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Evaluation of serum kisspeptin role in male infertility

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Abstract
Background: Kisspeptin are mainly expressed in discrete neuronal populations of the hypothalamus and have recently been emerged as an essential regulator of GnRH (gonadotropin-releasing hormone) neurons and, hence, are potent stimulators of gonadotropin secretion.
Objective: This study aimed to evaluate the level of serum kisspeptin concentration of azoospermic, oligospermic and normospermic men, and its role at reproductive axis and testicular function and the correlations between kisspeptin and (FSH, LH, Testosterone) hormones in these groups.
Subjects and Methods: This study was be carried out on ninety men (60 infertile men and 30 fertile men as a control group), their ages from (20-60) years were included in this study. Subjects were divided into the following groups according to semen analysis. Group A: Includes 30 normospermic fertile men served as control group. Group B: Includes 30 cases diagnosed as oligospermic men by low sperm count less than 15 million cells/ml, sperm motility less than 40 % and abnormal forms more than 96% each subject provided two semen samples,one month apart. Group C: Includes 30 cases diagnosed as azoospermic men (functional azoospermia) by absence of spermatozoa in two to three ejaculate one month apart after three days of abstinence following high speed centrifugation and pellet analysis by light microscopy. Measurement of serum kisspeptin concentrations were estimated by Enzyme Linked Immunosorbent Assay (ELISA).
Results: A significant decrease in mean serum kisspeptin concentration (ng/ml) ± SEM for oligospermic and azoospermic groups was observed when compared with the mean serum kisspeptin concentration of the normal group (P value<0.05). There was a significant positive correlation between kisspeptin and sperm motility in the serum of the normal and oligospermic groups (r=0.6457, p=0.0001) (r=0.6050, p=0.0004) respectively. There was a significant positive correlation between kisspeptin and testosterone in the serum of the normal, oligospermic and azoospermic groups (r=0.6309, p=0.0002) (r=0.5817, p=0.0007) and (r=0.6435, p=0.0001) respectively. There was a significant positive correlation between kisspeptin and FSH in the serum of the normal and oligospermic groups (r=0.6388, p=0.0001) (r=0.6545, p<0.0001) respectively. There was a significant positive correlation between kisspeptin and LH in the serum of the normal and oligospermic groups (r=0.6800, p<0.0001) (r=0.6016, p=0.0004) respectively.
Conclusion: The serum kisspeptin levels are significantly higher in the fertile as compared to infertile males (oligospermic and azoospermic males). Kisspeptin can be used as a diagnostic marker for diagnosis of infertility in males and differentiation between normospermic, oligospermic and azoospermic males. This study provides a link between the kisspeptin levels and male reproductive axis depending on the fertility status of the subjects so it's considered as a key contributory factor in the control of testosterone, FSH and LH levels in males.

Keywords: infertility, FSH, LH, testosterone, kisspeptin

Introduction
Infertility is defined as the inability to get pregnant during 12 months or more regular unprotected sexual intercourse. Fertility is initiated by hormonal signals from brain to the gonads which lead to leakage of gonadotropin hormones. Recently, Kisspeptin (Kp) and its receptor have been identified as vital upstream regulators, integrating central and environmental signals through gonadotropin-releasing hormone [1].
Spermatogenesis is a complex differentiative process starting from spermatogonial stem cells (SSCs), known as A-single (As) [2]. Sperm maturation occurs in the epididymis. Spermatogenesis continues throughout life and it is regulated by a complex assortment of hormones as well as numerous locally produced factors that include growth factors, cytokines, and chemokines, that act through autocrine and paracrine pathways [3].
The major hormonal control system of spermatogenesis is the hypothalamic-pituitary-gonadal axis, based essentially on the release of two gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH), under the stimulation of hypothalamic GnRHs. Leydig and Sertoli cells, the somatic

cells of the testis, are primary responders to circulating gonadotropin hormones and their failure to respond appropriately, results in male infertility [4].

Semen analysis is the most widely used biomarker to predict male fertility potential. It provides information on the functional status of the seminiferous tubules, epididymis and accessory sex glands, and its results are often taken as a surrogate measure of a man's ability to fathera pregnancy [5].

