IRAQI JOURNAL OF EMBRYOS AND INFERTILITY RESEARCHES (IJEIR)
Ghada et al., Vol. 10, Issue 2, Pp. 75-90 (2020) Original Research
DOI: http://doi.org/10.28969/IJEIR.v10.i2.r5.20 E-ISSN: 2616-6984 P-ISSN: 2218-0265

The Effect of BMI on Serum and Follicular Fluid Level of Kisspeptin in Infertile Women.

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ABSTRACT

Kisspepetin is considered as a key regulator of reproduction, through its direct role in controlling the hypothalamic pituitary gonadal axis necessary for controlling the ovulatory process and indirectly by impacting glucose heamostasis and metabolism. We aimed to examin the correlation between body mass index and level of Kisspeptin in serum and follicular fluid. This study is a cross sectional study done on (80) infertile Iraqi patients aged (18-40 years) scheduled for fresh intracytoplasmic sperm injection cycle. This study is conducted in the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies of Al-Naharin University, from September 2020 to May 2021. For each participant, the GnRH antagonist protocol was used to hyperstimulate the ovaries, and samples from serum and follicular fluid were obtained at day of oocytes retrieval for measuring Kisspeptin level using enzyme linked immunosorbent assay (ELIZA) technique. Serum and follicular fluid Kisspeptin were insignificantly increasing with the increase in the BMI (p value =0.406) (p value = 0.352) respectively. Serum and follicular kisspetin were positively and significantly correlated (r = 0.536, p value= 0.000). Serum and follicular fluid kisspeptin were numerically increasing with the body mass index of infertile women, although this increament is statically insignificant, serum kisspeptin can be used as a mirror image to follicular fluid kisspeptin as there was a statistical association between them in this study.

Received: 07-July-2020
Accepted: 30-July-2020
Published: 08-Aug-2020

How to cite:

Ghada Alanssari, Mufeda A. Jwad, Amal A. Mohammed; The Effect of BMI on Serum and Follicular Fluid Level of Kisspeptin in Infertile Women; Iraqi Journal of Embryos and Infertility Researches (IJEIR), (2020); 10 (2): 75-90.
Doi: http://doi.org/10.28969/IJEIR.v10.i2.r5.20

KEYWORD

Infertility, Kisspeptin, BMI, ICSI.

1. Introduction

Kisspeptin is a member of the neuropeptide family that is encoded by the *KISS1* gene. It binds to a G-protein-coupled receptor (GPR54/KISS1R), stimulating both the pulsatile and the surge secretion of gonadotrophin-releasing hormone (GnRH) from hypothalamic neurons. GnRH, in turn, will control the production of pituitary leutnizing hormone(LH) and follicular stimulating hormone(FSH) then the gonadal sexual steroids (Trevisan CM et al., 2018 [1]; Hu KL et al., 2018 [2]). Published scientific evidences stated that Kisspeptin plays a fundamental role in female reproduction via regulating the secretion of gonadotropins, ovulation, and the metabolic regulation of fertility (Hu KL et al., 2018 [2]). In humans, all Kisspeptins (KP) are produced from a common precursor, the pre-pro-Kisspeptin, composed of a 145-amino acid. This preproKisspeptin will further be cleaved into (KP-54, KP-14, KP-13, and KP-10) (Hussain MA et al., 2015[3]). When Kisspeptin binds to its receptors on GnRH neurons expressing Kiss1R mRNA, it exerts its stimulatory effect on GnRH release either directly or indirectly through the synaptic input from other neurons (Colledge WH. 2009[4]; Hrabovszky E et al., 2010 [5]; Skorupskaite K et al., 2014 [6]). Since GnRH neurons lack estrogen receptors α (ERα), while Kisspeptin-secreting neurons do, so Kisspeptin is believed to fill the gap between sex steroid hormones and the hypothalamus acting as an important mediator in regulating the feedback mechanism (Ikegami K et al.,2020[7]). Published literatures proved that estrogen exerts a negative feedback on kisspeptin secretion in the early follicular phase and a positive feedback mechanism in the late follicular phase inducing LH surge necessary for the final oocyte maturation and ovulation (Ikegami K et al., 2020[7]; Tang R et al., 2019[8]).

