

**Study of the relationship between the concentration of leptin ,Testosterone and insulin in the infertile men**

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Article**Information**

Received Date

15/1/2018

Accepted on

29/4/2018

Keywords

Infertility

Leptin

Insulin

Testosterone

Abstract

This study was carried out during the period 25/1/2017 to 20/3/ at AL-Sader Teaching Hospital in Najaf 2018, to investigate the effects of the relationship between the leptin hormone and infertile men, leptin hormone and two hormone (insulin and testosterone) and some demographic factors as age, weight, height and body mass index on leptin hormone. Fifty blood samples from infertile men and forty blood samples from fertile men were classified in to three aging groups ranged (20-35),(36-45) and (46-55) years ,the values of hormones (leptin ,insulin and testosterone) were examined to find out the correlation between hormone with each other and with infertile men. Significant differences were detected between ($p \leq 0.05$) in insulin and leptin levels. Besides significant reductions at ($p \leq 0.05$) in testosterone levels confined to age groups (20-35) and (36-45) in infertile men, as compared to fertile men. The result also revealed a positive relationship ($p \leq 0.05$) between the body mass index and leptin hormone.

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دراسة العلاقة بين تركيز اللبتين ،التستوستيرون والانسولين في الرجال غير الخصيين

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المستخلص

اجريت الدراسة الحالية في مستشفى الصدر التعليمي في النجف من 2017/ 1/25-2018/3/20 لتحقيق العلاقة بين هرمون اللبتين والعقم لدى الرجال ، هرمون اللبتين مع هرموني الانسولين والتستوستيرون ، و بعض العوامل الديموغرافية مثل العمر ،الطول ،الوزن و مسعر كتلة الجسم الذي يؤثر على هرمون اللبتين. خمسون عينة دم اخذت من الرجال الذين يعانون العقم واربعين عينة دم من الذكور الطبيعيين حيث استخدمت في هذه الدراسة التي صنفت الى فئات عمرية تتراوح (20-35)،(36-45) و (46-55) سنة ، قيم هرمونات (اللبتين و انسولين وتستوستيرون) حيث فحصت لتبين العلاقة بين الهرمونات مع بعضها ومع الرجال العقيمين ، النتيجة بينت وتم فحص هرمون اللبتين ،اظهرت النتائج فروق معنوية بين القيم المتمثلة بواسطة زيادة معنوية ($p \leq 0.05$) في مستوى اللبتين والانسولين وانخفاض معنوي ($p \leq 0.05$) في مستوى التستوستيرون عند الفئات العمرية (20-35) و (36-45) في الرجال العقيمين عند مقارنة مع الرجال الخصيين ، وكشفت النتائج علاقة موجبة ($p \leq 0.05$) بين مسعر كتلة الجسم وهرمون اللبتين .

Introduction

Infertility can be defined is the inability to conceive after one year of without interruption sexual intercourse (WHO,2010) Leptin is a protein that is produced from the white adipose tissue and newly discovered as a neurotransmitter hormone in various organs of the body which

including the reproductive system ,it is composed of fat cells that have an important role in body weight.(Najem *et al* .,2012). Leptin, a 16- Kilo Dalton (k.D) adipocyte-derived cytokine, leptin is circulated in blood and worked on the brain to energy expenditure and regulated food intake ,there for when fat mass increases , the leptin

concentrations increased which suppresses appetite until addition weight is lost, while when fat mass decreased, plasma leptin concentrations decreased which stimulated appetite and suppressed energy expenditure until fat mass was restored (Friedman, 2009).

The leptin hormones played an important role in transferring energy to the reproductive system and providing the sperms incapacitated with the energy it consumes in a process capacitation (Jorsaraei *et al.*, 2010). Testosterone is the primary men an anabolic steroid and sex hormone. In male, testosterone plays a key role in the involvement of men reproductive tissues such as the prostate and testis, as well as promoting secondary sexual characteristics such as increased bone mass and muscle, and the growth of body hair, in humans and most other vertebrates, testosterone is secreted primarily by the testicles of men and, to a fewer extent, the ovaries of woman. Small quantities are also secreted by the adrenal glands. On average, in adult men, levels of testosterone are about 7–8 times as great as in adult woman (Torjesen and Sandnes, 2009). Insulin is excreted of the beta cells in respond to different stimuli such glucose, arginine, sulphonyl urea's though physiologically glucose is the main determinative, different endocrine, pharmacological agents and neural can too exert stimulatory effects, insulin has been shown to play a central role in the organization of gonadal function; however, its significance in men fertility is not completely properly elucidated and understood. (Aquila *et al.*, 2005).