Kisspeptin is the product of the Kiss1 gene and the ligand for the seven-transmembrane G protein-coupled receptor GPR54, also named KISS1R.1. The Kiss1 gene encodes a 145 amino acid protein, which is hydrolyzed into four peptides of different lengths, including kisspeptin 54, kisspeptin14, kisspeptin13, and kisspeptin10; all these peptides have the same affinity for KISS1R.2. Expression of Kiss1 mRNA is detected in many regions of the mouse brain, including the anteroventral periventricular nucleus, periventricular nucleus, anterodorsal preoptic nucleus, and arcuate nucleus [6].

Kisspeptins, a family of neuropeptides encoded by the Kiss1gene, as natural ligands of the previously orphan G protein-coupled receptor, GPR54. They are mainly expressed in discrete neuronal populations of the hypothalamus and have recently been emerged as an essential regulator of GnRH (gonadotropin-releasing hormone) neurons and hence, are potent stimulators of gonadotropin secretion. Initially the known biologic function of the kisspeptin was to suppress the tumor metastasis. Kisspeptins are now regarded as the key players in the different aspects of the maturation and functioning of the reproductive axis, which include the sexual differentiation of the brain, puberty timing, regulation of secretion of gonadotropins and gonadal hormones, as well as control of fertility [7].

KissR1 (GPR54, AXOR12, or hOT7T175) as the receptor for kisspeptins also investigated the intracellular signaling pathways activated by this receptor. In mammals, the phospholipase C (PLC)/protein kinase C (PKC)/MAPK pathway was activated [8].

Initially, KISS1was thought to functions as a metastasis suppressor gene in human breast cancer, similar to its anti-metastatic roles observed in melanoma. However, a study was done using human 'breast' cancer MDA-MB-435 cells, which have been shown to have gene expression profile more closely resembles that of melanoma cell lines, rather than that of other breast tumor cell lines [9].

Kisspeptin immunoreactivity was reduced in both the arcuate nucleus and anteroventral periventricular in the hyperprolactinemic mouse model, suggesting that kisspeptin plays a role in mediating hyperprolactinemia-induced anovulation and hypogonadotropic hypogonadism. The administration of intraperitoneal injections of kisspeptin once daily into the hyperprolactinemic mice for 20 days induced ovulation, and increased circulating LH and FSH levels. The suppression of kisspeptin may mediate prolactin-induced inhibition of cycles [10].

This study aimed to evaluate the level of serum kisspeptin concentration of azoospermic, oligospermic and normospermic men, and its role at reproductive axis and testicular function and the correlations between kisspeptin and (FSH, LH, Testosterone) hormones in these groups.

Subjects and Methods

This study was carried out at Assisted Reproductive Technique Unit, International Islamic Center for Population Studies and Research, Al-Azhar University in the period between(2015-2016).

This study included ninety men (60 infertile men and 30 fertile men as a control group), their ages from (20-60) years were included in this study.

Subjects were divided into the following groups according to semen analysis:

- **Group A:** Includes 30 normospermic fertile men served as control group.
- **Group B:** Includes 30 cases diagnosed as oligospermic men by low sperm count less than 15 million cells/ml, sperm motility less than 40 % and abnormal forms more than 96% each subject provided two semen samples, one month apart.
- **Group C:** Includes 30 cases diagnosed as azoospermic men (functional azoospermia) by absence of spermatozoa in two to three ejaculate one month apart after three days of abstinence following high speed centrifugation and pellet analysis by light microscopy.

Semen analysis

Two semen analysis were carried out for every patient with one month interval. Semen samples collected in the laboratory by masturbation, no lubricant is used after abstinence period of 3 days in clean, dry, wide plastic container. History of previous semen analysis and their results were taken. Analysis performed after liquefaction at 37℃ by modified neubauer counting chamber (Haemocytometer) and examined by light microscopy. For azoospermia the test is repeated in two to three ejaculate one month apart after three days of abstinence following high speed centrifugation and pellet analysis by light microscopy.

