid carcinoma: 57.8 ± 2.4 % ($p < 0.001$). Thus, there was a clear association between expression of common leukocyte antigen CD 45^+ and progression of hyperplasia (Fig. 3).

The results of studying local cytokine levels showed the dependence of their levels on the type of hyperplastic process (Table 1). For instance, the level of IL-1β was greater than threefold higher in simple hyperplasia and greater than 20-fold higher in complex hyperplasia as compared to that of controls.

In atypical hyperplasia, the level of IL-1β was greater than twofold higher than that in women with complex EH

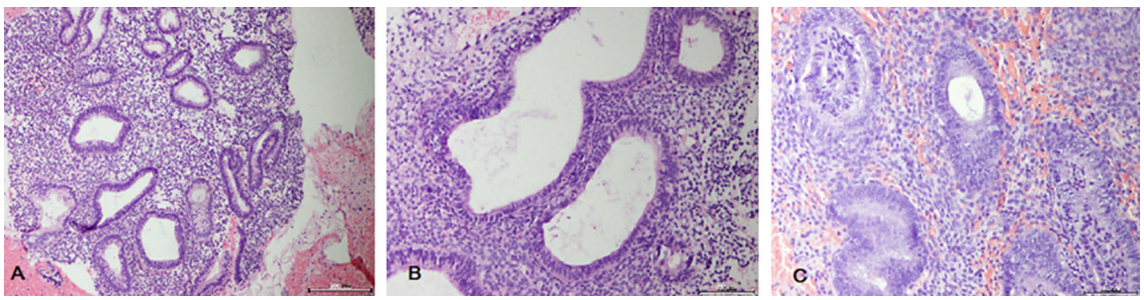

Fig. 1 Sections of formalin-fixed, paraffin-embedded samples of endometrial tissue with EH staining for the detection of inflammation in endometrial hyperplasia. **a** Simple hyperplasia (×200). **b** Complex hyperplasia without atypia with moderate inflammatory infiltration by macrophages and lymphocytes (×400). **c** Complex hyperplasia with atypia and prominent infiltration by macrophages and lymphocytes, rare plasma cells (×400). *Scale bar* 200 μm

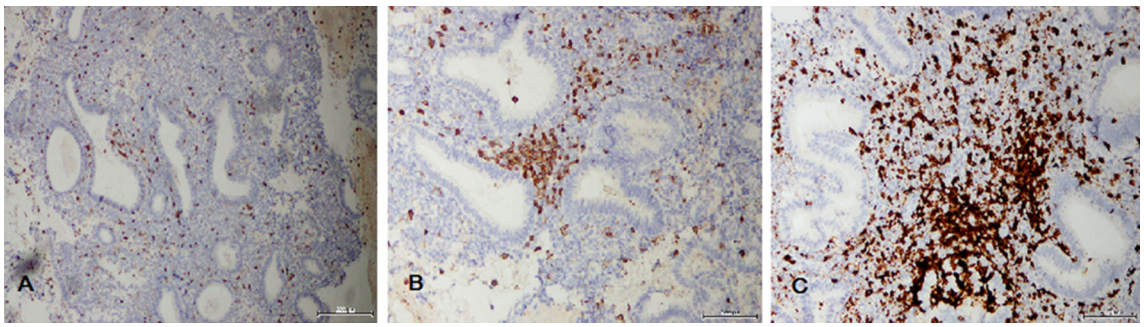

Fig. 2 Immunohistochemical staining for CD 45^+ expression. **a** Simple hyperplasia (×200). **b** Complex hyperplasia without atypia with moderate inflammatory infiltration by macrophages and lymphocytes (×400). **c** Complex hyperplasia with atypia with prominent infiltration by macrophages and lymphocytes, rare plasma cells (×400). EnVision™ FLEX+, High pH visualization system. *Scale bar* 200 μm

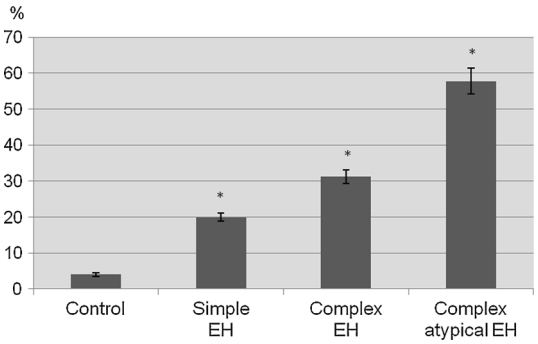

Fig. 3 Expression of CD 45^{+} (%) in endometrium of patients with different types of endometrial hyperplasia. *Asterisk* statistical significance was determined using a Student's *t* test ($p < 0.05$) compare to control

without atypia. Similar changes characterized the levels of IL-6: in simple EH, the IL-6 level was fivefold higher, in complex EH, 10-fold higher, and in complex atypical EH, 15-fold higher than that observed in the control group. Changes in levels of TNF-α were less significant. In patients with simple EH, the level of TNF-α was twofold higher, in complex EH, it was 2.5-fold higher, and in complex atypical EH, it was threefold higher compared to that of controls.

