

MORPHO-FUNCTIONAL CHANGES IN ENDOMETRIUM UNDER THE INFLUENCE OF CHRONIC ALCOHOLISM

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Alcoholism today is a global worldwide social and economic problem that affects almost every country. Up to 80% of men and 60% of women in different countries report episodes of alcohol abuse [2,21]. And even episodes of acute alcohol intoxication, which are associated with formidable changes on the part of many organs and systems, up to the development of coma, do not cause concern [7]. Therefore, chronic alcohol intoxication causes less fear in patients [21].

Women are a particularly vulnerable group in terms of alcohol abuse. It should be borne in mind that the development of chronic alcoholism in women requires much shorter periods of time than in men, which is due to lower body weight, hormonal factors, and social factors [22]. Conventionally, all these reasons can be divided into three groups: the first group includes the reasons associated with the direct action of ethanol, which is known to be a poison that affects all organs and systems [6]. The second group includes alcohol dependence, which aggravates the effects of ethanol, leads to often irreversible consequences on the part of the psyche and the nervous system. And, finally, the possibility of using alcohol substitutes, which also cause a complex of pronounced destructive changes in organs and tissues, is also especially dangerous. Thus, there are more pronounced changes on the part of the female body, which can develop with chronic alcohol abuse [5,25].

Alcohol abuse leads to a violation of the ovarian-menstrual cycle, disrupting both the physiological activity of the hypothalamic-pituitary system and the work of the ovaries, causing a whole complex of sclerotic-dystrophic changes in the ovaries [16]. Uterine mucosa is considered to be the target organ for these groups of hormones. In addition, ethanol has a direct effect on the endometrium, the vascular bed, thereby worsening its trophism. Disturbance of microcirculation can also be aggravated by the formation of thrombotic masses in the lumen of blood vessels due to hemolysis caused by the action of ethanol [18]. There are many studies to date, most of which have been conducted in animals. There is no sufficient number of works devoted to the study of the entire complex of changes directly in the female body [10]. And the data that would have been obtained precisely as a result of studying the changes caused by alcohol abuse in the female body can help solve many abnormal conditions developing in the female reproductive system, such as oligodysmenorrhea and amenorrhea. And more formidable ones include miscarriage and early menopause [9,16].

Considering all of the above, the purpose of this study was to identify changes in the endometrium that occur in chronic alcoholism.

Material and methods. The study included sectional material, selected from 60 women. All subjects were divided into two groups. The first group (30 women) consisted of women who, according to history data (interviews with relatives) and autopsy data (presence of alcoholic cirrhosis of the liver), had confirmed alcohol abuse. The control group consisted of women (30) who died from diseases not associated with reproductive diseases without accompanying signs of alcoholism (deaths as a result of car crash, accidents). Tobacco smoking, contraceptives (oral contraceptive pills), age of first sexual intercourse, somatic pa-

thology related (or no related) to alcohol consumption, numbers of pregnancies were not taken into account.

The material was fixed in 10% neutral buffered formalin, after which the selected samples were embedded in paraffin. At the next stage, sections with a thickness of 5×10^{-6} m were made from the prepared paraffin blocks. Subsequently, staining with hematoxylin and eosin was performed. Microscopic examination was carried out on an Olympus BX41 microscope, followed by morphometric examination using the Olympus DP-soft 3.12 software [4].

After determining the proliferative or secretory type of the endometrium, the following indicators were determined: the average diameter of the endometrial glands, the minimum diameter of the endometrial glands, the maximum diameter of the endometrial glands, gland wall thickness, the relative volume of the epithelium, and the thickness of the epithelium.

Statistical processing was performed using the methods of variation statistics. Correspondence of the distribution to the normal distribution was determined by the Shapiro-Wilk's test, which showed that the samples were close to the normal distribution. Statistical indicators are presented in the $M \pm \sigma$ format, where M is the arithmetic mean, σ is the standard deviation, Student's t -test. The statistical difference between the studied parameters was considered significant at p less than 0.05 [15].

The procedure was done strictly in compliance with the Helsinki Declaration after approval from the Regional Ethical Review Board at Odessa National Medical University, protocol 3, 17th October 2011.

Results and discussion. Our work confirm influence of alcohol in endometrium under with changes both in proliferative and in secretory stage (Fig. 1). Main focus of our work was directed on morphometric study for obtaining of relevant data and its results are presented in Table 1. As can be seen from the table, chronic alcoholism causes a whole complex of changes in the state of the endometrium, manifested in both the proliferative and secretory phases of the menstrual cycle.

