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Level of placental trophoblastic KISS1 protein is associated with high risk of recurrent spontaneous abortion in Iraqi women

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Abstract:

Introduction: Recurrent pregnancy loss is the syndrome that causes repeated miscarriage and/or stillbirth impairing the ability to have a live birth. More from research interest has been focused on the association studies between different factors and recurrent spontaneous abortion. The aim of this study was examine the role of alteration in levels of KISS1protein and in recurrent spontaneous abortion (RSA).

Methods: Level of KISS1 protein was detected by immunohistochemical examinations in samples from placenta and measurement the levels of Follicle stimulating hormone (FSH) , Luteinizing hormone (LH) by using Enzyme linked Fluorescent Assay (ElFA) technique of groups I (women pregnant were normal delivered pregnancy at delivery in the third trimester (n=15); II refer to normal pregnant in first trimester (n = 15) (I ;II control group)., III represented aborted women for the first time during the first trimester (n = 15.(and group (IV): (30) represented repeated aborted women during the first trimester

Results: Finding from this study revealed that the level of the protein was high in abortion groups and increased levels of LH and FSH as well.

Conclusion: Kisspeptine is implicated in RSA through inhibition of normal placentation and indirectly through stimulation of LH and FSH secretion.

Key words: *KISS1, Kisspeptine, Recurrent spontaneous abortion, LH, FSH*

Introduction:

Recurrent spontaneous abortion (RSA) is a reproductive problem that occurs in women in reproductive age with a frequency of 1 %–3 % (1). It is defined as two or more repeated pregnancy losses before the 20th week of gestation (2). The risk of miscarriage is enhanced by a variety of factors including chromosomal abnormalities, uterine abnormalities, hereditary thrombophilia, endocrinologic disorders,- immunologic factors, infections, and nutritional and environmental factors (3) .In the past years, research interest has been focused on the association between different genetic factor and recurrent spontaneous abortion.

Metastin, originally identified as metastatin, important in preventing cancer metastasis, has more recently been shown to be important in pregnancy. Roles indicated for metastin in pregnancy include regulating trophoblast invasion and migration during placentation. Metastin is encoded by a putative metastasis suppressor gene named KiSS-1 , its protein KISS consists of 54-amino acid residues and an amidated C-terminus (4)

The Kiss1 system has emerged in recent years as a fundamental player in the control of the reproductive axis, with essential roles in early differentiation and pubertal activation of the reproductive brain as well as key functions in the regulation of ovulation and the metabolic control of fertility (5). The first evidence for an essential role of kisspeptin signaling in the control of reproduction came from genetic studies in humans (6). The mechanisms whereby kisspeptins conduct their key reproductive roles are probably multifaceted, but compelling evidence has demonstrated that kisspeptins are capable of directly (and probably, also indirectly) stimulating GnRH neurons to secrete GnRH, as documented in various species (7;8)

Trophoblast invasion strongly resembles tumor metastasis as the invading trophoblasts utilize virtually the same molecular mechanisms for their migratory and invasive functions as tumor cells. These include expression of proteases

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that degrade the extracellular matrix (ECM) such as matrix metalloproteinases (MMPs), telomerase activity and immunosuppressive environmental conditions. Therefore, the normal trophoblast has been termed 'pseudo-malignant' (9)

Of particular importance to placentation , the involvement of KISS1 in cell migration, a process which is of crucial importance for trophoblast invasion. KISS1 has been shown to induce focal adhesion and stress fibre formation. Also to phosphorylate focal adhesion kinase and paxillin , the intracellular signals needed for cell migration, which may associate with integrins to inhibit migration (10).

A few previous studies have identified differences in gene expression between women with and without a history of RSA that have suggested potential involvement of immune, angiogenic and cell invasion regulators (11). Therefore, the main goal of this study was to investigate whether alteration in kisspeptine levels are involved in RSA in Iraqi population and to elucidate the possible mechanism by which kisspeptine induce its action.

Patient, Material and Methods:

Patients: This study was conducted during the period from July 2012 to June 2013. Seventy five women (Patients' ages ranged between 20-40 years) during first and third trimester included in the study patient have had curettage operation at the Obstetrics and Gynecology Department of AL-Yarmook Teaching Hospital, Dijlah hospital and Fatima AL- Zahraa hospital and then divided into four groups:

Group I: (15) women pregnant were normal delivered pregnancy at delivery in the third trimester (control group).

Group (II): (15) were normal pregnant in first trimester .

Group III: (15) represented aborted women for the first time during the first trimester.

Group (IV): (30) represented repeated aborted women during the first trimester .

None of them had any significant medical disease or anatomical uterine abnormality.

