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Review article

Kisspeptin as a potential biomarker throughout pregnancy

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ABSTRACT

Kisspeptins are a family of neuropeptides that are critical for the puberty initiation and female fertility. Plasma or serum kisspeptin is mainly derived from the placenta during pregnancy and plasma kisspeptin levels significantly increase across pregnancy. Plasma kisspeptin levels could be used as a potential biomarker for the detection of miscarriage, pre-eclampsia, gestational trophoblastic neoplasia (GTN), and fetal development. Kisspeptin may also be involved in the process of parturition by stimulating oxytocin secretion during term pregnancy. This review discussed the potential use of kisspeptin as a marker across pregnancy and highlighted the unresolved problems in this area.

Tweetable abstract: Plasma kisspeptin levels could be used as a potential biomarker across pregnancy.

Contents

Introduction

In the past decade, accumulating studies have demonstrated that kisspeptin is responsible for pulsatile and surge GnRH release, with essential roles in regulating the puberty onset, gonadotropin secretion, brain sex differentiation, ovulation triggering, and metabolic regulation of fertility [1–3]. Kisspeptin and its encoding gene, *KISS1*, were first identified in 1996 in Hershey, Pennsylvania, USA—the hometown of the famous Hershey's kisses chocolates [4], and the "SS" in *KISS1* was representative of "suppressor sequence" [4]. In human, *KISS1* is located on the long (q) arm of chromosome 1 at q32. *KISS1* gene encodes an unstable and biologically inactive intermediate prepropeptide of 145 amino-acids, which is further post-translationally processed to four biologically active peptides: kisspeptin-54, 14, 13, and 10 [5,6]. Kisspeptin-54, the major product of the human KISS1 gene, was also termed "metastin" for its ability to suppress tumor metastasis [6]. All of the peptides can activate their shared receptor, KISS1R, as they have a C-terminal region that contains an Arg-Phe-NH2 signal motif. Based on the structural similarities of these peptides and their shared origin as *KISS1* derived products, the term kisspeptin was globally used to

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define this family [1,5]. All these peptides can bind to and activate KISS1R with the same affinity and efficacy in both humans and rats [5]. The placenta-derived hormones, including HCG, are often used as a biomarker to help clinicians make consultations and manage disorders during pregnancy. Similar to HCG, both *KISS1* and *KISS1R* are highly expressed in the placenta [4,6,7], and kisspeptins could be isolated from human placental extracts [6]. Kisspeptin-54 was first observed to be present in human plasma in 2003 and its plasma levels consistently increased across the pregnancy in humans [8]. Accumulating data showed that plasma kisspeptin levels could be a potential marker to diagnose and/or predict pregnancy-related diseases, such as pre-eclampsia and miscarriage [9–11]. These findings may enable clinicians to manage clinical work better in the future. Furthermore, kisspeptin may also be involved in the process of parturition by stimulating oxytocin secretion during term pregnancy. This review summarizes our current understanding of the role of plasma or serum kisspeptin levels as potential biomarkers across pregnancy and discuss the possible role of kisspeptin in the process of parturition.