The average diameter of the endometrial glands (proliferative type) decreased by 13.7% (from $51.71 \pm 2.90 \times 10^{-6}$ m to $44.65 \pm 2.48 \times 10^{-6}$ m) with $p < 0.05$. The minimum diameter of the endometrial glands (proliferative type) was 9.7%, which is 2.23×10^{-6} m. The maximum diameter of the endometrial glands (proliferative type) changed from $72.14 \pm 2.21 \times 10^{-6}$ m in the control group to $64.13 \pm 3.90 \times 10^{-6}$ m in the comparison group, which was 11.1% ($p < 0.05$). The thickness of gland wall (proliferative type) decreased by 4.7% in the comparison group from $15.18 \pm 1.60 \times 10^{-6}$ m to $14.47 \pm 1.12 \times 10^{-6}$ m, the relative volume of the epithelium (proliferative type) by 5.4 % (from $54.43 \pm 1.79 \times 10^{-6}$ m to $51.48 \pm 2.56 \times 10^{-6}$ m).

The changes that were also observed in the secretory phase of the menstrual cycle were quite pronounced. At the same time, the average diameter of the glands decreased by 9.26% (from $101.55 \pm 3.12 \times 10^{-6}$ m in the comparison group to $92.15 \pm 4.10 \times 10^{-6}$ m in the group of women suffering from chronic alcoholism) with $p < 0.05$, the minimum diameter of the endometrial glands by 6.17% ($33.86 \pm 1.17 \times 10^{-6}$ m to $31.77 \pm 1.15 \times 10^{-6}$ m), maximum by 14.3%, from $127.98 \pm 2.10 \times 10^{-6}$ m to $109.66 \pm 4.13 \times 10^{-6}$ m, according to the above order ($p < 0.05$).

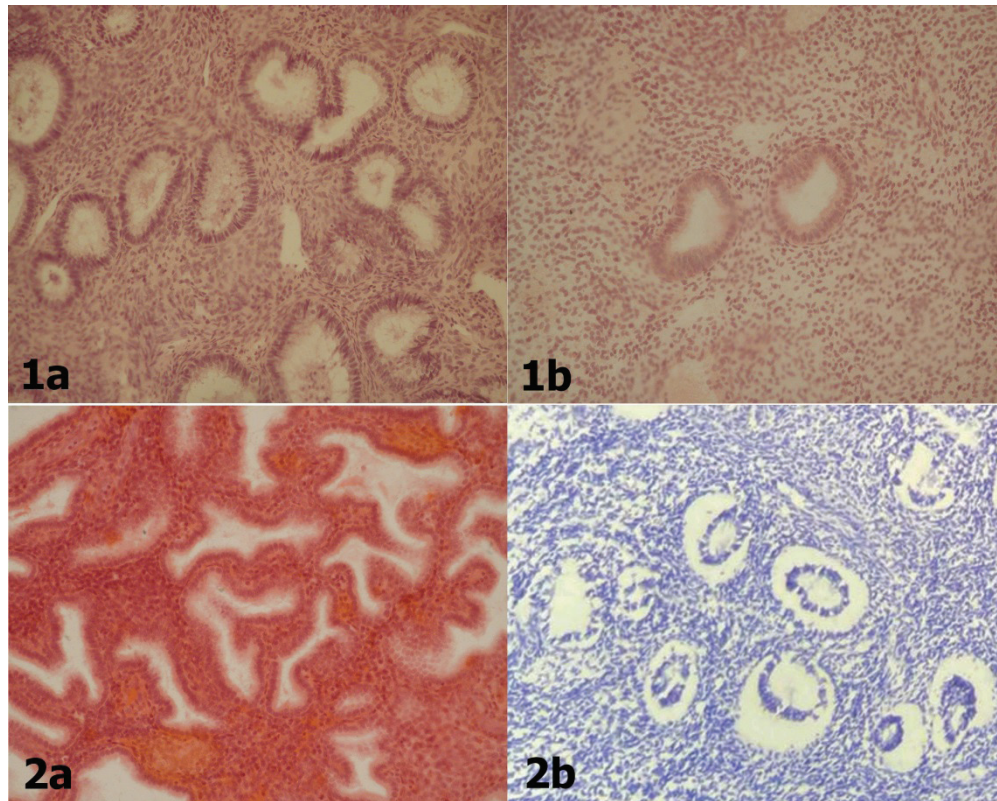


Fig. Endometrium of proliferative (1) and in secretory (2) types in comparison group (a) and under influence of alcohol (b). Hematoxylin and eosin, x200

Table 1. The studied indicators of the structure of the endometrium in the group of women who abused alcohol and in the comparison group