Aims of the study

Study of hormone leptin relationship with each other testosterone and insulin, and with infertility and fertility men.

Material and Methods

Studied groups

The samples were used in study included blood samples 50 of infertile men and 40 blood samples of fertile men that brought the hormones analysis.

Samples collection

Seminal fluid sample

The samples were collected using a masturbation method in a disposable container, it has a wide and inserted nozzle and without the use of any lubricating chemicals causes contamination of semen and affect its vitality, the samples were taken after conformation of sexual absences for a period of 3-5 days do not increase or decrease. The patients name, time and date were recorded in the sample, the sample was then placed in the incubator at 37c until it liquefaction and a general examination of the patients semen was then performed.

Blood samples

Blood sample were collected from the venous (5ml) were grouped from male of both healthy (control) and infertile. the serum acquired by reposing the blood sample in a clean dry plastic tube (without anticoagulant) and allowable to clot at 37c for 30 minutes before centrifugation. The tubes were centrifuge at 4000 rpm for 10 minute, serum was stored and grouped in deep freezing at -20c to the time of using.

Determine demographic factor

Weight determination

The weight of infertile men was measured as well as the measurement of control samples by using the electronic balance.

Length determination-

The length of the infertile men was measured as well as the measurement of control samples by tape measure length.

BMI determination-

The body mass index was measured according to the equation: the weight in kilograms /the length in squared meters Kilometer to square meter (km/m²)(champ *et al.*, 2008).

Seminal fluid analysis

The semen analysis examined the quantity and quality of the sperm produced by the man by taking a sperm samples and sent it to the laboratory , the things examined in the semen examination are the size of the sample ,liquefaction time , number of sperm ,movement of sperm, PH, and white blood cells number , where the specimens were divided into oligozoospermia ,which was a total count of sperm less than 20 million sperm(WHO,2010).And azoospermia are people who have not seen any sperm in each semen(Dohle *et al.*,2008) and Teratospermia people had a malformation in the shape of sperm.

Hormonal assay

Determine of the levels of leptin hormone The Determination of leptin hormone in human serum or plasma was performed according using the Enzyme Linked Immunosorbant Assay (ELISA) technique using a special kit . Inc USA. (Considine *et al.*,1996).in college of science .

Working steps

1. Determine wells for diluted standard ,blank and sample. prepare 7 wells for standard,1 wells for blank. Add 100microliter each of dilution of standard ,plank and sample into the appropriate wells. Cover with the plate sealer .incubated for 1hours at 37c.
2. Remove the liquid of each well, don't wash.
3. Add 100microliter of Detection Reagent A working solution to each well, cover the wells with plate sealer and incubate for 1hours at 37c.
4. Aspirate the solution and wash with 350microliter of 1xwash solution to each well using a squirt bottle, multi-channel pipette, auto washer, and let it sit for 1-2 minutes . remove the remaining liquid from all wells completely by snapping the plate on to absorbent paper. Totally wash 3 times .after the last wash, remove any remaining wash buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.
5. Add 100microliter of Detection Reagent B working solution to each wells ,cover the wells with the plate sealer and incubate for 30 minutes at 37c .

6. Repeat the aspiration/wash process for total 5 times as conducted in step 4.
7. Add 90 microliter of substrate solution to each well. Cover with a new plate sealer. incubate for 10-20 minutes at 37°C. protect from the light. the liquid will turn blue by the addition of substrate solution.
8. Add 50 microliter of stop solution to each well. the liquid will turn yellow by the addition of stop solution. mix the liquid by tapping the side of the plate.
9. Remove any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. then, run the micro plate reader and conduct measurement at 450 Nanometer (nm) immediately.
10. The standard curve is plotted as a relationship between standard and absorbent concentration.

Measurement range

The measurement range of the ELISA leptin: 2.2-8.6 Nano gram per milliliter (ng/ml).

Determine of the levels of insulin hormone

The determination of insulin hormone in human serum or plasma was performed according to using the Enzyme Linked Immunosorbent Assay (ELISA) technique using a special kit. Inc USA. (Chevenne *et al.*, 1994). in college of science

Determine of the levels of testosterone hormone

Testosterone hormone level was determined by ELISA technique using a kit provided by United States of America (USA). the working steps like insulin hormone procedure.

working steps

1. Place the desired number of coated strips into the holder.
2. Pipette 25 microliter of insulin standards, control and patients sera into appropriate wells.
3. Add 100 microliter of working insulin enzyme conjugate to all wells.
4. Thoroughly mix for 10 seconds.
5. Incubated for 60 minutes at room temperature (20-25°C).
6. Remove liquid from all wells. wash wells three times with 300 microliter of 1x wash buffer. Blot on absorbent paper towels.
7. Add 100 microliter of tetra methyl piperidine (TMP) substrate to all wells.
8. Incubate for 15 minutes at room temperature.
9. Add 50 microliter of stop solution to all wells. shake the plate gently to mix the solution.
10. Read absorbance on ELISA reader at 450 nm within 15 minutes after adding the stopping solution.
11. The standard curve is plotted as a relationship between standard and absorbent concentration.

