ASSOCIATION OF OBESITY WITH JAK 2 V617F GENE MUTATION IN RECURRENT PREGENCY LOSS

Submitted By

Mai Mahmoud AbdElhalim Shaker

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A thesis submitted in Partial Fulfillment
Of
The Requirement for the Doctor of Philosophy Degree
In
Environmental Sciences

Department of Environmental Medical Sciences
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APPROVAL SHEET

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I would like to dedicate this thesis to my family especially to the soul of my beloved parents.

Abstract

Background: Frustration affect couples when they lose their pregnancy several times successively. There are many factors that leads to Recurrent pregnancy loss, One of these factors is maternal obesity. In obese women leptin and IL-6 continuously turn on the activation of intracellular JAK-STAT3 signaling. This might lead to mutation in the gene encoding the Janus kinase2 (JAK2) that results in a replacement valine with phenylalanine at particular position 617(V617F). Which is main cause responsible for augmented rate of fetal loss in this situation.

Objectives: To explore the association of obesity with JAK2 V617F mutation in women with recurrent pregnancy loss.

Patients and Methods: This case control study included 250 female patients. The study group consists of 150 female patients with history of recurrent pregnancy loss for two consecutive times or more, aged (20-34) and having a body mass index of >24.9. The control group consists of 100 aged matched women have normal body mass index and as well as having a minimum of one live child birth and no past history of prior pregnancy loss or uncomplicated pregnancy. All women were investigated for the mutations by using the allele-specific multiplex PCR technique to genotype all participants in the study.

Results: Jak2v617 mutation was found in cases (9.3%) while no jak2 v617f mutation was found among the control group,($p< 0.001$). Patients in case group were obese with a mean BMI of (*30.9* ±4.3) vs mean BMI control group (22.4 ±1.1, $p<0.001$).History of live birth in case group (42.7%) vs 100 %in control,($P< 0.001$). consanguineous marriages were positive (57.4% vs 13 %, $p<0.001$). Only 13 patients of the case group experienced pregnancy loss after 12 weeks of gestation while the rest of the case group (137) including 14 patients who were positive for jak2 v617f mutation experienced pregnancy loss before 12 weeks of gestation ($p <0.06$).

Conclusion: In conclusion we found a correlation between the increased risk for the occurrence of RPL in obese women and V617F mutation in the Jak2 gene exon 12 and that obesity could be a risk modifier for somatic mutation.

Keywords: Recurrent pregnancy loss ·mutation · jak2. Obesity.

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List of Abbreviations

Abb.	Full term
Acl	AnticardioLipin
Apl	antiPhosphoLipid antibodies
APS	AntiPhospholipid Syndrome
AZF	Azoospermia Factor
BMI	Body Mass Index
CIS	Cytokine-Inducible SH2
CJD	Creutzfeldt–Jakob Disease
Cns	Central nervous system
DNA	DeoxyriboNucleic Acid
EPO	Erythropoietin
ET	Essential Thrombocythemia
F	Phenylalanine
GH	Growth Hormone
GWG	Gestational Weight Gain
HCG	Human Chorionic Gonadotropin
HDAC	Histone Deacetylase
HSG	Hysterosalpingography
HSV	Herpes Simplex Virus
IFN	Interferone
IL-6	Interleukin-6
IOM	Institute Of Medicine
IVIG	Intravenous ImmunoGlobulin
JAK	Janus Kinases
JH	Janus Homology
LAC	Lupus Anticoagulant
LDA	Low Dose Asprin
LepR	Leptin Receptor
LH	Luteinizing Hormone
LMWH	Low Molecular Weight Heparin
LPD	Luteal Phase Defect
MPN	MyeloP roliferative Neoplasm
MPN	Myeloproliferative neoplasm
Mrna	messenger RiboNucleic Acid

List of Abbreviations (Cont…)

Abb.	Full term
MTHFR	Methylene tetrahydrofolatereductase of Transcription
PCOS	PolyCystic Ovary Syndrome
PCR	Polymerase chain Reaction
PGD	Preimplantation Genetic Diagnosis
PIAS	Protein Inhibitors of Activated STAT
PRL	Prolactin hormone
PV	Polycythemia Vera
RPL	Recurrent Pregnancy Loss
SH	Src Homology
SOCS	Suppressors of Cytokine Signaling
STAT	Signal Transducers and Activators
TAD	TransActivation Domain
TIA	Transient Ischemic Attack
TORCH	Toxoplasmosis, Rubella, Cytomegalo virus, Herpes simplex
TSH	Thyroid Stimulating Hormone
UFH	Un Fractionated Heparin

INTRODUCTION

Recurrent pregnancy loss leaves a bad impact on patient psyche. It is also not one of the easy tasks to be dealt with in reproductive medicine. RPL is two to three consecutive miscarriages before 20 weeks' gestation ***(Regan et al., 2010).*** About 1–3% of women suffer from this medical condition during their reproductive period ***(Bettina et al., 2010)***. Until know up to 50% of RPL cases the definite underlying cause or pathophysiological mechanisms is still not determined ***(Karvela et al., 2008).***