The discovery that Kisspeptin and its receptor (KISS1R) are expressed in the human ovaries highlights its vital role in controlling follicular growth, oocytes maturation, steroidogenesis, and finally ovulation (Hu KL et al., 2018 [2]). however this expression was found to have a highly delicate expression pattern in human ovaries, and that its distribution in the ovary has a significant temporal specificity, proposing that in the ovary the Kisspeptin/ KISS1R system carry out many complex functions which differ according to the menstrual phases (Cao Y et al.,2019[9]). Kisspeptin indirectly affects the reproductive function by regulating insulin secretion and energy expenditure (Kołodziejski PA et al., 2018[10]), this is supported by scientific evidences which stated the presence of Kisspeptin expression in body organs specified for body metabolism like liver, pancrease and adipose tissues (Mancini A et al., 2021[11]). Moreover, Cockwell et al. found a positive correlation between BMI and KISS1 expression in omental adipose tissue in women (Cockwell H et al., 2013[12]). Kisspeptin had been correlated with leptin in regulating both the body metabolism and reproduction as Clarke et al. declared (Clarke H et al., 2015[13]). Kołodziejski et al found that in obese patient's serum Kisspeptin levels was correlated with insulin and glucagon levels, proposing that Kisspeptin might play a vital role in the regulation of pancreatic hormones secretion, and insulin resistance in peripheral tissues (Kołodziejski PA et al., 2018[10]). However, conflicting results were obtained regarding the correlation of Kisspeptin with glucose metabolism and metabolic parameters, several studies found a positive association between Kisspeptin and glucose metabolism, while other studies stated negative correlation between body mass index and serum Kisspeptin (Sithinamsuwan Ket al., 2020[14]). Therefore, this study investigates the correlation between obesity and Kisspeptin level which can

directly or indirectly affect sex hormones secretion responsible for regulating reproduction.

2. Patients and Methods

This study is a cross sectional study, conducted in the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies of Al-Naharin University, along a period of 9 months extended from (September 2020 to May 2021). A written informed consent has been taken from all the participants. The study was approved by the Local Medical Ethical Committee of high institution for infertility diagnosis and Assisted Reproductive Technologies of Al-Nahrain University.

Eighty infertile Iraqi women (n=80) were randomly selected for this study. The inclusion criteria for the participants were women aged (18-40) years, stimulated by the GnRH antagonist ovarian stimulation protocol and sechuled for fresh ICSI cycle. While the exclusion criteria were:

1- females who are diagnosed as poor responder as they have at least two of the following: (i) women with age ≥40 years; (ii) a previous history of poor ovarian response (oocyte retrieval less than 3 with a conventional induction method); (iii) decline in ovarian reserve (the number of antral follicles at transvaginal ultrasonography <5-7; serum AMH levels <0.5 to 1.1 ng/ml) (Celik N et al., 2018 [15]).

2- Women planned for ICSI program but with ovulation stimulation protocols other than GNRH antagonist protocol.

3- Females diagnosed with endocrine disease like thyroid disease, diabetes mellitus and hyperprolactinemia.

A complete history and physical examination had been taken from each participant including the measurement of the standing height (ht.) in meters and weight (wt.) in kilograms for calculating the body mass index(BMI) using the equation (BMI = wt./ ht^2) (Lim JU et al., [16]).

All females in this study were subjected to controlled ovarian hyper-stimulation (COH) by using the flexible antagonist protocol, starting with a daily dose of 150-450 IU r-FSH (Gonal F), then were monitored using the transvaginal ultrasound (TVS) for assessing the ovarian follicles growth. When follicles reached 13-14 mm in size, GnRH antagonist was given (Cetrotide®, Merck Serono), and when three or more follicles reached a diameter of 18 mm HCG (Ovitrelle® 250 microgram, Merck Serono) was administrated to induce the final oocytes maturation and ovulation.

