

## **BLEEDING WHEN WEARING INTRAUTERINE CONTRACEPTIVES AND THEIR RELATIONSHIP WITH THE NITRIC OXIDE SYSTEM**

***Khamdamova M.T.***

*Bukhara Medical University named after Abu Ali Ibn Sina*

**Annotation.** There is a decrease in HIF-1 $\alpha$  in women with IUDs, with the most profound differences found 6, 12 and 36 months after insertion of the IUD; it was revealed that the decrease in HIF-1 $\alpha$  reflects the state of women's adaptability to the conditions of formation of long-term adaptation to the IUD; in women with high levels of HIF-1 $\alpha$  (from 170% to 185%), adaptability to the IUD was manifested by the absence of clinical complaints about wearing a copper-containing contraceptive, and they did not experience complications associated with wearing the IUD. The absence of inflammation and bleeding in women with a high content of HIF-1 $\alpha$  is apparently due to the tolerance of the vessels of the uterus and its mucous membrane to the IUD; patients with low levels of HIF-1 $\alpha$  (from 120% to 140%) had the highest incidence of complications associated with wearing an IUD.

**Keywords:** HIF-1 $\alpha$ , nitric oxide system, IUD.

Among the protective factors at the cellular and systemic level, an important place is given to the angiohypoxic protein HIF - 1 (hypoxia - inducible factor - 1), [1,5,7,9,11]. It has been established that HIF - 1 coordinates the processes of proliferation, differentiation and cell survival, both at the stage of embryonic development and in the postnatal period [3,4]. It has been revealed that HIF - 1 regulates angiogenesis, erythropoiesis, glycolysis, vascular tone, through mechanisms of stimulation of nitric oxide (NO) production [2,10,12,13]. However, the role of NO and HIF-1 in the prevention of ischemic and reperfusion (IR) damage to the reproductive organs in their pathologies has not yet been fully studied. Some studies have shown that inhibition of NO synthesis prevents the formation of adaptive protection against IR damage to uterine tissue [3,14,15,16]. Of significant interest in this regard is work related to the wearing of intrauterine contraceptives [1,17,18]. The protective effects of NO are associated with its dilation of blood vessels in internal organs, enhancement of microcirculation processes, activation of enzymes of the antioxidant system, and stimulation of HIF-1 [7]. And the damaging one is IR by overexpression of NO, an increase in peroxynitrite (ONO<sup>-2</sup>), and activation of the pathological isoform of inducible NO synthase (iNOS) [3,19,20]. In this regard, it can be assumed that as a result of wearing an IUD, the development of various complications (expulsion, inflammatory diseases of the uterus, menstrual irregularities, heavy intrauterine bleeding) that are observed in 11 - 24% of women [2,21,22] lead to dysfunction uterus, to systemic hypoxia.

In connection with the above, the purpose of the study was to assess the effect of long-term wearing of an IUD on the hypoxic transcription factor HIF - 1 and the activity of the NO - system in erythrocytes.

**Research methods.** 150 women of reproductive age using copper-containing IUDs for 3 years were examined. The control group consisted of 40 women without ICH. The criteria for inclusion in the survey group were women of reproductive age from 20 to 43 years ( $33.6 \pm 2.15$  years), absence of inflammatory diseases of the pelvic organs and cancer. In the anamnesis, the examined women had from 1 to 7 pregnancies and from 1 to 4 births, and suffered from 1 to 5 abortions. According to the recommendations [3,4,15,16], copper-containing T-shaped IUDs (Cu T 380 A) were inserted after childbirth, taking into account the absence of infection and inflammation. The studies were carried out over time (6, 12, 24 and 36 months from the start of IUD insertion). Regularly, during the period of wearing the IUD, a clinical examination was carried out to determine the presence of pain, discomfort, volume and number of days of menstruation, etc. To determine the content of HIF-1 $\alpha$  [1,14,23], erythrocyte lysate was obtained. Proteins were separated on an 8% polyacrylamide gel (PAGE). The transfer of PAGE proteins to a nitrocellulose membrane was carried out by electroelution for 60 minutes. Preincubation of Western blots was carried out for 60 minutes in PBS containing 0.5% Tween - 20 and 5% skim milk. Then Western blots were incubated for 14 hours at 4°C in a solution of polyclonal antibodies (Santa Cruz Biotechnology) against HIF-1 $\alpha$  at a dilution of 1: 1,000. After washing, the blots were incubated for 60 minutes in a solution of secondary antibodies conjugated with nitrogen peroxidase (Santa Cruz Biotechnology) at a dilution of 1 : 5,000. Detection of HIF - 1 $\alpha$  was carried out in reaction with ECL - calculations on Kodak film, followed by densitometry on a DM - 1 penetrating densitometer (Russia). The results obtained were expressed as % of the total number of densitograms. The activity of markers of the NO system was assessed by the content of the sum of stable NO metabolites (NO - NO) [9], the activity of eNOS [11], iNOS and ONO-2 [1]. The NO - system was assessed spectrophotometrically using a UV - vis - 2100 dual beam spectrophotometer (LTD, China). The obtained data were processed using the computer program Statistika v.6. Significant differences were considered significant at  $p < 0.05$ .

