Estimation of Leptin and Leptin Receptor Concentrations in Seminal Plasma of Primary Infertile Men

Ruqaya M. AL-Barzinji* **BSc, MSc, PhD**
Ahmed A. AL-Naqshbandi** **BSc, MSc**

Abstract:

Background: There are many sources for Leptin secretion, and it is activated by binding with its receptor known as leptin receptor, that play a role in male infertility.

Objective: To assess the levels of leptin and leptin receptors in seminal plasma among primary infertile men and its impact on semen parameters.

Fac Med Baghdad 2017; Vol.59, No.2
Received: Mar.2017
Accepted: May 2017

Patients and Methods: A case control study of 75 primary infertile males and 40 healthy individuals who were enrolled in this study during March 2013 to May 2013. Estimation of age, body mass index (BMI), semen analysis, seminal plasma leptin, leptin receptor and testosterone hormone concentration were done for all study subjects.

Results: Highly significant difference found in mean of semen parameters of infertile male compared with healthy controls. Mean concentration of seminal plasma leptin and leptin receptor of infertile men were significantly were elevated, while serum testosterone concentration significantly decreased compared with healthy control.

Conclusion: There is emerging evidence that the leptin concentration negatively impacts fertility through its correlation with age, BMI, testosterone hormone and semen parameters.

Keywords: Leptin; Leptin receptor; Testosterone; semen quality.

Introduction:

Leptin, a key hormone in energy homeostasis and neuro-endocrine function, has a role in the pathogenesis of reproductive dysfunction in many states of energy imbalance [1]. Leptin is present in human Sertoli cells, seminiferous tubules, Leydig cells and germ cells. Indeed it is able to cross the testis blood barrier (TBB).Concentration of leptin in seminal plasma has a correlation with the sperm motility. By a high affinity binding protein, it's bound with a soluble leptin receptor [2]. It is the product of the ob gene and belongs to the class I cytokine superfamily of receptors. Leptin receptor mRNA is expressed in the anterior pituitary, in several areas of the brain and in other tissues, also has been detected in granulose and theca cells [3]. In human, two major forms of leptin receptors (ob-R) are expressed. The short form (ob-RS) and long form (ob-RL) [4]. Leptin act via hypothalamic receptor (LEPRb) to regulate energy balance [5]. Stopping of leptin signaling due to mutation of leptin or its receptor, results in increased food intake and decreased energy expenditure phenotype reminiscent of the neuro-endocrine starvation response (including hypothyroidism and infertility [6]. Sex steroids mainly testosterone are responsible for Leptin secretion by the adipocyte, also leptin secretion is regulated by other hormones, such as insulin and cortisol [7]. Testosterone as steroid hormones, is necessary for the maturity and maintenance of secondary sexual properties as well as beginning and maintenance of spermatogenesis [8].A person's weight can have a intense impact on fertility by presence correlation between body weight and sperm concentration [9]. The decrease in male fertility explained in relation to obesity, indeed obesity is considered as an etiology of male fertility [10]. There is a correlation between body weight and sperm concentration. It can disrupt the hormonal balance which is necessary for normal sperm production [11]. Semen analysis is of great importance in the initial investigation of male and its results are often taken as a surrogate measure of male infertility and pregnancy risks. It provides information on the functional status of The germ epithelium, epididymis and accessory sex glands [12]. Analysis of semen can give us information about problems in the genital organs of the male. The results of semen analysis have been used to categorize men into groups with different probabilities of achieving pregnancy within a certain time period. The aim of the basic semen analysis is to evaluate descriptive parameters of ejaculates obtained by masturbation. The qualities that are assessed are visual appearance, smell, liquidity, viscosity, volume, sperm concentration and total number of spermatozoa, sperm motility, and sperm vitality. Furthermore, differential count with respect to sperm morphology, assessment of sperm agglutination and assessment of presence of debris and other cell types in semen are also performed [13]. The aim of the study is evaluate the correlation between levels of leptin and leptin receptor in seminal plasma of primary infertile males and its effect to semen parameters.