Kisspeptin levels in the plasma and placenta across gestation

In humans, the plasma level of kisspeptin (kisspeptin-54) increases dramatically throughout pregnancy, with a 900-fold increase in the first trimester and over a 7,000-fold rise in the third trimester when compared to the non-pregnant women (Fig. 1) [8]. However, previous studies suggested that the *KISS1* mRNA levels in the early placenta and in the term placenta of pregnant women did not differ significantly [12]. Additionally, two independent studies suggested that the protein levels of kisspeptin-54 (using western blotting and immunohistochemistry) were much higher in the early placenta [13,14]. Interestingly, all these studies indicated that *KISS1R* mRNA and protein levels were higher in the early placenta than the term placenta [12–14]. When taking these data together, it seems that *KISS1* expression levels in the placenta may not necessarily reflect the plasma levels of kisspeptin. It is possible that the increased mass and trophoblast cells in the term placenta may lead to the increased circulation concentration of kisspeptin, despite the reduced *KISS1* expression levels. Recent studies in cows suggested that plasma kisspeptin levels consistently increased throughout pregnancy [15,16], indicating that the rise of plasma kisspeptin levels across pregnancy is conserved. However, a contrasting study indicated that plasma kisspeptin levels were very low and did not increase during pregnancy in several mammals, including sheep, cow, pig, rabbit, horse, rhesus monkey, and marmoset [17]. Based on these findings, it remains uncertain whether the increase in plasma kisspeptin levels across gestation is unique to human. While in other mammals, more related studies are needed to confirm the results. It needs to be noted that kisspeptins are a series of peptides, including kisspeptin-54, kisspeptin-14, kisspeptin-13, kisspeptin-10 [5,6]. These studies seemed to detect only kisspeptin-10 in the plasma. Additionally, the possibility that the antibody used could cross-react with other RF-amide-related peptides (RFRP), including prolactin-releasing peptide, RFRP1, RFRP3, et.al could not be excluded [10]. A previous study that used a Radioimmunoassay (RIA) kit with a high specificity and sensitivity to detect the plasma kisspeptins (include human kisspeptin-54, kisspeptin-14, and kisspeptin-10, rather than one kind of kisspeptin) demonstrated that plasma kisspeptin levels significantly increased in pregnant women compared with non-pregnant women [10]. In agreement, a very recent study that detected the plasma kisspeptin (kisspeptin-54) levels in pregnant women suggested the same results [9]. Therefore, the evidence that the increase in plasma kisspeptin levels across gestation seems convincing, at least in human. Since the processing time as well as the collecting method of samples can affect the concentration of kisspeptin in fluid samples [18], it would be advisable for future studies to use a standardized sample collecting method for the measurement of circulating kisspeptin levels. Future studies also need to address the seemingly incongruous findings between plasma kisspeptin levels and placental *KISS1* expression in humans.

Kisspeptin and early pregnancy viability

Miscarriage is the most common complication during early pregnancy affecting 20% of recognized pregnancies, with a potentially devastating impact on the health of pregnant women [19,20]. As miscarriages (except the threatened miscarriage) are sometimes irreversible, prevention is probably the only way to intervene in this problem. One possible way is to develop biochemical markers that have a high diagnostic accuracy to predict or diagnose the occurrence of miscarriage. Accumulating evidence suggested that plasma or serum kisspeptin (kisspeptin-10 or kisspeptin-54) levels showed equivalent or even better accuracy to discriminate confirmed miscarriage (spontaneous

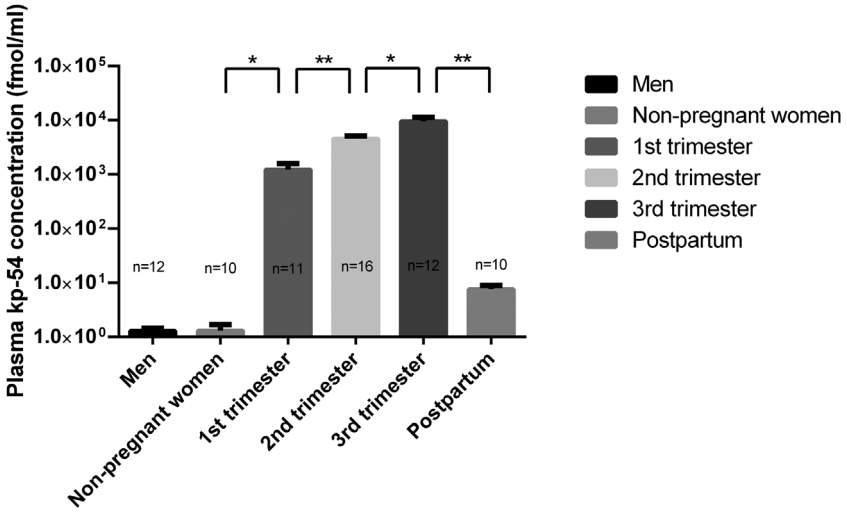

Fig. 1. Mean concentrations of kisspeptin-54 in men, nonpregnant women, in the first trimester (1 st), second trimester (2nd), and third trimester (3rd) of pregnancy, and postpartum. *, $P < 0.05$; **, $P < 0.01$.
Modified from [8].

