Original Article

Enzyme linked immunosorbent assay (ELSA) investigation for Human Kisspeptin and progesterone Levels in female having regular menstrual cycle

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Summary:

Background: Kisspeptin the product of the gene Kiss1 is a G-protein coupled receptor legend for GPR54. Kiss1 was originally identified as a human metastasis suppressor gene that has the ability to suppress melanoma and breast cancer metastasis. It is recently become clear that kisspeptin-GPR54 signaling has an important role in initiating GnRH secretion at puberty, the extent of which is an area of ongoing research.

Objective: The present study was designed to determine the change of plasma kisspeptin levels during the menstrual cycle

Materials & Methods: A total of 20 women from friends and relatives pool were involved in this study. Selected from frinds and relative .They were selected after measuring their progesterone level on day 21 of menstrual cycle (excluding women who have anovulate menstrual cycle; low level of progesterone) , and then on 1 -5 day of the next menstrual cycle. All measurements were done in Teaching laboratories in Baghdad Teaching Hospital in Baghdad/Iraq. Five mls of blood withdrawn from each selected women by veinpuncture of selected women. ELISA technique was used for the measurement of serum kisspeptin -1and progesterone level. Data were expressed as a mean ±SD. Results were evaluated using the student t-test for paired data. Conventional methods were used for the correlation and regression analyses.

Results: Results obtained showed that the levels of serum kisspeptin and progesterone were significantly higher on day 21of menstrual cycle than during menstruation period with $p<0.01$, also it was found a significant positive correlation of kisspeptin level with progesterone level ($r= 0.77$,$p<0.01$).

Conclusion: The results determine the role of kisspeptin on ovulation, and give a possibility for its beneficial manipulation of human fertility.

Keywords: progesterone, kisspeptin1, menstrual cycle, infertility

Fac Med Baghdad 2011; Vol. 53, No. 2
Received: May 2010
Accepted:Mar. 2011

Introduction:

GPR54 is a G protein- coupled receptor, which was originally identified as an "orphan "receptor in the rat (1). Although GPR54 shares a modest sequence homology with the known galanin receptors, galanin apparently does not bind specifically to this receptor (2).In 2001 , three teams of investigators discovered in quick succession that the natural ligand for GPR54 is a 54- amino-acid product of a gene called Kiss1 (3). The kiss1 gene was originally isolated as a tumor metastasis gene, and the peptide product was named metastin, reflecting its ability to suppress metastasis of melanomas, (The term, kisspeptin' refered to metastin and other biologically active fragments of metastin)(5). In 2003, two independent groups discovered almost simultaneously that disabling mutations of GPR54 are associated with delyed puberty and hypogonadotropic hypogonadism in men (6). This observation was corroborated by studies of mice bearing targeted deletions of GPR54, in which, it was noted that reproductive dysfunction is apparently the only remarkable phenotypic anomaly associated with the mutation (8). Thus, kisspeptin_GPR54 Signaling is essential to initiate gonadotropin secretion at puberty and support reproductive function in the adult. Kisspeptin expressing neurons are located in: Anteroventral periventricular nucleus (AVPV) ,periventricular nucleuse(PeN), Anterodorasl nucleus (ADP) and the arcute nucleus(Arc). Kisspeptin neurons reside in the nuclei such as Arc and AVPV and send projections into the site where there is an abundance of cell bodies. This anatomical evidence suggests that kisspeptin fibers appear in close anatomical relationship to GnRH (parvicellular) neurons. In fact, kisspeptin appears to act directly on GnRH neurons (via GPR54 to stimulate the secretion of GnRH(9). The physiologic mechanisms that govern the onset of puberty differ between the rodent and primate (10), yet recent evidence suggests that kisspeptin may also play a role in triggering the onset of puberty in the primate. First, central injections of kisspeptins stimulate luteinizing hormone (LH) in prepubertal, agonadal male monkeys, demonstrating that kisspeptin can override the central inhibition of gonadotrophin releasing hormone (GnRH) secretion characteristic of the prepubertal primate (11). Second, hypothalamic content of KiSS-1 mRNA increases across puberty in both the agonadal male and intact