**Results and discussion.** The initial content of HIF - 1 $\alpha$ , NO, the activity of enzymes eNOS, NOS, the concentration of ONO-2 in erythrocyte lysate both in women of the control group and before ICH were quite comparable. In the control group up to 36 months. observations there were slight fluctuations in these indicators and practically did not differ from the initial data (before ICH). However, in the ICH groups after 6 months there was a significant decrease in the concentration of HIF - 1 $\alpha$  compared to the control - by 20.1% ( $p < 0.05$ ), after 12 months it remained within the same limits as after 6 months of observation. After 24 months, the level of HIF-1 $\alpha$  was practically no different from the control data, and then after 36 months it was again reduced by 18.9% ( $p < 0.05$ ). It can be assumed that the decrease in HIF - 1 $\alpha$  reflects the state of adaptability of women in the observed groups to the conditions of formation of urgent and long-term adaptation to ICH. This is confirmed by the fact that we identified at least three groups: with a high level of HIF - 1 $\alpha$  - from 170 to 185% - 7 (14.0%) patients, moderate from 140 to 170% - 33 (66.0%) patients and low - from 120 to 140% - 10 (20.0%) patients.

It was in patients with low levels of HIF-1 $\alpha$  that the highest incidence of complications was observed, such as inflammatory diseases of the uterus - 3 cases (30%), heavy bleeding - 5 (50%), pelvic pain - 5 (50%). While in the group as a whole, out of the total number of examined patients, expulsion and pain syndrome were detected in 6 (12%), heavy uterine bleeding in 9

(18%) patients and inflammatory diseases of the uterus in 7 (14.0%). A decrease in the concentration of HIF - 1 $\alpha$  was simultaneously associated with a decrease, compared with data in the control, in such indicators of the NO - system as NO and eNOS against the background of the expression of iNOS and ONO<sup>2</sup>. Changes in the NO - system in the erythrocyte lysate were synchronous with the quantitative content of HIF - 1 $\alpha$ . At the same time, a correlation was revealed between the HIF - 1 $\alpha$  indicator with NO, eNOS, iNOS and ONO<sup>2</sup>, which averaged after 6 months of wearing an IUD -  $r = 0.61; 0.72; -0.72$  and  $-0.74$ . After 12 months of wearing an IUD,  $r = -0.63; 0.70; -0.71$  and  $0.76$ . After 24 months of wearing an IUD,  $r = 0.55; 0.60; -0.86$  and  $0.77$ . And after 36 months of using the IUD it is equal to  $r = 0.64; 0.76; -0.78$  and  $0.78$ , respectively. There is a high correlation between the parameters of the HIF - 1 $\alpha$  and NO - system, which means that during the adaptation of women to ICH, the processes of endothelial dysfunction are intensified, primarily due to the stimulation of the pathological isoform of NOS - iNOS, as well as overexpression of ONO<sup>2</sup>, which is highly cytotoxic, cytostatic and suppressive effects [1,24,25]. At the same time, attention is drawn to the fact that after 24 months the HIF-1 $\alpha$ , NO, eNOS activity, despite the high content of ONO<sup>2</sup>, apparently due to the activity of iNOS, were within control, which confirms what mechanisms In women with ICH, urgent and long-term resistance is formed, which is an indicator of adaptation. It should be emphasized that in women with high HIF - 1 $\alpha$ , adaptability to the IUD was manifested by a virtual absence of clinical complaints about wearing a contraceptive, they did not experience complications. The absence of inflammation and bleeding in this group was apparently due to the tolerance of the vessels of the uterus and its mucous membrane to the IUD. However, long-term wearing of an IUD for up to 36 months leads to a decrease in the adaptive capacity of HIF-1 $\alpha$ , which is consequently manifested by a decrease in NO, eNOS activity, an increase in the iNOS reaction rate, and the formation of cytotoxic ONO<sup>2</sup>. According to the literature, high concentrations of ONO<sup>2</sup> can cause the development of uterine and ovarian tumors in women [3,12].