Collection of samples

(5 ml) blood sample was taken by vein puncture at early morning after an overnight fast and was be left at room temperature for about one hour for coagulation. The samples were centrifugated at room temperature for 15 minutes to obtain serum supernatant (serum sample) which was then be transferred into an eppendorf tube and labeled and measured for hormonal assay and stored at (-20) until analyzed.

Fertility hormones (FSH, LH and Testosterone) were measured by enzyme-linked fluorescent assay (ELFA) by BioMérieux SA, France, VIDAS Kits assay.

Measurement of serum kisspeptin concentrations were estimated by ELISA technique (Enzyme Linked Immunosorbent Assay) with Glory Science Co., Ltd, TX 78840, USA Human Kisspeptin ELISA Kit.

Statistical Methods

The statistical analysis of the data was performed using the GraphPad Prism (version 5) and Sigma Plot version 12.5. The data were presented as mean ± standard error of the mean (SEM) for quantitative measures and both number and percentage for categorized data. The tests used were: X mean and SEM standard error of the mean to measure the central

tendency of data and the diversion around the mean. ANOVA or F-test: for testing statistical significant difference between means of more than two samples. Pearson's correlation test (correlation coefficient r): to test a positive or negative linear relationship between two variables (one dependent and the other is independent variable). Non-significant result is considered, if $p>0.05$. Significant result is considered, if $p\leq0.05$. Highly significant result is considered, if $p\leq0.01$.

Results

This study was conducted on 90 male Egyptian subjects. They were classified into three groups normal, oligospermic and azoospermic groups according to semen analysis.

Table 1: Demographic and clinical characteristics of the normal oligospermic and azoospermic groups:

characteristics	Normal (control) n=30	Oligospermic n=30	Azoospermic n=30	p-value
Age (years) Range Mean ± SEM	(26-52) 35.4 ± 1.14	(20-60) 34.1 ± 1.509	(25-45) 34 ± 1.006	0.6723 p>0.05
BMI (Kg/m^2) Range Mean ± SEM	(21.71-27.3) 24.13 ± 0.2328	(20.19-29.76) 25.95 ± 0.5080	(20.31-29.41) 25.12 ±0.4912	0.0136 p< 0.05
Volume(ml) Range Mean ± SEM	(2-6.5) 3.680 ± 0.1795	(0.5-6) 3.339 ± 0.2383	(1-5) 3.040 ± 0.1923	0.0930 p>0.05
Count(mil.c./ml) Range Mean ± SEM	(33-112) 66.27± 3.617	1-20) (11.90 ± 1.046	0	0.01> p<0.05
Motility (%) Range Mean ± SEM	(55-96) 82.77± 2.125	0-60) (21.80 ± 2.992	0	0.01> p<0.05

Table (1) showed demographic data of the studied subjects showed a non significantly decrease in mean age (years) ± SEM for oligospermic and azoospermic groups when compared with the mean age ± SEM of the normal group. There was a significantly increase in mean ± SEM of BMI (kg/m2) for oligospermic and azoospermic groups when compared with the mean ± SEM of BMI of the normal group. There was a non significantly decrease in mean ± SEM of seminal volume (ml) for oligospermic and azoospermic groups when compared with the mean ± SEM of seminal volume of the normal group. There was a highly significant decrease in mean ± SEM of sperm count (mil.c./ml) for oligospermic and azoospermic groups when compared with the mean ± SEM of sperm count of the normal group. There was a highly significant decrease in mean ± SEM of sperm motility (%) for oligospermic and azoospermic groups when compared with the mean ± SEM of sperm motility (%) of the normal group.