Measurement range

The measurement range of the ELISA insulin: <25 milli-international units per milliliter (µIU/ml).

Measurement range

The measurement range of the ELISA testosterone: 3.0-10.0 ng/ml.

Statistical Analysis

All the study data we are recorded and analyzed by T.test using Statistical package for social

Result and discussion

The mean of leptin hormone in infertile and fertile men .

The result in table 1 showed a significant increase at ($p \leq 0.05$) in leptin level in infertile men, and the

sciences (SPSS) program (Al-Rawi,2000).the result was consider significant when p value was ($p \leq 0.05$).

values of leptin in age groups (20-35) ,(36-45) and (46-55) were (0.1492 ± 4.1737), (1.4764 ± 4.1333) and (0.3250 ± 3.7400) respectively when compared with the fertile men has the values (0.1778 ± 2.6571), (0.1815 ± 2.6167) and (0.2462 ± 2.6250), respectively, (Table, 1).

Table (1). The means of Leptin in Fertile and Infertile men according age.

Age groups (years)	Mean \pm S.E.		T. test	P. Value
	Infertile N=50	Fertile N=40		
20-35	0.1492 ± 4.1737 *	0.1778 ± 2.6571	4.765	0.005
36-45	1.4764 ± 4.1333	0.1815 ± 2.6167	3.052	0.011
46-55	0.3250 ± 3.7400 *	0.2462 ± 2.6250	4.271	0.004

(*) statically significant differences at ($P \leq 0.05$) between fertile and infertile.

leptin level in infertile and healthy male.

Level of leptin in serum are a significantly increased in patients of male in comparison with those of fertile male and there for the results of the current study are consistent with the previous study conducted in Palestine, which indicated a rise in the level of leptin in infertile men (21.2-26.1) compared to fertile men (4.6-12.4)(Jaber ,2011) and the study conducted in Tikrit , which indicated a rise in the level of leptin hormone in infertile male (1.406-15.262) compared with fertile male (0.609-6.918) with significant statistical difference and under the probability of ($p \leq 0.01$) (Najem *et al.*, 2012) previous studies suggest that there is a link between elevated level of leptin and androgen hormone in infertile men , as the excessive difference in the level of leptin may

cause a decrease in the level of androgen hormone in obese men (Isidori *et al.* ,1999) as Behre and his group demonstrated in 2003, there was a close relationship between excessive leptin hormone and low testosterone level in infertile men.

Comparison leptin according to BMI.

The result in table 2 showed a significant increase at ($P \leq 0.05$) in leptin level within the body mass that ranges (30.1-35) and (above 35.1) it was (0.3136 ± 3.0125), (0.1581 ± 2.9000) in infertile men respectively compares with the fertile men (0.3614 ± 1.8750), (0.0947 ± 0.8250) .but there was no a significant difference ($P \leq 0.05$) in leptin level within the body mass that ranges (under 24) and (24.1-30) it was (0.1629 ± 2.4293), (0.1539 ± 2.7000) in infertile men respectively

compares fertile men (0.2230 ± 2.6545), (0.1412 ± 2.5529), respectively (Table, 2)

Table (2). The means of Leptin in infertile and fertile men according to BMI groups.

Groups BMI group (Kg/m ²)	Mean± S.E.		T. test	P. Value
	Infertile N=50	Fertile N=40		
Under 24	0.1629 ±2.4293	0.2230±2.6545	-.833-	0.424
24.1-30	0.1539±2.7000	0.1412±2. 5529	.944	0.359
30.1-35	0.3136 ±3.0125 *	0.3614±1.8750	2.285	0.026
Above 35.1	0.1581±2.9000*	0.0947±0.8250	10.745	0.002

(*) statistically significant differences ($P \leq 0.05$) between fertile and infertile men

leptin and BMI.

The current study showed a positive relationship between body mass index (BMI) and leptin hormone in infertile men with significant statistical difference ($p \leq 0.05$), the higher the concentration of BMI, the greater the concentration of leptin, as this study is consistent with the study conducted in France, which indicated a relationship between BMI and leptin hormone and a significant statistical difference ($p \leq 0.05$) (Couto-silva *et al.*, 2000).