The investigated indicator	Comparison group	Alcoholism
Average diameter of endometrial glands (proliferative type), $\times 10^{-6}$ m	51.71 \pm 2.90	44.65 \pm 2.48*
The minimum diameter of the endometrial glands (proliferative type), $\times 10^{-6}$ m	32.47 \pm 1.83	30.24 \pm 1.37
Maximum diameter of endometrial glands (proliferative type), $\times 10^{-6}$ m	72.14 \pm 2.21	64.13 \pm 3.90*
Gland wall thickness (proliferative type), $\times 10^{-6}$ m	15.18 \pm 1.60	14.47 \pm 1.12
The relative volume of the epithelium (proliferative type), %	54.43 \pm 1.79	51.48 \pm 2.56
Average diameter of glands (secretory type), $\times 10^{-6}$ m	101.55 \pm 3.12	92.15 \pm 4.10*
The minimum diameter of the endometrial glands (secretory type), $\times 10^{-6}$ m	33.86 \pm 1.17	31.77 \pm 1.15
Maximum diameter of endometrial glands (secretory type), $\times 10^{-6}$ m	127.98 \pm 2.10	109.66 \pm 4.13*
Gland wall thickness (secretory type), $\times 10^{-6}$ m	13.02 \pm 1.36	12.62 \pm 1.24
The relative volume of the epithelium (secretory type), %	61.24 \pm 1.11	52.81 \pm 1.09
Epithelium thickness, $\times 10^{-6}$ m	49.14 \pm 1.44	48.66 \pm 1.97

* $p < 0.05$ significant between groups with and without alcohol abuse

The indicator of the thickness of the wall of the glands also changed in the group of women who abused alcohol from $13.02 \pm 1.36 \times 10^{-6}$ m to $12.62 \pm 1.24 \times 10^{-6}$ m in the control group, which amounted to 3.07%. The relative volume of the epithelium decreased by 13.7% (8.43×10^{-6} m) in the study group compared to the comparison group. A change was also revealed in the thickness of the epithelium from $49.14 \pm 1.44 \times 10^{-6}$ m in the comparison group of women to $48.66 \pm 1.97 \times 10^{-6}$ m in the group of alcohol abusers. So, results of the morphometric study could be interpreted as tendency to atrophy in endometrium.

Alcohol abuse causes a series of reversible and, at later stages, irreversible changes in the body of women in general and, in particular, in the morphological and functional state

of the reproductive system [7,10]. The described effect may be due to both the indirect effect of ethanol on the state of the hypothalamic-pituitary system, leading primarily to ovarian hypofunction, manifested in a decrease in hormone production [11]. Changes in the endometrium in both the proliferative and secretory phases of the menstrual cycle are known to be caused precisely by the hormones of the hypothalamic-pituitary system (first of all, this is follicle-stimulating hormone (FSH) and luteinizing hormone (LH) of the pituitary and ovaries (estrogen, progesterone) [14].

In addition to the above effects, LH is a hormone that stimulates the maturation of the corpus luteum in the ovaries and the process of producing progesterone. Progesterone is of

great importance for the body of women, being the main hormone of the first trimester of pregnancy. Consequently, women who abuse alcohol should expect early miscarriage due to progesterone deficiency. Knowing this feature, it can be assumed that progesterone preparations should be recommended to correct this condition. In the course of the study, data were obtained indicating the effect of chronic alcoholism on the endometrium, which consisted in a significant ($p < 0.05$) decrease in the average diameter of the glands (secretory type), the minimum diameter of the glands (secretory type), the maximum diameter of the glands (secretory type), the relative volume of the epithelium (secretory type), the thickness of the epithelium [12,13].

Thus, the approach to the treatment of this category of patients should also be differentiated. In our opinion, it should consist both in the refusal to drink alcohol and in the selection of the correct hormonal therapy [8].

The hypoproduction of FSH entails a decrease in the production of estrogen by the ovaries. These effects can also be enhanced by the occurrence of dystrophic-sclerotic changes in the ovaries themselves. These abnormal processes are known to be manifested by the proliferation of connective tissue, disruption of the relationship between the cortex and medulla, a decrease in the size of all types of follicles (primary, secondary and tertiary), and even a decrease in the number of primordial follicles. The described changes inevitably lead to a decrease in the production of estrogen. The endometrium is regarded to be a target organ for estrogen. Proliferative changes occur under their influence [19]. Thus, hypoproduction or complete absence of estrogen by the ovaries, which is also due to dystrophic processes in them, can cause oligomenorrhea or even cause early menopause [17]. This fact could explain the changes obtained in the course of the study, namely: a decrease in the average diameter of the endometrial glands, the minimum diameter of the endometrial glands, the maximum diameter of the endometrial glands, wall thickness, the relative volume of the epithelium, which were calculated in the proliferation phase of the menstrual cycle [20]. Based on the study, it can be assumed that gynecologists sometimes need to look for concomitant factors that affect the female body for the correct selection of treatment of oligomenorrhea and early menopause [23].