miscarriage) from intrauterine pregnancy at the time of diagnosis [9,10,21]. In the study of Sullivan-Pyke et al. [9] (case-control study), the research group detected kisspeptin-54 in serum and plasma samples in intrauterine pregnant women (n = 20) and women with confirmed miscarriage (n = 20). While in the study of Jayasena et.al [10] (prospective cohort study), the authors detected kisspeptin-54, kisspeptin-14, and kisspeptin-10 in plasma samples in women with a singleton pregnancy (n = 899) and women with confirmed miscarriage (n = 50). Nevertheless, both studies performed adequate analysis to control and discuss possible confounders, including gestational and maternal age, and both suggested that kisspeptin levels were significantly lower in the miscarriage group than in the uncomplicated group. When compared with the serum hCG levels, the plasma kisspeptin levels had a higher diagnostic performance for miscarriage (ROC area under the curve: 0.899 ± 0.025, kisspeptin; 0.775 ± 0.040, hCG). Furthermore, combined kisspeptin and hCG measurement (OR 0.10; 95% CI 0.06–0.17; $P < .0001$) showed comparable diagnostic accuracy compared to kisspeptin measurement alone (OR 0.11; 95% CI 0.07–0.17; $P < .0001$) [10]. In the case-control study, the authors showed that serum kisspeptin and hCG levels have comparable diagnostic value (ROC area under the curve: 0.953, kisspeptin; 0.994, hCG) [9]. The gestational age of women enrolled in the case-control study is 6–10 weeks [9]. While the gestational age of women enrolled in the prospective cohort study is 8–14 weeks [10]. It is possible that the diagnostic value of circulating kisspeptin levels increases as the pregnancy progresses. These data strongly suggested that kisspeptin levels in the plasma could be considered as a new promising marker for early pregnancy viability. A prospective cohort study in the future is necessary to confirm the applicability of the serum or plasma kisspeptin assay for prediction or diagnosis of miscarriage.

Kisspeptin and pre-eclampsia

Pre-eclampsia, a gestational complication characterized by the de-novo development of concurrent hypertension and end-organ dysfunction after 20 weeks of gestation, remains the second leading cause of maternal death [22]. Recent studies implicated a possible role of kisspeptin in predicting and diagnosing this disease. For diagnosing role, accumulating data have shown that plasma kisspeptin (kisspeptin-10) levels were significantly lower in patients with pre-eclampsia than those in the normotensive pregnant women in the second and/or third trimester [23–26]. Additionally, plasma kisspeptin (kisspeptin-10) levels negatively correlated with proteinuria in 24 h (r = -0.299, $P < 0.01$) and with mean arterial pressure (r = -0.316, $P < 0.01$) in pre-eclampsia patients, suggesting that plasma kisspeptin (kisspeptin-10) levels were related to the severity of this disease [24]. Only one study suggested that plasma kisspeptins levels (including kisspeptin-54, kisspeptin-14, and kisspeptin-10) were not significantly different between patients with hypertensive diseases of pregnancy and normatensive controls [27]. However, this study did not match the gestation age of the two groups, with the pregnancy duration in pre-eclampsia group much longer than the normal control group (35.4 ± 1.1 vs 31.6 ± 0.5 weeks) [27]. This is important as the plasma kisspeptin levels significantly and consistently increased across gestation [8,10]. The same study also showed that treatment with kisspeptin had no significant effect on blood pressure in men or women [27], indicating that the changes of the plasma kisspeptin levels resulted from rather than resulted in the hypertensive diseases of pregnancy. For predicting role, serum kisspeptin (rather than plasma kisspeptin) levels in 11-14 weeks [28] or 16-20 weeks [29] of pregnancy were significantly lower in women who subsequently developed pre-eclampsia than in uncomplicated pregnant groups. In agreement, plasma kisspeptin concentrations (16-week of pregnancy) were lower in women who later developed pre-eclampsia compared with women with uncomplicated pregnancies [28,30]. Additionally, plasma kisspeptin (16 weeks) levels were negatively associated with 28- and 36-week blood pressure 30], indicating that plasma kisspeptin levels had the potential ability to predict the severity and/or outcome of the disease to some extent.