Subjects and Methods:

Sampling Size and Area: A case control study of 75 primary infertile male and 40 healthy individual were enrolled in this study during March 2013 to May 2013. Out of 75 patients, 9(12%) from Maternity Teaching Hospital/Erbil and 66 (88%) from private clinical laboratory/Erbil. Total of 40 apparently healthy individual were selected from males without any history of infertility problems or diseases who attended private

* *Hawler Medical University, College of Medicine; Microbiology department; Erbil, Iraq, Email:ruqayataher2012@gmail.com*
** *Rizgary Teaching Hospital, Department of Microbiology; Erbil, Iraq.*

clinical laboratory as control group.Seminal Fluid Sample Collection: The patients and healthy individuals were asked to bring semen sample which was collected in sterile, clean, wide-mouthed and labeled plastic container. Macroscopic and Microscopic Examination: After liquefaction time, according to guide line of WHO, seminal fluid was investigated for the macroscopically (appearance, volume, pH, viscosity) and microscopically (sperm count, sperm motility, sperm morphology) analysis [13]. The remaining specimens were centrifuged at 3000 round per minute for 15 minute, to obtain seminal plasma. Seminal plasma was dispensed into labeled and sterile Eppendrof tube, kept at -20 C˚, to estimate the level of leptin and leptin receptor. Blood Sample Collection: Five ml of peripheral venous blood was collected from each patient and control group performed by using disposable syringe and collected in tubes, allowed to clot at room temperature then centrifuged at 3000 round per minute for 15 minute, the sera obtained were dispensed into labeled and sterile Eppendrof tube, kept at -20 C˚, to estimate the level of testosterone hormone. Quantitative Estimation of Leptin, Leptin Receptor and Testosterone Level: Enzyme linked immuno-sorbent assay (biotek, USA) used for quantitative estimation of leptin, leptin receptor and testosterone. SPSS version 20 was used for statistical data analysis.

Results:

The infertile patients were divided in to two groups: 38 leukocytospermic men (24 normozoospermia, 10 oligozoospermia, 4 azoospermia) and 37 non-leukocytospermic men (23 normozoospermia, 10 oligozoospermia, 4 azoospermia). The age of fertile subjects ranged from 28-48 years with mean of 33.37±2.36 years; whereas the age of the infertile subject ranged from 20-43 years with mean of 30.21±0.61 years. The semen quality of infertile men was significantly lower compared to fertile. Significant differences in mean value of leptin, leptin receptor and testosterone were observed between fertile and infertile males (Table 1). There were significant differences between normozoospermia and oligozoospermia infertile men in following terms, spermatozoa concentration, spermatozoa motility and spermatozoa abnormal morphology. The difference in the levels of leptin, leptin receptor and testosterone among three study classes of leukocytospermic (Table 2) and non-leukocytospermic (Table 3) infertile male were statistically not significant. Men suffering primary infertility are categorized in to three groups according to age as shown in table (4). All investigated parameters reveal differences between age groups, but statistically not significant. Regarding to the BMI of the infertile men, age show a significant difference among different categorized BMI. Leptin, leptin receptor and testosterone found differences between them, but statistically not significant (Table 5).

Table 1: Comparison of Semen Parameters, Leptin, Leptin Receptor and Testosterone between Fertile and Infertile men

Parameters	Fertile men n=40 Mean±SE	Infertile men n= 75 Mean±SE	Probability
Age (years)	33.37±2.36	30.21±0.61	NS
Volume (ml)	3.12±0.09	2.77±0.15	HS **
Sperm concentration+	70.5±2.98	46.30±4.17	HS **
Sperm motility	71.25±2.18	51.46±3.06	HS **
Abnormal morphology	31.87±0.79	38.54±2.43	HS **
Leptin (ng/ml)	268.31±11.59	314.83±24.57	HS **
Leptin receptor (pg/ml)	1270.0±95.84	2250.5±114.88	S *
Testosterone (ng/ml)	11.64±0.66	7.62±0.30	HS **

P value ≥ 0.05: Non-significant, * P value < 0.05: Significant, ** P value < 0.01: Highly significant, +: $x10^6$ / ml

