MARKERS OF MENSTRUAL DISCHARGE IN THE DIAGNOSTICS OF GYNECOLOGICAL DISEASES

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Menstrual discharge is the complex in structure and biochemical indicators biological fluid which is cyclically secreted into the uterine cavity, and then released from its. Laboratory study of menstrual discharge is up till now poorly studied, non-invasive, promising method of early diagnosis of diseases of female reproductive organs. Investigation of menstrual discharge gives an integrated assessment of both local and systemic homeostasis at physiological and pathological processes of the genitals. In this article we made an attempt to summarize the data presented in current literature which relate to the above topics.

Keywords: menstrual discharge, Gynecological Diseases

Menstruation – physiological, cyclically repeated hemorrhage from the uterine lining occurring in women and females of certain mammal species from the reaching of the age of puberty until the end of reproductive life.

Menstruation usually begins between the ages of 12–14 years (menarche), is stabilized immediately or within a few months, lasts 3–5 (rarely to 7) days. The first experience of menstruation in 15 years and later, the painful period, as well as long period (more than 5–6 months) before a regular menstrual cycle is stabilized allow us to think about the inferiority of its regulation, which is often observed in the infantilism and genital hypoplasia.

The average volume of menstrual fluid during a monthly menstrual period does not exceed 70,0–80,0 ml. Changing in the amount of blood lost, and the frequency of menstruation may indicate the presence of some gynecological diseases.

Normal menstrual cycle is characterized by successive changes in the endometrium of four phases: desquamation, which is manifested in menstrual bleeding, regeneration, proliferation and secretion

The mucus dominates in the early days of the desquamation phase in the menstrual discharge. This mucus is rich by epithelial cells with a small admixture of blood, the content of which gradually increase. By the end of menstruation endometrial mucus dominates again in the menstrual discharge.

A characteristic morphological feature of desquamation phase is the presence in the menstrual discharge collapsed, penetrated with hemorrhage of star-shaped endometrial glands and glomus of the spiral arteries. On the first day of menstruation the separate groups of predecidual cells and tiny particles of the endometrium, having the viability and ability to implant can be still recognized in the compact layer in areas of hemorrhage.

Accumulate, and then arising from the uterus the menstrual discharge mixed with endometrial secretion, which has an alkaline environment, and as a rule, cannot coagulates and has the characteristic dark color.

Biochemical markers of the menstrual discharge have great interest for the diagnosis of pathology of the reproductive function of women. The α_2-microglobulin of fertility is the most studied among them and it is a dimeric glycoprotein with a molecular weight ranging from 42 to 56 Kd. Using the method of immune diffuse analysis, D.D. Petrunin and co-authors (1976) found the α_2-microglobulin of fertility in extracts of tissues of secretory endometrium and in the menstrual discharge during ovulatory menstrual cycles. It was found that α_2-microglobulin of fertility synthesize in the epithelium of endometrial glands during the luteal phase, as well as in decidual tissue of the placenta in the first trimester of pregnancy [12].

According to the content of the α_2-microglobulin of fertility in the menstrual discharge L.V. Posiseeva (1991) proposed to evaluate the functional state of the endometrium: thus, the number of α_2-microglobulin of fertility in menstrual discharge of women with two-phase menstrual cycle is hundred times higher than in peripheral blood serum and the majority of women (81,7 %) are in the range from 16000 to 70000 ng/ml [14].

Determining the level of the α_2-micro_globulin of fertility in the menstrual discharge may be one of methods to diagnose of a particular form of female sterility – habitual miscarriage in the early periods from 2 to 4 weeks of pregnancy. This form of sterility, the identification of which requires special methods of investigation, unfortunately, is not always objectively assessed in routine clinical practice. In cases of early, with subclinical variant of the course of spontaneous abortions, the concentration of α_2-microglobulin of fertility in the menstrual discharge is 50–100 times higher than the concentration of this protein in normal menstrual discharge, and reaches 80 000 ng/ml or more.

The analysis of the relationships between the characteristics of menstrual cycle, the functional changes of the endometrium and content α_2-microglobulin of fertility in the menstrual discharge making by L.V. Posiseeva and co-authors (1991) was the basis for the diagnosis of inadequate luteal phase. It was discovered that the level of α_2-microglobulin of fertility in the menstrual discharge in such cases reduces, and is 2000–12000 ng/ml [14].

