

Effect of Insulin Level on Recurrent Pregnancy Loss

تأثير مستوى الانسولين على فقدان الحمل المتكرر

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الخلاصة:

الهدف: ايجاد العلاقة بين ارتفاع مستوى الانسولين في مصل الدم للنساء اللاتي لديهن إسقاطات متكررة في مراحل الحمل المختلفة. **المنهجية:** دراسة من نوع عينات السيطرة اجريت في مستشفى الزهراء التعليمي للنسائية والأطفال في محافظة النجف الاشرف للفترة من الأول من شباط وحتى الأول من تشرين الأول سنة 2014 لمجموعه من النساء بلغن 160 أمراه على مجموعتين. وتتضمن الدراسة سحب عينات دم من تلك النساء اللاتي لديهن إسقاطات متكررة (مجموعة الحالات) وعينات أخرى من نساء حوامل في الشهر الأخير ليس لديها أي إسقاطات متكررة سابقه, بعد أخذ العينات خزنت بدرجه حرارة 20- درجة مئوية ثم بعد ذلك تم قياس نسبة الانسولين بطريقة الأليزا. **النتائج:** وجد ان مستوى الانسولين عالي عند النساء اللاتي لديهن اسقاطات متكررة خاصة في الثلث الاول من الحمل: فهناك فرق معنوي في مستوى الانسولين في الثلث الثاني من الحمل بالمقارنة مع الثلث الاول منه بينما ليس هناك فرق معنوي بين الثلث الاول والثلث الثالث منه. عدد النساء اللاتي لديهن اسقاطات متكررة في الثلث الاول من الحمل اكثر من النساء اللاتي لديهن اسقاطات في غير مراحل من الحمل. **الاستنتاج:** لوحظ ارتفاع نسبة الانسولين في الدم للنساء اللاتي لديهن إسقاطات متكررة سابقة بالمقارنة مع النساء اللاتي ليس لديهن إسقاطات متكرره سابقة. **التوصيات:** توصي الباحثتين بقياس مستوى الانسولين للنساء اللاتي لديهن اسقاطات متكررة , وتوصي ايضا باستخدام العوامل المحفزة للانسولين وتقليل وزن الجسم والتمارين الرياضية اليومية قبل الحمل.

Abstract

Aim of study; To find the relationship between rise plasma insulin level in women with recurrent pregnancy loss at different gestational age.

Methodology: A case control study done in Al Zahraa teaching hospital in Al-Najaf city from the first of February to the first of October in 2014 of 160 women divided in two groups . We estimate fasting serum insulin hormone in samples of women with recurrent pregnancy loss at different trimester(as a case group) and healthy pregnant women at the end of 3rd trimester(as a control group). The estimation of recurrent pregnancy loss in women at different trimester is associated with endocrine abnormalities where serum insulin level increase in a different way compared with normal healthy pregnant women.

Results: We found the level of insulin hormone was high in women with recurrent pregnancy loss especially in the 1st trimester ,therefore there is significant difference in insulin level in 2nd trimester compared to the 1st trimester while it is not significant between 1st and 3rd trimester, the number of women who lost their pregnancy during the 1st trimester was higher than those losing during other trimesters.

Conclusion : Current study showed that, in women with recurrent pregnancy loss, fasting insulin level is high compared with normal healthy pregnant women.

Recommendations: We recommend that measuring fasting insulin level is usefull in all women with recurrent pregnancy loss The use of insulin sensitizing agent (metformin) and decrease body weight with regular daily exercise before embarking in pregnancy

Keywords; insulin ,pregnancy loss.

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INTRODUCTION

Recurrent pregnancy loss is a distinct disorder defined by two or more failed clinical pregnancies. A fetal loss up to 24 completed weeks of gestation is defined as a miscarriage. **Intra uterine death:** It is a clinical term for death of baby in the uterus, during pregnancy and before birth. The term used for pregnancy losses that happen after the 24th week of gestation⁽¹⁾.

Incidence: Recurrent pregnancy loss is the most common complication of pregnancy. Approximately 50% of all conceptions are lost, the majority of which is unrecognized clinically. Farther more,15-25%(almost one in 6)of all clinically recognized pregnancies end in pregnancy loss⁽¹⁾.

