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Analysis of the reason of abnormal uterine bleeding induced by copper corrosion of IUD Cu

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Summary

Objectives: This study aimed to analyze relationship of the copper corrosion of copper intrauterine device (TCu220 IUD) and abnormal uterine bleeding. *Materials and Methods:* Sixty-four patients of abnormal uterine bleeding (too much blood volume, shorten cycle, lengthen period or irregular vaginal bleeding) and 72 cases of normal menstrual cycle and quantity in the present hospital, which were removed of IUD due to non-medical reasons, were enrolled, and 36 regular menstruation cases without placing IUD were selected as control group, in which had assessed in vitro copper ion release of TCu220 IUD and content of copper ions and VEGF in endometrial tissue of each group of women. *Results:* Daily Cu I UD copper dissolution quantities of abnormal uterine bleeding women was significantly higher than that of regular menstruation women ($p < 0.05$). Copper ion content and the expression of VEGF in endometrial tissue of abnormal uterine bleeding women was significantly higher than that of regular menstruation women endometrial tissue ($p < 0.05$), and the endometrial VEGF expression had a positive correlation with copper ion concentration in endometrial tissue. *Conclusion:* High dissolution quantity of Cu IUD may lead to increase of copper ion content in endometrial tissue and may cause VEGF secretion in the endometrium, and then the occurrence of abnormal uterine bleeding.

Key words: Intrauterine device; Uterine bleeding; Copper corrosion quantity; Vascular endothelial growth factor.

Introduction

Intrauterine device (IUD) is the most widely used method of contraception among women of reproductive age [1, 2], which has advantages, such as simple, economic, safe, reliable [3], practical, effective, and reversible [4]. IUD is divided into two categories: one category is the inert IUD, and the contraceptive effect of inert IUD is poor, which has been gradually phased out; the other is the active IUD, because the copper erosion in the uterine cavity may release copper ion, the contraceptive effect is improved obviously, which has now basically replaced the inert IUD [5]. However after using IUD, some women may have an abnormal menstrual period, excessive menstruation, increased vaginal bleeding combination with dripping wet symptom [6], which has seriously influenced the lives of women and reproductive health, and has aroused widespread concern, and the literatures also include many solutions [7, 8]. Placement of a levonorgestrel IUD is recommended for women with idiopathic abnormal uterine bleeding [9]. The reason why there are such symptoms, studies have shown that after placement of Cu IUD, endometrial microvascular commonly undergoes abnormal changes in structure and in function, and changes of endometrial vascular abnormalities are more significant in those with IUD bleeding [10], but the understanding of the pathogenesis still requires more study. There was one study which suggests that the angiopoietin/Tie-2 system promotes vascular remodelling in the endometrium and the changes of Ang-1, Ang-2, and Tie-2 may contribute to abnormal uterine bleeding in some IUD users [11]. TCu220I UD is the clinical commonly used IUD in recent years. Based on the TCu220 IUD as an example, this research performed an in vitro assessment of release of copper ion, and detected content of copper in endometrial tissue and vascular endothelial growth factor (VEGF) in IUD women; an analysis of the role of Cu IUD copper dissolution quantity as the cause of uterine abnormal bleeding, at the same time, provided the theory basis for development of a new type of IUD.

Materials and Methods

Subjects

From August 2009 to December 2011, the women of gynecological clinic in Hebei People's Hospital were enrolled, including 64 patients with abnormal uterine bleeding (study group 1) (copious blood volume, shorten cycle, lengthened period or irregular vaginal bleeding) and 72 cases of normal menstrual cycle and quantity in the present hospital (study group 2), which had removed IUD due to non-medical reasons, and the wearing device time ranged from seven months to eight years. All subjects had the IUD removed at three to seven days after menorrhea and un-

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derwent curettage of the endometrium. Thirty-six normal menstrual cycle cases without placing IUD were selected as control group, which underwent curettage of endometrium three to seven days after menorrhea. The collected specimens were sealed and stored in a -20°C refrigerator. This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of Hebei People's Hospital. Written informed consent was also obtained from all participants.

Determination of copper ion content

Using the electronic balance to weigh the wet weight of endometrium, adding 1.5 ml nitric acid for complete digestion, and adding deionized water to the volume of ten ml, the copper ion content in the endometrium was determined by atomic absorption spectrophotometer.

In vitro release test

The removed TCu220 10 ml IUD was placed in a brown bottle with cover containing ten ml simulated uterine solution, which was places in the 37 ± 1°C constant temperature water bath oscillator, with speed of 120 ± 1 rpm/min. Changing fresh simulated uterine solution once every three days for a total of three times, the replaced liquid was collected, the absorption value was determined by atomic absorption spectrophotometer, and the average value was taken.