Assessment of α_2-microglobulin of fertility in the menstrual discharge of women with abnormal hormonal status, with perinatal loss in their anamneses, showed that the average α_2-microglobulin of fertility was 3 times lower than in the control group. During the correlation analysis a direct connection was noted between the number of α_2-microglobulin of fertility in the menstrual discharge, level of progesterone during the second phase of the cycle, luteinizing hormone in periovulatory period and an inverse relationship between the content α_2-microglobulin of fertility and prolactin level [14].

E.G. Shvarev (1993) showed that in the menstrual discharge of fertile women, as a rule, α_2-microglobulin of fertility was determined, in contrast to samples of endometrial swabs in women with endometrial hyperplasia and endometrial cancer [18]. Resynthesis of α_2-microglobulin of fertility in endometrial secretion of patients in postmenopause with endometrial cancer of second pathogenic variant [2, 3] is an early marker of the sensitivity of adenocarcinoma to hormone therapy [20].

Soluble antigen of leukocyte-2 was first described by D.D. Petrunin (1982). In the endometrium, menstrual discharge and cervical mucus soluble antigen of leukocyte-2 was detected in small quantities and its index was not associated with the phase of the menstrual cycle and the characteristics of ovaries function. An important property of soluble antigen of leukocyte-2 is its high resistance to enzymatic action. With its high resistance to the action of proteolytic enzymes, soluble antigen of leukocyte-2 can carry out its biological function in the focus of destruction and inflammation, where lysosomal proteolytic enzymes are concentrated from the leukocytes [13].

L.V. Posiseeva and co-authors (1995) proposed a method for diagnostics of chronic endometritis on the basis of determining the level of soluble antigen of leukocyte-2 in menstrual discharge. So, the content of soluble antigen of leukocyte-2 in menstrual discharge is equal to or greater than 640 mcg/ml indicated the presence of chronic metroendometritis [16].

In a number of publications there are data on the study of fibrinolytic proteins in the menstrual discharge. So, S.A. Cederholm-Williams et all. (1984) studied the content of fibrinolytic proteins in the menstrual discharge and in the peripheral blood serum in women with normal menstrual blood loss (80 ml) in 1–2 days desquamation phase. In this case, the active plasmin, which slows down platelet aggregation and formation of fibrin fibers, was absent in the peripheral blood serum, while in menstrual discharge its concentration reached 1,8 mmol/l. The authors pointed out the lack of α_2-antiplasmin in menstrual discharge, which prevents the binding of plasminogen with fibrin and has antiplazmin action, it was shown that in the peripheral blood serum its concentration did not exceed 1 μm (Table 1) [4, 11].

Table 1

The activity of fibrinolytic proteins in menstrual flow, and peripheral blood serum
S.A. Cederholm-Williams, C.P. Margaret Rees, A.C. Turnbull (1984)

	Menstrual Discharge	Peripheral Blood Serum
Active plasmin (mmol/l)	0,83 ± 0,97	0
Plasminogen activator (IU/ml)	1,04 (0–3,5)	0,15 (0–0,2)
α_2-antiplasmin (%)	0	100 ± 20%

The concentration of α_2-macroglobulin involved in the physiological regulation of blood coagulation, clot lysis and complement did not practically differ in the menstrual discharge and in the peripheral blood serum (3,49 and 3,68 mmol/l, respectively) [4].

In addition to studying of the fibrinolytic activity of proteins, the researchers compared the concentrations of albumin, IgG and α_2-antiplasmin, which rates were similar both in the menstrual discharge and in the peripheral blood serum (Table 2).

In the few studies devoted to the study of lipid peroxidation and oxidative modification of proteins at the obstetrical and gynecological pathology, the most often object of study was peripheral blood serum. In the scientific literature there are practically no studies on the levels of markers of oxidative stress in the menstrual discharge – in the biological fluid, flowing directly from the uterus.