Causes of early pregnancy loss: It is important to identify and focus on specific risk factors or causes of recurrent pregnancy loss as evidence shows that greatest reductions in recurrent loss pregnancy in the past have occurred with target strategies⁽²⁾**1-chromosomal abnormalities:** This is an abnormality with the genetic material of an embryo and usually occurs when the chromosomes from the egg and the sperm fail to line up correctly this can result from flawed genetic material (translocation) from the mother or father that does not necessarily affect the mother or father' s health but can prevent an embryo from developing correctly, because the baby only inherits half of each parents genes there is still chance of creating a healthy baby if the defect gets bypasses or spontaneously corrects itself when the embryo is created. **2-Uterine abnormalities:** Abnormalities of the uterus are well established as a cause of pregnancy loss . these abnormalities may be the result of trauma (as Asher man' s syndrome) or abnormal development . the developmental anomalies include the septate , bicornuate unicorn ate and didelphys uterus.**3- Endocrine causes:** like hypo or hyperthyroidism a very common endocrine issue affecting many women symptoms can be very subtle and can present with recurrent miscarriages only. **Other causes of endocrine like diabetes And hyperprolactinemia** ,It has been reported that hyperprolactinemia is a cause of recurrent miscarriage and that treatment with bromocriptine which suppresses prolactin secretion by the anterior pituitary, significantly reduces the miscarriage rate⁽³⁾ .**4- Luteal phase abnormalities:**Insufficient progesterone secretion by the corpus luteal or placenta, also termed a luteal phase defect, has been suggested to cause miscarriage. Deficient progesterone production,however,may be the consequence rather than the cause of early pregnancy loss⁽⁴⁾.**5- Immu nological disorders** Antiphospholipid syndrome is an autoimmune disorder defined by the presense of characteristic clinical features antiphospholipid antibodies, the most common and serious complications of this disorder are venous and arterial thrombosis, the increased thrombotic potential in women with antiphospholipid syndrome is associated with recurrent pregnancy loss after 10 weeks gestation⁽⁵⁾.**6- bacterial infection:** Any sever infection that leads to the bacteremia or

viremia can cause an early sporadic miscarriage, recent study has reported that positive immunoglobulin G(IgG) serology for CLAMYDIA TRICHOMATIS is associated with early but not late miscarriage, the authors suggest that even in the absence of detectable organism, previous CLAMYDIA infection increases the miscarriage risk possibly by triggering chronic inflammation⁽⁶⁾.**7- Thrombophilia**

The pregnancy is a hypercoagulable state secondary to an increase in the levels of some coagulation factors, and a decrease in the levels of anticoagulant proteins, and an increase in fibrinolysis. Thrombophilic defect is an abnormality in the coagulation system that predisposes individual to thrombosis, the presumed hypothesis is that some cases of recurrent miscarriage and later pregnancy complications are caused by an exaggerated hemostatic response during pregnancy, leading to thrombosis of the uteroplacental vasculature and subsequent fetal demise⁽⁷⁾.**8- Environmental:**Smoking, alcohol and recreational drugs have all been associated with increased miscarriage rates, so cessation of such substances is recommended.**9- Un explained causes.**Causes of late pregnancy loss:There are several factors associated with fetal loss some of these may have a direct link to fetal loss (for example abruption or cord event) and others have indirect cause like obesity, so the most common causes of late pregnancy loss are the following^(1,2).

(I) - maternal age; In < 20 years may be associated with preterm birth, fetal growth restriction, pre-eclampsia. In > 40 years is a risk for Diabetes, chromosomal abnormalities, preterm birth, pre-eclampsia, growth restriction, multiple pregnancies.

(II)- hypertensive disorder such; Chronic hypertension, mild and severe pregnancy induced hypertension.

(III)-Endocrine and autoimmune disease; A-Insulin dependent diabetes and non-insulin dependent diabetes may be associated with Pre-eclampsia, thromboembolic disease, macrosomia, fetal growth restriction; B-SLE (systemic lupus erythematosus) is a risk for superimposed PET, abruption, FGR(fetal growth restriction), renal dysfunction, thromboembolic events. C-Thyroid disorders is a risk for; preterm birth, neurodevelopmental problems in offspring of hypothyroid mothers.