Nowadays, Obesity is a major health burden on governments and becoming a global problem across the world ***(Anonymous, 2001).*** It is documented that obese mothers act like a risk factor for adulthood obesity in their new coming offspring ***(Parsons et al., 2001).*** Obesity promotes to a poor pregnancy outcome, for example spontaneous or unexplained intrauterine fetal death ***(Froen et al., 2001).***

Poor pregnancy outcome affects the mother and offspring due to pre pregnancy maternal obesity for example diabetes, hypertension and many thrombophlic problems. Off spring of many obese mothers are more liable to face difficulties during birth, macrosomia, and prenatal death ***(Nuthalapaty and Rouse, 2004).***

Gene mutations can be turned on by any change in the home environment and also the external environment in which human grow ***(Lobo, 2008).***

Many hormones depend on their mechansime on Jinas Kinas, JAK-signal transducers and activators of transcription STAT which greatly affects fat cell function. In Obesity leptin and IL-6 increase in obese women continually activating intracellular JAK-STA3. Leptin works mainly on central nervous system, IL-6 works on peripheral organs and they can switch targets. Continuous JAK-STAT3 activation by leptin and IL-6 lead to the increased expression of the negative regulator SOCS3. SOCS3 in then have a negative feedback on leptin and IL-6 signaling regulating it plus antagonizing insulin action which leads to gaining more weight and insulin resistance ***(Wunderlich et al., 2013).***

JAK2 a tyrosine kinase has a main role in signal transduction in many hematopoietic growth factor receptors. The(V617F) mutation is due to valine-to-phenylalanine substitution at position 617this mutation happened due to a gain-of-function mutation in the gene encoding the Janus kinase2 (JAK2) leading to continues activation tyrosine kinase and mutation, V617F, in the JH2 pseudo-kinase domain of JAK2 gene is responsible for offspring loss. This mutation was found in patients suffering from thrombocythemia. Thrombocythemia during pregnancy leads to sudden pregnancy loss and decreased percentage in live rates ***(Randone et al., 2011, ElMagraby and Bedwey, 2012).***

Thrmobophilia leads to thrombosis of the utero-placental circulation due to cascading that ocurrs in haemostatic response ***(Lockwood and Wendel, 2011).*** Disturbance and decrease in placental perfusion may lead to recurrent pregnancy loss ***(Lockwood and Wendel, 2011).***

AIM OF THE STUDY

To explore the association of obesity with JAK2 V617F mutation in women with recurrent pregnancy loss.

Chapter 1

RECURRENT PREGNANCY LOSS

Patients usually suffer from bad psyche when faced with successive events of recurrent pregnancy loss (RPL). Its one of the challenging areas in reproductive medicine. It is always a priority for the clinician to choose the suitable investigations to be able to identify the the main cause for RPL and to determine which guidelines of treatment will be given to the patient ***(Niekerk et al., 2013).***

Defining RPL as a as a separate, special medical problem which require specific diagnostic testing and therapeutic interference is based on history of high risk for previous events of RPL and the high chance of finding a effective etiology for the disease. Although there is no definite published data have estimated the chance of finding a cause for RPL in a specific community with 2 against 3 or more miscarriages, A previous study suggested that the risk of pregnancy loss in the following pregnancies is 30% after 2 losses, versus 33% after 3 recurrent losses in couples with no history of any live birth this was reported by ***The American College of Obstetricians and Gynecologists (2001).*** This highly recommends an investigations to be done for couples after just 2 losses with no history of previous live births. Patient should start investigation early in pregnancy if the fetus cardiac activity was evident, the age of the woman is more than 35 years, or the couple faced difficulty in trying to conceive. The high basic rate of spontaneous unexplained and recurrent pregnancy losses in the general population, the defect in constant definition for RPL, limitation in reaching the tissues effectively to allow study of the disorder, and the major good prognosis for the ability of

having a live birth among patients with RPL all add up to frustrate any achievements in diagnostic and therapeutic recommendations ***(Ford and Schus, 2009).***

Nowadays, there are a several causes for RPL. These include chromosomal abnormality in parents, thyroid mal function, uncontrolled diabetes mellitus, specific inborn uterine anatomic anomalies and antiphospholipid antibody syndrome (APS). Other widespread potential causes include some of endocrine disorders, inborn or acquired thrombophilic diseases, disorders in the immune system, infections, and environmental factors. After exploration for these probable etiologies, nearly half of all cases will remain with unexplained reasons ***(Ford and Schus, 2009).***

Definition

According to the Royal College of Obstetricians and Gynaecologists (RCOG) Green-top Guideline (Number 17), " RPL is defined in the form of spontaneous loss of a pregnancy before the developing fetus was able to reach viability at 24 weeks of gestation" ***(Regan et al., 2010).*** It takes into consideration pregnancy losses from the conceiving time till 23 weeks of gestation had been completed. RPL is two or more consecutive miscarriages. The World Health Organization suggests a way to define viability. Particularly in some countries mainly the developing countries or when a couple are not sure about the gestation weeks, the baby's birth weight of 500 g should be used as a tool to define viability. The American Society for Reproductive Medicine count on ultrasound scan and lab work inform of histopathology in defining RPL as