Role of Sildenafil Citrate on early unexplained recurrent pregnancy Loss. A randomized controlled study

A Thesis

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Mahmoud Yakout Mohammed

Contents

List of Abbreviations

Abbr.	Full-term
AFC	**:** Antral follicle count
ANA	**:** Antinuclear antibody
APS	**:** Antiphospholipid syndrome
cGMP	**:** Cyclic guanosine monophosphate
CI	**:** Confidence interval
EDRF	**:** Endothelium-drived relaxing factor
EISA	**:** enzyme-linked immunosorbent assay
FGR	**:** Fetal growth restriction
FSH	**:** Follicle stimulating hormone
Hb	**:** Hemoglobin
hCG	**:** Human chorionic gonadotropin
HPV	**:** Human papillomaviruses
HSG	**:** Hysterosalpingography
IFN	**:** Interferon
Ig	**:** Immunoglobulin
IL	**:** Interleukin
IVF	**:** In vitro fertilization
LGL	**:** Large granular lymphocytes
LH	**:** Luteinizing hormone
LMP	**:** Last menstrual period
LMWH	**:** Low-molecular weight heparin

MRI : Magnetic resonance imaging
NK : Natural killer
NO : Nitric oxide
PCOS : Polycystic ovary syndrome
PGD : Preimplantation genetic diagnosis
PGS : Preimplantation screening
pNK : Peripheral blood NK cells
PR : Progesterone receptors
RM : Recurrent miscarriage
RPL : Recurrent pregnancy loss
RR : Risk ratio
RSM : Recurrent unexplained spontaneous miscarriage
SC : Sildenafil citrate
SD : Standard deviation
sGC : Soluble guanylate cyclase
SPSS : Statistical package for social science
TPO : Thyroid peroxidase
TSH : Thyroid stimulating hormone
TVS : Transvaginal ultrasound
uNK : Uterine natural killer
URA : Unexplained recurrent abortion

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Abstract

Background: Recurrent unexplained pregnancy loss is frustrating for patient and clinician which is defined as loss of three or more consecutive pregnancies before 20 weeks' gestation. Multiple etiologies for recurrent unexplained spontaneous miscarriage have been reported including autoimmune, endocrine, anatomic, genetic factors. Aim of the Work: to assess the efficacy of sildenafil therapy in prevention of recurrent miscarriage. **Patients and Methods:** a randomized controlled clinical trial was conducted in Ain-Shams University Maternity Hospital on 90 women with a history of recurrent early unexplained spontaneous miscarriage. **Results:** There was no statistically significant difference between the study groups regarding demographic data (age, parity and BMI). There was also no statistically significant difference between both study groups regarding transvaginal measurement of cervical length. This study results showed statistically significant decrease in group I from the group II according to uterine artery resistant index at 20 weeks Mean±SD (0.54±0.14 versus 0.66±0.24). This study shows statistically significant decrease in group I from the group II according to uterine artery pulsatility index at 20 weeks Mean±SD(0.62±0.17 versus 0.77±0.12). This study shows highly statistically significant difference between groups according to fetal viability till 20 weeks. Also this study showed beneficial effect of sildenafil citrate in the management of early recurrent unexplained pregnancy loss. **Conclusion:** our study showed that use of sildenafil citrate decrease rate of early recurrent unexplained pregnancy loss.

Key words: Sildenafil Citrate, unexplained recurrent pregnancy Loss.

Introduction

Recurrent unexplained spontaneous miscarriage is defined as three consecutive pregnancy loss prior to 20 weeks from the last menstrual period. 1% to 2% of women experience unexplained Recurrent Spontaneous miscarriage (URSM) ***(Patki and Chauhan, 2016).*** Multiple etiologies for URSM have been reported including autoimmune (20%), endocrine (17%-20%), anatomic (10%-15%), genetic (2%-5%) factors and infection (0.5%-5%). However, about 40% - 50% of URSM are of unknown etiology and are classified as URSM. Human endometrial microarray analyses between unexplained recurrent miscarriage and elective terminations in women with previous living offspring identified many significantly dysregulated genes in recurrent unexplained spontaneous miscarriage patients that had immune function or were cell-signaling associated ***(López-Tello et al., 2017).***

Treatment of Recurrent unexplained spontaneous miscarriage is a real challenge ***(Tzioras et al., 2009)*** and in most cases is unsuccessful as identifiable causes can be found only in 30-50% of women ***(Li et al., 2002)*** and the rest remain unexplained. As the fetus is secured from the humoral immunity during normal pregnancy, cell-mediated immunity (cells and cytokines) was considered an important etiologic factor in URSM. Previously, it was reported that 37.1% of women with

URSM have elevated peripheral blood NK cells. Natural killer (NK) cells are a dominant lymphocyte subset, accounting for more than 70% of lymphocytes at the maternal–fetal interface. This subset plays an important role in maintaining maternal–fetal immune tolerance and regulating trophoblast invasion ***(Dan et al., 2015).*** Accumulating evidence suggests that unexplained recurrent miscarriage is related to abnormal NK cell numbers and activity. Studies have indicated that an elevated proportion and cytotoxicity of peripheral blood NK cells (pNK) is associated with infertility and the failure of *in vitro* fertilization (IVF) ***(Karami et al., 2012).*** Studies have also revealed a close correlation between an increased number of uterine NK cells (uNK) in the mid-secretory phase and the occurrence of unexplained RM. The relationship between NK cells and pregnancy outcome as well as the associated mechanisms ***(Thum et al., 2005)*** remains the subject of intense debate ***(Liu et al., 2014).***