D-Thrombophilia is a risk for thromboembolic disease, PET and abruption.

E-Cholestasis of pregnancy may be associated with sudden unexpected fetal death.

(IV) - Marked obesity (BMI > 30 kg/m²) may be associated with thromboembolic disease, PET and abruption. thromboembolic disease, PET and abruption.

(V)- Multiple gestation (twin or triplet) is a recognized cause of; preterm birth, fetal growth restriction, anti-partum hemorrhage, congenital abnormalities, twin to twin transfusion (if mono-chorionic twins), cerebral palsy. (VI)- Ethnicity; Black women have been shown to have high risk of pre-eclampsia, diabetes and sickle cell disease.

(VII)- Smoking cigarettes > 10 is a risk for preterm birth, fetal growth restriction, cognitive impairment in offspring.

Pathophysiology of insulin resistance; Insulin hormone it is an endocrine hormone that secreted in to the maternal and to lesser extent in to the fetal blood stream where they act as placental signals and nourish the fetus, making them possible candidates for the endocrine control of the placenta. Decrease insulin sensitivity or increase insulin resistance is defined as the decrease biological response of nutrient to a given concentration of insulin at the target tissue, e.g. liver, muscle or an adipose tissue. Obesity is the most common risk factor related to decrease insulin sensitivity. Insulin resistance is often associated with a

hypercoagulable state (impaired fibrinolysis) and increased inflammatory cytokine levels insulin resistance has been demonstrated to increase expression of plasminogen activator inhibitor-1 (PAI-1). Plasminogen activator inhibitor-1 (PAI-1) activity is known to elevate levels of serum insulin and it induces a hypofibrinolytic state. This creates a thrombotic milieu at the maternofetal interface with high risk of miscarriage. Insulin resistance is known to play a critical role in the ovarian androgen excess and therefore might promote miscarriage by increasing circulating testosterone concentration. Insulin resistance/hyperinsulinemia is frequently associated with 40-50% of women having polycystic ovary syndrome especially obese women⁽⁸⁾. Furthermore, obese women with polycystic ovary syndrome are more likely indicative of insulin resistance than lean women with or without polycystic ovary syndrome. Insulin resistance/hyperinsulinemia correlate with implantation disturbances and cause infertility in polycystic ovary syndrome. However, few potential mechanisms of insulin resistance may contribute to abortion.

First: insulin resistance may cause excessive transport of glucose to the fetal environment leading to an increased loss in the first pregnancy.

Second: Hyperinsulinemia can elevate plasminogen activator inhibitor-1 (PAI-1) concentrations, which can lead to thrombotic induction and placental insufficiency⁽⁹⁾.

Third: Increased insulin concentrations are responsible for decreased concentrations of serum glycogen and insulin growth factor binding protein 1 (IGFBP-1), which have been observed in the first trimester of polycystic ovary syndrome patients. Decrease concentrations of these proteins suggest deficient endometrial development during the peri-implantation period⁽¹⁰⁾.

Association of insulin resistance and obesity; Insulin resistance (IR) is a physiological condition in which cells fail to respond to the normal actions of the insulin hormone. The body produces insulin, but the cells in the body become resistant to insulin and are unable to use it as effectively, leading to hyperglycemia. Beta cells in the pancreas subsequently increase their production of insulin, further contributing to hyperinsulinemia. It is well known that insulin resistance commonly coexists with obesity. However, causal links between insulin resistance, obesity, and dietary factors are complex and controversial. It is possible that one of them arises first, and tends to cause the other; or that insulin resistance and excess body weight might arise independently as a consequence of a third factor, but end up reinforcing each other. Some population groups might be genetically predisposed to one or the other. Another possible explanation is that both insulin resistance and obesity often have the same cause, a systematic overeating, which has the potential to lead to insulin resistance and obesity due to repeated administration of excess levels of glucose, which stimulate insulin secretion; excess levels of fructose, which raise triglyceride levels in the bloodstream; and fats, which can be easily absorbed by the adipose cells and tend to end up as fatty tissue in a hypercaloric diet⁽¹¹⁾. The most common type of insulin hormone resistance is associated with overweight and obesity in a condition known as the metabolic syndrome. Insulin resistance often progresses to full type 2 diabetes mellitus (T2DM) or latent autoimmune diabetes of adults^(12,13). This is often seen when hyperglycemia develops after a meal.