In addition, an imbalance in the concentration of FSH and LH can lead to the development of follicular and corpus luteum cysts, which, although functional, are sometimes associated with the development of complications and require urgent surgical treatment [3].

Also important is the information available today on the proven carcinogenicity of ethanol. That, in combination with impaired immune surveillance that occurs in people who abuse alcohol, can cause the development of malignant neoplasms, including those with localization in the organs of the female reproductive system [1,24].

An interesting fact is the different variability of all the studied parameters in the group of women suffering from alcoholism and in the control group. So, the most pronounced were the changes in the maximum diameter of the glands, which were observed both in the proliferative phase and in the secretory phase. The relative volume of the epithelium decreased as much as possible only in the secretory phase and was relatively stable in the proliferative phase. The least variable indicator was the thickness of the gland wall in both proliferative and secretory types.

Conclusions: Based on the study, it can be assumed that alcohol abuse has a significantly significant effect on the female reproductive system as a whole, in particular, on the morpho-functional state of the endometrium, which is manifested by its statistically reliable thinning, hypoplasia of the glands, which was determined both in proliferative and in the secretory phase of the menstrual cycle. Results of the morphometric study have to be interpreted as tendency to atrophy in endometrium with decreased by 13.7% the average diameter of the endometrial glands (from $51.71 \pm 2.90 \times 10^{-6}$ m to $44.65 \pm 2.48 \times 10^{-6}$ m), reduced diameter of the endometrial glands from $72.14 \pm 2.21 \times 10^{-6}$ m to $64.13 \pm 3.90 \times 10^{-6}$ m, abridged the relative volume of the epithelium.

The study shows the importance of an individual approach in working with this cohort of patients, which may consist in proper history taking (to confirm alcohol abuse), detection of pathomorphological changes in the endometrium, which is important for the correct diagnosis and selection of the most effective treatment.

Conflict of Interest Statement. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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SUMMARY

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According to data that would have been obtained precisely as a result of studying the changes caused by alcohol abuse in the female body which can help solve many abnormal conditions developing in the female reproductive system, such as oligodysmenorrhea and amenorrhea, the purpose of this study was to identify changes in the endometrium that occur in chronic alcoholism.

The study included sectional material, selected from women who, according to history data (interviews with relatives) and autopsy data (presence of alcoholic cirrhosis of the liver), had confirmed alcohol abuse. Microscopic examination of endometrium was carried out followed by morphometric examination with determining: the average diameter of the endometrial glands, the minimum diameter of the endometrial glands, the maximum diameter of the endometrial glands, gland wall thickness, the relative volume of the epithelium, and the thickness of the epithelium for the proliferative or secretory type of the endometrium.

Based on the study, it can be assumed that alcohol abuse has a significantly significant effect on the female reproductive system as a whole, in particular, on the morpho-functional state of the endometrium, which is manifested by its statistically reliable thinning, hypoplasia of the glands, which was determined both in proliferative and in the secretory phase of the menstrual cycle. Results of the morphometric study have to be interpreted as tendency to atrophy in endometrium with decreased by 13.7% the average diameter of the endometrial glands (from $51.71 \pm 2.90 \times 10^{-6}$ m to $44.65 \pm 2.48 \times 10^{-6}$ m), reduced diameter of the endometrial glands from $72.14 \pm 2.21 \times 10^{-6}$ m to $64.13 \pm 3.90 \times 10^{-6}$ m, abridged the relative volume of the epithelium.

The study shows the importance of an individual approach in working with this cohort of patients, which may consist in proper history taking (to confirm alcohol abuse), detection of pathomorphological changes in the endometrium, which is important for the correct diagnosis and selection of the most effective treatment.

Keywords: endometrium, pathology, morphometry, alcoholism.

РЕЗЮМЕ

МОРФОФУНКЦИОНАЛЬНОЕ СОСТОЯНИЕ ЭНДОМЕТРИЯ ПОД ВЛИЯНИЕМ ХРОНИЧЕСКОГО АЛКОГОЛИЗМА

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Целью исследования явилось определение изменений эндометрия, возникающих при хроническом алкоголизме.