After (34-36) hours following the HCG injection, Oocytes retrieval was performed using a transvaginal ultrasound guided oocyte retrieval. Venous blood sample was taken at day of oocytes retrieval, then was put in dry sterile plain tube, allowed to coagulate for 30 minutes at room temperature then centrifuged at 5000 rpm for 10min, the supernatant serum was aspirated and put in small tube to be stored in the freezer at -20 C^{o} for subsequent analysis of Kisspeptin. Follicular fluid was collected using double lumen from the very first aspirated follicles of the infertile females undergoing fresh ICSI cycle, then was put in plain sterile tube to be centrifuged at 5000 rpm for 10 min, the supernatants were taken to other sterile tube and stored at –20°C for subsequent assay for Kisspeptin level. follicular fluid samples which were heavily contaminated with blood were all discarded. Kisspeptin in serum and follicular fluid was measured using commercially available human Kisspeptin 1 (KISS-1) kit which uses enzyme – linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology. the ELISA kits used were human Kisspeptin 1(KISS-1) ELISA KIT (cat. No. YHB1811Hu, SHANGHAI YEHUA Biological Technology) with sensitivity of 10.1ng/L, intra-assay coefficients of variation of less than

10% and inter-assay coefficients of variation of less than 12%.

Statistical analysis

The collected data were introduced into SPSS version 24 statistical software program. Descriptive statistic was presented using tables (mean ± standard deviations, frequency and percentage), and graphs. Person correlation was used to find out significance of correlation between related numerical data. Chi-square test was used to find out significance of association between related categorical data. Independent two sampled. (T test) and one-way ANOVA were used to find out significance of differences between means of related numerical data. Mann Whitney test and cross Kruskal Walis test were used to find out significance of differences between related variables if the distribution of dependent variables were found to be not normally distributed according to Shapiro Wilk test. P value less than (0.05) was considered as a discrimination point of significant.

3. Results

The mean age of the entire study sample (n=80) was (31.4±6.37 years) as shown in figure (1).

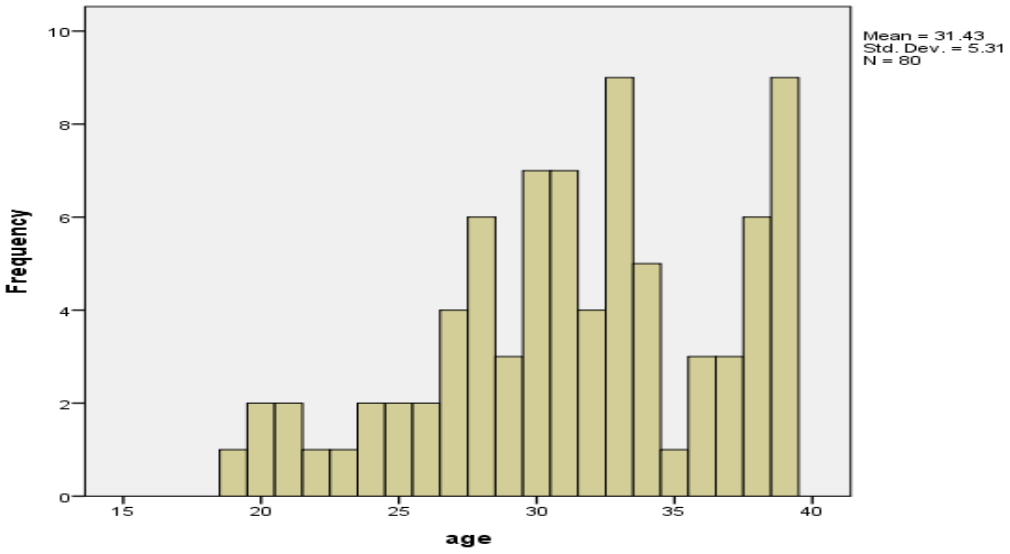


Figure 1): Age distribution of all studied sample

Table (1) showed the demographic features of the study sample, the mean age of the participants was (31.4 ± 6.37), the mean BMI was (26.6 ± 3.93) kg/ m^2, and the mean duration of infertility was (8± 4.75) years.

