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Endometrial Interleukin-6 in Unexplained Infertility

Mortada El-Sayed Ahmed
Department of Obstetrics and Gynecology, Ain Shams University, Cairo, Egypt.
Email contact: mortadaelsayed@yahoo.com

Abstract

30-40% of infertile couples are diagnosed as unexplained infertility, and, failure of implantation is claimed to be the etiology behind many cases diagnosed as unexplained infertility. Emerging evidence links deficiency and excess of Interleukin-6 (IL-6) to reproductive impairment; this leads to the interest in how IL-6 contributes to fertility. This is a case-control study of total 80 women; 40 women with primary unexplained infertility and 40 control fertile women to compare mid-secretory endometrial IL-6 in normal fertile women and women with unexplained infertility. Mid-luteal endometrial IL-6 was significantly higher in the control group (41.6 ± 19.8 pg/dl) than in infertile group (15.5 ± 5.5 pg/dl) (P < 0.0001, CI 95%; -32.57 to -19.63). Receiver-operating characteristic (ROC) curve analysis showed the best cutoff value of >22.6 pg/dl for discrimination between fertile and infertile women. In conclusion, Women with unexplained infertility show lower levels of mid-luteal endometrial IL-6 when compared to fertile women. This implies that IL-6 might have an important role in human embryo implantation.

Keywords

Infertility; endometrial; Interleukin-6; immunoglobulin

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I. Introduction

Unexplained infertility is referred to as a diagnosis (or more is lack of diagnosis) reached in couples where all standard investigations for infertility are normal. Usually, there is disagreement among practitioners concerning this subject, but 30-40% of infertile couples are diagnosed under the category of unexplained infertility [1]. Successful implantation needs a synchronized dynamic process where both the embryo and endometrium are well developed to allow their coalescence during a short window of endometrial receptivity. Cytokines and immune cells are intimately involved in both aspects of the process [2]. Endometrial priming for optimizing the implantation window has been subject of research for decades. The embryo factor is thought to account for only one-third of implantation failure while endometrial receptivity accounts for two-thirds of implantation failures [3]. Emerging evidence links deficiency and excess of Interleukin-6 (IL-6) to reproductive impairment; this lead to the interest in how IL-6 contributes to fertility [4]. IL-6 is a cytokine that induces immunoglobulin production in activated B cells; it was also found to have a variety of functions outside the B-lymphocyte system. IL-6 expression has been detected in human endometrium, with highest levels detected during the luteal phase [3]. Serum IL-6 and cervical mucus in women with unexplained infertility were found to be higher when compared to fertile women [5, 6], also, increased IL-6 trans-signaling was claimed to be associated with unexplained infertility [4]. During the implantation window, IL-6 receptors are found in both the endometrium and the blastocyst; this suggests a paracrine/ autocrine role of IL-6 during the implantation period. Experiments on mice with disrupted IL-6 gene showed compromised growth and development of the blastocyst itself [3, 7]. This suggests the role of IL-6 deficiency in infertility even after successful implantation. This case-control study was conducted to evaluate mid-secretory endometrial IL-6 in normal fertile women and women with unexplained infertility.

II. Material and Methods

This is a case-control study of 40 women with primary unexplained infertility recruited from outpatient infertility clinic and 40 control fertile women, recruited from outpatient family planning clinic of Ain Shams University Hospital during the period from October 2017 to February 2019. Approval of the Research Ethics Committee was obtained and written informed consent was obtained from all patients before participation. The required sample size was calculated using the G*Power software version 3.1.7 (Universität Düsseldorf, Germany). The primary outcome measure was the differences between the two groups as regards mid-luteal IL-6. It was estimated that a sample of 40 patients in either study group would have a power of 82% (type II error, 0.18) to detect a statistically significant difference between the two study groups for a medium-to-large effect size of Cohen's d = 0.65, which is equivalent to a difference of 0.65 SD in the outcome measures. This difference was chosen as it