VEGF detection

Immunohistochemical SP method was used for detection and tissue sections were dewaxed routinely with anti VEGF antibody according to the instruction's of the kit. Result determination: the cells with the brown yellow reactants in the cytoplasm, which staining was significantly higher than that of the background, were VEGF positive cells. Each specimen was randomly taken at five high power fields (*400 times), with application of image analysis system, and the average optical density of intracellular positive products was measured.

Statistical analysis

The experimental results were expressed as the mean + standard deviation, measurement data were assessed with *t*-test, and data were analyzed by correlation analysis. A $p < 0.05$ indicated significant differences.

Table 1. — *Comparison of Cu^{2+} content/VEGF in endometrium and daily copper dissolution quantity of Cu IUD ($\bar{x} \pm s$).*

Items	Cu^{2+} content in endometrium (μmol/L)	Daily copper dissolution quantity of Cu IUD	VEGF expression in endometrium
Control group (n = 36)	1.72 ± 0.46	-	0.283 ± 0.046
Study group 1 (n = 64)	9.05 ± 1.54	18.02 ± 3.6	0.553 ± 0.116
Study group 2 (n = 72)	6.73 ± 0.92	15.17 ± 3.1	0.425 ± 0.149

Results

Content of copper ion

The endometrial copper ion content of study group 1 and group 2 women were, respectively, 9.05 ± 1.54 μmol and 6.73 ± 0 /L 92U mol/L, and the difference was statistically significant ($p < 0.05$). The copper ion content in endometrial tissue of women in the control group was 1.72 ± 0.46 μmol/L, which was less than that of the study group ($p < 0.05$). The copper ion content in endometrial tissues of all study group patients had nothing to do with the insertion time (Table 1).

In vitro release of Cu IUD

Most of the removed Cu IUD had integrity of copper wire, with shades of black surface; the surface of some copper wire included peeling and a lackluster copper color exposed at the peeling site; most copper wire was visible with uneven white or grey white sediments, and there was rare phenomenon, such as copper wire fracture, and copper wire falling off. Daily Cu IUD copper dissolution quantity of group 1 and group 2 was 18.02 ± 3.6 g and 15.17 ± 31 g, respectively, with significant difference ($p < 0.05$, Table 1).

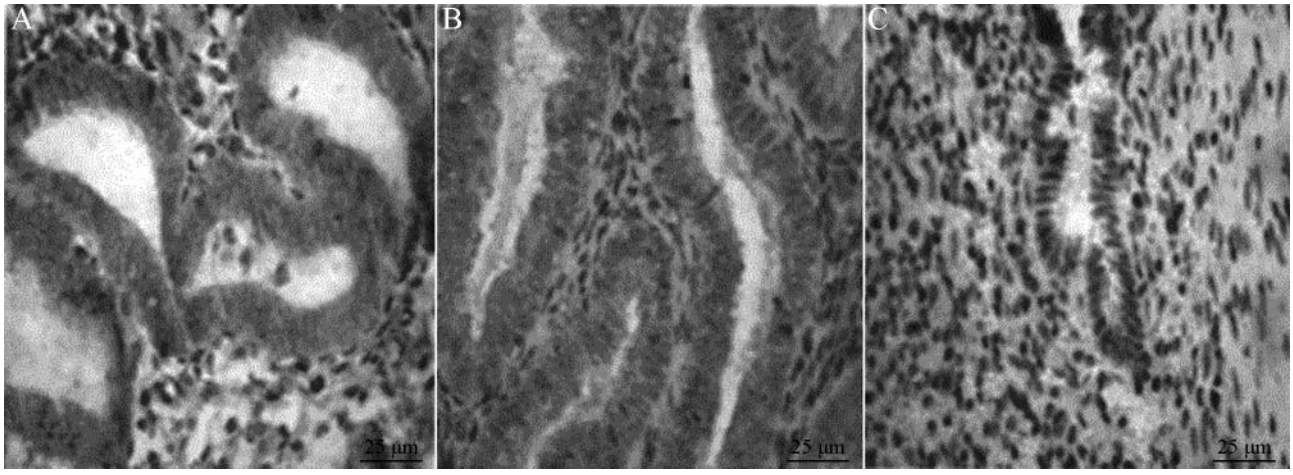

Figure 1. — VEGF expression of the three groups. A: control group; B: study group 1; C: study group 2.

Expression of VEGF

The endometrium of each group all had expression of VEGF. Expression of VEGF (0.553 ± 0.116) of study group 1 (Figure 1B) was significantly higher than that of study group 2 (Figure 1C) (0.425 ± 0.149) and the control group (Figure 1A) (0.283 ± 0.046), and the difference was statistically significant ($p < 0.05$). VEGF expression of study group 2 was significantly higher than that of the control group ($p < 0.05$, Table 1).

The relationship between VEGF and copper content

The endometrial VEGF expression was positively correlated with the content of copper ion ($r = 0.885$, $p < 0.05$).

