Comparison of Tuba Uterina Leukemia Inhibitory Factor Levels in Pregnant and Non-Pregnant Women: A Preliminary Report

Gürkan **KIRAN**[1], Hakan **KIRAN**[2], Kenan **ERTOPÇU**[3], Metin **KILINÇ**[4], Alanur Menekşe **GÜVEN**[1], Mehmet Ali **VARDAR**[5]

[1]*Department of Obstetrics and Gynecology, Kahramanmaraş Sütçüimam University School of Medicine, Kahramanmaraş, Turkey*
[2]*Prof. Dr. Turan Çetin Women Health and IVF Center, Adana, Turkey*
[3]*Ege Maternity Hospital, Family Planning Center, İzmir, Turkey*
[4]*Department of Biochemistry, Kahramanmaraş Sütçüimam University School of Medicine, Kahramanmaraş, Turkey*
[5]*Department of Obstetrics and Gynecology, Çukurova University School of Medicine, Adana, Turkey*

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Abstract

Objective: The aim of this study was to compare the levels of leukemia inhibitory factor (LIF) in isthmic tubal tissue of pregnant and non-pregnant women. Because of the effects of LIF on successful fertilization and early embryonic development, we postulated that there should be increased LIF concentration in pregnant tubal specimens.
Materials and Methods: Isthmic tubal tissues from 20 women who desire tubal ligation were obtained by mini laparotomy. Study participants were divided into the following 2 distinct groups: (1) normal pregnant women who desire dilatation and currettage and tubal ligation (n=10), (2) non-pregnant women who desire tubal ligation only (n=10). LIF levels were detected in the homogenized fluid by an enzyme-linked immunosorbant assay (ELISA).
Results: There was no significant difference in tubal tissue LIF levels between the groups.
Conclusion: Our study has shown that tubal LIF secretion was normal during early weeks of pregnancy. It is possible that there may be an increase in endometrial LIF production during implantation while the tubal secretion decreases and it may be concluded that paracrine factors are more effective than endocrine factors in the regulation of secretion of LIF.

Keywords: leukemia inhibitory factor, fallopian tubes, first trimester

Özet

Gebelerde ve Gebe Olmayan Kadınlarda Tuba Uterina Leukemia Inhibitory Faktör Düzeylerinin Karşılaştırılması: Bir Ön Çalışma Raporu

Amaç: Bu çalışmanın amacı, gebe ve gebe olmayan kadınlarda istmik tubal dokuda leukemia inhibitory factor (LIF) düzeylerini karşılaştırmaktı. LIF'in başarılı fertilizasyon ve erken embryonik gelişim üzerindeki etkilerinden dolayı, gebelerden elde edilen tuba örneklerinde LIF konsantrasyonlarının daha yüksek olacağı düşünüldü.
Materyal ve Metot: İstmik tubal dokular, tüp ligasyonu isteyen 20 kadından mini-laparotomi ile elde edildi. Hastalar iki gruba ayrıldı: (1) dilatasyon küretajla birlikte tüp ligasyonu talebi olan normal gebe kadınlar (n=10), (2) tüp ligasyonu isteyen gebe olmayan kadınlar (n=10). LIF düzeyleri homojenize sıvıda ELISA yöntemi ile saptandı.
Sonuçlar: Gruplar arasında tubal doku LIF düzeyleri açısından anlamlı fark saptanmadı.
Tartışma: Çalışma gebeliğin erken haftalarında tubal LIF sekresyonunun normal olduğunu göstermektedir. İmplantasyon döneminde endometriyal LIF üretimi artarken tubal dokuda sekresyonun azalması söz konusu olabilir ve LIF sekresyonunun düzenlenmesinde parakrin faktörlerin endokrin faktörlerden daha etkili olduğu söylenebilir.