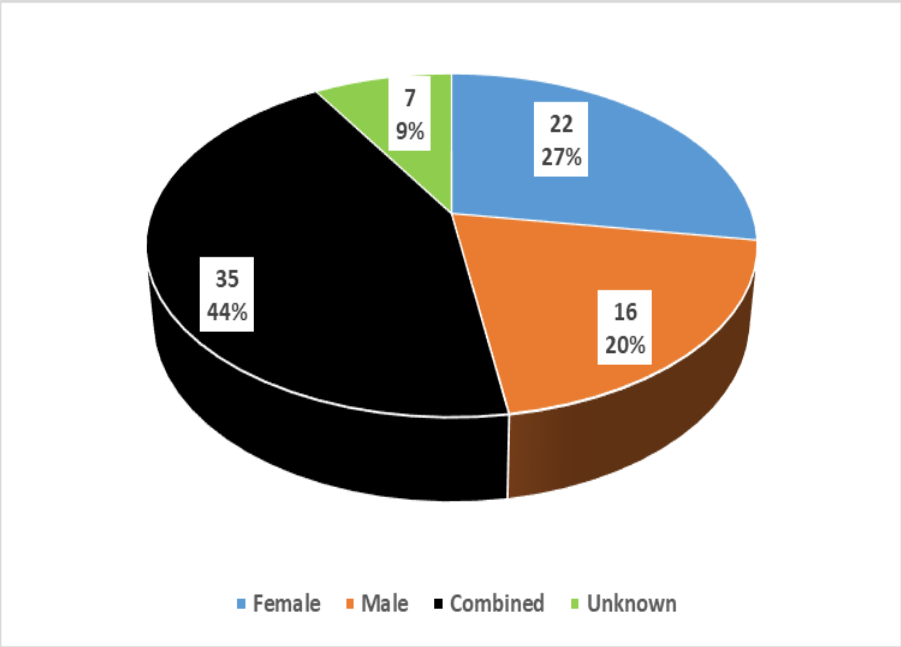
(Table 1): Demographic and baseline hormonal levels of infertile women characteristics.

Variable	Mean ± SD
Demographic characteristics	
Age (years)	31.4±6.37
BMI (kg/m2)	26.6 ±3.93
Duration of infertility (years)	8±4.75
Baseline hormonal levels characteristics	
FSH (IU/L)	6.24±2.17
LH (IU/L)	5.11±3.21
E2 (CD3) (pg/ml)	5.67±34.78
E2 (at trigger) (pg/ml)	1473.36±1538.73

SD= slandered deviation, FSH= follicular stimulating hormone, LH= luteinizing hormone, E2= estradiol, CD= menstrual cycle

Causes of infertility

In this study (44%, n=35) of the infertile couples had both male and female's (combined) factors of infertility, (27%, n= 22) complained from female causes only, (20%, n= 16) suffered from male factor solely, and finally (9%, n= 7) of this study sample had unknown cause for their infertility as shown in figure (2).



***(Figure 2)*:** Caused of infertility among all (80) studied cases in percentage.

Basal hormones measurements

In table (2) the basal hormonal level of the study participants were described in term of the (mean± standard deviation), the basal hormones were drawn at the second or third day of the menstrual cycle before starting the antagonist protocol. The means of estradiol level (E2) at the day of HCG administration (at trigger day) were also measured. FSH mean was found to be (6.24±2.17 IU/L), LH mean was (5.11 ± 3.21 IU/L), E2 (CD2-3) mean was (5.67 ± 34.78 pg/ml), and finally E2 at trigger day mean was (1473. 36 ±2511.09 pg/ml).