The development of endometrial hyperplasia also led to an increase in proteolytic activity in the uterine flushings (Table 2). The activity of elastolytic enzymes was increased 7.5-fold in simple EH and 8- to 9-fold in both forms of complex hyperplasia. The level of TLA was 2.5- to 3-fold higher than control levels.

Antitrypsin activity, which characterizes the ability to inhibit trypsin-like proteases in uterine flushings, did not change significantly in any of the EH groups compared with that of the control group. After a slight increase in simple EH, this parameter decreased back to control values. At the same time, locally secreted acid-stable protease inhibitors were decreased two to threefold in simple and complex hyperplasia (not determined in complex atypical EH) as compared with the level in the control group. Thus, the study of the activity of proteases and their inhibitors in uterine flushings found that the development of EH is accompanied by imbalance, which characterized by increases in TLA and ELA, and a decrease in activity of locally synthesized ASI. The most significant changes were identified in complex atypical endometrial hyperplasia.

Discussion

It has previously been reported that endometrial hyperplasia is almost exclusively associated with a relative excess of estrogen [4, 8, 15]. Our results indicate that absolute or relative hyperestrogenia plays a strong initial

Table 1 Level of cytokines in uterus lavage fluid in patients with endometrial hyperplasia

Groups	*n*	IL-1β (pg/mg)	IL-6 (pg/mg)	TNF-α (pg/mg)
Control	18	3.56 ± 0.93	4.36 ± 1.19	5.04 ± 1.68
Simple EH	41	12.8 ± 3.32[a]	22.55 ± 3.96	9.96 ± 1.52[a]
Complex EH	36	80.31 ± 8.92[a,b]	44.32 ± 9.77[a,b]	12.41 ± 1.85[a]
Complex atypical EH	12	174.72 ± 15.14[a,b]	67.81 ± 16.39[a,b]	15.59 ± 2.66[a,b]

Statistical significance was determined by two-tailed paired Student's *t* test ($p < 0.05$)

EH endometrial hyperplasia, *IL-1β* interleukin 1β, *IL-6* interleukin 6, *TNF-α* tumor necrosis factor alpha

[a] Compared with the control

[b] Compared with the simple EH group

Table 2 Proteolytic enzymes and their inhibitors in uterine flushings in women with endometrial hyperplasia (EH)

Groups	*n*	ELA (nM/mg/min)	TLA (nM/mg/min)	ATA (mIU/mg)	ASI (mIU/mg)
Control	18	2.62 ± 0.41	11.33 ± 1.30	174.21 ± 14.52	88.14 ± 16.11
Simple EH	41	19.8 ± 3.32[a]	28.12 ± 4.77[a]	226.91 ± 33.50	23.2 ± 4.9[a]
Complex EH	36	24.3 ± 5.5[a]	36.41 ± 6.27[a]	192.70 ± 26.85	31.30 ± 13.0[a]
Complex atypical EH	12	22.71 ± 5.02[a]	25.57 ± 3.39[a]	152.72 ± 32.70	0[a,b]

Statistical significance was determined by two-tailed paired Student's *t* test ($p < 0.05$)

ELA elastase-like activity, *TLA* trypsin-like activity, *ATA* antitrypsin activity, *ASI* acid-stable protease inhibitors

[a] Compared with the control

[b] Compared with the simple EH group

role in the development of simple EH. The most significant changes to estrogen and progesterone levels in the blood serum and uterine flushings, and levels of receptor expression for steroid hormones in endometrial tissue, occur when simple EH develops, but these parameters do not change dramatically during the progression of EH. Logical assessment suggests that hyperestrogenia plays the most important role in the initial stages of EH development, but that conversion of simple EH to complex and complex atypical EH depends on other mechanisms that may underlie the pathogenesis of this disease.

