Y CHROMOSOME MICRODELETIONS IN HUSBANDS OF WOMEN WITH RECURRENT UNEXPLANIED FIRST TRIMESTER ABORTIONS

Protocol of Thesis

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INTRODUCTION

Spontaneous pregnancy loss is a surprisingly common occurrence, with approximately 15% of all clinically recognized pregnancies resulting in pregnancy failure. Recurrent pregnancy loss (RPL) has been inconsistently defined. When defined as 2 or more consecutive pregnancy losses prior to 20 weeks from the last menstrual period, it affects approximately 1% to 2% of women. Various etiologies implicated in RPL, include factors known to be causative, as well as those implicated as possible causative agents**.** Spontaneous pregnancy loss can be physically and emotionally taxing for couples, especially when faced with recurrent losses**.** Whereas approximately 15% of all clinically recognized pregnancies result in spontaneous loss, there are many more pregnancies that fail prior to being clinically recognized. Only 30% of all conceptions result in a live birth ***(Ford et al., 2009)***.

The American College of Obstetricians and Gynecologists (2001) recognizes only two types of tests as having clear value in the investigation of recurrent miscarriage: (1) parental cytogenetic analysis, and (2) lupus anticoagulant and anticardiolipin antibodies assays. Cytogenetics, in addition to providing a potential explanation for the abortions, may identify couples at risk of giving birth to an infant with a deleterious unbalanced chromosomal translocation ***(Cunningham et al., 2005)***.

To investigate whether Y chromosome microdeletions are associated with recurrent miscarriage or not is attracting a growing attention and studies are giving contradictory results, for example ***Dewan et al. (2008)*** reported the prevalence of Y-chromosome microdeletions in recurrent pregnancy loss couples to be 86%. Also ***Karaer et al. (2008)*** concluded that the prevalence of the Y chromosome microdeletions in AZF (Azoospermia Factor) region was much higher in men from

couples with recurrent pregnancy loss than men in fertile couples and stated that Y-chromosome microdeletions in AZF region may be a possible etiologic factor of recurrent pregnancy loss, while other studies showed no relationship between AZF microdeletions and recurrent spontaneous abortion ***(Lu et al., 2008).***

After the Klinefelter syndrome, Y chromosomal microdeletions are the most frequent genetic cause of male infertility ***(Vicdan et al., 2004).***

Deletions of the distal euchromatic region of the Y chromosome (Yq11) are associated with spermatogenic failure. The locus, named azoospermia factor (*AZF*), extends from the proximal to the distal end of the q region of the Y chromosome ***(Luddi et al., 2009)***. Analysis of these deletions demonstrates in the AZF region four nonoverlaping loci, AZFa, AZFb, AZFc and AZFd which is localized between AZFb and AZFc ***(SaoPedro et al., 2003 and Ferlin et al., 2007)***.

The *AZFa* interval is estimated to span 792 kb and includes two widely expressed functional genes: *USP9Y* (a Y-linked gene encoding the ubiquitin-specific peptidase) ***(Krausz et al., 2006),*** *DDX3Y* (the DEAD [Asp–Glu–Ala–Asp] box polypeptide) ***(Kamp et al., 2001)***, and Y-linked gene formerly known as *DBY* ***(Sargent et al., 1999 and Kamp et al., 2001).***

USP9Y spans 170 kb of DNA, consists of at least 46 exons, and occupies a small part of the *AZFa* interval. It encodes a protein reported to function as ubiquitin C-terminal hydrolase and is ubiquitously expressed. Deletions affecting *USP9Y* have been associated with azoospermia or severe oligospermia ***(Brown et al., 1998 and Sun et al., 1999).***

Complete deletion of the *AZFa* region is relatively rare (deletions in the q region of the Y chromosome are found in less than 2% of men with spermatogenic defects) but is well

documented and always associated with the Sertoli-cell-only syndrome ***(Ferlin et al., 2007).***

Deletion of these loci results in spermatogenic arrest and is associated with azoospermia and oligozoospermia ***(Bache et al., 2004; Hellani et al., 2006; Vutyavanich et al., 2007 and Ferlin et al., 2007)***. These AZF genes code for RNA binding proteins and may be involved in regulation of gene expression, RNA metabolism, packaging and transport to cytoplasm, and RNA splicing ***(Zamani et al., 2006)***.