The correlation of Kisspeptin with hormones

The results in table (3) showed that serum Kisspeptin had a positive significant association with follicular fluid Kisspeptin (r = 0.536, p value= 0.000). Serum Kisspeptin hadfound to be negatively and insignificantly correlated with basal hormones (FSH, LH and E2) drawn at menstrual cycle day 3 as well as E2 measured at day of HCG administration (trigger day) (p value > 0.05) for all. Follicular Kisspeptin showed positive and insignificant correlations with both FSH and E2 (CD3) (r= 0.032, p value= 0.776) (r= 0.106, p value=0.500) respectively, and a negative insignificant correlation with LH and E2 at trigger day (p value >0.05) for both.

Kisspeptin and Body Mass Index

When correlating the level of Kisspeptin in the serum of the whole participants in this study with their BMI, results in table (4) showed that despite the increase in the mean of the serum Kisspeptin level with the increase in the BMI, but this increment did not reach a statistical significance (P= 0.406).

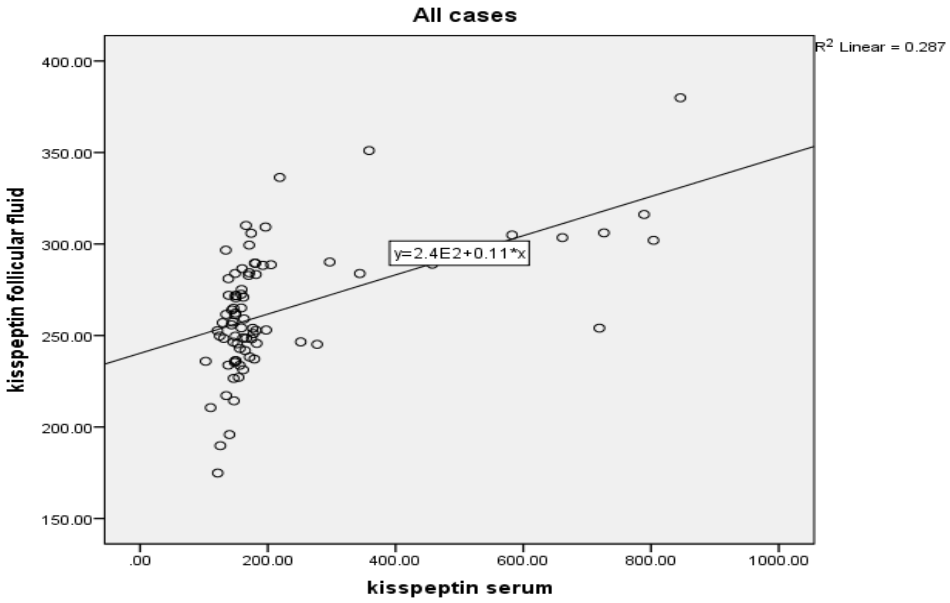
Similarly, the follicular fluid Kisspeptin level which also increased with the rise in the BMI of the study sample, but did not reach a statistical significance (P= 0.352) as shown in table (4).

Body mass index (BMI) by measuring height (ht.) in meters and weight (wt.) in kilograms and applying the equation (BMI = wt./ ht^2), BMI is categorized into four groups according to the conventional WHO classification: underweight (<18.5 kg/m^2), normal weight (18.5–24.9 kg/m^2), overweight (25–29.9 kg/m^2), and obese (≥30 kg/m^2) .

(Table 3): Kisspeptin correlation with hormones.

		Kisspeptin in follicular fluid (ng/L)	FSH (IU/L)	LH (IU/L)	E2 (CD3) (pg/ml)	E2 (at trigger) (pg/ml)
Kisspeptin in serum (ng/L)	**R**	0.536	-0.025	-0.095	-0.041	-0.077
	Pv	0.000	0.828	0.403	0.792	0.496
Kisspeptin in follicular fluid (ng/L)	**R**		0.032	-0.086	0.106	-0.066
	Pv		0.776	0.450	0.500	0.560

SD= slandered deviation, FSH= follicular stimulating hormone, LH= letunizing hormone, E2= estradiol, CD= menstrual cycle, pv= p value.



(Figure 3): The correlation of serum and follicular Kisspeptin in the studied sample.

(Table 2): Kisspeptin Level in Serum and Follicular Fluid and Body Mass Index in all Patients Enrolled.

