Morphological characteristics of endometrium in patients of reproductive age with viral hepatitis type C

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Abstract

Aim: The purpose of the study was to investigate the morphological features of endometrium in patients with viral hepatitis type C.

Materials and methods: A morphological survey was conducted involving endometrium sections stained with hematoxylin and eosin and Van Gieson’s picrofuchsin collected from 140 women of reproductive age, among them 70 patients were diagnosed with chronic hepatitis type C (CHC) infection with minimal (40 patients) and moderate activity (30 patients), and 70 women suffered from endometrial proliferative diseases without viral hepatitis (comparison group). In addition, 36 women with CHC and 36 women without CHC underwent immune histochemical test (IHC) of paraffin sections aimed at investigating the expression level of estrogen (ERα) and progesterone (PR) receptors and proliferation marker Ki-67. Endometrial biopsy samples were collected on the 10th to 14th day and the 15th to 18th day of the menstrual cycle.

Results: Focal changes of endometrium in the form of uneven entering of the endometrium into the next phase of the menstrual cycle with the dominating proliferative phase were found in chronic hepatitis C patients. The number of patients having proliferative activity in the endometrium combined with inflammation increased as viral hepatitis became more active. Immune histochemical test showed the prevalence of the expression levels of ERα and PR receptors in the endometrial stroma. Increased expression of Ki-67 was observed mainly in the glandular epithelium and accrued together with the increment in the CHC activity.

Conclusion: Endometrial pathology in patients of reproductive age with chronic hepatitis type C is characterized primarily by proliferative forms with the increasing mismatch between the nature of structural changes and mucous phase of the menstrual cycle prompting suggestions that direct effect of hepatitis C virus on endometrium is possible.

Introduction

Long-term observation (over 10 years) provides some researchers with a basis to indicate the increase in the number of patients with endometrial pathology in the group of women of reproductive age [1,2]. Viral diseases account for substantial share in the structure of endometrial pathology [2,3]. The results of surveys demonstrating the induction of pronounced inflammatory and proliferative processes caused by hepatitis C virus are especially noteworthy [4,5]. Given the data on low efficiency of the IVF programs among patients with chronic hepatitis C (CHC) [6], both hormonal association of proliferative processes with a chronic liver failure, and direct effect of the virus on endometrial tissue cannot be excluded. Despite vast opportunities of the up-to-date ultrasonic research [7], results of morphological analysis of the endometrium including immune histochemical test in patients with blood borne diseases to a large extent promote verification of the uterine factor of infertility and determination of the optimized IVF protocols and efficient preparing for pregnancy strategy [8].

The purpose of the study was to investigate the morphological features of the endometrium in patients with viral hepatitis type C.

Materials and methods of research

A morphological survey of endometrium sections was conducted involving 70 female patients having chronic hepatitis type C with minimal (40 patients) and moderate activity (30 patients) and 70 women diagnosed with endometrial proliferative diseases without viral hepatitis (comparison group). Endometrial biopsy samples were received during diagnostic hysteroscopy and uterine curettage. Chronic hepatitis C was verified by ELISA (antibody validation) and quantitative PCR. Withdrawal criteria were age under 19 and above 35 years; hepatitis of another etiology; HIV infection; malignant neoplasms of any etiology; severe background somatic pathology (2nd- to 3rd-grade functional failure);
any urinary tract infection and associated active inflammation. Average age of patients of the main survey groups and comparison group was 36 (32±39) and 37.5 (34±39) years, respectively, \( p > 0.05 \).

Taking into account higher activity of the receptor system in the late proliferative phase and early secretory phase, morphological survey was conducted on the 10th to 14th day and 15th to 18th day of the menstrual cycle with average duration of 29±1 days. The comparison groups did not differ from each other with regard to the number of surveys conducted in the certain phase of the menstrual cycle, \( p > 0.05 \).

Endometrial biopsy samples received during hysteroscopy were fixed in neutral buffering 10% formalin (pH 7.4) for 24 h. After dehydration, the material was embedded into a homogenized paraffin mix (Histomix Extra, BioVitrum, Russia) at 80 °C temperature. Sections of 4±1 micron thickness were received using pendulum-type microtome MZP-01 Technom. Two sections from each block were placed on the slide with a colored edge (BioVitrum, Russia). The sections were stained with hematoxylin and eosin and Van Gieson’s picrofuchsin. Along with morphological analysis of endometrium, immune histochemical test (IGH) of paraffin sections was carried out for 36 women suffering from CHC with minimal (18 patients) and moderate (18 patients) activity and 36 women without CHC.