The change of *KISS1* expression in placenta tissue seems to be different from plasma or serum kisspeptin levels in the cases of pre-eclampsia. Higher expression of *KISS1* mRNA and/or protein levels in pre-eclampsia patients than in normal term pregnant group was observed [25,31,32]. Only one study showed that *KISS1* expression in placenta tissue was reduced in pre-eclampsia patients [14]. The inconsistency is probably attributed to the discrepancy of the onset time for pre-eclampsia as elevated *KISS1* expression occurs only in early-onset preeclampsia (before gestational week 34) and not late-onset preeclampsia (after gestational week 34) [11]. Importantly, the discrepancy of gestation age between the uncomplicated group and the pre-eclampsia group should also be taken into consideration since the expression of *KISS1* changes across pregnancy [14,33].

The dysregulation of matrix metalloproteinase-9 (MMP9) in the placenta has a negative effect on the invasion and maturation of the placenta and was associated with the occurrence of pre-eclampsia [34,35]. Therefore, that kisspeptin down-regulates the expression of MMP9 in placenta and subsequently the invasion and maturation of placenta may underlie the relationship of the locally overexpressed *KISS1* level and subsequent pre-eclampsia [32,36]. Kisspeptin-10 had an antiangiogenic effect and showed the ability to inhibit new vessel sprouting from placental arteries [37,38]. The defect of angiogenesis was thought to be involved in the pathogenesis of pre-eclampsia [39,40] and related to the severity of the disease and perinatal outcome [41,42]. Additionally, localization of KISS1R has been found in the smooth muscle of vessels, including the aorta, coronary artery, and umbilical vein [38,43]. It seemed that kisspeptin could have a cardiovascular effect on the body by binding to the receptor in vessels, and abnormal plasma kisspeptin levels may act as a potential factor involved in the promotion of pre-eclampsia. However, administration of kisspeptin was not associated with significant changes in blood pressure or heart rate in healthy men and/or women [27,44]. Taking these data together, it seems that locally produced kisspeptin could directly act on the placental tissues and exert an anti-angiogenesis effect in patients with pre-eclampsia. While the changes in plasma kisspeptin levels probably result from rather than result in this disease.

Kisspeptin and gestational trophoblastic neoplasia (GTN)

The highest *KISS1* expression levels in placenta were found during the first trimester in human [13,14] and embryo day 12.5 in rodents [17,45], which coincides with the time of peak trophoblast invasion when regulation of this process is of critical importance [17]. In human, *KISS1* was mainly expressed in the villous trophoblast and kisspeptin inhibited trophoblast migration through a paracrine/autocrine manner within the placenta tissue [13,46,47]. Therefore, the change of local expression of *KISS1* in the placenta may be involved in placental invasive disease.

Gestational trophoblastic neoplasia (GTN) is a set of malignant placental diseases, including invasive mole, choriocarcinoma, placental site trophoblastic tumor and epithelioid trophoblastic tumor [48,49]. Serum hCG levels were largely used to predict the prognosis of malignant GTN before, during, or after chemotherapy [50,51]. Previous studies suggested that *KISS1* and *KISS1R* expression was significantly increased in mole (benign GTN cells), but reduced in choriocarcinoma cells (malignant GTN cells) [12]. The results indicated that down-regulation of the placental expression of *KISS1* might be responsible for the malignant

invasion. Intriguingly, the plasma kisspeptin levels (including kisspeptin-54, kisspeptin-14, and kisspeptin-10) in patients with malignant GTN were elevated [52]. This study supported that the placental expression levels of *KISS1* may not necessarily reflect the plasma levels of kisspeptin. Additionally, the plasma kisspeptin levels fell during and after treatment with chemotherapy in GTN patients [52], indicating that plasma kisspeptin levels could be used to predict the prognosis of malignant GTN. Furthermore, the plasma kisspeptin levels positively correlated with plasma hCG levels ($r(2) = 0.99$, $P < 0.0001$) in patients with malignant GTN before, during, or after chemotherapy [52], indicating that plasma kisspeptin and hCG were derived from the same tissue and showed similar secretion pulse in the cases of malignant GTN. It would be of great interest for future studies to compare plasma or serum kisspeptin levels with serum hCG levels in the sensitivity and specificity of predicting the prognosis of malignant GTN.