Table 2: Comparison of Semen Parameters, Leptin, Leptin Receptor and Testosterone between Seminal Classes of Leukocytospermic Infertile Men

Parameters	Normozoospermia n=24 Mean±SE	Oligozoospermia n= 10 Mean±SE	Azoospermia n= 4 Mean±SE	Probability
Volume (ml)	2.91±0.28	2.05±0.35	2.5±0.28	NS
Sperm concentration+	67.16 a ±4.89	10.4 b ±2.39	0.00±0.00	HS**
Sperm motility	60.0 a ±3.41	41.0 b ±8.02	0.00±0.00	HS**
Abnormal morphology	38.54 a ±2.39	44.5 b ±2.03	0.00±0.00	HS**
Leptin (ng/ml)	252.49±31.70	313.00±56.21	327.83±73.93	NS
Leptin receptor(pg/ml)	2161.4±257.50	1982.2±299.54	2172.6±426.51	NS
Testosterone(ng/ml)	8.92±1.07	8.59±1.97	7.21±1.38	NS

P value ≥ 0.05: Non-significant, ** P value < 0.01: Highly significant, Different letters means significant difference, +: $x10^6$ / ml

Table 3: Comparison of Semen Parameters, Leptin, Leptin Receptor and Testosterone between Seminal Classes of non-Leukocytospermic Infertile Men

Parameters	Normozoospermia n=23 Mean±SE	Oligozoospermian=10 Mean±SE	Azoospermia n= 4 Mean±SE	Probability
Volume (ml)	3.06±0.3	2.6±0.47	2.75±0.52	NS
Sperm concentration +	69.26 a ±5.57	16.4 b ±7.86	0.00±0.00	HS**
Sperm motility	62.60 a ±3.93	57.0 b ±7.31	0.00±0.00	HS**
Abnormal morphology	35.0 a ±2.30	38.5 b ±2.79	0.00±0.00	HS**
Leptin (ng/ml)	322.28±50.45	369.33±69.17	433.23±193.21	NS
Leptin receptor (pg/ml)	2333.9±136.63	2363.1±292.33	2764.2±762.03	NS
Testosterone(ng/ml)	9.96±1.51	8.43±2.53	7.99±1.33	NS

P value ≥ 0.05: Non-significant, ** P value < 0.01: Highly significant, Different letters mean significant difference, +: $x10^6$ / ml

Table 4: Comparison of Semen Parameters, Leptin, Leptin Receptor and Testosterone among Infertile Men According to Age

Parameters	Age < 29 n= 39 Mean±SE	Age 30-39 n=32 Mean±SE	Age > 40 n= 4 Mean±SE	Probability
Volume (ml)	2.64±0.16	3.14±0.27	2.50±0.28	NS
Sperm concentration $^{+}$	47.53±5.68	46.84±6.59	30.00±19.09	NS
Sperm motility	54.23±4.18	47.65±4.62	40.0±13.38	NS
Abnormal morphology	37.38±3.21	41.34±3.95	27.50±9.68	NS
Leptin (ng/ml)	296.34±26.35	307.99±39.03	349.80±128.49	NS
Leptin receptor (pg/ml)	1967.7±121.39	2200.2±137.57	2411.0±301.04	NS
Testosterone(ng/ml)	8.67±0.97	9.04±1.11	6.447±0.89	NS

P value ≥ 0.05: Non-significant, $^{+}$: $x10^6$ / ml

Table 5: Comparison of Age, Semen Parameters, *Leptin, Leptin Receptor and Testosterone*among Infertile Men According to BMI

