# ASSOCIATION OF OBESITY WITH JAK2 V617F GENE MUTATION IN RECURRENT PREGNANCY LOSS

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## ABSTRACT

Maternal obesity is one of the factors that have a role in recurrent pregnancy loss ( RPL) . Increased rate of fetal loss in obese pregnant women might be due the mutation in Janus kinase2 gene (V617F). Our aim to explore the association of obesity with JAK2 V617F mutation in recurrent pregnancy loss. The study is carried out on 250 women, the case group have history of recurrent pregnancy loss with a body mass index (BMI) of >24.9 $kg/m^2$.Control group consists of women with normal body mass index with at least one live child birth and with no history of pregnancy loss. All of the subjects were investigated for the mutations by using the allele-specific multiplex PCR. In the 250 women in the study, women who were obese and tested positive for JAK2 V617F were 14 %. The case group revealed 9.3% patients who were for JAK2 V617F and had a BMI with mean of 30.9 ±4.3 ,history of live birth were 64 out of 150 with a mean of 1.3 ±0.5. In conclusion the study was able to find a correlation between the increased risk for the occurrence of RPL in obese women and the investigated V617F mutation in the Jak2 gene exon 12 and that obesity could be a risk modifier for that somatic mutation.

**Keywords:** Recurrent pregnancy loss, Mutation, Jak2, Obesity.

## 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*., 2011). About 1–3% of women suffer from this medical condition during their reproductive period (Toth *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).

There is a bad outcome that affects the mother and the offspring due to pre pregnancy maternal obesity for example diabetes, preeclampsia and thrombophlic disorders. Off spring of 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 *et al*., 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 infact 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 is a tyrosine kinase has 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; Elamgrabey and Badewy, 2012).

Some of the genetic mutations are acquired among a person's lifetime and are found only in some cells. These kinds of changes, which are called somatic mutations. In the *JAK2* gene theses Somatic mutations found are correlated with essential thrombocythemia,. ( James *et al*.,2008).

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

## SUBJECTS AND METHOD

This case control study included 250 patients. The study group consists of 150 women with history of recurrent pregnancy loss for two consecutive times or more, aged (20-34) and having a body mass index of more than 24.9 $kg/m^2$. The control group consisted of 100 aged matched women having normal body mass index less than 24.9 $kg/m^2$ with at least one live child birth and no history of spontaneous miscarriages or uncomplicated pregnancy. All subjects were referred from the outpatient clinic of the Prenatal Diagnosis and Fetal Medicine Department, National Research Centre. During the period

from June 2014 to May 2015. An informed consent has been obtained from all patients according to the National Research Centre Ethical Committee.

**Method:**

Five ml venous blood samples were withdrawn from all subjects under complete aseptic conditions and collected in a polypropylene tube containing 0.5 M EDTA (pH 8.0) to prevent clotting and nuclease activity.

**DNA extraction:**

DNA genome was extracted from peripheral blood leukocytes of all candidates in the study using the QIAGEN DNA Extraction Kit (Qiagen, Germany).

**Detection for the V617F jak2 gene mutation:**

V617F mutation is detected by using an allele-specific multiplex PCR having a one common reverse and other two separate forward primers was being used. 150ng / ml of the DNA genome extract will act as a template in the total volume of 25 μl containing the following: 1.5 mmol/L $MgCl_2$, 20 pmol/L of each primer, 0.2 mmol/L dNTPs, and 1 U of Taq polymerase (Fermentas, Germany). Amplification using 40 cycles with an annealing temperature of 60°C was performed.

The target of the first forward primer is to amplify a 364-bp fragment for both the mutant and the wild-type alleles but the second forward primer was put specifically to detect the presence of mutated allele and it has an extra mismatch near the 3′ end. The mutation produce a 203-bp product exclusively if V617F mutation is present (Horn *et al*. 2006 )

**JAK2 Primer Sequences:**

| **Allele-specific PCR (3)** |
|---|
| • **Forward** (internal control 5_-ATCTATAGTCATGCTGAAAGTAGGAGAAAG-3_ |
| • **Forward** (V617F specific)) 5_-AGCATTTGGTTTTAAATTATGGAGTATATT-3_’ |
| • **Reverse**-3_ 5_-CTGAATAGTCCTACAGTGTTTTCAGTTTCA-3_ |

## RESULTS

All patients included in the cases group had two or more RPL ranging from 2 to 11 abortions .Their ages ranged from 20-35 years mean 26.8± 4.3 . Our case group consisted of 150 women with history of RPL who were obese and tested for JAK2V617F mutation verses control group which had no history of RPL, and had at least one living child. History of live birth was present in 64 patients in case group (42.7% vs 100 %in control, P 0.001). Jak2v617 mutation was found in 14 patient (9.3%)in cases while no jak2 v617f mutation was found among the control group, p< 0.001) . Patients in case group were found to be obese with a mean BMI of (30.9 ±4.3 vs mean BMI control group22.4 ±1.1, p<0.001) .Consanguinity was positive in 82cases and 13of the controls (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).

