大韓不妊學會雜誌: 第21卷 第3號 1994
Kor. J. Fertil. Steril., Vol. 21, No. 3, 1994, 12

A Decrease in the Circulating Levels of Immunoreactive Insulin-like Growth Factor Binding Protein-1(IGFBP-1) after Endometrial Ablation

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자궁내막 박리후 Immunoreactive Insulin-like Growth Factor Binding Protein-1(IGFBP-1)의 혈중치의 감소에 관한 연구

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=국문초록=

자궁내막이 생체내 insulin-like growth factor binding protein-1(IGFBP-1)의 혈중치를 유지하는데 얼마나 관여하는지를 평가해 보았다. Immunoreactive IGFBP-1의 혈중치의 측정을 위하여, 월경과다를 주소로 내원한 19명의 환자를 대상으로, gynecologic resectoscopy로 자궁내막 박리를 시행하였다. 자궁내막 박리를 시행한 환자의 혈중 IGFBP-1의 평균치는, 시행전과 비교할 때 감소된 소견을 보였으며, 월경주기와는 상관관계가 없었다. 이러한 소견으로 보아, 자궁내막이 혈중 IGFBP-1의 생성원의 하나로 사료되었다.

INTRODUCTION

The endometrial/decidual IGFBPs, called placental protein 12(PP12) and α1-PEG, are found in endometrial/decidual stroma, are regulated by progesterone and are structurally and genetically identical to IGFBP-1, as expressed by the human hepatoma cell line(Guidice, 1991). It has been suggested that cyclic secretion of IGFBP-1 in secretory endometrium during the menstrual cycle and the presence of IGF-1 receptors in the endometrium(Rutanen, 1991) suggests an important local role for IGFs in human endometrial/decidual function. Most of the circulatory(>90%) IGF-1 and IGF-2 are carried by IGFBP-3 complexed with an acid labile unit(150 k-Da)(Daughaday, 1982) and only a minor proportion of IGFs(<10%) are carried by IGFBP-1. Recently, it has been suggested that circulatory IGFBP-1 also has important systemic effects, such as the pathogenesis of anovulation(Pekonen, 1989). Most of the serum IGFBP-1 comes from the liver : however, a small amount also comes from the ovarian follicles(Matikainen, 1992) and uterus(Suikkari, 1987). Several studies have been done with regard to the changes occuring during the menstrual cycle : most studies suggest that is no change in the levels of IGFBP-1 across the menstrual cycle despite cyclic secretion of this protein by the secretory endometrium(Guidice, 1991 : Suikkari, 1987 : Rutenen, 1984 : Seppala, 1988). Suikkari et al. report that the serum level of this protein does not follow the cyclic changes in the levels of the major reproductive

hormone, such as gonadotropin, estradiol and progesterone but noted a significant decrease in serum levels after hysterectomy.

To further understand the correlation between the levels of IGFBP-1 and the phase of the menstrual cycles we have documented the levels of IGFBP-1 after isolated removal of the endometrium.

MATERIALS AND METHODS

Subjects

The study patients comprised 19 premenopausal women, aged from 35 to 47 years, with irregular uterine bleeding requiring endometrial ablation. Seven patients had uterine myoma, six had bloody dyscrasia, three had cystic endometrial hyperplasia and three had recurrent abnormal uterine bleeding refractory to repeated hormonal therapy and curettage. Among the patients with hemorrhagic disorders, three had idiopathic thrombocytopenic purpura, and three had aplastic anemia. The types of myoma were intramural in three cases and submucosal in four cases (two sessile and two pedunculated). None of the patients received any hormonal therapy immediately prior to or during this study. The control group consisted of 14 patients who underwent operative hysteroscopy for a mild degree of uterine synechiae(nine patients), uterine septum(three patients) or an endometrial polyp(two patients). This study was approved by our institutional Medical Research Council.

Blood samples were taken pre-operatively and on the third post-operative day in all patients. To minimize hormonal influences and to observe the immediate effect of loss of endometrium on circulating levels of IGFBP-1, we shortened the time interval between surgery and the second blood sampling to three days. All blood samples from both study subjects and controls were drawn between 08 : 00 and 11 : 00 A.M. in a fasting state to minimize the effect of diurnal variation. The sera were separated immediately and stored at -70℃ until assayed.