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could be regarded as a clinically relevant difference to seek in this pilot study. This calculation used a two-sided unpaired t test and assumed a two-tailed type I error of 0.05, i.e., a confidence level of 95%. Women with normal ovulatory cycles (as proven by folliculometry and/or mid-luteal serum progesterone ≥ 10 ng/ml), good ovarian reserve (as proven by day3 FSH < 10 IU/L) [8], normal hormonal profile (serum PRL < 20 ng/mL, and TSH 0.4-4.2 mcU/mL), patent tubes (as proven by Hysterosalpingography and/or laparoscopy with +ve dye test), no recognizable cause of infertility by clinical examination and/or ultrasound, normal semen analysis of the partner [9], and infertility for at least one year of unprotected intercourse were included in the study. Women were asked to abstain from intercourse or use a barrier method of contraception during the study cycle. Folliculometry for determining the ovulation was done during the study cycle, and mid-secretory (5-9 days post-ovulation) endometrial office biopsy using ENDOCELLTM endometrial cell sampler (WALLACH Surgical Devices, USA) was obtained. Levels of IL-6 were measured in the conditioned medium sampled at the 4-h time point using AviBion Human IL-6 ELISA kit by (Oregenium Laboratories, Vantaa, Finland) according to the manufacturer's instructions. The characteristics of the ELISA as reported by the manufacturer are sensitivity <7pg/ml; range 7.8-500 pg/ml; Intra-Assay-Precision ≤ 9.4%; Inter-Assay-Precision ≤ 8.6%. Fresh culture medium (DMEM/F12) was used to dilute the standards and used as a zero standard. Endometrial biopsies were rinsed in a mixture of DMEM/F12. All the biopsies were used for short-term culture. This was rinsed twice in DMEM/F12, pre-warmed to 37 C, cut into approximately 2-3 mm pieces and placed in 6 ml of DMEM/F12 in a 6-well plate. This was then cultured at 37 C in a 5% Co2 humidified incubator for 4 hours then aliquots of culture fluid were removed. Cellular debris was removed by centrifugation. The supernatants were stored at -20 C until assayed by ELISA.

Data were analyzed using IBM© SPSS© Statistics version 22 (IBM© Corp., Armonk, NY, USA) and MedCalc© version 13 (MedCalc© Software bvba, Ostend, Belgium). The D'Agostino-Pearson test was used to examine the normality of numerical data distribution. Normally distributed numerical variables were presented as mean and SD, and intergroup differences were compared using the independent-samples (unpaired) t-test. The Welch test was used in place of the t test whenever equality of variance could not be assumed. Categorical data were presented as number and percentage. Correlations among numerical variables were tested parametrically using the person product-moment correlation. Receiver-operating characteristic (ROC) curve analysis was used to examine the value of mid-luteal phase IL-6 for discrimination between cases and controls. The DeLong method was used to compare the area under various ROC curves (AUC). A two-sided p-value <0.05 was considered statistically significant.

III. Results

Eighty women were included in the study, 40 women with primary unexplained and 40 fertile women as control. There was no significant difference in the demographic characteristics between both groups (Table 1). Women in the study group had normal hormonal assay (Table 2). Mid-luteal

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endometrial IL-6 was significantly higher in the control group (41.6 ± 19.8 pg/dl) when compared to the infertile group (15.5 ± 5.5 pg/dl) (P < 0.0001, CI 95%; -32.57 to -19.63) (Table 3). Receiver-operating characteristic (ROC) curve analysis showed the best cutoff value of >22.6 pg/dl for discrimination between fertile and infertile women using mid-luteal phase endometrial IL-6 (Table 4).

IV. Discussion

Several studies have investigated the endometrial expression of many physiological signals during the luteal phase as markers for endometrial receptivity in humans [10]. Cytokine-network disorders at the utero-placental unit have been implicated in the etiology of human implantation failure [11-12]. Leukemia inhibitory factor (LIF), a cytokine belonging to the IL-6 family, has demonstrated its importance for implantation and trophoblast development [13, 14]. IL-6 was found to be expressed at lower levels in the endometrium reaching maximum levels at the mid-luteal phase [15], this implies the importance of the IL-6 cytokine family in the process of implantation. IL-6 itself is claimed to play a role in implantation; even pregnancy losses have been associated with deficient levels of IL-6 [16-18]. Little studies investigated mid-luteal endometrial IL-6 and their results were consistent with these of this study where it showed decreased levels of IL-6 in mid-secretory endometrium of women with unexplained infertility [15], even inadequate expression of IL-6 in endometrial tissue might predispose to recurrent miscarriage which implies the role of this cytokine in normal development of blastocyst and not only implantation [19].