Kisspeptin and fetal development

Emerging studies suggested that plasma kisspeptin levels across pregnancy were able to predict the birth weight. In an uncomplicated pregnancy, plasma kisspeptin (kisspeptin-10) levels in the first trimester (gestation age week 8–14) were significantly lower in pregnancies with small for gestational age neonates (defined as customized birth weight below the 10th centile) [53]. In agreement, kisspeptin levels (16 weeks) in maternal plasma were positively associated with birthweight in uncomplicated pregnancy ($r = 0.16$, $P < 0.0001$) [30]. In patients diagnosed with pre-eclampsia, plasma kisspeptin (kisspeptin-10) levels were directly correlated with the estimated fetal weight in utero during the second and the third trimesters ($r = 0.760$, $P = 0.001$, and $r = 0.920$, $P = 0.0001$, respectively) [26]. These data strongly suggested that plasma kisspeptin levels were an emerging marker for the evaluation of birth weight in uncomplicated pregnancy or patients with pre-eclampsia. However, a previous study suggested no significant correlation between plasma kisspeptin levels (including kisspeptin-54, kisspeptin-14, and kisspeptin-10) in all trimesters and birth weight at delivery in patients with pre-eclampsia or the uncomplicated group [23]. The inconsistency probably resulted from the different specificity of the antibody used to detect plasma kisspeptin levels.

Kisspeptin and parturition

A recent study showed that intracerebroventricular administration of kisspeptin-10 increased the firing rate of oxytocin neurons in the hypothalamus in late-pregnant rats (days 18–21 of gestation) but not in early- or mid-pregnant rats [54]. Oxytocin is principally synthesized in magnocellular neurons of the supraoptic nucleus and paraventricular nucleus in the hypothalamus and released from the posterior pituitary gland to act in the periphery. This hormone is best known for its role in parturition (by inducing uterine contraction) and lactation [55,56]. This is interesting. Note that the hypothalamic *KISS1R* expression in rats is unaltered during the pregnancy [57]. It remains unknown why kisspeptin-10 can bind to and activate its receptor during the late pregnancy but not the early-pregnancy or mid-pregnancy in rats. Future studies need to address this effect of kisspeptin in other mammals and potential mechanisms. Notably, continuous exposure to kisspeptin may lead to the desensitization of the KISS1R [58,59]. It is likely that continuous high kisspeptin levels during pregnancy are not able to activate KISS1R in the hypothalamus or fetus. But it surely needs further evidence.

Unanswered questions and future research

Although the kisspeptin levels during pregnancy have been fully discussed in this review, many questions remain to be addressed.

The concentrations of kisspeptin detected in plasma samples were significantly higher than those detected in serum [18]. Additionally, the collecting methods, processing times, and storage conditions of samples will also affect the concentrations of kisspeptins. Therefore, a standardized sample processing method for the collection of samples should be established if kisspeptin levels were considered as a marker for pregnancy or other use. It remains possible that the plasma kisspeptin (kisspeptin-10 or