Also Scientists ***(Bernstein et al., 2006)*** showed that during the luteal phase of normal menstrual cycle, uterine artery impedance to blood flow decreases and there is an increase in uterine and sub-endometrial blood flow which reaches its maximum level during the period of implantation. Other studies ***(Detti et al., 2006)*** showed that uterine artery perfusion regulates uterine receptivity, influences the success of implantation, maintains early pregnancy and that impaired uterine perfusion

plays a central role in the pathogenesis of unexplained recurrent spontaneous miscarriage (URSM) ***(Abdel-Razik e al., 2014).***

Treatment of URSM is a challenging issue. The currently available lines of treatment according to simplicity of use, reliability and degree of invasiveness include corticosteroids, aspirin, heparin and immunoglobulins (besides good antenatal care), but up to now there are no prospective randomized studies, powerful enough, to determine a significant difference between these therapeutic protocols, with any of the above mentioned pharmacological agents ***(William, 2006).*** Several therapies have been advocated in patients with a history of URSM and an elevated level of peripheral blood NK cells. Intravenous gamma immunoglobulin (Ig) ***(Coulam and Roussev, 2003)*** is one of these treatment lines. However, a recent large placebo-controlled study found limited efficacy of intravenous gamma Ig in treating URSM patients. Another proposed therapy which is reported to increase implantation rates in these patients is intravenously administered intralipid ***(Stephenson et al. 2010).***

Other treatment modalities are sildenafil citrate (SC) that increases blood flow to the uterus and increases lining thickness in non-pregnant women with the history of URSM. ***El Far et al., (2014)*** have recently reported novel preliminary as well as first longitudinal clinical study of 50 cases from

Egypt, they were the first to show the use of sildenafil citrate as novel antiabortive agent in cases of URSM by restoring and augmenting the capacity of antioxidants as well as modulating lipid peroxidation and nitrosative stress and improvement of vasoconstriction through increasing blood flow causing relaxation of uterine arteries.

ESHRE 2017 GUIDLINES for unexplained recurrent pregnancy loss reported that Glucocorticoids are not recommended as a treatment of unexplained RPL or RPL with selected immunological biomarkers ***(Laskin et al., 1997)***, Heparin or low dose aspirin are not recommended, as there is evidence that they do not improve live birth rate in women with unexplained RPL ***(Pasquier et al., 2015, Schleussner et al., 2015).***

Sildenafil Citrate (Viagra®), a vasodilator, is also described as an anti-inflammatory agent ***(Bogdan et al., 2012; Check, 2012; Raposo et al., 2013; Zhang et al., 2013)*** Although SC has been developed for erectile dysfunction, it is now used for other medical indications such as cardiovascular conditions and diabetes mellitus, depression, pulmonary hypertension, pre-eclampsia, IUGR, infertility patients with Asherman's syndrome, inflammation, chronic heart failure and renal insufficiency and hypertensive disorders. In pregnant women, treatment with Sildenafil may improve blood flow to the placenta and fetus and is currently being investigated as a treatment in fetal growth restriction (FGR) ***(von Dadelszen et***

al., 2011; Dastjerdi et al., 2012). In pregnant mice, Sildenafil citrate enhances fetal growth, even in the absence of abnormal placental circulation ***(Dilworth et al., 2013).***

Sildenafil citrate (VIAGRA) augments the vasodilatory effects of NO. Vaginal sildenafil improves uterine artery blood flow and sonographic endometrial thickness. While improving uterine blood flow in the proliferative phase, NO may have detrimental effects at the level of the endometrium during the implantation window. The NO- mediated release of cytokines such as tumour necrosis factor- from activated natural killer cells has been implicated as a cause of implantation failure ***(Rashidi et al., 2012).*** NK cytotoxicity has been reported to be predictive of subsequent pregnancy loss in women who had recurrent unexplained spontaneous miscarriage. The administration of SC from day 5 of menstrual cycle to ovulation found to cause significant increase in subendometrial vascularity in apparently fertile women without effecting an increase in endometrial thickness or volume. ***El-Far et al (2014):*** used sildenafil citrate intravaginal and showed significant increase in blood flow in uterine arteries; as intravaginal administration of SC was found to decrease the incidence of systemic side-effects by delivering the medication in close proximity to the target organ.

Past studies ***(Valdes et al., 2009)*** provide evidences that nitric oxide (NO) generated *in vivo* from the essential amino acid L-arginine by the vascular endothelium plays a major role in vascular smooth muscle relaxation. Subsequently, it results in a decrease in vascular resistance which leads to an increase in uterine artery blood flow observable in early pregnancy. Impaired L-arginine-nitric oxide pathway has been suggested to be a subtle cause of URSM and treatment with nitric oxide donors is reported as a significant success in some studies ***(El-Far et al., 2009).*** Based on these observations, an attempt was made to study uterine arteries and sub-endometrial blood flow during the luteal phase in normal fertile women and in patients with unexplained recurrent miscarriage ***(Abdel Razik et al., 2014).***