Parameters	BMI < 24.9 n= 24 Mean±SE	BMI 25-29.9 n=33 Mean±SE	BMI > 30 n=18 Mean±SE	Probability
Age	27.16^{a} ±1.086	30.93^{bc} ±0.72	32.94^{c} ±1.29	HS**
Volume (ml)	2.93±0.29	2.75±0.17	2.69±0.39	NS
Sperm concentration $^{+}$	52.91±8.12	51.75±5.90	46.72±7.59	NS
Sperm motility	58.33±4.43	53.78±4.87	51.66±5.75	NS
Abnormal morphology	34.79±3.46	36.60±3.73	38.22±4.88	NS
Leptin (ng/ml)	269.48±24.45	279.61±34.82	317.64±54.61	NS
Leptin receptor(pg/ml)	1886.8±133.72	2085.6±123.67	2149.0±225.17	NS
Testosterone(ng/ml)	8.694±1.14	9.420±1.25	8.55±1.33	NS

P value ≥ 0.05: Non-significant, ** P value < 0.01: Highly significant, Different letter means significant difference, $^{+}$: $x10^6$ / ml

Discussion:

The mean age of primary infertile men was 30.21 years which is consistent with study done by Gowri *et al.* (2010)[14]. It has been found that the average age of infertile men in current study seem to be young; this may due to seeking for treatment at early stage of marriage. Significant decreased sperm quality in infertile men was found when compared to fertile men, concordant to study of Al-Dakhly *et al.* in 2007[15].Zorn *et al*. in 2007 demonstrated that leptin level was increased in infertile males and adversely affected sperm quality through regulation of Leydig cell function[16]. This result concordant to Zorn and co-worker, we found increasing concentration of leptin in infertile men with worse quality of seminal parameters. Moreover, Leptin can exert their function through expression of functional leptin receptor [17]. Similarly the study detected increased leptin receptor concentration in semen of infertile men. Testosterone is necessary for normal sperm development. The present results showed that there was a significant decline testosterone level in sera of infertile men associated to poor sperm quality compared to healthy control and this is in agreement with result of Khatoon *et al*. (2012)[18]. Higher number of patients with normozoosperia were recorded in both leukocytospermic and non-leukocytospermic infertile male concordant to Al-Dakhly *et al.* (2007) [15]. These results revealed significant differences in the seminogram between seminal classes in terms of sperm concentration, motility and morphology in leukocytospermic and non-leukocytospermic infertile men, same conclusion observed by Masroor et al. (2013) [19]. Normal leptin secretion is necessary for normal reproductive function, in this study, a trend was observed for non-significant higher leptin concentration in seminal plasma of azoospermic men, that may be caused by circulating leptinthat is capable of crossing the blood-testis barrier by a leakage[20]. Jorsaraei et al. (2010) also reported that the source of leptin in seminal plasma is likely from seminal vesicle, prostate tissue or testis tissue in addition to spermatozoa [21]. This finding is similar to conclusion of Ma *et al.* [22], who showed that sperm negative patient, has a higher leptin level than that of sperm positive patient. Normozoospermic infertile men accompanied by rise in testosterone level more than oligozoospermic and azoospermic subject is concordant with Sheikh *et al.* (2005) [23]. The effects of age on fertility are real and may be greater than has been thought and it's well known that the fertility declines with age [24]. Previous studies documented that age is associated with diminished in semen volume, sperm concentration, motility and morphology [25]. Similarly, this study concluded that increasing age is associated with decreased seminogram and older men have lower semen volume and sperm parameters. Older age in current study revealed an increasing leptin level and its receptor in which associated with decline in testosterone level [26]. Obesity is defined when the BMI greater than 30 kg/m^2, it cause many pathophysiological problems and adversely affects male fertility by endocrine, thermal and genetic mechanisms. In obese men, more androgen is converted to estrogen via aromatization in peripheral fat. Consequently, serum testosterone level is reduced. In obese men, the scrotum remains in closer contact with surrounding tissue, predisposing to increased scrotal temperature that may adversely affect semen parameters [27]. Results of this study indicated negative effect of BMI on sperm quality and obese men have lower sperm parameters, this finding is supported by other researchers [28] [29].Obese men in current study associated with increased leptin and leptin receptor level, concordant to Roberts et al. (1997)[30]. Increasing in leptin and its receptor level associated with decline in testosterone level[31].In this result, infertile obese men were associated with decreased testosterone, consistant with results of Pauli et al. (2013)[32].