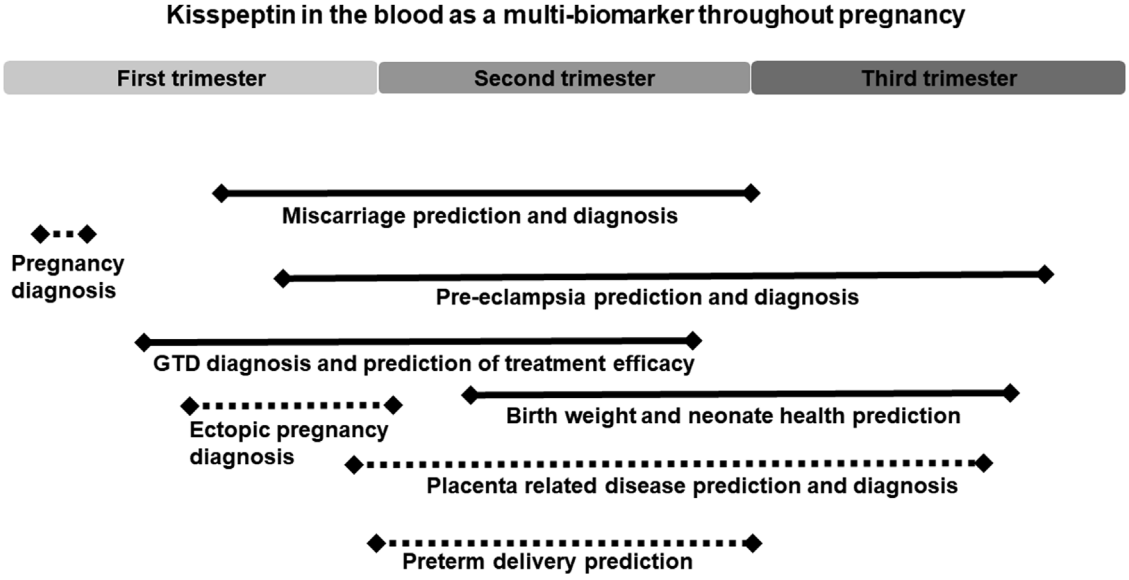

Fig. 2. Plasma or serum kisspeptin levels in the plasma as a multi-biomarker throughout human pregnancy.
Plasma or serum kisspeptin level was considered as a potential biomarker to predict and diagnose miscarriage in the first and second trimester. It was also suggested to predict and diagnose pre-eclampsia in all trimesters. In patients with GTD, plasma or serum kisspeptin level could help to diagnose the disease and predict the prognoses during and after treatment. Whether plasma or serum kisspeptin levels could be used as biomarkers in the diagnosis of early pregnancy or prediction of preterm delivery and other placenta related diseases has not been investigated. Solid arrows stand for actions of kisspeptin that have been indicated in human. Dotted arrows reflect potential biomarkers of kisspeptin across pregnancy that need further evidence. GTD, gestational trophoblastic disease.

kisspeptin-54) measured in pregnant patients is not, in fact, kisspeptin but a cross-reacting peptide/s. Furthermore, since all kisspeptins have the high-affinity binding and the activation of KISS1R [5,6], it is better to detect all or most of these peptides rather than one of them. Though a measurement method of great sensitivity and specificity to detect kisspeptin levels (including kisspeptin-54, kisspeptin-14, kisspeptin-10) has been used [10,44], the kisspeptin-13 levels were omitted. We still don't know the ratio of these plasma or serum kisspeptins. Although plasma or serum kisspeptin levels significantly increased in early pregnancy [8,10], the exact time that plasma kisspeptin levels start to increase has not been explored (Fig. 2). It would be interesting to compare the sensitivity and specificity in the diagnosis of early pregnancy between the plasma or serum kisspeptin levels and hCG levels. These questions should be addressed in future studies.

For the miscarriage, pre-eclampsia, GTN, or fetal development, a prospective cohort study is necessary to explore the sensitivity and specificity of plasma kisspeptin levels in the diagnosis of these diseases or the prediction of newborn health. A large-scale study to define the normal range of plasma or serum kisspeptin levels during pregnancy at different gestation age will be greatly helpful. Furthermore, it is also interesting to compare the kisspeptin assay with other potential biomarkers (such as hCG) or the combination of them in predicting the pregnancy outcome.

Additional studies should explore the kisspeptin levels in other placenta related diseases (Fig. 2). Future studies focused on the kisspeptin levels in pregnant women with assisted reproduction will also be of great interest. Additionally, the applicability of the serum or plasma kisspeptin assay in other pregnancy outcomes, such as multiple pregnancy and ectopic pregnancy, has not been investigated, to the best of our knowledge.

Conclusion

In this review, we provided a concise overview of the available evidence indicating a role of kisspeptin as an emerging marker in the gestational stage. Those studies expanded our understanding of the kisspeptin in predicting the health state of mothers as well as the fetus. Admittedly, the conclusive demonstration of plasma kisspeptin levels as a potential marker for a series of pregnancy-related disease is still pending. Many unsolved problems do exist and are far from elucidated. Future studies are needed to determine whether plasma kisspeptin levels could be largely used in the diagnosis and prediction of the health state of mothers and the fetus or neonates.

Contribution to authorship

Kai-Lun Hu is responsible for the conception, analyzing, and writing up of the work; Hongcui Zhao and Yang Yu infused some key ideas for this work; Rong Li is responsible for the conception, checking and revising the work.

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