Table 2: Biochemical hormonal levels of normal oligospermic and azoospermic groups:

Variables	Control n=30	Oligospermic n=30	Azoospermic n=30	p-value
Testosterone (ng/ml) Range Mean ±SEM	(1.37-13.2) 6.313 ± 0.5552	(3.1-12.9) 7.362 ± 0.5484	(1-12) 4.422 ± 0.4476	0.0005 p<0.05
FSH (mlU/ml) Range Mean ±SEM	(4.3-20) 11 ± 0.7408	(3-27) 10.75 ±0.9675	(2.4-18.8) 9.458 ± 0.7104	0.3616 p>0.05
LH (mlU/ml) Range Mean ±SEM	(3.3-18.4) 8.983 ± 0.7398	(3.2-13.4) 7.295 ±0.5396	(3.2-11.7) 5.975 ± 0.3943	0.0017 p<0.05

Table (2) showed a significantly increase in mean serum testosterone concentration (ng/ml) ± SEM for oligospermic group when compared with the mean serum testosterone concentration of the normal group while there was a significantly decrease in mean serum testosterone concentration (ng/ml) ± SEM for azoospermic group when compared with the mean serum testosterone concentration of the normal group. There was a non significantly decrease in mean serum FSH concentration (mlU/ml) ± SEM for oligospermic and azoospermic groups when compared with the mean serum FSH concentration of the normal group. There was a significantly decrease in mean serum LH concentration (mlU/ml) ± SEM for oligospermic and azoospermic groups when compared with the mean serum LH concentration of the normal group.

Table 3: Kisspeptin (ng/ml) for normal oligospermic and azoospermic groups:

Kisspeptin (ng/ml)	Normal n=30	Oligospermic n=30	Azoospermic n=30	p-value
Range Mean ±SEM	(42.9-451) 115.3 ± 16.89	(54.5-197) 90.77 ± 6.338	(38-104.6) 69.72 ± 2.982	0.0118 p<0.05

Table (3) showed a significant decrease in mean serum kisspeptin concentration (ng/ml) ± SEM for oligospermic and azoospermic groups when compared with the mean serum kisspeptin concentration of the normal group.

Table 4: Correlations between kisspeptin and other clinical data of studied groups:

Kisspeptin (ng/ml)	Normal		Oligospermic		Azoospermic	
	r	p-value	r	p-value	r	p-value
Age (years)	-0.2163	0.2509	-0.1427	0.4519	0.3373	0.0684
BMI (kg/m2)	-0.08447	0.6572	0.2139	0.2564	-0.07143	0.7076
Volume(ml)	-0.1272	0.5030	-0.01797	0.9249	0.1759	0.3525
Count (mil.c./ml)	0.6457	0.0001	0.6050	0.0004		
Motility (%)	0.1720	0.3635	0.3075	0.0983		
Testosterone (ng/ml)	0.6309	0.0002	0.5817	0.0007	0.6435	0.0001
FSH (mlU/ml)	0.6388	0.0001	0.6545	<0.0001	0.1519	0.4231
LH (mlU/ml)	0.6800	<0.0001	0.6016	0.0004	0.3271	0.0777

Table (4) showed anon significant positive correlation between kisspeptin and age in the serum of the azoospermic group (r=0.3373, p=0.0684). There was a non significant positive correlation between kisspeptin and BMI in the serum of the oligospermic group (r=0.2139, p=0.2564). There was a non significant positive correlation between kisspeptin and seminal volume in the serum of the azoospermic group (r=0.1759, p=0.3525). There was a significant positive correlation between kisspeptin and sperm motility in the serum of the normal group and oligospermic group (r=0.6457, p=0.0001) (r=0.6050, p=0.0004) respectively. There was a non significant positive correlation between kisspeptin and sperm motility in the serum of the normal group and oligospermic group (r=0.1720, p=0.3635) (r=0.3075, p=0.0983) respectively. There was a significant positive correlation between kisspeptin and testosterone in the serum of the normal group, oligospermic group and azoospermic group (r=0.6309, p=0.0002) (r=0.5817, p=0.0007) and (r=0.6435, p=0.0001) respectively. There was a significant positive correlation between kisspeptin and FSH in the serum of the normal group and oligospermic group(r=0.6388, p=0.0001) (r=0.6545, p<0.0001) respectively. While there was a non significant positive correlation between kisspeptin and FSH in the serum of the azoospermic group (r=0.1519, p=0.4231). There was a significant positive correlation between kisspeptin and LH in the serum of the normal group and oligospermic group (r=0.6800, p<0.0001) (r=0.6016, p=0.0004) respectively. While there was a non significant positive correlation between kisspeptin and LH in the serum of the azoospermic group (r=0.3271, p=0.0777). There was a non significant negative correlation between kisspeptin and age in the serum of the normal group and oligospermic group (r=-0.2163, p=0.2509) (r=-0.1427, p=0.4519) respectively. There was a non significant negative correlation between kisspeptin and BMI in the serum of the normal group and azoospermic group (r=-0.08447, p=0.6572) (r=-0.07143, p=0.7076) respectively. There was a non significant negative correlation between kisspeptin and seminal volume in the serum of normal group and oligospermic group (r= -0.1272, p=0.5030) (r= -0.01797, p=0.9249) respectively.