Assays

The level of IGFBP-1 was measured in the serum samples using an IGFBP-1 immunoenzymometric assay kit, which uses antihuman IGFBP-1 mouse monoclonal antibody(Medix Biomedica, Finland). This assay has intrassay and interassay coefficient variances of 2.5% and 6.4%, respectively and a detection limit of 0.4 ng/ml. Serum estradiol and progesterone were measured using radioimmunoassay kit (Diagnostic Product Corp., Los Angeles, CA).

Endometrial ablation

The procedure was scheduled during the post-menstrual proliferative phase or during the anovulatory cycles in 13 patients and during the luteal phase in six patients. All of the control subjects were in the proliferative phase. The menstrual cycle phase was defined by clinical history, hormonal assay and histologic postoperatively.

The instrument used for endoscopic resection of endometrium was a gynecologic resectoscope(Richard Wolf, Germany), which has a 24 Fr. outer sheath and a 90-degree angle wire loop electrode. Electric energy was provided by a Valleylab Force-2 electrosurgical generator(Valley-lab Inc. Boulder, CO) with power settings at 30-50 watts of cutting current. The uterine cavity was distended with 32% dextran contained in a hand-held 50 ml syringe through a side channel of the resectoscope. The total volume of dextran never exceeded 500 ml(average;375 ml).

The technique used included shaving of the endometrium from the cornual portion and the fundal surface down to the level of the internal os at a thickness of approximately three to four mm. A ball electrode was used for adequate hemostasis. Sessile and pedunculated myomas were morcellated together with the endometrium. A pediatric Foley cather was placed in the uterine cavity after the procedure in all patients for hemostasis for up to 24 hours. All tissue specimens were sent to the pathology laboratory and immediately frozen or permanently sectioned for tissue pathology. All patients tolerated the procedures well, and there were no significant complications. All patients began taking GnRH analogue or progesterone 1 week after the operation.

Statistical analysis

The Wilcoxon signed rank test was used to assess differences in the pre-operative and post-operative values of serum IGFBP -1. The correlation between IGFBP-1 and estradiol and progesterone was assessed by the Spearman rank correlation test. $P<0.05$ was considered significant.

RESULTS

During the follow-up period of three months, amenorrhea developed in 13 patients(76.5%), hypomenorrhea in two patients and no changes in the menstrual amount was noted in two patients(two patients were lost during the follow-up period). We evaluated the adequacy of endometrial eradication by a through pathologic examination. The surgical specimens of the patients revealed endomyometrium showing an adequate depth of surgical resection.

Serum levels of IGFBP-1 in the control group did not alter significantly after the operation(from 8.04 ± 2.92 ng/ml to 7.50 ± 2.88 ng/ml, mean SEM, $p>0.05$). In the study group, the pre-operative serum IGFBP -1 levels were 10.66 ± 2.45ng/ml(mean$\pm$ SEM) and the levels fell to 4.74 ± 0.91 ng/ml on post-operative day three($p<0.05$) (Fig. 1). The post-operative serum IGFBP-1 levels decreased significantly during the proliferative phase as well as the secretory phase.

There was no apparent difference in the extent of decrement of this protein between the two phases of the menstrual cycle. No correlation between the levels of estradiol or progesterone and that of IGFBP-1 was observed(Fig. 2).

DISCUSSION

IGFBP-1, which has a mol wt of 25,000 based on its aminoacid sequences(Ballard et al., 1989) is a minor binding protein in adult serum but is found in highest concentration is found in amniotic fluid(Rutanen, 1991), and its cDNA has been cloned and sequenced by several groups (Julkunen, 1988;Leeetal, 1988;Brinkman, 1988;Brewer, 1988;Murphy, 1990). It is known to be secreted by the endometrium (Rutanen, 1986) and liver(Povoa, 1985)

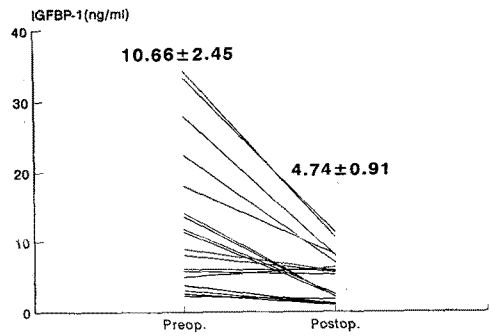

Fig. 1. Changes in circulating levels of IGFBP-1, before and after endometrial ablation. Numerics in figure represent mean$\pm$ SEM. Pre-operative serum IGFBP-1 levels were significantly higher than those on of post -operative day 3($p<0.05$).