Conclusion:
Seminal plasma concentrations of leptin and their receptor play a vital role in infertility and inverse effect on semen parameters.

Author contribution:
Ruqaya M. Ghareeb Taher AL-Barzinji: Supervisor
Ahmed Abdul-Qader Abdul-Salam AL-Naqshbandi: MSc Student

References:
1-Hofny E.R.M., Ali M.E, Abdel-Hafez H.Z., Kamal E.E, Mohamed E.E, Abd el-azeem.; et al. (2010). Semen parameters and hormonal profile in obese fertile and infertile males. Fertility and Sterility, 94(2):581-584.
2-Quinton N.D, Smith R.F, Clayton PE. Gill, MS, Shalet S, Justice SK (1999). Leptin binding activity changes with age: The link between leptin and puberty. The Journal of Clinical Endocrinology and Metabolism, 84(7):2336-2341.
3-Finn PD, Cunningham MJ, Pau KYF, Spies HG, Clifton DK and Steiner RA. (1998). The stimulatory effect of leptin on the neuroendocrine reproductive axis of the monkey. Endocrinology, 139:11(4652-4662).
4-Wang Y, Kuropatwinski KK, White DW, Hawley TS, Hawley RG, Tartaglia LA and Baumann H (1997). Leptin receptor action in hepatic cells. The Journal of Biological Chemistry, 272:26(16216-16223).
5-Knobelspies H, Zeidler J, Hekerman P, Bamberg-Lemper S and Becker W (2010). Mechanism of attenuation of leptin signaling under chronic ligand stimulation. Bio-Medical Central Biochemistry, 11(2):1-12.
6-Munzberg H, Bjornholm M, Bates SH and Myers JM.G. (2005). Leptin receptor action and mechanisms of leptin resistance. Cellular and Molecular Life Sciences, 62(6):642-652.
7-Wauters M, Considine RV and Gaal FV (2000). Human leptin: From an adipocyte hormone to an endocrine mediator. European Journal of Endocrinology, 143(3):293-311.
8-Al-Daghistani HI, Hamad AR, Abdel-Dayem M, Al-Swaifi, M. and Abu-Zaid, M (2010). Evaluation of serum testosterone, progesterone, seminal antisperm antibody, and fructose levels among Jordanian males with a history of infertility. Biochemistry Research International, doi:1155/2010/409640.
9- Al-Qadhi H I. , Jawad H M and Al-Nasiri U S(2016). Response of Overweight Patients with Oligozoospermia to Coenzyme Q10 Treatment. Journal of Pharmacy and Biological Sciences, 11(2): 63-68
10-Cabler S, Agarwal A, Flint M and Du Plessis SS (2010). Obesity: Modern mans fertility nemesis. Asian Journal of Andrology, 12(4):480-489.
11-Sudha G., Reddy KSN and Reddy K.K. (2009). Association between body mass index and infertility: A cross sectional study. Asia-Pacific Journal of Social Sciences, 1(1):73-81.
12-Esteves SC and Agarwal A (2011). Impact of the new WHO guideline on diagnosis and practice of male infertility. The Open Reproductive Science Journal, 3(5):7-15.
13-World Health organization, (2010). WHO laboratory manual for the examination and processing of human semen, 5th ed. Geneva.
14-Gowri V, Venkiteswaran KP, Al-Zakwani I, Mathew J, Abdul-Rahman K and Al-Marhoon M (2010). Comparison of the demographics, semen parameters and hormone profiles in men with primary and secondary infertility. Sultan QaboosUniversity Medical Journal, 10(3):350-353.
15-Al-Dakhly MR, Tawadrous GA, Mostafa T, Roaia MM, El-Nashar ARM., Shedeed SA, et al. (2007). Assessment of seminal plasma laminin in fertile and infertile men. Asian Journal of Andrology, 9(1):63-67.
16-Zorn B, Osredkar J, Meden-Vrtovec H and Majdic G (2007). Leptin levels in infertile male patients are correlated with inhibin B, testosterone and SHBG but not with sperm characteristics. International Journal of Andrology, 30(5):439-444.
17-Caprio M, Isidori AM, Carta AR, Moretti C, Dufau, M. and Fabbri A (1999). Expression of functional leptin receptors in rodent Leydig cells. Endocrinology, 140(11):4939-4946.
18-Khatoon M, Chaudhari AR and Singh R (2012). Effect of gender on antisperm antibodies in infertile couples in central India. Indian Journal of Physiology and Pharmacology, 56(3):262-266.
19-Masroor S, Muneshwar JN and Zingade US (2013). Estimation of seminal MDA levels in infertile patients. IOSR Journal of Dental and Medical Sciences, 4(4):18-23.
20-Banks WA, McLay RN, Kastin AJ, Sarmiento U and Scully S (1999). Passage of leptin across the blood-testis barrier. American Journal of Physiology, 276(6pt1):E1099-E1104.
21-Jorsaraei SGA, Shibahara H, Ayustawati, Hirano Y, Suzuki T, Marzony ET et al. (2010). The leptin concentrations in seminal plasma of men and its relationship to semen parameters. Iranian Journal of Reproductive Medicine, 8(3):95-100.
22-Ma Y, Chen B, Wang H, Hu K and Huang Y (2011). Prediction of sperm retrieval in men with non-obstructive azoospermia using artificial neural network: Leptin is a good assistant diagnostic marker. Human Reproduction, 26(2):294-298.
23-Ekwere PD, Archibong EI, Bassey EE Ekabua, JE, Ekanem EI and Feyi-Waboso P (2007). Infertility among Nigerian couples as seen in Calabar. Port Harcourt Medical Jpurnal, 2(1):35-40.
24-Azab S, Sayed M, Raafat M and Saleh OM (2011). Male infertility and chronic hepatitis C virus infection in Egyptian patients: Is there and correlation? Australian Journal of Basic and Applied Sciences, 5(12):2942-2951.
23-Sheikh M, Khan MS, Danyal A, Zaidi SSH, Begum B and Turabi A (2005). Azoospermia and oligozoospermia, semen and hormonal analysis of patients. The Professional, 12(1):80-84.
24-Ng KK, Donat R, Chan L, Lalak A, Di Pierro I and Handelsman DJ (2004). Sperm output of older men. Human Reproduction, 19(8):1811-1815.
25-Feki NC, Abid N, Rebai A, Sellam, A, Ben A, Yed B, Guermazi M et al. (2009). Semen quality decline among men in infertile relationships: Experience over