Thus, the research results showed that during the dynamics of wearing an IUD, fluctuations in the level of HIF - 1 $\alpha$  in the erythrocyte lysate and indicators of the NO - system are observed. Fluctuations in HIF - 1 $\alpha$  and NO - system indicators are synchronous, which can be assumed to reflect the adaptation and response of the female body to IUD, oxygen supply to tissues, regulation of vascular tone, which is important for the functioning of the uterus. A decrease in HIF - 1 $\alpha$ , as well as an imbalance in the NO - system, is characterized by increased damage to cellular systems at the level of the uterus, which is manifested by the development of complications. At the same time, a clear relationship was revealed between the degree of disruption of the NO - system, decrease in HIF - 1 $\alpha$  and the severity of complications of wearing an IUD. The revealed pattern of dependence of the development of complications on changes in the level of HIF - 1 $\alpha$  and NO - systems allows them to be used as markers for prognosis and assessment of the implementation of preventive and therapeutic measures depending on the duration of the IUD in the uterus.

### **Conclusions:**

1. In women with ICH, synchronous fluctuations in the level of HIF-1 $\alpha$  and parameters of the NO system (NO content, eNO and iNOS activity, ONO<sup>2</sup> concentration) are observed in erythrocyte lysates; a decrease in HIF-1 $\alpha$  is associated with a decrease in the level of NO, eNOS activity, iNOS expression and ONO<sup>2</sup> concentrations.
2. A correlation was revealed between the level of HIF - 1 $\alpha$  NO - system

Literature

1. Doroney H. F., Interni Hent hypoxia conditioning prevents endothelial dysfunction and improves nitric oxide storage in spontaneously hypertensive rats // *Exp. Biol. Med.*- 2011. – Vol. 36. – P. 867 – 873.
2. Calvert S. W., Cahill I, Yamaguchi – Okava M., Zhang I. H. Oxygen treatment after experimental hypoxia – isolumia in neonatal rats alters the expression of HIF – 1 and its downstream target genes // *I. Appl Physiol.*-2006. - №101.-P. 853 – 865.
3. Semeza G.L Regulation of Oxygen Homeostasis by Hypoxia – Inducible Factor//*Physiology.* – 2009. - №24. - P.97 – 106.
4. Ferrandina G., Ranelletti F. O., Gallotta V. et al Expression of cyclooxygenase – 2(COX - 2), receptors for estrogen (ER), and progesterone (PR) PS3,ki 67, and neu protein in endometrial cancer // *Gynecol.Oncol.* – 2005. –Vol. 98. - P.383 – 389.
5. Khamdamov I.B. Clinical evaluation of the effectiveness of the traditional approach to the treatment of hernias of the anterior abdominal wall in women of fertile age // *Doctor's Bulletin.* – Samarkand 2022. No. 2.2 (104).-P.65-70.
6. Khamdamov I.B., Mirkhodzhaev I.A. Khakimov M.Sh. Khamdamov B.Z. Evolution of the use of polymer implants for hernioplasty // *Tibbiyotda Yangi kun.* – Tashkent; 2021,- No. 2 (34) P.-107-111.
7. Khamdamov I.B., Khamdamov A.B. Differentiated approach to the choice of hernioplasty method in women of fertile age (Clinical and experimental study) // *Tibbiyotda Yangi kun.* – Bukhoro, 2021.-No. 6 (38/1).-P. 112-114.
8. Khakimov M.Sh., Urmanova N.M., Khudoiberdiev S.S., Khamdamov I.B. Possibilities of allohernioplasty in women of fertile age // *Nazariy va clinic tibbiyot journals.* Tashkent.-2022.-No.3.P.89-93.
9. Khamdamov I.B., Khamdamov A.B. Fertil yoshdagi ayollarda endovideo surgeon hernioplasty // *Tibbiyotda yangi kun.* Bukhoro, 2021.-№6 (38/1) -S. 25-27.
10. Khamdamov I.B. Experimental determination of the extensibility of the anterior abdominal wall tissues at different times of pregnancy using various approaches to hernioplasty // *Academicia: An International Multidisciplinary Research Journal* Vol. 12, Issue 04, April 2022 SJIF 2022 = 8.252 R.193-201 (Scopus).
11. Khamdamov I.B. Improving tactical approaches in the treatment of hernias of the anterior abdominal wall in women of fertile age // *Tibbiyotda Yangi kun.* Bukhoro, 2022.-№10(48)- pp. 338-342.
12. Khamdamov I.B. Morphofunctional features of the abdominal press in women of reproductive age // *Tibbiyotda Yangi kun.* Bukhoro, 2022.-№3(41)- pp. 223-227.
13. Khamdamova M.T. Ultrasound features of three-dimensional echography in assessing the condition of the endometrium and uterine cavity in women of the first period of middle age using intrauterine contraceptives // *Biology va tibbiyot muammolari.* - Samarkand, 2020. - No. 2 (118). - P.127-131.
14. Khamdamova M. T. Ultrasound assessment of changes in the endometrium of the uterus in women of the first and second period of middle age when using intrauterine and oral contraceptives // *Биомедицина ва амалиёт журналі.* – Ташкент, 2020. - №2. - 8 часть. - С.79-85.
15. Khamdamova M. T. Anthropometric characteristics of the physical status of women in the first and second period of middle age // *A new day in medicine.* Tashkent, 2020. - № 1 (29). - С.98-100.