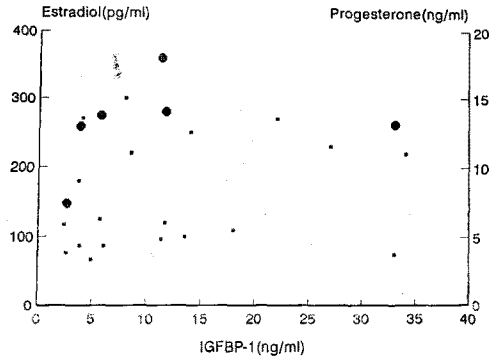

Fig. 2. The relation between serum estradiol level(■) or progesterone level(●) and preoperative IGFBP-1 level. There was no correlation between the parameters($p > 0.05$, Spearman rank correlation test).

and has also been detected in human preovulatory follicular fluid, the lutea cells of hyperstimulated preovulatory follicles, and the corpora lutea(Martikainen, 1992). IGFBP-1 mRNA is expressed abundantly only in the stromal cells of the late secretory endometrium (Julkunen, 1990;Irwin, 1993), although IGFBP-1 has been localized in decidualized stromal cells, and in some cases, in endometrial epithelial cells on immunohistochemistry(Waites, 1988; Wahlstrom, 1982, 1984;Waites, 1989). There has been some heterogeneity observed in terms of tissue localization of IGFBP-1 and its mRNA in vivo and even in some vitro experiments due to the short intracellular half life of mRNA and IGFBP-1(Julkunen, 1988, 1990). Also, it has not been detected in explant cultures of leiomyomata and myometrium(Guidice, 1993). Circulating levels of this binding protein exibit circardian variations and appear to be regulated by insulin(Siukkari, 1989) and nutrient intake(Busby, 1988 ; Baxter, 1988), and its serum half life is approximately seven to eight minutes(Lewitt, 1992). Fasting in humans and rats increase the serum levels of IGFBP-1 and −2(Busby, 1988;Orowski, 1990;Murphy, 1991). Most of the serum IGFBP-1 is reported to be derived from the liver, but a small portion also comes from the ovarian follicles(Martikainen, 1992) and uterus(Seppala, 1988). In addition to its local action in the endometrium its systemic action has also been considered recently to be important(Pekonen, 1989; Thissen, 1994).

Rutenen et al. observed a slight correlation between endometrial and serum pp12, inspite of a strong correlation between the endometrial pp12 and serum progesterone (Rutenen, 1984). However other reporters have not found any changes in the serum levels of IGFBP-1 across the menstrual cycle(Guidice, 1991;Seppala, 1988). Suikkari et al. also could not find out any correlation between the serum levels of IGFBP-1 and the ovarian steroids and noted a significant decrease in serum levels of this protein during the initial postoperative period(Seppala, 1988). In our study a significant decrease in the serum levels of this protein was observed three days post-operatively. This findings suggests that the endometrium at all phases of the menstrual cycle is significantly related with the maintaining of serum IGFBP-1 levels in vivo. Since the half life of serum IGFBP-1 is short(7 to 8 minutes)(Lewitt, 1992), the post-operative three day value is considered to reflect the expression of the new products after operation. To minimize the diurnal variation and nutritional effect, most the of blood samplings were scheduled in the early morning around 8:00 A.M. after an overnight fast. The drawback of this study seems to be a lack of long-term results, as our patients started to take GnRH analogue or progesterone a week after surgery for inhibition of new endometrial growth. Although endometrial production of IGFBP-1 has been thought to be relatively small in comparision with production by the liver, it is concluded from this study that the endometrium, irrespec-

tive of its phase, proliferative or secretory, plays a significant role in maintaining the serum levels of IGFBP-1 in vivo.

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PRECIS

The circulating level of IGFBP-1 decreased after endometrial ablation, indicating that the endometrium contributes to the circulating levels of this protein.