Discussion

Kisspeptin might be a key contributory factor in the control of testosterone, FSH and LH levels in males. A study of Ramzan *et al.* [11] provides a link between the kisspeptin levels and male reproductive axis. Several studies were carried out to determine the role of kisspeptin in the regulation of the reproductive axis. However, information regarding the kisspeptin concentration in infertile males was lacking. There's only one study was done to explain the role of the serum kisspeptin levels in infertile males so we studied the effect of kisspeptin on reproductive hormones to clarify it's role in infertility.

In the current study there was highly significant difference as regards sperm motility where the mean of sperm motility for normal, oligospermic and azoospermic groups was 82.77 ± 2.125,21.80 ± 2.992 and 0.00 respectively (P < 0.0001). This was in agreement with Al-Nahi [12] who reported that sperm motility was significantly lower in infertile men when compared with control (P < 0.05) where the mean ± SD of sperm motility for control and infertile group was 76 ± 9.9 and 24.32 ± 15.47 respectively. Also this was in agreement with Sati and Huszar [13] who demonstrated that sperm motility correlates well with fertility and pregnancy rates after intrauterine insemination. Based on 358 semen samples from a group of men reflecting the general male population and the concentration of motile spermatozoa was the most significant and independent and computer-assisted semen analysis parameter in predicting the chance of natural conception.

In the present study there was highly significant difference as regards sperm count where the mean of sperm count for normal, oligospermic and azoospermic groups was 66.27 ± 3.617, 11.90 ± 1.046 and 0.00 respectively (P < 0.0001). This was in agreement with Al-Nahi [12] who reported that sperm count was significantly lower in infertile men when compared with control (P < 0.05) where the mean ± SD of sperm count for control and infertile group was 62.6 ± 13.47 and 32.6 ± 21.12 respectively.

In this study there was no significant difference as regards seminal volume where the mean of seminal volume for normal, oligospermic and azoospermic groups was 3.680 ± 0.1795, 3.339 ± 0.2383and 3.040 ± 0.1923 respectively. (P > 0.05)

In our study there was no significant difference as regards age where the mean of age for normal, oligospermic and azoospermic groups was 35.4 ± 1.14, 34.1 ± 1.509 and 34 ± 1.006 respectively (P > 0.05). This was in agreement with Saleh *et al.* [14] study who reported that there was no significant difference in mean age between the fertile and oligospermic men where the mean age ± SD of fertile and oligospermic men was (33.10±7.17) and (33.40±6.21) year respectively.

In the present study there was a significant difference in testosterone mean for normal, oligospermic and azoospermic groups (p-value < 0.05), where the mean ± SEM of testosterone for normal, oligospermic and azoospermic groups was 6.313 ± 0.5552, 7.362 ± 0.5484 and 4.422 ± 0.4476ng/ml respectively. This was in agreement with Al-Nahi [12] who recorded that there was a significant difference in testosterone mean between fertile and infertile men where significant decrease in testosterone value was recorded in this study. Also this was in agreement with Saleh *et al.* [14] who recorded that the mean value of serum testosterone concentration of oligospermic men was significantly lower than that of fertile men where the mean ± SD of testosterone for fertile and oligospermic men was (8.0 ± 0.78) and (4.91 ± 1.73) ng/ml respectively (p value < 0.040).