Pancreatic B-cells are unable to produce sufficient insulin to maintain normal blood sugar level (euglycemia) in the face of insulin resistance. The inability of the B-cells to produce sufficient insulin in a condition of hyperglycemia is what characterizes the

transition from insulin resistance to T2diabetes mellitus⁽¹⁴⁾. Insulin itself leads to a kind of insulin of insulin resistance ,every time a cell is exposed to insulin, the production of GLUT4 (type four glucose receptors) on the cells membrane decrease somewhat⁽¹⁵⁾. In the presence of a higher than usual level of insulin (generally caused by insulin resistance), this down regulation act as a kind of positive feedback, increasing the need for insulin . Exercise reverses this process in muscle tissue⁽¹⁶⁾. but if it is left unchecked, it can contribute to insulin resistance. Insulin resistance is often found in people with visceral adiposity (i.e., a high degree of fatty tissue within the abdomen as distinct from subcutaneous adiposity or fat between the skin and the muscle wall, especially elsewhere on the body , such as hips and thighs), hypertension, hyperglycemia and dyslipidemia involving elevated triglycerides, small dense low density lipoprotein particles, and decreased HDL (high density lipoprotein cholesterol levels). Insulin resistance is also often associated with a hypercoagulable state (impaired fibrinolysis) and increased inflammatory cytokine levels.

PCOS and Insulin Resistance: Polycystic ovary syndrome (PCOS) patients are known to have a high incidence of insulin resistance and glucose intolerance and tend to be at high risk of hypertension, diabetes mellitus and cardiovascular disease. Insulin resistance, probably via hyperinsulinemia, results in a general augmentation of steroidogenesis and LH release in PCOS. The profound insulin resistance and defects in insulin secretion, together with obesity, explain the substantially increased prevalence of glucose intolerance in PCOS. A number of obese PCOS patients display a particular metabolic pattern including an atherogenic lipid profile (dyslipidemia), glucose intolerance and an increased fasting insulin level, which is known to be closely linked with an insulin resistant state. (Approximately 75% of patients with PCOS are insulin resistant).

PATIENTS AND METHODS

This is a prospective case control study done in Al Zahraa teaching hospital in Al-Najaf city from the first of February to the first of October during year 2014 and it consists of 160 pregnant women enrolled in this study and divided into two groups : the first group was 80 pregnant women with pregnancy loss at different trimester as a (case group). patients with polycystic ovary syndrome and patients with diabetes are excluded after done for them oral glucose tolerance test and the second group was 80 healthy pregnant women at the of third trimester with no previous history of pregnancy loss as a (control group) . All patients are agreed to participate in this study by verbal consent. and then complete history from each patient was recorded on specially prepared data sheets including : (number of pregnancy loss and time of pregnancy loss , age, parity, gravidity and time of previous pregnancy loss). A fasting venous blood sample was aspirated from each patient at time of admission and from the control group.

Blood samples were collected in a tube chilled into ice then centrifuged, clear plasma was separated and plasma insulin hormone was assayed by Enzyme Linked Immuno Sorbent Assay (ELISA) technique.

Using insulin level kit. Then to evaluate serum insulin level in both groups. where the normal range of serum insulin level is 2-25 μ I/ml.

STATISTICAL ANALYSIS

Statistical analysis was done by using SPSS (statistical package for social sciences) version 20, in which we use independent sample T-test, ANOVA (analysis of variance) and person correlation coefficient for numerical data. We set up p value < 0.05 as significant.

RESULTS:

The results of this study consist of 160 women, 80 cases and 80 controls. The age, BMI and insulin level of these women had been shown in table 1 and 2.