At the present time, special emphasis has been focused on the role of endometrial inflammation, which may accompany hyperplasia and its transformation. Chronic endometrial inflammation is a known cause of disorders of uterine function, implantation failure, carcinogenesis, etc. [16]. Indeed, uterine inflammation induces inflammatory cells and alters the expression and subtle balance of various molecules, contributing to endometrial hyperplasia [17]. In connection with all the above the role of nonspecific inflammatory markers, such as cytokines, matrix metalloproteases, proteases, and tissue protease inhibitors, in the development of EH is actively being studied, along with the occurrence and progression of malignant processes in the uterus [18–20].

Progression of EH requires the involvement of biologically active substances, most of which participate in the inflammatory process. In addition, in some cases chronic inflammation bears the primary responsibility for hyperplastic disorders and subsequent carcinogenesis [21]. Chronic inflammation can initiate tumor onset and development by means of several mechanisms. One is the induction of DNA damage by leukocytes and other phagocytic cells through their generation of the reactive oxygen and nitrogen species that are produced normally by these cells to fight against infection [22]. Repeated tissue damage and regeneration of the tissue, in the presence of free radicals released from inflammatory cells, interacts with DNA in proliferating epithelium resulting in permanent genomic alterations, which lead to atypical changes [23]. Inflammation is also responsible for production of angiogenic factors, such as vascular endothelial growth factor, which provides vascularization of neoplastic tissue, and production of matrix metalloproteases, which are necessary for invasive growth and metastasis [24, 25].

Even when the primary reason driving the neoplastic process is hormonal imbalance, an inflammatory component can be involved in pathogenesis. These pathogenetic links between the inflammatory process and carcinogenesis became the foundation of the concept of cancer-related inflammation [26]. Several reports indicate that in estrogen-dependent endometrial hyperplasia the same mechanisms take place. For example, estrogen acting through estrogen receptor 1 regulates matrix metalloproteinase expression, resulting in elevated levels [27]. An increase in the number of infiltrating macrophages and its contribution to the tumor inflammatory microenvironment may result in the development of the type I endometrial carcinoma [28].

Nowadays, the role of proteases and their inhibitors in the development of endometrial hyperplasia, and in the initiation and progression of malignant processes in the mucosa of the uterus, is actively being studied [29, 30]. Proteolytic enzymes secreted by activated leukocytes are capable of destroying connective tissue and epithelial basement membrane, which disrupts the histoarchitectonics of the endometrial tissue and contributes to disorders of the proliferation and differentiation processes in endometrium [31].

In our study, we focused on the activity of non-specific proteases and inflammatory cytokines such as interleukin 1β, IL-6, and TNF-α as candidate biomarkers of chronic uterine inflammation, which plays an important role in the transformation of endometrial hyperplasia into a malignant neoplastic process. The relationship between CD 45^+ expression and clinicopathological indicators was also examined in EH. Elevation of inflammatory cytokines levels, the activity of TLA and ELA, and increased CD 45^+ expression are all incontestable evidence for the involvement of the inflammatory process.

Our results demonstrate that inflammatory changes depend on the type of EH: from the minimal symptoms observed in simple hyperplasia to the greatest manifestations seen in atypical hyperplasia. We can assume that EH is associated with the development of the inflammation. Endometrial inflammation promotes maintenance and, perhaps, the progression and transformation of the hyperplastic process. In recent years, a higher level of importance has been given to the inflammation associated with tumor growth [32], which is described as one of the common symptoms of cancer [33]. Of particular note is the concept that "cancer associated inflammation" may influence the genetic restructuring of tumor tissue and promote the processes of invasion and metastasis. The results obtained in our study indicate the important role played by inflammation in the hyperplastic processes of the endometrium, which can be interpreted in terms of the development of inflammation associated with endometrial hyperplasia. These findings support the hypothesis that the inflammatory pathway may contribute to a susceptibility to endometrial cancer [34].

Based on these results, we propose a scheme describing the involvement of the inflammatory process in the development of EH (Fig. 4). We have suggested that development of an imbalance of steroid hormones, with an