Table 2

The concentration of proteins in the menstrual discharge, and in peripheral blood serum S.A. Cederholm-Williams, C.P. Margaret Rees, A.C. Turnbull (1984)

	Menstrual Discharge		Peripheral Blood Serum	
	g/l	mmol/l	g/l	mmol/l
IgG	12,5 ± 2,3	84,5	12,0 ± 2,3	81,1
albumin	43,6 ± 11,8	641	44 ± 8,8	647
fibrinogen	3,71 ± 2,3	10,9	2,25 ± 0,54	6,62
plasminogen	0,17 ± 0,05	1,88	0,15 ± 0,03	1,67
α_2-antiplasmin	0,56 ± 0,12	0,81	0,70 ± 0,14	1,0
α_2-macroglobulin	2,51 ± 0,37	3,49	2,65 ± 0,61	3,68

It should be noted that in the case of oxidative stress there is not an isolated damage of proteins, lipids, nucleic acids, as there is a close structural and functional interaction in biological membranes of cells between them [10]. And at the same time the antioxidant system of the body is involved in the process that regulates lipid peroxidation and oxidative modification of proteins. Increase of reactive oxygen species in cells is accompanied by a relative decrease in the level of the main non-enzymatic and enzymatic components of antioxidant protection [1, 9, 10].

In the pathogenesis of both benign and malignant tumors the processes of lipid peroxidation have a considerable place. Taking in the consideration the instability of the primary products of lipid peroxidation, the object of the study were secondary or end products, including malon dialdehyde, which has the most damaging effect on the cell.

The researches of L.V. Dikareva and co-workers (2009) have shown that the oxidative stress is pathogenetically important in the formation of the endometrial hyperplasia, and that the oxidative stress is characterized by the depletion of antioxidant protection, and by the levels of catalase and the content of total antioxidant activity as well. The level of catalase in the menstrual discharge in patients with uterine myoma with the normal structure of the endometrium (with the slow growth rate) was practically the same as in healthy women. The level of catalase in the menstrual discharge in the group of patients with uterine myoma with in combination with the endometrial hyperplasia has a clear downward trend – 0,7 ± 0,03 y.e. ($p < 0,001$) [5].

The level of malon dialdehyde in the menstrual discharge in patients with uterine myoma with the normal structure of the endometrium in combination with ovarian tumors increased to 1,7 ± 0,04 nmol, and in patients with uterine myoma in combination with the endometrial hyperplasia, the level reached maximum values (2,8 ± 0,08 nmol) compared with the level of healthy women ($p < 0,01$, $p < 0,001$) [5].

The content of carbonyl groups of the protein in menstrual discharge in patients with uterine myoma in combination with the endometrial hyperplasia was also increased: from 4,3 to 8,2 nmol/mg (5,7 ± 0,63 nmol/mg), which was higher compared with the similar index in healthy women [5].

A fundamentally new scientific direction in the diagnosis is the theory of V.N. Shabalin and S.N. Shatokhina of the functional morphology of the biological fluid, evaluation of its self-organization processes (the method of wedge dehydration, Litos – system). In the biological fluid there are highly dynamic changes of molecular composition, the changes in nature of the interaction of various components under physiological and pathological conditions, which are the basis for the diagnosis of various diseases at the earliest stages of development [17].

To identify the endometrial hyperplasia L. Dikareva and co-workers (2008) were the first to suggest making a structural analysis of the menstrual discharge and endometrial secretion, and described three facies morphotype: radial mixed and triradial. It is shown that the pathognomonic marker of formation of endometrial pathology is a decrease in the material the area of radial cracks with simultaneous increase of the area of triradial cracks [8].

E.G. Shvarev and co-workers (2008, 2011) studied in comparison by microscopy the structural features of facies of the menstrual discharge in patients with inflammatory diseases of the small pelvis and in patients with ovarian tumors. It was found that in 38% of cases the emergence of «tongue» structures in the peripheral zone of facies was marked in women with inflammatory diseases and the combination of «tongue» structures in the peripheral and the triradial cracks in the central part of facies was marked in 62% of cases [19]. Increasing the area of triradial cracks points to the likelihood

of forming of ovarian tumors in the patients, what requires in-depth checkup [21].

S.Yu. Sotnikova and co-workers (2001) proposed a way to diagnostics of internal endometriosis by determination of number of CD95 + lymphocytes in the menstrual discharge. So we can determine the relative number of CD95 + lymphocytes in the women's menstrual discharge and if the values of this parameter equal to 15% and less we can with 80% accuracy the internal endometriosis was revealed [22].

Using of the menstrual discharge in diagnostics of gynecological pathology allows noninvasive, in small amounts of biological fluid with minimal material costs (!) in a women's clinic, in a short time to get objective information about the status of the female reproductive system. Atraumatic sampling of the material, ease of processing and storage give a wide opportunities to do an effective prophylactic medical examination of women. To date the menstrual discharge is by-way, but it has a large amount of information on the functional and morphostructural status of female genital biological fluid. The ability to decode this information is one of the major aims of modern medical practice.

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