Table (1) Mean and standard deviation of controls

| | Minimum | Maximum | Mean | Std. Deviation |
|-----------------------------|---------|---------|----------|----------------|
| Age/years | 22.00 | 35.00 | 28.8205 | 3.12773 |
| Insulin level(μ Iu/ml) | 10.770 | 16.883 | 12.72923 | 1.482723 |
| BMI(kg/m ²) | 19.00 | 32.00 | 26.13 | 2.89 |

table 1; show that the mean age of women was 28.8 ± 3.12 years and the mean insulin level was 12.72 ± 1.4 . The body mass index of control group was 26.13 ± 2.89 Kg/m²

Table(2) Mean and standard deviation of cases

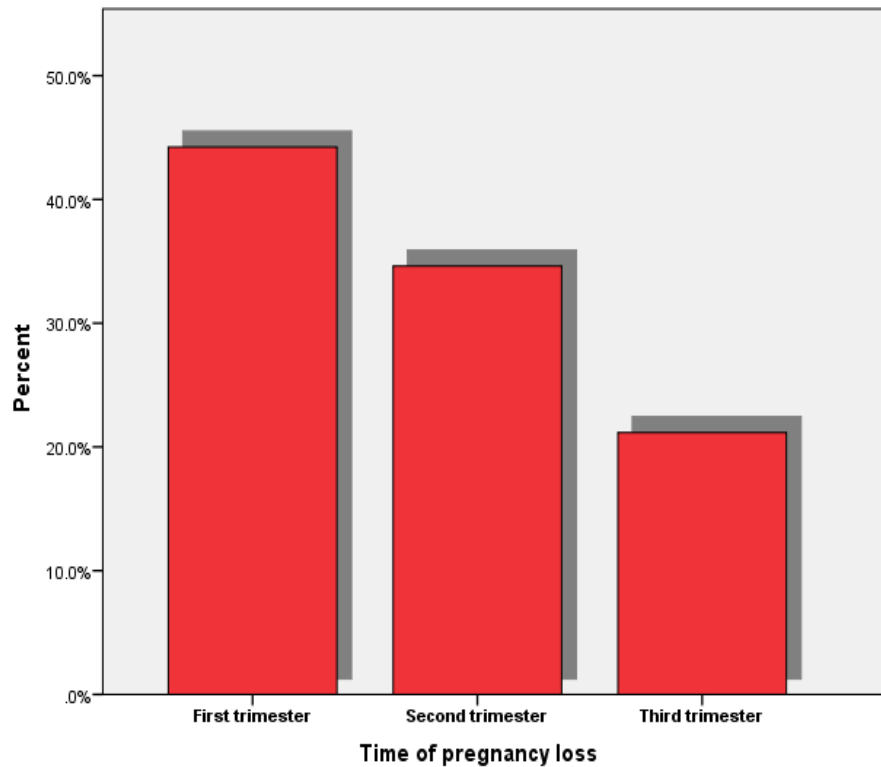
| | Minimum | Maximum | Mean | Std. Deviation |
|-----------------------------|---------|---------|----------|----------------|
| Age/years | 15.00 | 35.00 | 27.8462 | 4.36737 |
| Insulin level(μ Iu/ml) | 9.787 | 54.213 | 26.80717 | 10.267065 |
| BMI(kg/m ²) | 19.00 | 25.00 | 22.67 | 2.45 |

table 2 ; show that the mean age of women was 27.84 years and the mean insulin level was 26.8 ± 10.26 . The body mass index of cases group was 22.67 ± 2.45 Kg/m²

Table(3) Comparison between cases and controls in different variables.

| Variables | Cases(n=80) Mean \pm SD | Controls(n=80) Mean \pm SD | P value |
|-----------------------------|-----------------------------------|------------------------------------|------------------|
| Age/years | 27.84\pm4.36 | 28.82\pm3.12 | 0.679 |
| Insulin level(μ Iu/ml) | 26.8\pm10.267 | 12.729\pm1.482 | <0.001 |
| BMI(kg/m ²) | 22.67\pm2.45 | 26.13\pm2.89 | 0.031 |

table 3 ; show that there were significant difference in insulin level and BMI between cases and controls (as we show in this table the mean of insulin level in case group more than the upper normal limit compared with control group where the insulin level within normal range) while there were no significant difference between the two groups in age.