Material and methods: After taking informed consent from the patients

Blood sample for hormonal Study:

6ml of peripheral blood sample were taken for serum hormonal measurement the sample were obtained without anticoagulant collected in dry tube .In the lab. serum was separated by centrifugation and stored at 2-8°C until hormonal level measurement.

Follicle stimulating hormone (FSH) , Luteinizing hormone (LH) were measured. In this study, the use of technology Enzyme linked Fluorescent Assay (ElFA) technique and Mini VIDAS name of the device of the company bio Merieux.The assay principle combines an enzyme immunoassay competition method with final fluorescent detection.

Sera from women in these groups were negative for specific IgM and IgG for rubella virus, human cytomegalovirus, and Toxoplasma gondii and negative for specific IgM for Herpes simplex virus.

Trophoblastic tissue from each woman, two to three samples were taken from different sites of the uterus during evacuation operation; thus, 2-3 paraffin embedded blocks were prepared for each patient. Sections from each block were stained with heamatoxylin and eosin for histopathological examination (only the sections trophoblastic tissue were included in the stud).

For Immunohistochemistry technique (IHC), KISS1 protein, was detected by immunohistochemical staining with 1/150 diluted anti-kisspeptin antibodies (Abcam, UK)

Based on staining intensity and number of positive cells, all sections were evaluated according to a score from 0 to 9. Samples were scored negative if the net result of intensity x number of positive cells equals to 0, 1, and 2. Whereas, samples were positive when the net result equals to 3, 4, 6, and 9 . (12)

Statistics: Data were expressed as mean±SE, *P<0.05 results were analyzed by one sample t-test and comparison between groups by one way ANOVA.

Result:

Results revealed that abortion groups have LH high hormone levels of 1.57 ± 0.16 in group III and the highest level was 1.8 ± 0.1 in groups IV. These levels were significantly higher from the control groups I and II (0.8 ± 0.12 and 0.188 ± 0.04; P<0.05 and P<0.01 respectively). These results are explained in figure (1).

While results indicated that the abortion groups III and IV have elevated FSH hormone levels (expressed as IU/L) were 1.5 ± 0.15 and 1.6 ± 0.096 successively and these levels were significantly higher than those of the control groups I and II (0.65 ± 0.103 and 0.31 ± 0.07; P<0.05 and P<0.01 respectively). These results are explained in figure 2.

Characteristics of the groups are shown in Table1, group I have 84% of patient samples were negative score and 16 % positive score. In contrast to group I, groups II and III demonstrated a negative scores of 62% and 68 % respectively. Positive scores were high in group II 38% and 32% in group III compared to control group (group I) (P<0.05).

Figure (3) illustrated the level of the KISS1 protein in the groups under investigation. It is clear from the figure that group I has the lowest level of protein. On the other hand, groups II and III demonstrated a high level of the protein.

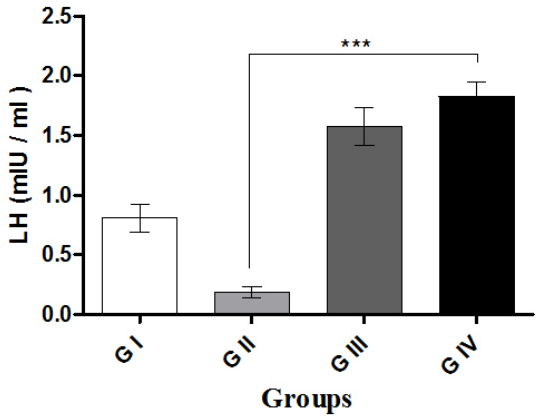

Figure 1: Effectof LH hormoneon Recurrent Spontaneous Abortion. The levels of the hormone in the abortion groups (III and IV) were found to be greater than those of the control groups (I and II).

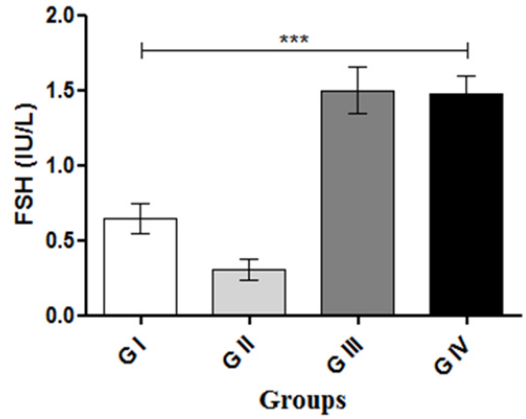

Figure 2: Effect of FSH hormone on Recurrent Spontaneous Abortion. Levels of the hormone in abortion groups (III and IV) were found to be higher than those of the control groups (I and II).