In the current study there was no significant difference in FSH mean for normal, oligospermic and azoospermic groups (p-value > 0.05), where the mean ± SEM of FSH for normal, oligospermic and azoospermic groups was 11 ± 0.7408, 10.75 ± 0.9675 and 9.458 ± 0.7104 ng/ml respectively. Our study doesn't agree with Al-Nahi [12] who recorded that there was a significant difference in mean of FSH between fertile and infertile men, a significantly higher serum levels of FSH in the infertile men was shown when compared with the levels in proven fertile controls.

In our study there was a significant difference in LH mean for normal, oligospermic and azoospermic groups (p-value < 0.05), where the mean ± SEM of LH for normal, oligospermic

and azoospermic groups was 8.983 ± 0.7398, 7.295 ± 0.5396 and 5.975 ± 0.3943 ng/ml respectively. This was in agreement with Al-Nahi [12] who recorded that there was a significant difference in LH mean between fertile and infertile men where elevated levels of LH in azoospermic males when compared to normal fertile men. Our results were in agreement with Saleh *et al.* [14] who recorded that the mean values of serum LH concentrations of oligospermic men were significantly higher than those of fertile controls where the mean ± SD of LH for fertile and oligospermic men was (3.67 ± 1.34) and (4.52 ± 2.35) mIU/ml respectively (p value < 0.044).

In addition, this result was in agreement with Bhale and Mahat [15] who recorded that there was a significant difference in LH mean for normal, oligospermic and azoospermic groups.

Kisspeptin neurons exist in close apposition with GnRH neurons in the hypothalamus of a range of species, and GnRH neurons express the kisspeptin receptor. It stimulates GnRH neurons leading to GnRH release in both in vitro and in vivo studies, an effect which is inhibited by the administration of GnRH antagonists and it's administration both centrally and peripherally leads to an increase in circulating lutenizing hormone (LH) levels in both animal and human studies [16].

In the current study there was a significant difference in kisspeptin mean for normal, oligospermic and azoospermic groups (p-value < 0.05), where the normal group has more kisspeptin level than the oligospermic group, also the oligospermic group has more kisspeptin level than the azoospermic group. The mean ± SEM of kisspeptin for normal, oligospermic and azoospermic groups was 115.3 ± 16.89, 90.77 ± 6.338 and 69.72 ± 2.982 ng/ml respectively.

This was in agreement with Ramzan *et al.* [11] who reported that the serum kisspeptin concentrations in fertile subjects were significantly higher as compared with infertile normozoospermic, azoospermic, asthenozoospermic, asthenoteratozoospermic, oligozoospermic, oligoasthen-azoospermic and oligoasthenoteratozoospermic males which was shown that the serum kisspeptin concentrations might be decreased in infertile males as compared to the fertile males. They concluded that the concentration of kisspeptin is significantly lowered in infertile males than the fertile controls and might be used as a diagnostic tool for infertility and treatment of infertility disorders. They suggested that the kisspeptin has a central role in the control of reproduction and is critical for the normal development and maintenance of the reproductive axis.

Kisspeptin plays a key role in maintaining reproductive function and fertility in humans. Humans with mutations in Kiss1 or KISS1R, resulting in low concentrations of circulating LH and FSH and failure to advance through puberty [17].

Kisspeptin-producing neurons have been demonstrated as existing in the hypothalamus and in particular the pre-optic region and infundibular nucleus in humans, corresponding to the arcuate nucleus in rodents which acts as a crucial gatekeeper of reproductive function in the human lifespan, down-regulating fertility at times of physical strain, such as over-exercise or weight loss, and controlling normal physiological development at puberty also suggests that the influence extends beyond that of puberty and the age of reproduction. This confirms that the administration of kisspeptin stimulates gonadotrophin release and can modulate reproductive pathology [18].

In our study there was positive significant correlation between sperm count and kisspeptin in the serum of the normal group (r=0.6457, p=0.0001) and there was positive significant correlation between sperm count and kisspeptin in the serum of the oligospermic group (r=0.6050, p=0.0004).

In our study there was positive significant correlation between testosterone and kisspeptin in the serum of the normal group (r=0.6309, p=0.0002), there was positive significant correlation between testosterone and kisspeptin in the serum of the oligospermic group (r=0.5817, p=0.0007), also there was positive significant correlation between testosterone and kisspeptin in the serum of the azoospermic group (r=0.6435, p=0.0001).