Исследование проведено на секционном материале, полу-

ченном от женщин, злоупотреблявших алкоголем, что установлено по данным анамнеза (беседа с родственниками) и результатам вскрытия (наличие алкогольного цирроза печени). Проведено микроскопическое исследование эндометрия с последующим морфометрическим исследованием с определением показателей: средний диаметр, минимальный диаметр и максимальный диаметр эндометриальных желез, толщина стенки желез, относительный объем эпителия и толщина эпителия для пролиферативного или секреторного типа эндометрия.

На основании проведенного исследования следует предположить, что злоупотребление алкоголем оказывает значимое влияние на женскую репродуктивную систему в целом, в частности на морфофункциональное состояние эндометрия, что проявляется его статистически достовер-

ным истончением, гипоплазией желез и определяется как в пролиферативной, так и в секреторной фазе менструального цикла. Результаты морфометрического исследования следует интерпретировать как тенденцию к атрофии эндометрия при уменьшении на 13,7% среднего диаметра желез эндометрия (с $51,71 \pm 2,90 \times 10^{-6}$ м до $44,65 \pm 2,48 \times 10^{-6}$ м), уменьшении диаметра эндометриальных желез с $72,14 \pm 2,21 \times 10^{-6}$ м до $64,13 \pm 3,90 \times 10^{-6}$ м, уменьшении относительного объема эпителия.

Данное исследование указывает на значимость индивидуального подхода к работе с этой группой пациентов, что заключается в правильном сборе анамнеза (для подтверждения злоупотребления алкоголем), выявлении патоморфологических изменений эндометрия для правильной диагностики и выбора наиболее эффективного лечения.

რეზიუმე

ენდომეტრიუმის მორფოფუნქციური მდგომარეობა ქრონიკული ალკოჰოლიზმის გავლენის პირობებში

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კვლევის მიზანს წარმოადგენდა ქრონიკული ალკოჰოლიზმის პირობებში ენდომეტრიუმში განვითარებული ცვლილებების გამოვლენა.

კვლევა ჩატარდა იმ ქალების სექციურ მასალაზე, რომლებიც ჭარბად მოიხმარდნენ ალკოჰოლს, რაც დადგენილია ანამნეზური მონაცემებით (საუბარი ნათესავებთან) და გაკვეთის შედეგების მიხედვით (ღვიძლის ალკოჰოლური ციროზის არსებობა). ჩატარებულია ენდომეტრიუმის მიკროსკოპიული შესწავლა, შემდგომი მორფომეტრიული კვლევით და შედეგი მანვენებლების განსაზღვრით: ენდომეტრიული ჯირკვლების საშუალო, მინიმალური და მაქსიმალური დიამეტრი, ჯირკვლის კედლის სისქე, ეპითელიუმის შეფარდებითი მოცულობა და ეპითელიუმის სისქე ენდომეტრიუმის პროლიფერაციული ან სეკრეციული ტიპისათვის.

ჩატარებული კვლევის საფუძველზე შესაძლებელია ვარაუდი, რომ ალკოჰოლის ჭარბად მოხმარება მნიშვნელოვან გავლენას ახდენს ქალის რეპროდუქციულ სისტემაზე მთლიანად, კერძოდ, ენდომეტრიუმის მორ-

ფოფუნქციურ მდგომარეობაზე, რაც ვლინდება მისი სტატისტიკურად სარწმუნო გათხელებით, ჯირკვლების ჰიპოპლაზიით და აღინიშნება მენსტრუალური ციკლის როგორც პროლიფერაციის, ასევე, სეკრეციის ფაზაში.

მორფომეტრიული კვლევის შედეგები ინტერპრეტირებული უნდა იყოს, როგორც ენდომეტრიუმის ატროფიის ტენდენცია ენდომეტრიუმის ჯირკვლების საშუალო დიამეტრის შემცირებისას 13,7%-ით ($51,71 \pm 2,90 \times 10^{-6}$ მ-დან $44,65 \pm 2,48 \times 10^{-6}$ მ-მდე), ენდომეტრიული ჯირკვლების დიამეტრის შემცირებისას $72,14 \pm 2,21 \times 10^{-6}$ მ-დან $64,13 \pm 3,90 \times 10^{-6}$ მ-მდე, ეპითელიუმის შეფარდებითი მოცულობის შემცირებისას. კვლევა მიუთითებს სწორი დიაგნოსტიკისა და ეფექტური მკურნალობის არჩევის მიზნით პაციენტების ამ ჯგუფთან ინდივიდუური მუშაობის მნიშვნელობაზე, რაც გამოიხატება ანამნეზის სწორ შეგროვებაში (ალკოჰოლის ჭარბად მოხმარების დადასტურებისათვის) და ენდომეტრიუმის პათომორფოლოგიური ცვლილებების გამოვლენაში.