For IHC reactions, paraffin sections were processed according to the standard technique using Leica Bond-MAX slide stainer with mouse monoclonal antibodies to Estrogen Receptor 6F11, Progesterone Receptor 16 (Leica Bond) and FLEX Monoclonal Mouse Anti-Human Ki-67 Antigen Clone MIB-1 Ready-to-Use (Dako Autostainer/Autostainer Plus). To assess the endometrial receptivity, expression levels of estradiol receptors alpha (ER-\( \alpha \)) and progesterone receptors (PR) were investigated; in order to specify the degree and nature of proliferative activity in the endometrium, the nuclear Ki-67 protein reflecting the number of mitotically active cells and being an informative criterion of their proliferative activity [9] was studied. Micrographs were taken with Zeiss Axio Scope.A1 microscope.

The results of IHC reactions were estimated with regard to intensity of brown coloring per 100 cells in 10 high power (400× magnification) fields. In relation to estrogen and progesterone receptors, the H-score method was used according to the formula: \( HS = a + 2b + 3c \), where \( a \) is the percentage share of poorly stained cells; \( b \) is the percentage share of moderately stained cells; \( c \) is the percentage share of strongly stained cells; and \( 1, 2 \) and \( 3 \) are the staining intensity expressed in scores. The degree of manifestation of the estrogen and progesterone expression was interpreted as follows: 0–10 scores – lack of expression, 11–100 scores – weak expression, 101–200 scores – moderate expression and 201–300 scores – pronounced expression. For Ki-67 marker, only the percentage of intensively stained cell nuclei was considered [10].

Statistical data were processed using application programs for IBM SPSS Statistics 22. Methods of valuation of mean values and standard errors, Student t-test for parametric and Mann–Whitney U test for nonparametric samples were used.

**Discussion of results**

Analysis of histologic specimen revealed that the morphological pattern of endometrium in patients with CHC and in the comparison group on the 10th to 14th day of the menstrual cycle corresponded to the late proliferative phase. Endometrial samples of patients of the comparison group (without CHC) obtained on the 15th to 18th day of the menstrual cycle morphologically corresponded to the early secretory phase. In 40% patients with CHC (28 patients out of 70), endometrial samples collected on the 15th to 18th day did not correspond to

![Figure 1. Endometrium of patient Zh.](https://example.com/image1.png)

the early secretory phase: there were no typical tortuosity, extension of lumen and subnuclear vacuolization of prismatic glandular epithelium; mitoses were found in the glandular epithelium. The stated histologic pattern of the endometrium was typical for the late proliferative phase (Figure 1).

In all, 32 women out of 70 who suffered from CHC (45.7%) had dense endometrial stroma with edemas; at the same time, this sign was more often observed in the 2nd group of patients with moderate-grade CHC (18 patients, 64.3%) compared to the 1st group (14 patients, 35%) and comparison group (20 patients, 28.6%), \( p = 0.028 \) and \( p = 0.023 \), respectively; this fact evidences the irregular entering of the endometrium into the next proliferative phase. In six patients of the 2nd survey group (21.4%), glomuses consisting of spiral vessels were found indicating incomplete ejection of functional layer of the endometrium within the previous cycle which underwent involution [11].

Focal nature of changes in the endometrium was confirmed for all chronic hepatitis C patients (100%). In 30% and 42.9% of cases in the 1st and 2nd groups, focal glandular hyperplasia of the endometrium with large and cystically dilated glands was found, this figure was higher for the moderate CHC group (8 women, 28.6%) compared to the data for CHC with minimal activity (4 women, 10%) and the comparison group (5 women, 7.1%), \( p = 0.036 \) and \( p = 0.028 \), respectively.

Endometrial polyps were diagnosed in 22 women with CHC (31.4%) being characterized by prevalence of glandular and fibrous structure with cystic transformation of the glands: in six cases (50%) in the 1st survey group and eight cases (80%) in the 2nd survey group. The fibrous component in the polyp’s stroma and massive fibrosis of arterial walls of the polyp’s pedicle being the sign of its long existence were discovered. In the comparison group (without CHC) in 75% of cases functional type polyps were detected, the state of the glands corresponded exactly to the day of the menstrual cycle.

The morphological survey findings were qualified as a special feature due to the intensifying proliferative processes in the endometrium in women of reproductive age with CHC.