Evidence still needs further studies to explore the role of IL-6 and its family in the process of implantation, in spite of several studies implying a deregulation of endometrial LIF production in unexplained infertility and multiple failures of implantation [20] and deficiency of endometrial IL-6 in women with unexplained infertility [15, 21], others have denied decrease in endometrial IL-6 expression in unexplained infertile women with multiple implantation failures [22]. Our study suggests, based on the decreased levels of mid-secretory endometrial IL-6 in women with unexplained infertility, that IL-6 cytokine family might play an important role in the process of human embryo implantation. Further studies are needed to investigate the impact of these cytokines deficiency on human fertility and the possible role of recombinant IL-6 or LIF in the management of women with unexplained infertility and implantation failure.

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V. CONCLUSION

Women with unexplained infertility show lower levels of mid-luteal endometrial IL-6 when compared to fertile women. This implies that IL-6 might have an important role in human embryo implantation.

VI. References

[1] Management of symptomatic [1] Ray A, Shah A, Gudi A, Homburg R (2012). Unexplained infertility: an update and review of practice. Reprod Biomed Online, 24(6), 591-602.

[2] Robertson SA, Moldenhauer LM (2014). Immunological determinants of implantation success. Int J Dev Biol, 58(2-4), 205-217.

[3] Achache H, Revel A (2006). Endometrial receptivity markers, the journey to successful embryo implantation. Hum Reprod Update, 12(6), 731-746.

[4] Prins JR, Gomez-Lopez N, Robertson SA (2012). Interleukin-6 in pregnancy and gestational disorders. J Reprod Immunol, 95(1-2), 1-14.

[5] Naz RK, Butler A (1996). Interleukins-6 and -8 levels in sera and cervical mucus of fertile, idiopathic infertile, and immunoinfertile women: implication in infertility. Am J Reprod Immunol, 35(6), 534-540.

[6] Demir B, Guven S, Guven ES, Atamer Y, Gul T (2009). Serum IL-6 level may have role in the pathophysiology of unexplained infertility. Am J Reprod Immunol, 62(4), 261-267.

[7] Salamonsen LA, Dimitriadis E, Robb L (2000). Cytokines in implantation. Semin Reprod Med, 18(3), 299-310.

[8] Practice Committee of the American Society for Reproductive Medicine (2015). Diagnostic evaluation of the infertile female: a committee opinion. Fertil Steril, 103(6), e44-50.

[9] Cooper T, Noonan E, von Eckardstein S, Auger J, Baker H, Behre H, Haugen T, Kruger T, Wang C, Mbizo M, Vogelsong K (2010). World Health Organization reference values for human semen characteristics Hum Reprod Update, 16(3), 231-245.

[10] Giudice LC (1999). Potential biochemical markers of uterine receptivity. Hum Reprod, 14 (Suppl 2), 3-16.

[11] Giudice LC (1994). Growth factors and growth modulators in human endometrium: their potential relevance to reproductive medicine. Fertil Steril, 61(1), 1-17.

http://www.ijogr.com/

[12] Hunt JS (1989). Cytokine network in the uteroplacental unit: Macrophage as a pivotal regulatory cell. J Reprod Immunol, 16(1), 1-17.

[13] Sharkey AM, Smith SK (2003). The endometrium as a cause of implantation failure. Best Pract Res Clin Obstet Gynaecol, 17(2), 289-307.

[14] Cheng JG, Rodriguez CI, Stewart CL (2002). Control of uterine receptivity and embryo implantation by steroid hormone regulation of LIF production and LIF receptor activity: towards a molecular understanding of ‘‘the window of implantation’’. Rev Endocr Metab Disord, 3(2), 119-126.