Table 1: Human suppressor metastatin level in trophoblasts of aborted and control cases

Study groups	Cases N	Trophoblasts	
		Negative (%)	positive (%)
GI	15	84	16
GIII	15	62	38
GIV	30	68	32

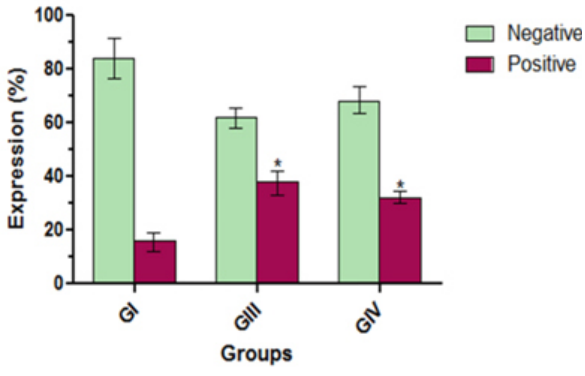

Figure 3 : Human suppressor metastatin in normal pregnancy and aborted women. Score was sorted into negative and positive based on the intensity and number of positively stained cells. Group I has lower percent of positive score compared with groups II and III

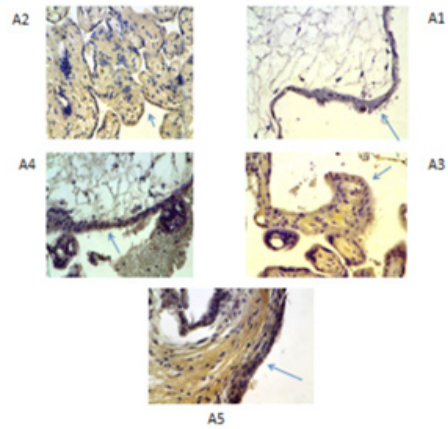

Figure 4: Photomicrographs of different kinds of staining intensity of KISS1 level in trophoblasts (×400). (A1–A3) Immunostaining of KISS1: negative staining (A1), weak staining (A2), moderate staining (A3) and strong staining (A4)

Discussion:

Successful pregnancy outcomes are dependent on trophoblast invasion into the uterine vasculature and on the development and maintenance of an adequate uteroplacental circulatory system (13). As a regulatory factor for trophoplast invasion during placentation process we investigated the levels of KISS1 protein in normal pregnant women and those with abortions. Moreover, it has been reported that KISS1 is highly expressed in the synctiotrohpbalst (ST) in normal human placenta with increased KISS1 mRNA levels in early placenta (14). Finding of this study indicated that the levels of KISS1 is markedly high in cytoplasm of trophoplacental cells at first trimester abortion (mean age) . Despite several studies have been conducted on the implication of placental Kp in RSA, data remains contradictive. Recent report has shown decreased expression of KISS1 in women with RSA (15). It has been suggested that lower levels of KISS1 within the placenta in pathological pregnancies may signal low invasive capacity, where reduced invasive capacity would be expected (16). However, it is hypothesized that KISS1 is highest during the period of maximal trophoblast invasion which suggest that high invasive capacity is counteracted by the inhibitory effect of high levels of kisspeptin, as a result low invasive capacity. Alternatively, smaller less invasive placentae may develop which have a reduced capacity to produce KISS1 (16). Therefore, we aimed to measure plasma levels of kisspeptine in third trimester normal pregnancy and compared with abortion groups. Finding of our research revealed that level of KISS1 protein was strongly elevated in first trimester abortion groups compared with third trimester normal pregnancy group. For estimation of this protein level in first trimester normal pregnancy was assumed it to be doubled the level of third trimester in normal delivery. Our assumption is supported by recent studies performed by Cartwright and Williams., (10) in which the authors demonstrated that expression of KISS1 gene was high in early pregnancy abortion, in women underwent elective surgical termination of pregnancy, and reduced to be around 50% of the expression level at third normal trimester pregnancy. The present findings revealed that the level of KISS1 protein in abortion groups is more than double of the amount in third trimester normal delivery group. Current findings are in agree with recent studies by (17) where there was an elevation in the level of KISS1 protein in early pregnancy trophoplast and the level of KISS1 in missed abortion was high compared with normal pregnancy.