Ramzan *et al.* [11] showed that kisspeptin potentially increase the serum testosterone levels in normal males. Serum testosterone levels were significantly lower in all infertile groups than fertile group while FSH is lower significantly in normozoospermic and asthenozoospermic males as compared to fertile and didn't differ between the other infertile groups and fertile males.

Spermatogenesis is assessed by sperm counts, motilities, and morphologies which is reinitiated and maintained at normal levels in men by introducing human chorionic gonadotropin (hCG) to stimulate leydig cell function. This restores the intratesticular testosterone concentration with undetectable FSH levels in blood after short-term suppression of exogenous testosterone while FSH alone could restore the sperm output [11].

Clarkson and Herbison [19] showed the existence of a male-specific neonatal kisspeptin GnRH neuron signaling mechanism that drives the neonatal testosterone surge, which initiates sexual differentiation of the brain through its receptor GPR54 is critical for puberty and adult fertility because of the growing evidence that kisspeptin neurons may regulate the activity of the GnRH neurons prior to birth. Embryonic GnRH neurons express GPR54 and can respond to kisspeptin, and kisspeptin neurons project to GnRH neurons in embryonic male. This provides that male-specific elevations in embryonic testosterone levels give rise to kisspeptin neurons that then activate GnRH neurons to evoke the neonatal testosterone surge and brain sexual differentiation.

In the present study there was positive significant correlation between FSH and kisspeptin in the serum of the normal group(r=0.6388, p=0.0001), there was positive significant correlation between FSH and kisspeptin in the serum of the oligospermic group (r=0.6545, p<0.0001) but there was positive non significant correlation between FSH and kisspeptin in the serum of the azoospermic group (r=0.1519, p=0.4231).

In the current study there was positive significant correlation between LH and kisspeptin in the serum of the normal group (r=0.6800, p<0.0001), there was positive significant correlation between LH and kisspeptin in the serum of the oligospermic group (r=0.6016, p=0.0004) but there was positive non significant correlation between LH and kisspeptin in the serum of the azoospermic group (r=0.3271, p=0.0777).

Ramzan *et al.* [11] found that kisspeptin is a powerful stimulator of LH release, the sensitivity of LH release to the

stimulatory effect of kisspeptin is manifold high as compared to the sensitivity of FSH release in response to kisspeptin.

Skorupskaite *et al.* [20] found that kisspeptin stimulates the secretion of LH in the human, kisspeptin-54 was first administered in healthy men by intravenous infusion for 90 min and resulted in a robust and dose-dependent increase in LH, and less marked rises in FSH and testosterone. The potency of kisspeptin to stimulate the secretion of gonadotrophins and its effect on the release of LH has been consistently observed when kisspeptin is administered.

Conclusion and Recommendations

The serum kisspeptin levels are significantly higher in the fertile as compared to infertile males (oligospermic and azoospermic males). Kisspeptin can be used as a diagnostic marker for diagnosis of infertility in males and differentiate between normospermic, oligospermic and azoospermic males. This study provides a link between the kisspeptin levels and male reproductive axis depending on the fertility status of the subjects so it's considered as a key contributory factor in the control of testosterone, FSH and LH levels in males. There was a highly significant positive correlation between kisspeptin and LH. There was a significant positive correlation between kisspeptin and FSH which may explain the role of kisspeptin in initiation of puberty. There was a significant positive correlation between kisspeptin and testosterone which is important for spermatogenesis and secondary sex characters in human males. Further studies for use of kisspeptin as a therapeutic target for the treatment of infertility disorders such as delayed puberty and hypogonadotropic hypogonadism will allow modulation of the HPG axis that may open new therapeutic strategies. Other studies using kisspeptin antagonist to clarify the effect of kisspeptin deficiency in LH and FSH release and demonstrate its effect in hypogonadotropic hypogonadism. Further studies on kisspeptin gene polymorphism for demonstration the effect of mutation of kisspeptin gene in infertility.

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