16. Khamdamova M.T. Age-related and individual variability of the shape and size of the uterus according to morphological and ultrasound studies // News of dermatovenereology and reproductive health. - Tashkent, 2020. - No. 1-2 (88-80). - P.49-52.
17. Khamdamova M. T. Anthropometric characteristics of the physical status of women in the first and second period of middle age // Тиббиётда янги кун. Ташкент, 2020. - № 1 (29). - С.98-100.
18. Khamdamova M.T. Age-related and individual variability of the shape and size of the uterus according to morphological and ultrasound studies // News of dermatovenereology and reproductive health. - Tashkent, 2020. - No. 1-2 (88-80). - P.49-52.
19. Khamdamova M.T. Ultrasound features of three-dimensional echography in assessing the condition of the endometrium and uterine cavity in women of the first period of middle age using intrauterine contraceptives // Biology va tibbiyot muammolari. - Samarkand, 2020. - No. 2 (118). - P.127-131.
20. Khamdamova M. T. Ultrasound assessment of changes in the endometrium of the uterus in women of the first and second period of middle age when using intrauterine and oral contraceptives // Biomedicine va amaliyot journals. – Tashkent, 2020. - No. 2. - Part 8.- С.79-85.
21. Khamdamova M.T. Features of ultrasound parameters of the uterus in women of the first and second period of middle age using injection contraceptives // Tibbiyotda yangi kun. - Tashkent, 2020. - No. 2/1 (29/1). - pp.154-156.
22. Khamdamova M.T. Features of ultrasound images of the uterus and ovaries in women of the second period of middle age using combined oral contraceptives // Tibbiyotda yangi kun. - Tashkent, 2020. - No. 2 (30). - pp. 258-261.
23. Khamdamova M.T. Individual variability of the uterus and ovaries in women who use and do not use various types of contraceptives // Tibbiyotda yangi kun. - Tashkent, 2020. - No. 3 (31). - pp. 519-526.
24. Khamdamova M. T. Echographic features variability in the size and shape of the uterus and ovaries in women of the second period of adulthood using various contraceptives // Asian Journal of Multidimensional Research - 2020. – N9 (5). - P.259-263.
25. Khamdamova M. T. Somatometric characteristics of women of the first and second period of adulthood using different contraceptives with different body types // The american journal of medical sciences and pharmaceutical research - 2020. – N8 (2). - P.69-76.