A possible explanation for the increased level of KISS1 in the pathophysiology of RSA may relate to the simultaneous increase in KISS1 receptor (KISS1R), which may serve to maximise the activity of KISS1 and thereby inhibit trophoblast migration, which is crucial to the successful development of the placenta. KISS1 has also been shown to be angiostatic (18) and therefore increased overall KISS1 activity due to increased KISS1R may result in inhibition of angiogenesis within human placental vessels leading to the development of RSA

Considering the association between abnormalities with age, the level of KISS1 within abortion groups (groups II and III) was accelerated in group II , characterized by lower mean age (25 years) and decreased number of abortions, compared with group III that characterized by higher mean age (33 years) and increased incidence of abortion among all groups. Interestingly, the difference between both groups was not significant. It is interesting to note that in women with RSA at first time (mean age 25) and those with repeated miscarriage (mean age 33), there was increased levels of KISS1 suggesting that these differences are apparent early.

Although Kp were originally defined as metastasis suppressors, most of the recent attention has been directed at their central role in reproduction. These features make it tempting to predict that alterations of this system might result in substantial perturbations of the gonadotropic axis. In this scenario, it is appealing to hypothesize that dysregulated gonadotropin secretion may reflect altered patterns of kisspeptin inputs to GnRH neurons (19). To investigate more the possible mechanism of action of Kp, we measured the levels of both LH and FSH in all study groups. Results obtained showed that increase levels of these hormones in patient groups compare with controls groups.

In addition to central actions, kisspeptins have been detected in the ovary and in the circulation, although the physiologic relevance of the latter, except during gestation, when a dramatic rise of circulating kisspeptins is detected (Horikoshi et al., 2003), remains obscure. Nonetheless, few studies have evaluated kisspeptin concentrations in nonpregnant women. Increased plasma kisspeptin concentrations were found in girls with premature thelarche (20). Moreover, the secretagogue activity of kisspeptins on gonadotropin secretion has been demonstrated in a wide variety of species after administration via different routes and/or at low doses (21;22).

Our findings revealed a positive correlation between kisspeptin concentrations and LH and FSH suggesting an indirect mechanism of action of kisspeptine in the development of RSA.

It is concluded from present study that KISS1 is strongly involved in RSA in Iraqi population through its action on GnRH and placenta. Of significant importance, the high level of KISS1 protein in women experiencing first abortion can be used as a parameter to predict abnormalities in following pregnancies. Future testing for KISS1 is therefore an important step in the pregnancy evaluation and treatment-decision pathway. Moreover, the high incidence of abortion found in group III, with level of KISS1 protein, suggesting that KISS1 gene is not a dominant factor but abortion probably due to other factors that may have more influence abortion in these patients such as the chromosomal abnormalities.

Limitations of the present study are that the increase in KISS1 levels in RSA may represent a secondary change rather than being responsible for the primary pathogen-

esis of RSA; however, the confirmation of higher levels of KISS1 in early placenta pregnancy samples indicates that these findings are likely to be important in the early placental changes associated with RSA which requires more molecular studies to dissect the molecular mechanisms by which by which KISS1 protein influence normal pregnancy. Furthermore, the present sample size is relatively small (n=15) in some groups and these findings need to be replicated in a much larger sample size to validate the present findings.

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مستوى البروتين في الارومه الغاذيه مع ارتفاع مخاطر الإجهاض التلقائي المتكرر في النساء العراقيات

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الخلاصة:

فقدان الحمل المتكرر هو الذي يسبب متلازمة الإجهاض المتكرر أو ولادة جنين ميت تكرار هذه قد تقل النسبه في القدرة على الحصول على ولادة حية. الكثير الأبحاث ركزت على دراسه العلاقة بين العوامل المختلفة والإجهاض العفوي المتكرر. وكان الهدف من هذه الدراسة دراسة دور تغيير في مستويات KISS1protein والإجهاض التلقائي المتكرر (RSA).

تم الكشف عن مستوى البروتين KISS1 من خلال استعمال طريقه التحليل الكيميائي المناعي في عينات من المشيمة من المجموعتين الأولى (الحوامل في الثلث الأول من الحمل (ن = 15)، والثاني يشير إلى الحمل طبيعي في الثلث الأول من الحمل (ن = 15) وتم اعتبارهما مجموعات سيطره واما المجموعه الثالثه فكانت تمثل النساء اللاتي اجهضن للمره الاولى خلال الاشهر الثلاثه الاولى من الحمل (ن = 15) والمجموعه الرابعه تمثل النساء اللاتي تعرضن للاجهاض المتكرر

بيت النتائج من هذه الدراسة أن مستوى البروتين كان مرتفعا في مجموعات الإجهاض وكذلك زيادة في مستويات LH FSH لذلك نستنتج من هذه النتائج دور البروتين kisspeptein في الاجهاض التلقائي المتكرر من خلال تثبيط المشيمه الغاذيه وبصورة غير مباشرة من خلال تحفيز افراز هرمون المحفز للجريبات وهرمون الحليب