Anahtar sözcükler: lösemi inhibe edici faktör, fallop tübü, erken gebelik

Corresponding Author: Dr. Gürkan Kıran
Kahramanmaraş Sütçüimam Üniversitesi Tıp Fakültesi
Kadın Hastalıkları ve Doğum AD
Yoruk Selim Mah. Hastane Cad. No: 32, 46050
Kahramanmaraş, Türkiye
Phone : +90 344 221 23 37/307
Fax : +90 344 221 23 71
E-mail : gurkankiran@yahoo.com

Introduction

Implantation is the process by which the blastocyst becomes connected with the maternal endometrium/decidua (1). This is a critical step in the establishment of pregnancy. The endometrium must be appropriately prepared for implantation after ovulation in each reproductive cycle, and to this end it undergoes considerable remodelling during the cycle. For most of the human menstrual cycle, the endometrium is hostile to embryo implantation, and there is a brief period in time in which the endometrium becomes receptive, the so-called "window of receptivity" in each cycle. The endometrium then returns to a non-receptive or refractory period for the remainder of the cycle (2).

Successful implantation is most likely a function of both embryonic and maternal processes. There are many factors involved in the regulation of blastocyt implantation. One of these factors is leukemia inhibitory factor (LIF) (1). LIF is a pleiotrophic cytokine that has some effects in various tissues and involved in proliferation, differentiation and cell survival (3,4). In ovine and murine embryo experimentation the addition of recombinant LIF to *in vitro* culture media increased implantation and pregnancy rate (5). There may be an increase in LIF secretion in tubal tissue during early period of pregnancy, as well.

In the present study, we hypothesized that LIF secretion in fallopian tube could be elevated during early weeks of pregnancy.

Materials and Methods

The study was conducted at the University Hospital in Kahramanmaraş. Permission to use the tubal tissues was provided by the instutitional review board. Written informed consent was obtained from each woman before surgery. Segments of human fallopian tubes were obtained from specimens after tubal ligation. From January 2003 to September 2003 inclusive, isthmic tubal tissues from 20 women who desire tubal ligation were obtained by mini-laparotomy. Study participants were divided into two groups: (1) normal pregnant women who desire dilatation and currettage and tubal ligation (n=10), (2) non-pregnant women who desire tubal ligation only (n=10). All pregnant subjects were at 6-8th weeks of gestation. Gestational age was confirmed by ultrasound examination. Isthmic tubal segments were obtained from all subjects. Exclusion criteria included a history of ectopic pregnancy, pelvic infection or tubal operation. All samples were collected in saline and kept -20°C for further ELISA testing.

LIF immunoassay. All samples were washed with saline and weighed. Then the samples were homogenized in phosphate buffered distilled water (0.01 M, pH: 7.0) in 1:5 ratio (weight/volume, g/ml) with 12000 rpm. LIF levels were detected in the homogenized fluid by an enzyme-linked immunosorbant assay (ELISA) method using an ELISA from Med Systems Diagnostics GmbH, Cat. No: BMS 242, Vienna, Austria. The sensitivity for LIF was less than 1.0 pg/ml. The intra- and inter-assay coefficients of variation were 5.5% and 7.0%, respectively. The results were given as pg/mg wet tissue.

Statistical analysis. Because of the non-normal distribution of data, all values were expressed as median and range. Biochemical and clinical data were evaluated by Mann-Whitney U-test. Differences were considered significant for a p value <0.05. Analyses were performed by using SPSS software, version 9.0 for Windows (SPSS Inc., Chicago, IL).

Results

We examined the tubal LIF secretory capacity in both pregnant and non-pregnant women. We found that there was an extensive LIF secretion by tubal tissue in all women. There was no statistically significant difference between the groups (p=0.579). The clinical characteristics and biochemical data of the subjects are presented in Table 1.

Discussion

There are many mechanisms of biochemical changes in the endometrium throughout the menstrual cycle, implantation, and maintenance of pregnancy. Evidences support a central role of cytokines and growth factors in endometrial physiology: in cyclic mitosis and differentiation of endometrial cellular components, early pregnancy establishment, tissue shedding in the absence of implantation and endometrial regeneration (6). There are some similar changes for preparing to implantation other than endometrium in genital organs, for example in fallopian tubes. The human fallopian tube is the physiologic site of fertilization and early embryo development (7-9).