The nodal form of adenomyosis in patients with CHC was detected in only 6 patients out of 70 (8.6%), with an obvious prevalence of the given pathology in women with CHC of the 2nd activity grade: four patients (14.3%) compared to two patients (5%) with CHC of the 1st activity grade, \( p = 0.036 \). The diagnosed adenomyosis was of the 2nd grade and below, that is,
The number of patients having a combination of proliferative processes and inflammation (17.7% in the common group with CHC) increased being consistent with the activity of viral hepatitis: 15% in the 1st group and 21.4% in the 2nd group, respectively, \( p = 0.036 \). Paraffin sections of the endometrium of the most of chronic hepatitis C patients (40 patients out of 70, 57.1%) showed focal lymphocyte collection located mostly around the glands and seldom around blood vessels as well as focal fibrosis of the stroma being the evidence of chronic inflammation [12].

Immune histochemical test of paraffin sections demonstrated that the expression level of ER\(\alpha\) receptors in the endometrial stroma during the proliferative processes both in the main groups and the comparison group showed statistically significant difference compared to the same parameter in the glands: 100 (71.5\(\pm\)172.5) and 255 (132.5\(\pm\)276) scores, \( p = 0.026 \) in the 1st group; 177 (122.5\(\pm\)230) and 269 (164\(\pm\)296.5) scores, \( p = 0.038 \) in the 2nd group; and 265 (210\(\pm\)298) and 295 (220\(\pm\)298) scores, \( p = 0.048 \) in the comparison group, being the typical feature of proliferative diseases of the endometrium [13] (see Table 1).

A significant prevalence of PR receptors expression in the stroma in comparison with expression of these receptors in the endometrium glands in patients with CHC was also detected: 256 (211\(\pm\)275) and 292 (61\(\pm\)295) scores, \( p = 0.012 \) in the 1st survey group; 272 (247.5\(\pm\)286) and 296 (294\(\pm\)298) scores, \( p = 0.042 \) in the group with CHC of the 2nd activity grade. In the comparison group (without CHC), indicators of the PR expression in the stroma and glands had no significant differences: 275 (234\(\pm\)298) and 290 (275\(\pm\)297) scores, \( p = 0.053 \) (see Table 1).

It is necessary to emphasize that significant differences of the expression of both estrogen and progesterone receptors were obtained only in the minimal activity CHC group in comparison with the group of patients suffering from moderate CHC and the comparison group (without CHC) prompting suggestions that some additional (not associated with hormones) evolution factors of proliferative processes in the endometrium exist in patients with CHC (see Table 1). Findings of the carried survey give the reason to assume that disturbances in the receptor system of the endometrium caused by immediate effect of hepatitis C virus can lead to an insufficient susceptibility of the endometrium to exogenous hormonal influence.

### Table 1. Valuation of sensibility of estrogen and progesterone receptors (H-score) and proliferative activity Me (25\(\pm\)75).

|       | 1st group, \( n = 18 \) | 2nd group, \( n = 18 \) | Control group, \( n = 18 \) | \( p \), significance of differences |
|-------|--------------------------|--------------------------|-----------------------------|----------------------------------|
| **ER-\(\alpha\)** (scores) | | | | |
| Stroma | 100 (71.5\(\pm\)172.5) | 177 (122.5\(\pm\)230) | 265 (210\(\pm\)298) | P1.2 = 0.008, P1.3 = 0.002, P2.3 = 0.024, P1.2 = 0.036, P1.3 = 0.021, P2.3 = 0.043 |
| Glands | 255 (132.5\(\pm\)276) | 269 (164\(\pm\)296.5) | 295 (220\(\pm\)298) | |
| **PR** (scores) | | | | |
| Stroma | 256 (211\(\pm\)275) | 272 (247.5\(\pm\)286) | 275 (234\(\pm\)298) | P1.2 = 0.011, P1.3 = 0.007, P2.3 = 0.079, P1.2 = 0.043, P1.3 = 0.041, P2.3 = 0.83 |
| Glands | 292 (61\(\pm\)295) | 296 (294\(\pm\)298) | 290 (275\(\pm\)297) | |
| **Ki-67 (%)** | | | | |
| Stroma | 21 (5.5\(\pm\)33.5) | 13.6 (2.5\(\pm\)29.5) | 11.8 (6\(\pm\)16.5) | P1.2 = 0.028, P1.3 = 0.016, P2.3 = 0.032, P1.2 = 0.012, P1.3 = 0.036, P2.3 = 0.002 |
| Glands | 24.4 (13\(\pm\)48) | 53 (7\(\pm\)80) | 18.2 (12\(\pm\)28) | |
Preconception care of women with CHC, especially in case of infertility, should be accompanied with a morphological survey of the endometrial biopsy samples and improvement of its quality with the view of achieving higher efficiency of the IVF programs in this category of women.

Declaration of interest
The authors declare no conflict of interests.

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