Figure(1) Patients distribution according to their time of loss

The highest percentage of recurrent pregnancy loss was found among women in their first trimester(45%) whereas it was (33.7%) for women in the second trimester. However it was much less in women during their third trimester (21.3%).

Table(4) Comparison between three trimesters in patient group.

| Estimated variables | Trimester | | | P value |
|-------------------------|--------------|--------------|-------------|---------|
| | First (n=36) | Second(n=27) | Third(n=17) | |
| Age/years | 27.86±3.64 | 28.44±5.53 | 26.81±3.76 | 0.632 |
| Insulin level(μIu/ml) | 31.182±7.1 | 22.158±9.82* | 25.05±13.39 | 0.021 |
| BMI(kg/m ²) | 23.21±2.2 | 22.34±2.7 | 23.54±1.9 | 0.899 |

table 4;show that there were significant difference in insulin level in the second trimester compared to the first trimester while it is non-significant between the first and third trimester. Also non-significant difference between groups regarding age and BMI.

DISCUSSION

Recurrent pregnancy loss is one of the most frustrating and difficult areas in reproductive medicine because the etiology is often unknown and there are a few evidence based diagnostic and treatment strategies. Dysregulation of insulin metabolism and /or in the placenta may be implicated in the pathogenesis of various disorders during pregnancy such as recurrent miscarriage, gestational diabetes,intrautrine growth retardation and pre-eclampsia.

Patients in this study were classified as having recurrent pregnancy loss which was associated with endocrine abnormalities, these abnormalities obvious in their insulin level, with significantly higher level than the level found in the control group (table 3) and this finding agrees with Corina-Alina Ispasoiu and Iverson CL study^(17,18). Also in this study we aimed to identify the age factor and its relation with recurrent pregnancy loss, as we know that incidence of recurrent pregnancy loss increases with increase maternal age but in our study we take the range of maternal age between 15 years and 35 years, therefore we noticed there is no significant difference in age between cases and control group and this is consistent with study of Corina-Alina Ispasoiu study⁽¹⁷⁾.

Another remarkable factor introduced in this study is the effect of the body mass index which is associated with metabolic glucose intolerance, type 2 diabetes mellitus and dyslipidemia and non-metabolic disorders. However, more than its total amount, the distribution of adipose tissue throughout the body is a better predictor of the risk to the development of those disorders. Fat accumulation in the abdominal area and in non-adipose tissue is associated with an increased risk to develop metabolic and non-metabolic derangements. So in our study the body mass index introduced was up to 25 in the case group and we notice a significant difference between the case group and control group and this is inconsistent with Rayah S Baban, et al⁽¹⁹⁾ study. In addition we found that the percentage of pregnancy loss increases in the 1st trimester compared with the 2nd trimester and the latter is more compared with the loss in the 3rd trimester and this is consistent with Craig, et al study⁽⁹⁾. There is a significant difference in insulin level in the 2nd trimester compared with the 1st trimester while there is no significant difference between the 1st and 3rd trimester and this goes with other study. Lowering of insulin level with insulin sensitivity agents (metformin) enhances uterine vascularity and reduces uterine vascular resistance and decreases rates of early pregnancy loss in a series of small studies⁽²⁰⁾.

CONCLUSION

Current study showed that, in women with recurrent pregnancy loss, fasting insulin is high compared with normal healthy pregnant women.

RECOMMENDATION

We recommend that measuring fasting insulin level is useful in all women with recurrent pregnancy loss. Also because most pregnancy losses occurred during the first trimester, it is highly recommended that insulin hormone evaluation tests are provided as early as possible when pregnancy occurs.

Insulin elevation during pregnancy may serve as markers for detecting and monitoring pregnancy complications especially if cases of recurrent pregnancy loss (RpL) have no specific medical cause for pregnancy loss. The use of insulin sensitizing agent (metformin) and decrease body weight with regular daily exercise before embarking in pregnancy, also from recommendation. Furthermore comprehensive studies are needed in the future.

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