**Table1:** Frequency Distribution of JAK2 V617F Mutation and Live birth

| | Case (n=150) | | Control (n=100) | | Total | |
|---|---|---|---|---|---|---|
| **Variables** | **No.** | **%** | **No.** | **%** | **No.** | **%** |
| **Live birth** | | | | | | |
| Yes | 64 | 42.7 | 100 | 100.0 | 164 | 65.5 |
| No | 86 | 57.3 | 0 | 0.0 | 86 | 34.5 |
| **JAK2 V617F** | | | | | | |
| Positive | 14 | 9.3 | 0 | 0.0 | 14 | 5.6 |
| Negative | 136 | 90.7 | 100 | 100.0 | 236 | 94.4 |

In the whole studied groups, more than one half ( 65.5% ) had a history of a live births. (42.7%) of women had live births in the case group while all women in the control group had history of normal live births( 100% ). In the whole studied groups 5.6% had JAK2 V617F gene mutation ( 9.3 % ) were positive for JAK2 V617F gene mutation while in the control group all of the women were negative for JAK2 V617F gene mutation .

**Table 2:** Comparison between the case and control groups as regard the JAK2 V617F mutation

| **Variables** | **Case (n=150)** | | **Control (n=100)** | | **Total** | | **$\chi$ 2** | **P value** |
|---|---|---|---|---|---|---|---|---|
| | **No.** | **%** | **No** | **%** | **No** | **%** | | |
| JAK2 V617F | | | | | | | | |
| positive | 14 | 9.3 | 0 | 0.0 | 14 | 5.6 | 9.9 | 0.001 |
| negative | 136 | 90.7 | 100 | 100.0 | 236 | 94.4 | | |

JAK2 V617F mutation was present in the case group (9.3 %) but was not present in the control group with significant p value of < 0.001.

**Table 3:** Comparison between the studied groups regarding the Age, live birth and BMI

| | Case | Control | $\chi^2$ | P |
|---|---|---|---|---|
| **Age** | | | | |
| Range | 20 - 35 | 20 -31 | 1.8 | 0.07 |
| Mean ± SD | 26.8 ± 4.3 | 25.9± 3.1 | | |
| **Live birth** | | | | |
| Range | 1.0 -3.0 | 1.0 - 4.0 | 6.7 | <0.001 |
| Mean ± SD | 1.3 ± 0.5 | 2.1± 0.9 | | |
| **BMI** | | | | |
| Range | 6.4 - 43.2 | 19.5- 25.5 | 19.3 | <0.001 |
| Mean ± SD | 30.9 ± 4.3 | 22.4 ± 1.1 | | |

Mean age was about 26 years in both studied groups , live birth was more prominent in the control group with a range of ( 1-4) live births and the body mass index was much higher in the case group ranging (19.5-25.5) $kg/m^2$

**Table4:** Comparison between JAK2 V617F and obesity

| | Jak | | | | Total | | $\chi^2$ | P |
|---|---|---|---|---|---|---|---|---|
| | Positive | | negative | | | | | |
| | no | % | no | % | No | % | | |
| **Obesity** | | | | | | | | |
| Normal | 0 | 0 | 100 | 42.4 | 100 | 40 | 9.88 | 0.001 |
| Obese | 14 | 100 | 136 | 57.6 | 150 | 34.4 | | |
| **Total** | 14 | 100 | 236 | 100 | 250 | 100 | | |

In the whole study population (250 women), women who had normal BMI were negative for jak mutation while women who were obese and tested positive for JAK2 V617F mutation were 14. Giving a significant difference $p < 0.001$.

## DISSUCSSION

RPL is a traumatic event for the patient to go through and its challenging target for the clinician too. It is tough experience for the couple because they hardly get clear-cut answers for the repeated failure to maintain pregnancy and not even able to put your hand on the best guidelines for a fail-safe treatment. Nowadays, the list making the main etiologies for RPL has been changing . It is recorded that the disorders which promote to venous thrombosis, are all named "thrombophilias," which have a role in the pathogenesis or the process of fetal loss. The huge differences between different theories as well as keeping in mind the correlation between such disorders and unexplained pregnancy loss suggests the presence of underlying risk modifiers (Mercier *et al* ., 2007).

An effort was done to try to reduce any error ,women in our study had at least one history of live birth, This strategy was preformed to rule out that the patient might had any anatomical factor that might be a cause for the repeated losses. The current study took in consideration that the participants have had the same partner during their marital life to be able to exclude paternal chromosomal abnormality that might affect the results. The presence of high incidence of thromboembolic attacks due to JAK2V617F gene mutation and pregnancy loss in obese women and a possible relationship between these parameters is a matter of concern. In our study women who were positive for JAK2V617F mutation in the case group 9.3 % in relation to the control group had no positive incidence for the mutation giving a significant p value of 0.001 Mercier *et al.,* (2007) supported our results when he mentioned the