The introduction of testicular sperm extraction (TESE) and intracytoplasmic sperm injection (ICSI) into the treatment of male factor infertility has permitted the use of sperm from oligospermic or azoospermic patients to achieve successful fertilization and pregnancies ***(Vernaeve et al., 2004; Nagvenkar et al., 2005 and Poongothai et al., 2009)***. However, this procedure might have a potential risk of transmitting genetic abnormalities to the offspring, since the natural selection process of sperm cells is bypassed ***(SaoPedro et al., 2003; Vicdan et al., 2004; Şamli et al., 2005; Zamani et al., 2006; and Vutyavanich et al., 2007).***

In view of the genetic risks for the next generation, the importance of careful evaluation of karyotypes and AZF microdeletions in male infertility prior to assisted reproduction by ICSI is evident ***(Şamli et al., 2005 and Hellani et al., 2006)***.

Dewan et al. (2006) concluded that Y-chromosome microdeletion testing particularly of the AZFc region in the evaluation of RPL couples when all other tests fail to reveal the etiology**.**

AIM OF THE WORK

This study intends to calculate the prevalence of Y-chromosome microdeletions in the husbands of women with history of recurrent unexplained first trimesteric abortions.

PATIENTS AND METHODS

Type of the study: A case control study.

This study will be conducted in Ain Shams university maternity hospital and the patients will be picked up from the recurrent pregnancy loss clinic. This will be done in collaboration with the molecular pathology department research laboratory of the German University in Cairo.

Sample Size Justification:

A previous study showed that (16%) from couples with recurrent pregnancy loss had microdeletions in 1 or more of the 4 segments studied, whereas none of the fertile men had any microdeletions ***(Karaer et al., 2008).***

Using the PS programs for sample size calculation, the criterion for significance (alpha) has been set at 0.05 (2-tailed), any effect in either direction will be interpreted. For our study, assuming an abnormal test for Y-chromosome microdeletions in 16% of males in cases arm of the study versus 0% in controls, it was calculated that, with a sample size of 50 men per cases arm and 50 men per the control arm, will give a power of 95% to yield a statistically significant result.

Group I: **Cases:** 50 men from couples with a history of recurrent unexplained first trimesteric miscarriage, (defined as at least two or more unexplained miscarriages).

Inclusion criteria in Group I:

- Females aged between 18-40 years old. With recurrent 2 or more unexplained miscarriage.
- Male partner aged between 30-45 years old.

Exclusion criteria:

- Known cause of recurrent miscarriage (e.g. Antiphospholipid Antibody Syndrome, uterine abnormality etc).

Group II: **Controls:** group of 50 men from couples with at least one live birth and no history of miscarriages.

Methodology:

Patients enrolled in the study will undergo complete clinical examination and detailed medical history will be obtained. Blood samples for basic investigations and Blood sample will be taken for ***DNA extraction.*** Each patient will have a Case Record Form (CRF) in which the following data will be recorded.

All the patients will be subjected to:

1. History taking (Personal, past present, antenatal record).
2. General abdominal and local examination.
3. BMI measurement (kg/m^2).
4. Investigations which will include (CBC, liver function test, fasting blood sugar and post prandial blood sugar also lupus anticoagulant, anticardiolipin markers, day 2 LH and FSH and midluteal serum progesterone).
5. Pelvic Ultrasound to exclude any uterine abnormalities.

DNA extraction: A blood sample will be taken in an (EDTA) tube and specimen will be sent to lab within 24 hours of collection. Analysis will be done by amplification of this DNA

using a set of primers that will target different genetic deletions on the chromosome using Real Time Polymerase chain reaction (PCR).