Variable	Weight status	N	Mean	SD	Mean rank	P value
Serum kisspeptin	**Normal**	30	191	112	38.33	0.481*
	Overweight	35	225	191	39.60	
	Obese	15	262	211	46.93	
Follicular fluid kisspeptin	**Normal**	30	260	24		0.352**
	Overweight	35	261	37		
	Obese	15	275	41		

N= number, SD= standard deviation, *Kruskal Walis test, **one-way ANOVA.

4. Discussion

Obesity is one of the leading cause of decrease potential for fertility. Obese women showed less reproductive outcomes regardless of the way of conception. Increased body mass index (BMI) is associated with poorer fertility outcome as it is responsible for menstrual dysfunction like oligo - ovulation, and decrease in the implantation rate which resulted from the disturbance in the normal endometrial hormonal balance (Cena H et al., 2020 [17]; Starosta A et al., 2020[18]; Butler AE et al., 2020[19])

Statistic of this study showed that the mean BMI of the study sample was > 25 kg/m which mean that most participants lie in the overweight category. This result is similar to the result of a cross sectional study done by Rashad et al., where the majority of patients in that study were overweight or obese (67.7%) (Rashad NM et al., 2019[20]).

In the present study, results demonstrate that serum Kisspeptin level has a positive insignificant correlation with the BMI of this study sample. However, this correlation might reach a statistical significance with larger sample size. This result is online with the result declared by Ibrahim et al. (2020) who stated a positive insignificant differences in serum Kisspeptin levels among overweight, obese and non-obese healthy and PCOS patients, and agrees with Rafique and Latif (2015) who obtained the same result in normal and overweight Saudi women (Ibrahim RO et al., 2020 [21]; Rafique N et al., 2015[22])

On the contrary, Kołoziejski et al. (2018) and Asalah et al. (2021), they found that Kisspeptin level was significantly higher in normal-weight women as compared to obese women (Kołodziejski PA et al., 2018[10]; Asalah AK et al., 2021 [23]).

In the present study, follicular KP had a positive, insignificant correlation with the BMI, similar to serum KP. For the best of our knowledge no similar study had been done regarding this correlation.

Scientist believed that Kisspeptin upregulate reproduction not only by regulating GnRH secretion in the hypothalamus but also by transmitting metabolic signals to the brain centers. This is supported by many published literatures like, Cockwell et al. who declared a positive correlation between BMI and KISS1 expression in omental adipose tissue in women (Cockwell H et al., 2013[12]).

Furthermore, Kisspeptin has been linked to leptin in regulating both the body metabolism and reproduction. Scientists proposed that since the hypothalamus lack leptin receptors while it possess Kisspeptin ones, so leptin regulates reproduction by transmitting energy signals through Kisspeptin neurons in the hypothalamus (Clarke H et al., 2015[13]). In the same prospective, Ibrahim et al. mentioned in their study that the association between obesity and Kisspeptin level is highly affected by alteration in plasma triglyceride levels, and that hyper-triglyceride induced lipotoxic inflammation in the hypothalamus (Ibrahim RO et al., 2020 [21]).

However, performing similar study on a larger sample size will certainly further clarify the correlation of serum and follicular KP with the BMI. Furthermore we recommend that studying the correlation of kisspeptin with leptin will help in better understanding the role of kisspeptin in regulating body weight and consequently fertility problems related to obesity.

6- Conclusion

Obesity might have a positive numerical correlation with serum and follicular kisspeptin level , and subsequently with fertility potential, although this association is not statically significant. Serum kisspeptin can be used as a mirroe image for follicular kisspeptin as they were statically correlated.

Acknowledgment

We would like to acknowledge the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies/ Al Nahrain University, Baghdad, Iraq

Funding

This work received no funding .

Author Contribution

Ghada Alanssari, performed the study, Mufeda A. Jwad, Amal A. Mohammed, supervised the work .

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The study was approved by the Ethical Approval Committee.

Financial Disclosure

There is no financial disclosure.

Ethical Clearance

The study was approved by the Ethical Approval Committee

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