The means of insulin in infertile and fertile men.

The result in table 3 showed a significant increase at ($P \leq 0.05$) in the level of insulin in infertile men within the age groups (20-35), (36-45) and (46-55) it was (1.1311 ± 13.1158), (1.9290 ± 15.2333) and (2.2106 ± 13.3000) compares with the fertile men (0.6711 ± 6.2526), (0.5909 ± 5.0333) and (2.6585 ± 8.0500), respectively, (Table, 3).

Table(3). The means of Insulin in Infertile and Fertile men.

Traits	Groups Age groups (years)	Mean± S.E.		T. test	P. Value
		Infertile N=50	Fertile N=40		
Insulin	20-35	1.1311±13.1158*	0.6711± 6.2526	-6.087	0.000
	36-45	1.9290±*15.2333	0.5909 ± 5.0333	-5.783	0.002
	46-55	2.2106±13.3000 *	2.6585 ±8.0500	-3.198	0.017

(*) statistically significant differences ($P \leq 0.05$) between fertile and infertile men .

The correlation between leptin and insulin in fertile and infertile men .

The result in table 4 showed no a significant positive relationship between leptin and insulin in infertile men ($r=0.553$) and showed no a

significant positive correlation between leptin in infertile and insulin in fertile men($r=0.553$), also showed a significant positive correlation between leptin in fertile and insulin in infertile men ($r=0.553, p=0.01$) and a significant positive relationship between leptin in fertile and in

infertile and a significant positive relationship between leptin in fertile and in infertile men ($r=0.553, p=0.05$), while notice a significant negative relationship between leptin and insulin in fertile in men($r=0.553, p=0.01$).

Table (4). correlation between leptin and insulin in fertile and infertile men.

	Correlations			
	Leptin infertile	Leptin fertile	Insulin infertile	Insulin fertile
Leptin infertile	1	*0.129	0.099	0.058
Leptin fertile	*0.129	1	**0.468	** -0.483-
Insulin infertile	0.099	**0.468	1	* -0.373-
Insulin fertile	0.058	** -0.483-	* -0.373-	1

** correlation is significant $p \leq 0.01$ level .

*correlation is significant $p \leq 0.05$ level.

$R^2=0.553$ T. test=0.205

The correlation between leptin and insulin .

The current study a highly a significant increase insulin level in infertile men within age group (20-35)(36-45)(46-55). The result of this study accordance with other studies (Abundis and Ortiz,2001) which explained that increased insulin secretion leads to increased leptin in the fatty tissue .also the result of this study agreement with (Pieloud *et al.*,2005tt) who explain a high insulin hormone lead to lowered testosterone level independently of hypothalamus – pituitary – axis (leydig cells) . While the result of this study disagreement with (Murray *et al.*, 1983) who explain decreased insulin lead to decrease leydig cells number impaired , leydig cells function . The significances of insulin has been demonstrator in male rat reproductions by usage

streptozotocin ,to depletes the β -cells of the pancreas, and thereby inducing Insulin depended diabetes mellitus (IDDM) (Murray *et al.*, 1983). The decrease of insulin in these rates conduce a declines in leydig cell number as well as a detriments in leydig cell function. this consequence interpreted to a decreases in serum testosterone levels and androgen biosynthesis . subsequent decline in testosterone in IDDM and the weakened leydig cell function could be explained by the absenteeism of the direct stimulatory impacts of insulin on leydig cells, as well as to insulin-dependent decreases in Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) (Wang *et al.* , 1998). Reports were also reported that insulin plays a central role in the regulating of the (HPTA) by

the reducing in secretion of LH and FSH in diabetic male as well as in knockout mice lacked the insulin receptor in the hypothalamus (Baccetti *et al* ., 2002). The present study showed a positive correlation between insulin and leptin in infertile men .the result of this study agrees with most studies(Abundis and Ortiz,2001) which found a positive relationship between leptin hormone and insulin hormone , the increase in secretion of insulin leads to increment the formation messenger Ribonucleic acid (m RNA)for leptin in adipose tissue . also agree with(Paz *et al* ., 1977) who explain leptin and insulin are strongly associated with increase in obesity , it is plausibly there is a cross over from perimetric circulation to the reproductive tract , insulin and insulin like peptides in human semen have formerly been proposed to be secreted by the seminal vesicles .

