Assessment of Sub-Endometrial Blood Flow and Endometrial Leukemia Inhibitory Factor as a Marker for Endometrial Receptivity in Women with Unexplained Infertility

Thesis

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Abstract

Background: Infertility is customarily defined as the inability to conceive after 1 year of regular unprotected intercourse. The infertility evaluation is typically initiated after 1 year of trying to conceive, but in couples with advanced female age (> 35 years), most practitioners initiate diagnostic evaluation after an inability to conceive for 6 months. **Aim of the Work:** To assess endometrial receptivity in women with unexplained infertility using sub-endometrial vascular flow resistant index and endometrial leukemia inhibitory factor (LIF). **Patients and Methods:** This clinical controlled trial was conducted at Ain Shams University Maternity Hospital during the period from August 2014 to September 2017 on 140 patients divided into two equal groups: **Group I (study group):** women with unexplained infertility defined as inability to conceive inspite of regular marital life for at least 12 months. **Group II (control group):** matched women with infertility due to tubal factor, recruited from outpatient gynecology or infertility clinic at Ain Shams University Maternity Hospital. **Results:** A cutoff value for LIF staining score of ≤1 predicted unexplained infertility with a sensitivity of 73.33% and specificity of 70.67%; whereas a cutoff of > 0.71 for subendometrial blood flow RI predicted unexplained infertility with a sensitivity of 70.67% and specificity of 86.67%. A cutoff of ≤10 mm for endometrial thickness had the highest sensitivity of 100%, but lacked specificity (only 16%). **Conclusion:** Leukemia inhibitory factor may be a predictor for unreceptive endometrium in cases of unexplained infertility. Subendometrial blood flow RI and endometrial thickness may be used rather than LIF IHC (due to its invasive nature)for the prediction of endometrial factor status in cases of unexplained infertility because of the statistically significant negative correlation between the above parameters. **Recommendations:** Further studies are needed to evaluate the predictive value of the multiple logistic model including (endometrial thickness, subendometrial blood flow color Doppler and LIF IHC score and even other proposed cytokines as VEGF, IL6 and integrins) in unexplained infertility.

Key words: sub-endometrial blood flow, endometrial leukemia, inhibitory factor, endometrial receptivity, unexplained infertility

INTRODUCTION

Unexplained infertility refers to the absence of a definable cause for a couple's failure to achieve pregnancy after 12 months of attempting conception despite a thorough evaluation, or after six months in women 35 and older ***(ASRM, 2008).***

Unexplained infertility affects 15% of couples. Infertility rates are influenced by a woman's age. Although the rate is approximately 10% at 30 years of age, it can be as high as 40% by the time a woman reaches 40 years of age ***(Ray et al., 2012).***

Diagnosis of unexplained infertility includes a semen analysis, assessment of ovulation, a hysterosalpingogram, and if indicated, tests for ovarian reserve and laparoscopy. When the results of a standard infertility evaluation are normal, practitioners assign a diagnosis of unexplained infertility. Although estimates vary, the likelihood that all such test results for an infertile couple are normal (i.e, that the couple has unexplained infertility) is approximately 15% to 30% ***(ASRM, 2006).***

In the absence of a correctable abnormality, the therapy for unexplained infertility is, by default, empiric. Proposed treatment regimens include intrauterine

insemination (IUI), ovulation induction with oral or injectable medications, combination of IUI with ovulation induction, and assisted reproductive technologies (ART) ***(ASRM, 2006)***.

Embryo implantation represents the most critical step of the reproductive process in many species. It consists of a unique biological phenomenon, by which the blastocyst becomes intimately connected to the maternal endometrial surface ***(Aplin, 2000)***.

The endometrium is normally a non-receptive environment for an embryo, except during implantation window. Implantation window is a period during which the endometrium is optimally receptive to implanting blastocyst. Implantation of the human embryo may occur only during a regulated "implantation window" on days 6-10 postovulation, and surrounded by refractory endometrial status ***(Aboubakr et al., 2004).***

The use of digitally analyzed power angiography, a new non-invasive technology to assess blood flow and vascular characteristics, could provide information regarding the local angiogenic processes occurring in the endometrium. Local angiogenesis is essential for implantation and gestation ***(Nardo et al., 2005)***.