LIF is a secreted glycoprotein that displays multiple activities in various tissues and cell types (10, 11). Some of these activities are the induction of differentiation in myeloid leukemia cell lines, the induction of the acute-phase response in hepatocyte cultures, the suppression of differentiation in normal embryonic stem cells, the stimulation of calcium release from bones and the establishment of the cholinergic phenotype in rat symphathetic neurons (12-15). In a recent study, it has been shown that LIF increased long-term sperm motility and survival *in vitro* in a concentration-dependent manner (16).

Table 1. Clinical characteristics and biochemical data of the subjects

	Pregnant (n=10)		Non-pregnant (n=10)		
Characteristic	Median	Min-max	Median	Min-max	p
Age (years)	31	25-37	38	33-43	0.003†
Gravidity	4	2-8	4.5	1-11	0.809
Parity	3	2-7	2.5	0-5	0.539
LIF (pg/mg)	0.052	0.017-1.770	0.053	0.003-0.682	0.579

†Age in non-pregnant group was significantly higher than pregnant group (p<0.05)

LIF was first recognized as playing a critical role in embryo implantation when female mice deficient in the LIF gene ($LIF^{-/-}$) failed to become pregnant (2). It may be an active participant in the biochemical processes of implantation and the regulation of the expression of LIF may have an important role in the physiological and pathological processes of human implantation, too. It is actively secreted by decidual tissue (17). It has been shown that LIF was expressed throughout menstrual cycle, but there was a striking increase in the LIF messenger ribonucleic acid (mRNA) levels in the mid- and late-secretory phase samples. Endometrial LIF expression was not dependent on the presence of pregnancy, and that the highest level of expression occurred in the mid- and late-secretory phase, indicates that expression was under maternal control, possibly as a direct response to the increase in circulating estrogen and progesterone levels, which occurred during the secretory phase (1). In another study, it has been shown that there was a disregulation of LIF production in the endometrium during both the proliferative and the secretory phases of the cycle in infertile women. The lack of surge of endometrial LIF production was determined during the secretory phase in these women (18).

It has also been shown that in periovulatory and luteal phases, endometrial LIF expressions of fertile women were statistically higher than those of infertile women while expressions were comparable in the follicular phase between both groups (19). Quantitative endometrial LIF deficiency is associated with failure of successful implantation (20).

Although there are many studies which investigate the effects of LIF on endometrium, there are very few studies about its secretion and function in tubal tissue. It has been shown that LIF mRNA and tubal secretion of LIF was markedly elevated in association with ectopic pregnancy (7). It has been shown that in ectopic pregnancy serum LIF concentration was lower than that in intrauterine pregnancy (21).

In our study we collected the samples from non-pregnant women in early proliferative phase. Keltz et al. have shown that the level of LIF mRNA varied minimally in tubal samples throughout the menstrual cycle in non-pregnant women. High constitutive secretion of LIF in the ampullary portion may be important in the support of early pluripotent embryonic cells and may lead to greater viability of the early embryo (7). We did not find a significant difference in LIF levels in isthmic tubal tissue between pregnant and non-pregnant women. In pregnant group, women were younger than the other group. The difference was statistically significant but in our opinion, this difference was negligible. Because, it has been shown that tubal LIF mRNA levels did not vary with age during the reproductive years except perimenopausal period (7).

In conclusion, our study has shown that tubal LIF secretion was normal during early weeks of pregnancy. It is possible that there may be an increase in endometrial LIF production during implantation while the tubal secretion decreases. Because there is no significant change in hormonal mileu during early weeks of pregnancy, it may be postulated that signals originated from early embryo or paracrine factors are more effective than endocrine factors in this fine regulation of LIF secretion.

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