Leptin and insulin are important organizers of men reproduction by the hypothalamic pituitary-testicular axis (HPT axis) both peripherally and centrally ,in additional to being attended in human semen with important regulative role for fertilization and sperm function (Lampiao *et al*

., 2009: Ando and Aquila ,2005). Moreover , both hormones are secreted and synthesized by ejaculates spermatozoa in an autocrine style (Ando and Aquila ,2005).exogenous additional of leptin and insulin to ejaculates semen has been show to augments progressive motility, acrosome reaction, total motility and nitric oxide producing in human spermatozoa (Lampiao and Plessis,2008). Sertoli cells have been demonstrated to secrete and synthesize insulin hormone (Schoeller *et al* ., 2012).

The means of testosterone in infertile and fertile men.

The result in table 5 showed a significant increase at ($P \leq 0.05$) in testosterone level in infertile men within the age group(46-55) it was (0.5498 ± 5.6250) compares with the fertile men (1.5562 ± 3.4000) ,while no a significant difference ($P \leq 0.05$) in testosterone level in infertile men within the age groups (20-35),(36-45) it was(0.3617 ± 5.0316),(0.3745 ± 4.5833) compares with the fertile men (0.3440 ± 5.568),(0.9031 ± 5.1833) ,respectively,(Table, 5).

Table(5). The means of Testosterone in Infertile and Fertile men.

Traits	Groups Age groups (years)	Mean± S.E.		T. test	P. Value
		Infertile N=50	Fertile N=40		
Testosterone	20-35	0.3617 ± 5.0316	0.3440 ± 5.568	-1.151	0.265
	36-45	0.3745 ± 4.5833	0.9031 ± 5.1833	-0.543	0.610
	46-55	0.5498 ± 5.6250 *	1.5562 ± 3.4000	1.368	0.026

* Statistically Significant differences ($P \leq 0.05$) between Infertile and Fertile groups.

The correlation between leptin and testosterone in fertile and infertile men.

The result in table (6) showed a significant negative correlation between leptin and

testosterone in infertile male within age group (46-55) ($r=.317, p=0.05$), also showed no a significant positive correlation between leptin in infertile and testosterone in fertile and between testosterone between fertile in infertile ($r=.317$), while notice no a significant negative correlation

between leptin in fertile and testosterone in fertile ,also between testosterone and leptin in fertile male , also showed a significant positive correlation between leptin in infertile and fertile male.

Table (6) correlation between leptin and testosterone in fertile and infertile men .

	Correlations			
	Leptin infertile	Leptin fertile	testosterone infertile	testosterone fertile
Leptin infertile	1	*0.129	*-0.216	0.037
Leptin fertile	*0.129	1	-0.028-	-0.045-
testosterone infertile	*-0.216	-0.028-	1	0.023
testosterone fertile	0.037	-0.045-	0.023	1

*correlation is significant $p \leq 0.05$ level .

$$R^2=0.317 \quad T. \text{ test}=0.112$$

The correlation between leptin and testosterone.

The current study shown a high testosterone level in patient within age groups (46-55).The result of this study agrees with the work of (Behre *et al.*, 1997) who find significantly high concentration testosterone in patients. Also the result of this study agrees with (Hmanns and Hafez , 1981) who found increases concentration testosterone level in patients.While the result of this study disagreement other study (Aggerholm *et al.* , 2008) who explain the decrease serum testosterone level observed in obese men.

The current study shown a highly negative correlation between leptin and testosterone in infertile men within age group (20-35),(36-45).

this result of this study agrees with (Hanafy *et al.* , 2007) who measured the serum leptin concentrate in 80 men (infertile oligozoospermia (n=50) and fertile normozoospermia as a control (n = 30). and studied its correlation with testosterone .they found that the serum leptin concentration correlation inversely with the testosterone .moreover, this result agrees with the result obtain by (Isidori *et al.* , 1999) who investigate the correlation linking leptin and androgens in men. Also agrees with (Hanafy *et al.* , 2007) that explain a negative relationship between serum total testosterone and leptin concentration was found .This study demonstrated that circulation fat mass (F.M) and leptin were adversely appertained with total testosterone and free testosterone and deduced that increase circulating in men obesity .

Howsoever, there is consonance excitement in the literature , with great circumstantially supporting , for hypothesis that alteration of sperm parameter connected with fatness can be imputed to unsuitable repress of the hypothalamic pituitary gonadal axis (HPGA) by elevation estrogen derivative from peripheral aromatization, and results reduced testosterone production reversed in low level of intratesticular testosterone and

circulates testosterone (Hammound *et al* ., 2006). The testosterone level was significantly decrease as leptin level increased. The result of this study agrees with (Madah *et al* ., 2001) who investigated the relationship of sex hormones, leptin and anthropometric indices in 186 adult male and influence of average weight loss on these differences in obese individuals.

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