mutation was present more frequently in patients with past history of pregnancy loss (1.06%) than in control group (0.20%). Also Melillo *et al*.(,2009 ) stated that fetal loss encountered more in females with the JAK2 V617F mutation (mutated vs. unmutated: 9/25, 36.0% vs. 2/24, 8.3%, P 0.037), and this correlation was further confirmed by multivariate analysis (OR: 6.19; 95% CI: 1.17– 32.61, P 0.038). The frequency of having at least one healthy live birth in the case group was 42.7% with a Mean of 26.8 ±4.3 .While in the control all of them had a history of at least 1 life birth 100% with a Mean of 2.9 ± 0.1 with high significant difference of p value of <0.001. In the case group of the current study the patients BMI had mean of 30.9 ±4.3 and a median of 30.8 while in the control group the candidates BMI had a mean of 22.4 ±1.1 and a median of 22.6 with high significant difference of p value of <0.001, this is supported by study made by Sugiura *et al*.,(2014) stating that Obesity may elevate the risk of pregnancy loss in sporadic pregnancies. ,Obese women with a body mass index (BMI) > 30 kg/$m^2$ can be an independent risk factor for having future pregnancy losses with odds ratio 1.7–3.5 in females having early RPL.

In the current study only 13 patients( 9.6 %) in the case group experienced pregnancy loss after 12 weeks of gestation and while the rest of the case group including 14 patients who were positive for jak2 v617f mutation experienced pregnancy loss before 12 weeks of gestation (p 0.0612). Dahabreh *et al*.,(2008 ) stated that out of 389 cases, 92% faced early miscarriage and 14.1% suffered from late miscarriage while 6.1% had experienced both , Also Passamonti *et al* .,(2007 )documented in his study that complications occurred in the mothers were 9%, but the complications

happened in the fetus were 40% out of the investigated 96 pregnancies. 27% out of 96 pregnancies had a first trimester abortion with only 4 % had a second trimester abortion while the rest lies between 3% still birth and 4 % growth retardation.

In conclusion the study was able to find a correlation between the increased risk for the occurrence of RPL in obese women and the investigated V617F mutation in the Jak2 gene exon 12 and that obesity could be a risk modifier for that somatic mutation. However it is recommended for others who are interested in this point to do the study on a bigger number of candidates to support our results and to settle the issue of JAK2V617F could be predictor for recurrent pregnancy loss and that the obesity is a modifying risk factor .

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# دراسة مدي ارتباط السمنة بطفرة أف٦١٧ف في جين الجاك ٢ في حالات فقدان الحمل المتكرر

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## المستخلص

فقدان الحمل المتكررهي تجربة مؤلمة للمرضى، يتم تعريف فقدان الحمل المتكرر وهو فقدان للجنين مرتان متتليتان او اكثر قبل ٢٠ أسبوع من الحمل، من العوامل التي لها دور في حالات فقدان الحمل المتكرر هو بدانة الأم قبل الحمل مما قد يؤدي الي واحدة من الطفرات الجينية المرتبطة فقدان الحمل المتكرر هي طفرة أف٦١٧ف في جين الجاك٢ ويسبب تخثر الأوعية الدموية بداخل الرحم والمشيمة مما يؤدي الي ضعف التروية المشيمة وهو الحدث الرئيسي المسؤول عن زيادة معدل فقدان الجنين، الهدف من هذة الدراسة استكشاف مدي ارتباط السمنة بطفرة أف٦١٧ف في جين الجاك ٢ والتى تؤدي بدورها الي مشكلة فقدان الحمل المتكررلبعض السيدات

طبقت الدراسة على٢٥٠ سيدة، مجموعة الدراسة: تتضمن ١٥٠سيدة من اللاتى يعانين من فقدان الحمل المتتكر مرتان او اكثر قبل الاسبوع ٢٠ من الحمل، بناء مؤشر كتلة الجسم،٢٤العمر

المحدد من ٢٠–٣٧ سنة. المجموعة الضابطة تتكون من السيدات لهن نفس العمرو لا يعانين من تاريخ مرضي لفقدان الحمل المتكرر او اضطربات في الحمل .تم تحديد االطفرة من خلال تكبير بطفرة أف٦١٧ق باستخدام تقنية تفاعل تفاعل البلمرة المتسلسلة.

أوضحت نتائج الدراسه ان في العينة ككل نسبة السيدات الاءى يعانون من السمنة وحاملون لطفرة كانوا ١٤ ٪ ، مجموعة الدراسة اظهرت ٣,٩٪ من السيدات كانوا حاملون للطفرة ومتوسط بناء مؤشر كتلة الجسم ٩,٣٠ ± ٤,٣ ،٦٤ لديهم ابناء علي قيد الحياة من١٥٠ سيدة بمتوسط ١,٣ ± ٠.٥٪. في مجمل الدراسة استخلصنا وجود علاقة بين زيادة خطر حدوث حالات فقدان الحمل المتكرر في النساء البدينات وبين الطفرة أف٦١٧ق ، وأن السمنة يمكن أن تكون عامل مساعد لحدوث هذه الطفرة الجسدية.