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female monkey, suggesting that increased production of kisspeptin could contribute to activating the neuroendocrine reproductive axis at puberty in this primate species (12). Hypothalamic levels of GPR54 mRNA also increase as a function of pubertal maturation – but only in the intact female – indicating that this is a steroid-dependent phenomenon and unlikely to be a centrally mediated 'triggering' event for puberty (13). Kisspeptine-1 and progesteroneserum levels were studied previously and the study showed that plasma kisspeptin levels are altered in patients with malignant gestational trophoblstic neoplasias (GTN), nonpregnant and pregnant volunteers. For these groups, a specific radioimmunoassay (RIA) for human kisspeptin and investigated kisspeptin immunoreactivity (IR) human chorionic gonadotropin (HCG) and progesterone level were measured. It was found that kisspeptin IR was elevated in patients with malignant GTN compared with controls and positively correlated with plasma HCG levels. Chemotherapy treatment reduced kisspeptin IR and HCG levels in this patient (14). According to our knowledge, there is no published literature focusing on serum kisspeptin level in Iraqi female , therefore the present work is the first study concerning on serum kisspeptin and progesterone levels, their correlation in women and its relation with infertility.

Subjects and methods:

Subjects: A total of 20 women with selected from relatives and friends pool regular with menstrual cycles were involved in this study, these women selected after measuring their progesterone level on day 21 of menstrual cycle was evaluted(excluding women who have anovulatery menstrual cycle, low level of progesterone) , then their level on 1 -5 day of the menstrual cycle (during their menstruation period) . the subjects were selected from friends and relatives .Their age range between 25 -40 years with a mean of (34.5 ± 9.1) all measurements were done in Teaching laboratories in Baghdad Teaching Hospital in Baghdad/Iraq.

Collection of blood samples: Venous blood (5 ml) was collected during the follicular (day 1-5) and luteal phases (day21) of the cycle. Serum was immediately separated and stored at -20 ◦c until the time for Enzyme linked immune assay (ELSA) .

Methods: Hormone assay, Enzyme linked immunoassay technique was used in the determination of studied hormones (progesterone & kisspeptin)

Progesterone & kisspeptin assay: a progesterone Enzyme immunoassay kits was purchased from PHOENIX PHARMACEUTICALS, INC.

Statistical analysis: Results were analyzed by one – way analysis of student t-test, and rank correlation coefficient (r), $p<0.05$ is considered significance.

Results:

The level of Kisspeptin-1 showed a reduction in women during menstruation period when compared with the level 21 day of menstrual cycle, and the difference reaches highly statistical significance ($p< 0.01$). Mean (±SD) levels of the hormonal parameters in healthy women with regular menstrual cycle are shown in table (1).

Table 1: Mean (±SD) levels of the studied hormones (serum kisspeptin & serum progesterone) in women on 21 day of menstrual cycle and during their menstruation period

Studied hormone	on 21 day of menstrual cycle	1st5th menstruation period
Kisspeptin ng/ml	65 ± 30.1*	20 ± 15
Progesterone ng/ml	18.4 ± 7.2*	2.2 ± 1.4

*** p<0.01**

Kisspeptin level & Progesterone level are significantly higher on 21 day of menstrual cycle than during menstruation period with $p<0.01$ The correlation coefficient was calculated to determine the relationship between kisspeptin-1 and progesterone concentration based on mean values obtained during the luteal phase of menstrual cycle. The results showed a positive correlation between progesterone and kisspeptin-1(Figure1; $r= 0.77$, $p <0.01$)

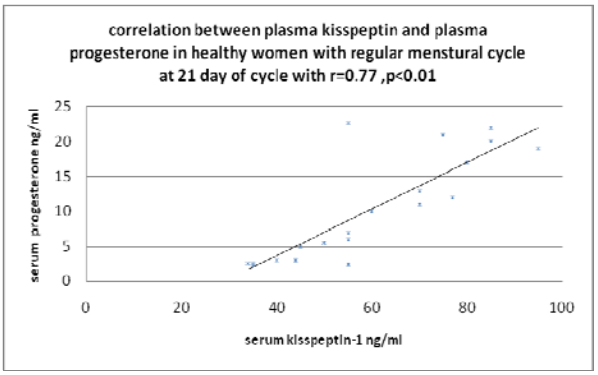

Figure 1: shows that kisspeptin had stimulatory effect on ovulation through increasing level of progesterone

Discussion:

Infertility is a devastating condition that affects million couples worldwide. This research shows that Kisspeptin may offer a promise as a kind of treatment for infertility. Kisspeptin is a small peptide produced by a gen called kiss-1. Since the discovery of the role of GPR45 in 2003(15) .The kiss 1 gene was originally isolated as a tumor metastasis gen and the peptide product were named metastin, reflecting its ability to suppress metastasis of melanomas. The data (table1) in Present study demonstrated that plasma kisspeptin-1 is

high on 21 day of menstrual cycle and decreased during the period of menstruation (about every 28 days, some blood and other products of the disintegration of the inner lining of the uterus (the endometrium) are discharged from the uterus, a process called menstruation).To discuss the relationship between kisspeptin -1 and progesterone level ,it is important to know that Kisspeptin-1 which is a newly identified peptide plays a major role in the ovulation through the stimulation of the secretion of progesterone , kisspeptin's mechanism action appears to directly activate GnRH neurons. Evidence for this involves the persistence of a neural response to kisspeptin levels even in the presence of TTX(a neurotoxin that blocks nerve signals). It is not clear how the expression of this gene is regulated by steroids, nor is it clear whether it plays a major role in the steroid feedback regulation of GnRH secretion (16). Several groups of studies have now shown that kisspeptin, administered either centrally or peripherally, stimulate gonadotropin secretion. Gottsch *et al.* (2004)(17) reported that extraordinarily low doses of kisspeptin , injected into the lateral ventricle of the mouse, can elicit a rapid and robust secretary burst of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Similar observations (but with higher doses of kisspeptin) were reported in the rat (18), sheep (19, 20), monkey (21) and, most recently, the human male (22, 23). Moreover, as demonstrated in the mouse, rat, and monkey, the GnRH antagonist acyline can block the kisspeptin _induced release of LH and FSH (24)′ This suggests that kisspeptin_1 stimulated gonadotropin release is depend on the release of GnRH and does not reflect a direct action of kisspeptin on the pituitary. The apparent lack of kisspeptin's effect on the pituitary was confirmed in the rat by Matsui *etal* (25), who reported that another GnRH antagonist, cetrorelix, also blocks the kisspeptin _induced release of LH and FSH(26). Some studies (27) reported that kisspeptin led to a 48-fold increase in LH and 16-fold increase in FSH; Arising level of LH causes the developing egg with the follicle to complete the first meiotic division (meiosis), forming a secondary oocyte . After about two weeks ; there is a sudden surge in the production of LH . This surge in LH triggers ovulation: the release of the secondary oocyte into the fallopian tube. Under the continued influence of LH, the now-empty follicle develops into a corpus luteum (hence the name luteinizing hormone for LH). Stimulated by LH, the corpus luteum secretes progesterone which continues the preparation of the endometrium for a possible pregnancy inhibits the contraction of the uterus and inhibits the development of a new follicle (28). To confirm that the elevation kisspeptin-1 level which is observed (table1)is intertwined persistence with level of circulating progesterone on 21 day of menstrual cycle (the highest level of progesterone during period of menstrual cycle), kisspeptin -1 was measured during luteal phase of menstrual cycle and compared with its level during follicular phase (lowest or undetectable level of progesterone); the ovaries of sexually –mature females secrete : a mixture of estrogens of which 17 –estradiol is the principle and all are steroids , high level of estrogenes suppress the release of GnRH providing a negative feedback control of hormone level. It works like this: Secretion of GnRH depends on certain neurons in the hypothalamus which express a gen (kiss-1) encoding a protein of 145 amino acids. From this are cut several short peptides collectively called kisspeptin. These are secreted and bind to G-protein –coupled receptors on the surface of the progesterone GnRH neurons stimulating them to release GnRH. However, high levels of estrogen (or progesterone or testosterone) inhibit the secretion of kisspeptin and suppress further production of those hormones. Progesterone production is stimulated by lutenizing hormone (LH), which is also stimulated by GnRH, elevated levels of progesterone control themselves by the same negative feedback loop used by estrogens (and testosterone)(29). An important significant positive correlation that observed in the present study between serum kisspeptin -1 levels and progesterone values (fig.1) may pointed to new recent function of kisspeptin -1 in improve the ability of the corpus luteum to secretes progesterone. This finding has not been published before. In summary, the present study showed that there is a significant positive correlation between progesterone levels and kisspeptin levels in women who have normal ovulation. These correlations suggest that kisspeptin may produce solution for certain cases of infertility, but further study dealing with infertile women (who have low progesterone level) injection of appropriate dosage of kisspeptin -1 may produce useful treatment for these infertile cases. This research shows that kisspeptin may be offer promise as a treatment for infertility.

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