[15] Sherwin JR, Smith SK, Wilson A, Sharkey AM (2002). Soluble gp130 is up-regulated in the implantation window and shows altered secretion in patients with primary unexplained infertility. J Clin Endocrinol Metab, 87(8), 3953-3960.

[16] Tabibzadeh S, Kong QF, Babaknia A, May LT (1995). Progressive rise in the expression of interleukin-6 in human endometrium during menstrual cycle is initiated during the implantation window. Hum Reprod, 10(10), 2793-2799.

[17] Sakurai T, Takai R, Bürgin H, Ishihara K, Sakamoto Y, Amano J, Higuchi Y, Chiba S, Singer T, Kawamura A, Suzuki M, Müller L (2012). The effects of interleukin-6 signal blockade on fertility, embryo-fetal development, and immunization in vivo. Birth Defects Res B Dev Reprod Toxicol, 95(4), 304-317.

[18] Dimitriadis E, White CA, Jones RL, Salamonsen LA (2005). Cytokines, chemokines and growth factors in endometrium related to implantation. Hum Reprod Update, 11(6), 613-630.

[19] Jasper MJ, Tremellen KP, Robertson SA (2007). Reduced expression of IL-6 and IL-1alpha mRNAs in secretory phase endometrium of women with recurrent miscarriage. J Reprod Immunol, 73(1), 74-84.

[20] Hambartsoumian E (1998). Endometrial Leukemia Inhibitory Factor (LIF) as a Possible Cause of Unexplained Infertility and Multiple Failures of Implantation. Am J Reprod Immunol, 39(2), 137-143.

[21] Junovich G, Azpiroz A, Incera E, Ferrer C, Pasqualini A, Gutierrez G (2013). Endometrial CD16(+) and CD16(-) NK cell count in fertility and unexplained infertility. Am J Reprod Immunol, 70(3), 182-189.

[22] Wu M, Yin Y, Zhao M, Hu L, Chen Q (2013). The low expression of leukemia inhibitory factor in endometrium: possible relevant to unexplained infertility with multiple implantation failures. Cytokine, 62(2), 334-339.

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Tables:

Table 1 Characteristics of fertile and infertile women

Variable	Fertile (n=40)	Infertile (n=40)	p-value
Age (years)	27.2 ± 5.4	27.3 ± 4.9	0.939
BMI (kg/m^2)	26.4 ± 3.8	25.4 ± 3.4	0.197
Parity	1.7 ± 0.7	N/A	-
Duration of infertility (years)	N/A	3.2 ± 1.4	-

Table 2 Hormonal assay in women with unexplained infertility

Variable	Mean ± SD
Day 3 FSH (mIU/ml)	6.72 ± 1.50
Day 3 LH (mIU/ml)	4.10 ± 1.68
Day 3 E2 (pg/ml)	41.95 ± 10.66
sPRL (ng/ml)	11.16 ± 3.62
TSH (mIU/l)	1.98 ± 0.49
Mid-luteal progesterone (ng/ml)	15.29 ± 4.27

FSH: Follicular stimulating hormone, LH: Luteinizing hormone, E2: Estradiol, sPRL: serum Prolactin. TSH: Thyroid stimulating hormone.

Table 3 Comparison of mid-luteal phase IL-6 in control and infertile women

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Variable	Infertile (n=40)	Control (n=40)	p-value	CI 95%
IL-6 (pg/dl)	15.5 ± 5.5	41.6 ± 19.8	**<0.0001**	-32.57 to -19.63

Table 4 Receiver-operating characteristic (ROC) curve analysis for discrimination between fertile and infertile women using mid-luteal phase IL-6

ROC index	Value
Area under the ROC curve (AUC)	0.902 (95% CI, 0.827 to 0.977)
p-value (AUC0=0.5)	**<0.0001**
Youden index J	0.75
Best cutoff criterion (pg/dl)	>22.6
Sensitivity (%)	85 (95% CI, 70.2 - 94.3)
Specificity (%)	90 (95% CI, 76.3 - 97.2)
Positive predictive value (%)	89.5 (95% CI, 75.2 - 97.1)
Negative predictive value (%)	85.7 (95% CI, 71.5 - 94.6)

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