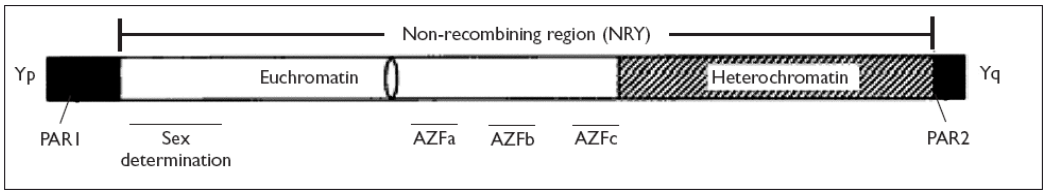

Diagram 1: Shows the schematic structure of the Y-chromosome ***(Foresta et al., 2001)***.

Test result:

1. Abnormal: the presence of 1 or more deletions in specific regions of the Y chromosome (designated AZFa, AZFb, and AZFc)

2. Normal: the absence of deletions in the regions of the Y chromosome under test.

The absence of a detectable microdeletion(s) does not rule out the presence of other genetic or non genetic factors that may be the cause of clinical findings.

Statistical Analysis:

Statistical analysis will be performed. For each group, we will describe continuous-scale demographic variables by calculating the characteristics (e.g. minimum, 25^{th} quartile, median, 75^{th} quartile and maximum). For these variables, we will verify initial-state group comparability by analysis of the variance (independent-groups Student t-test). In order to analyze the distribution of ordinal and nominal demographic variables in the groups, we will use Pearson Chi square test.

Pearson correlation test will be used for between-group correlations of demographic and clinical data when a parametric distribution of variables is confirmed.

Statistical analyses will be performed using SPSS for Windows release 19.0 (SPSS Inc., Chicago, Ill., USA). A p value < 0.05 will be considered significant.

Ethical and Legal Aspects

1. Good Clinical Practice (GCP)

The procedures set out in this study protocol, pertaining to the conduct, evaluation and documentation of this study, are designed to ensure that the investigators abide by the principles of good clinical practice and the ethical principles laid down in the current revision of the Declaration of Helsinki ***(Angel 1988, Goodyear et al., 2009)***.

2. Delegation of Investigator Responsibilities

The investigator will ensure that all persons assisting with the study are adequately informed about the protocol, any amendments to the protocol, the study variants, and their information collection-related duties and functions. The publication of this research is an intellectual property of Ain Shams University and the molecular pathology department in the German University in Cairo and any publications should be done only after the agreement of both sides.

3. Patient Information and Informed Consent

Before being enrolled to the clinical study, the patient must consent to participate after the nature, scope, and possible consequences of the clinical study have been explained in a form understandable to her. An informed consent document, in Arabic language, contains all locally required elements and specifies who informed the patient. After reading the informed

consent document, the patient must give consent in writing. The patient's consent must be confirmed at the time of consent by the personally dated signature of the patient and by the personally dated signature of the person conducting the informed consent discussions.

If the patient is unable to read, oral presentation and explanation of the written informed consent form and information to be supplied to patients must take place in the presence of an impartial witness. Consent must be confirmed at the time of consent orally and by the personally dated signature of the patient or by a local legally recognized alternative (e.g., the patient's thumbprint or mark). The witness and the person conducting the informed consent discussions must also sign and personally date the consent document. The original signed consent document will be retained by the investigator. The investigator will not undertake any measures specifically required only for the clinical study until valid consent has been obtained.

4. Confidentiality

Only the patient number and patient initials will be recorded in the CRF, and if the patients name appears on any other document (e.g., pathologist report), it must be kept in privacy by the investigators. The investigator will maintain a personal patient identification list (patient numbers with the corresponding patient names) to enable records to be identified.

5. Protocol Approval

Before the beginning of the study and in accordance with the local regulation followed, the protocol and all corresponding documents will be declared for Ethical and Research approval by the Council of OB/GYN Department, Ain Shams University.

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الجزيئات المحذوفة من الكروموسوم (واي) فى أزواج النساء ذوي الإجهاض المتكرر الغير مبرر فى الأشهر الثلاثة الأولى من الحمل

بروتوكول رسالة

توطئة للحصول على درجة الدكتوراه فى
أمراض النساء والتوليد

مقدمة من

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