Endometrial blood flow reflects properly the uterine receptivity because the endometrium is the site where embryonic implantation takes place ***(Merce, 2002).***

Many endometrial derived cytokines and growth factors play an important role in the initial process of successful implantation in human. Any failure in the production or regulation of these cytokines or growth factors may be a cause of unexplained infertility, among these cytokines, is leukemia inhibitory factor (LIF) ***(Sharkey et al., 2003).***

Maternal LIF affects trophoblast growth and development and is essential for implantation and has been described as a marker of the embryo implantation process. LIF is expressed on endometrium of uterus. Low levels of LIF are found in the proliferative phase and maximal expression is found during the mid-secretory phase which occurs between days 5 and 10 following the luteinising hormone (LH) surge ***(Manwu et al., 2013).***

AIM OF THE WORK

To assess endometrial receptivity in women with unexplained infertility using sub-endometrial vascular flow resistant index and endometrial leukemia inhibitory factor (LIF).

Chapter (1)

UNEXPLAINED INFERTILITY

Introduction:

Unexplained infertility is a subject on which agreement is rarely found among practitioners. Unexplained infertility usually refers to a diagnosis (or lack of diagnosis) made in couples in whom all the standard investigations such as tests of ovulation, tubal patency and semen analysis are normal. Unexplained infertility is a term that has been applied to as many as 30-40% of infertile couples ***(Ray et al., 2012).***

According to the American Society of Reproductive Medicine ***(ASRM, 2015),*** diagnostic evaluation for infertility should include assessment of ovulatory function, structure, and patency of the female reproductive tract, and semen analysis of the husband. However routine laparoscopy should not be performed in the evaluation of the infertile female but may be warranted when there is a strong suspicion of advanced stage endometriosis, tubal occlusive disease, or peritoneal factors. The practice committee also reported that ovarian reserve testing, post coital testing, and endometrial biopsy should not be performed as a part of the routine diagnostic evaluation of the infertile female. Even highly sophisticated tests may fail to

detect very subtle abnormalities responsible for subfertility. Although estimates vary, the likelihood that all such tests for an infertile couple are all normal (i.e. that the couple has unexplained infertility) is approximately 15% ***(Guzick et al., 1994).***

Definition

The 'diagnosis' of unexplained infertility is made when tubal patency (hysterosalpingogram and/or laparoscopy), normal ovulatory function (basal body temperature, cervical mucus changes, serum LH surge, folliculometry or mid-luteal progesterone) and normal semen analysis are established. Infertility is usually described as 1 year of unwanted non-conception with unprotected intercourse in the fertile phase of the menstrual cycle ***(Evers, 2002),***

According to guidelines from the National Institute for Health and Clinical Excellence ***(NICE, 2013),*** A woman of reproductive age who has not conceived after 1 year of unprotected vaginal sexual intercourse, in the absence of any known cause of infertility, should be offered further clinical assessment and investigation along with her partner. In the general population, of couples attempting conception, 84% will conceive after 1 year and 92% will conceive after 2 years ***(Ray et al., 2012).***

Prevalence

The proportion of couples suffering from unexplained infertility is popularly quoted as 16% but the range varies from 0% to 37%. The prevalence of unexplained infertility is still truly debatable because the diagnosis of unexplained infertility is due to lack of a specific test or may be due to misdiagnosis. The most frequent reasons for the latter are endometriosis, mild degrees of tubal infertility, premature ovarian failure and immunological causes. Although these subtel causes may be responsible for so-called unexplained infertility, it may not be in the best interests of the patient to subject them to all these invasive tests. The result may well only fulfil clinical curiosity without helping in better clinical decision making ***(Ray et al., 2012).***

Possible Etiologies

1- Hormonal disorders:

• Subtle ovulatory dysfunction:

Subtle ovulatory dysfunction has been postulated as an etiology of unexplained infertility. A history of regular menstrual cycles provides an indication that ovulation is most likely taking place. Nevertheless, current diagnostic tests for ovulation provide only indirect evidence of ovulation and can mil confirm the actual release of the oocyte. The Luteinized

unruptured follicle syndrome (LUF) has been suggested to be a cause of infertility in couples in whom the hormonal evaluation is normal, yet conception doesn't take place. Although this condition arises sporadically with some frequency, no evidence that it occurs consistently ***(Harrison et al., 2000).***

In the absence of an ideal method, the measurement of midluteal progesterone levels in plasma is probably the most effective compromise to indicate that ovulation has occurred using hormonal method. Because progesterone is released in a pulsatile manner, more than one assay may be needed to determine serum levels accurately. The value of >3 ng/ ml correlates with a finding of secretory endometrium and has been used for presumptive indication of ovulation ***(Blacker et al., 1997).***

Hull (1992) found that a value 10 ng/ ml or more closely correlate with subsequent conception, suggesting that lower level, while indicative of ovulation, may be associated with subtle ovulatory inadequacy. Ovulatory dysfunction not detectable by current diagnostic techniques may be an etiology of unexplained infertility.