Discussion

Bleeding irregularities, such as intermenstrual spotting or heavy or prolonged menstrual bleeding, are common among Cu-IUD users and are one of the leading reasons for method discontinuation [12]. The study found that the concentration of copper ion in endometrium of women with IUD was higher than that in the women without IUD. After insertion, the daily dissolution quantity of copper in the in vitro simulated uterine fluid of uterine bleeding women was higher than that of without abnormal bleeding women, and the copper ion content in endometrial tissue of the former was significantly higher than the latter, suggesting that high concentration of copper ion in endometrial tissue may be the cause of abnormal uterine bleeding after insertion of IUD.

TCu220 IUD has a full length 29 mm, a width of 19 mm, a diameter vertical arm 1.8 mm, with 0.4 mm diameter copper wire around the copper, copper surface area being 220 mm^2, and there is nylon tile fiber in the end of the longitudinal rod. No difference was found whether the insertion was immediate or delayed [13]. However, immediately after the insertion, a burst release of copper ions occurs, which may be associated to a variety of side effects [14]. Under normal circumstances, the human endometrium rarely has copper ion content, and the copper concentration may increase after insertion of Cu IUD. Cu IUD may dissolve and release the copper ion in the uterine cavity, leading to destruction of endometrial enzyme system activity, thus affecting glycogen metabolism and the formation of endometrial DNA, estrogen, and progesterone, and leading to interference of endometrial cell, thus playing the contraceptive effect [15]. After insertion of IUD in the uterus, due to its wide contact area with endometrium or larger support, uterine bleeding may be caused, combined with mechanical compression of the device, which may lead to the damage of uterine endothelial cells, the release of a large number of kinins, prostaglandins, and plasmin activity factor, an increase of uterus fibrinolytic system activity, and the vascular permeability, resulting in menorrhagia and menstaxis [16]. Studies have found that the higher the Cu^{2+} concentration, the better the contraceptive effect, but the side effects are more serious, which can easily cause phenomena such as bleeding and menstaxis [7].

There have been reports in the 80's of the last century that, in the no vascular corneal of rabbits, Cu^{2+} can attract the migration of endothelial cells, and hence cause the formation of new capillaries to promote angiogenesis [17]. Later, a large number of experimental studies have confirmed this finding [18]. Human uterine cavity is a complex environment, because of the difference of race and age, composition of the uterine fluid also has a very large variance. The study results of Bastidas *et al.* and Cai *et al.* showed that the kind and concentration of protein in the uterine fluid has a great influence on the dissolution behavior of Cu in IUD, however, the influence of other components in the simulated uterine fluid, such as inorganic chlorine salt, salt, glucose, and so on, on the release of the Cu^{2+} in the Cu-IUD need further study [19, 20].

VEGF is considered to be the most specific angiogenic factor. In 1989, Ferrara *et al.* [21] first isolated and purified this kind of glycoprotein in a bovine pituitary follicle astrocytes culture liquid. VEGF is polypeptide growth factor and combines with heparin, after high affinity binding with KDR and flt- 1 in endothelial cells, which can be taken as the specific mitogen of the endothelial cells to induce hyperplasia of the endothelium, the formation of the capillary loops, and at the same time, increase the permeability of micrangium, through inducing the generation of the mesenchyme, to promote neovascularization [22, 23]. The expression level of VEGF can reflect the level of vascular endothelial cell proliferation and vascular construction. There are studies considering that hypervascularity may be the reason of abnormal uterine bleeding after insertion of IUD [24]. The results of this study suggest that, after insertion of IUD, the change of the microenvironment in the uterine cavity can lead to the enhanced expression of VEGF, while the expression of VEGF may be stronger, indicating that the abnormal bleeding after insertion of IUD may be related to the increased secretion of VEGF in the endometrium and increased density of microvascular. There was one reason which was considered as significantly higher immunoreactivity for VEGFR-1 and VEGFR-3 [25]. The associated research in the study showed that compared to after insertion of IUD and before insertion of IUD, the density of microvascular and the expression of VEGF had all increased [26], which was consistent with the present study. The result of this study also showed that in the endometrium, the expression of VEGF is positively correlated with the content of Cu^{2+}, indicating that the high concentration of Cu^{2+} may cause the high expression of VEGF, causing abnormal bleeding after insertion of IUD. Studies also found that Cu^{2+} is closely related to the expression of VEGF [27, 28], and through influencing the regulatory factor, hypoxia-inducible factor 1 (HIF-1), Cu^{2+} can stimulate

the endothelial cells to upregulate the expression of VEGF [28, 29], and through the HIF-1, which can influence the expression of multi vasculogenesis related gene. Therefore, Cu^{2+} can promote the formation of the new vessels and VEGF can promote the proliferation of endometrial cells and induce angiogenesis, but the new vessels are not mature and can cause blood vessels leakage [30].

This study only assessed the TCu220 IUD; for the IUD of different surface area, the relationship between the copper erosion and the abnormal uterine bleeding need further study.

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