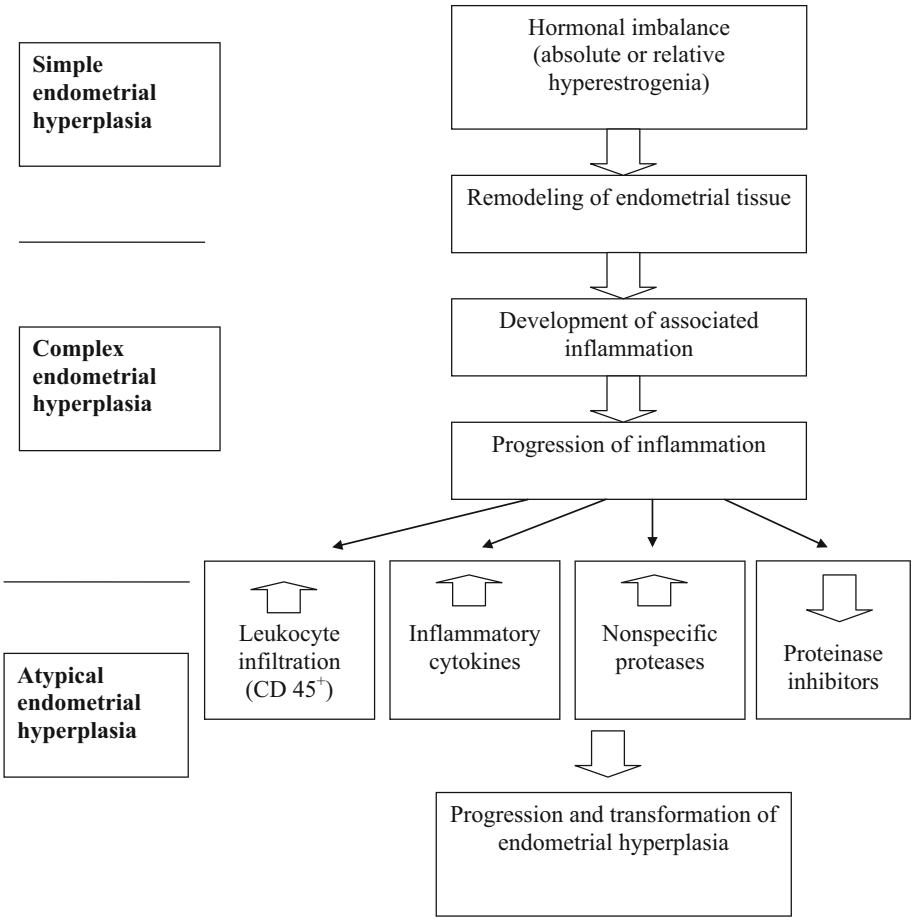

Fig. 4 Pathogenesis of involvement of the inflammatory process in the development of endometrial hyperplasia

absolute or relative prevalence of estrogen, is really a triggering factor and a key mechanism in the development of hyperplastic processes in simple EH. This is demonstrated by the relative hyperestrogenemia and marked increase in levels of sex hormones in uterine flushings, with the predominance of estrogen along with the prevalence of expression of estrogen receptors in the stroma and endometrial glands in simple EH. However, endometrial tissue remodeling, which occurs in association with hormonal imbalance, was accompanied by the development of inflammation. Moreover, the severity of inflammatory manifestations in the endometrium is magnified by the morphological reorganization of the endometrium and the more severe clinical symptoms of EH. The degree by which inflammatory changes increase is characterized by greater common leukocyte antigen CD 45^+ expression in the endometrium, which increases the level of pro-inflammatory cytokines, elevates the of activity of non-specific proteases, and reduces the secretion of local acid-stable inhibitors. Moreover, the degree of absolute or relative hyperestrogenia is leveled. Progressive reduction of the expression of receptors for steroid hormones in the endometrium takes place; a certain prevalence of expression of the progesterone receptor (especially in the glandular tissue) is noted in atypical EH.

Thus, the studies conducted demonstrate that the initial development of simple endometrial hyperplasia is associated with systemic, and especially local, hyperestrogenemia. They also show that the progression of endometrial hyperplasia depends on the appearance of a hyperplasia-related inflammatory component, initiated by the excessive extracellular matrix remodeling caused by estrogens. This study adds to the growing evidence that inflammation plays an important role in the development of endometrial hyperplasia. The activity of non-specific proteases and inflammatory cytokines such as IL-1β, IL-6, and TNF-α may be used as biomarkers of endometrial hyperplasia related inflammation and is recommended for further research.

Conclusion

In conclusion, our results demonstrate that, while hormonal imbalance is an important progressive factor in simple EH, the role of the inflammatory process increases in complex and atypical EH. This inflammatory process, which follows

EH, was interpreted in our investigation such as "endometrial hyperplasia associated" or "endometrial hyperplasia related" inflammation. Development of these inflammatory changes in endometrial hyperplasia may be considered a factor responsible for the promotion and progression of pathology, as well as a risk factor contributing to the malignancy in endometrial hyperplasia. In this study, we have confirmed a role for non-specific proteases and the inflammatory cytokines IL-1β, IL-6, and TNF-α in endometrial hyperplasia related inflammation. Further research examining the roles of these biomarkers for clinical use is required.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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