12 years in the south of Tunisia. Journal of Andrology, 30(5):541-547.

26-Al-Harithy RN (2004). Relationship of leptin concentration to gender, body mass index and age in Saudi adults. Saudi Medical Journal, 25(8):1086-1090.

27-American society for reproductive medicine (ASRM) (2008). Obesity and reproduction: An educational bulletin. Fertility and Sterility, 90(3):s21-s29.

28-La-Vignera S, Condorelli RA, Vicari E and Calogero E (2012). Negative effect of increased body weight on sperm conventional and non-conventional flow cytometric sperm parameters. Journal of Andrology, 33(1):53-58.

29-Najafi M, Sreenivasa G, Aarabi M, Dhar M, Babu S and Malini SS (2012). Seminal malondialdehyde levels and oxidative stress in obese male infertility. Journal of Pharmacy Research, 5(7):3597-3600.

30-Roberts SB, Nicholson M, Staten M, Dallal GE , Sawaya AL, Heyman MB et al.

(1997). Relationship between circulating leptin and energy expenditure in adult men and women aged 18 years to 81 years. Obesity Research, 5(5):459-463.

31-Sih R, Morley JE, Kaiser FE, Perry HM, Patrick P and Ross C (1997). Testosterone replacement in older hypogonadal men: A 12 month randomized controlled trial. Journal of Clinical Endocrinology and Metabolism, 82(6):1661-1667.

32-Pauli EM, Legro RS, Demers LM, Kunselman AR, Dodson WC and Lee PA (2013). Diminished paternity and gonadal function with increasing obesity in males. Fertility and Sterility, 90(2):346-351.