Role of leptin in infertile men before and after treatment with clomiphene citrate and vitamin E

Ahlam H. Essa[1] and Waleed H. Yousif [2]

[1]High Institute of Infertility Diagnosis and Assisted Reproductive Technology,[2] College of Applied Biotechnology, Al-Nahrain University, Baghdad, Iraq.

Abstract:

Background:

Leptin is an adipocyte –secreted protein that participates in the regulation of energy homeostasis. It prevents the body from storing fat, controls hunger and cravings, regulates food intake and energy expenditure, provides the body with an index of nutritional status and controls the whole body fat metabolism. Leptin is a product of ob gene created by adipocytes. It seems to signal metabolic information to the reproductive system.

Objective:

The aim of this study is to evaluate the relationship between serum leptin and infertility in oligozoospermic men.

Materials and Methods:

Seventy men were investigated; fertile normozoospermic as a control (n = 35) and infertile oligozoospermic (n =35). The patients underwent estimation of body weight (kg), height (cm), body mass index (BMI), semen analysis, serum FSH,LH, testosterone and leptin, lipid profile and serum malondialdehyde (MDA). The infertile group was given a treatment course of clomiphene citrate (50 mg) and vitamin E (400mg) and then all the previous parameters were reevaluated after 3 months .

Results:

Mean body weight was significantly higher in infertile oligozoospermia compared to controls.The height showed no significant difference between the two groups.Hormonal profile revealed significant (P=0.0001) difference in FSH between control group and oligozoospermic group (7.04± 4.20 versus 3.35± 1.47 mIU/ml), but after treatment with clomiphene citrate and vitamin E there is a slight non-significant (P=0.231) decrease in the level of FSH in oligozoospermic group.The level of testosterone showed a statistically significant (P=0.0001) difference between fertile normozoospermic (5.52 ±1.29 ng/ml) and infertile oligozoospermic (2.40 ±0.96 ng/rnl).This hormone showed a significant (P=0.0001) increase in its level after treatment with clomiphene citrate and vitamin E (3.48± 1.56 ng/ml). Other hormone showed non significant difference between the two groups pre and post treatment. Lipid profile showed no significant difference between the two groups . There was no significant (P=0.477) difference in serum MDA between the control and the infertile groups (4.69± 1.32 vs. 5.18± 1.19 µmol/L) respectively,but after treatment the results showed a mild significant (P=0.026) decrease (4.30± 0.67 µmol/L) in comparison to the level in the control group and also a significant (P=0.0001) decrease in comparison to the level before treatment. Infertile oligozoospermic had significantly (P<0.0001) higher serum leptin level (8.03± 1.22 ng/ml) than control (3.62 ± 1.00 ng rnl).

Conclusion:

Serum leptin demonstrated a significant positive correlation with age, body weight, BMI and a significant inverse correlation with serum testosterone. It had nonsignificant correlation with the height and sperm concentration. These results are suggestive of a link between the adipocyte derived hormone leptin and male reproduction.

Key words: Leptin, Male infertility, FSH, LH.

Introduction

In our life millions of men and women use condoms, diaphragms, or any way of contraception to avoid pregnancy, meanwhile about (9- 14)% of couple can not conceive, to them these seem tragic, sadness which could lead to disruption of marriage[1]. Infertility is a term used to define the in ability to conceive despite regular unprotected sexual intercourse over a specific period of time, usually either 1or 2 years; in fact, around 10- 15% of couples have to wait more than 12 months, the time proposed by the WHO as the maximum normal limit before achieving pregnancy[2]. There is anincrease in the percentage of infertility problem in the last years, but this may be due to recent advances in infertiltiy treatment and access of patients to such information have led to early presentation of these patients and their request for treatment.This may give a false impression of an increasing infertility problem. However, there is concern that male fertility is declining due to environmental factor.Male factors alone constitute (25 -30)% of infertility[3]. and they contribute to another 30% in combination with female factor. Known etiology of male infertility can be divided according to affecting factor into pretesticular, testicular and post testicular factors. Primary testicular disorders are most commonly responsible include cryptorchidism, testicular tortion or trauma, genetic defect, varicocele, gonadal dysgenesis, infection as mumps[4]. The most common pretesticular causes, hypogonadisim, estrogen excess, hyperprolactinemia, medical cause as liver disease[5], and post testicular causes due to obstruction of reproduction channel either congenital or acquired. Other causes are anti-sperm anti-bodies and obesity [6] .

Leptin is an adipocyte –secreted protein that participates in the regulation of energy homeostasis It prevents the body from storing fat, controls hunger and cravings, regulates food intake and energy expenditure, provides the body with an index of nutritional status and controls the whole body fat metabolism[7]. Leptin is a product of ob gene created by adipocytes. It seems to signal metabolic information to the reproductive system

Male ob/ob mice treated with leptin have elevated serum level of FSH, increased testicular and seminal vesicle weight and elevated sperm count, leptin has been shown to stimulate GnRH as well as LH and FSH secretion. *In vitro* results suggest that testosterone may be important regulator of leptin secretion[8]. A strong association between serum level of testosterone and leptin was reported in untreated and testosterone treated hypogonadal men[9].The aim of the present study was to assess the potential contribution of leptin to the male infertility by studying serum leptin, gonadotrpins, testosterone, lipid profile, malondialdehyde and the effect of medical treatment (clomiphene citrate and vitamin E) on the above mentioned parameters

Materials and Methods

Sample collection

This prospective study enrolled a total of 70 males attending outpatient clinics at High Institute of Infertility Diagnosis and Assisted Reproductive Technology, Alnahrain University. The mean age of the fertile men was 34.37 ±6.73 (range 23 -45 years) and for the infertile is 33.86 ±5.99 (range 21- 45 years). All of them had been married for more than 2 years. They were divided into: fertile normozoospermic (n = 35), infertile oligozoospermic (n = 35) groups. Individuals with chronic disease e.g. diabetes, liver or renal diseases, patient with infection as history of mumps, orchitis or any previous operation, obstructive azoospermic, highly obese subjects cases were excluded. A detailed medical history, physical examination, estimation of body weight (kg) and height (cm) were carried out. Body mass index (BMI) was calculated by dividing the weight (kg) by square of the height (meters).

Semen samples collection:

Ejaculates were obtained in the morning (7.00 hours and 9.30 hours) after 5 days of sexual abstinence. The samples were examined immediately after liquefaction according to WHO guidelines.

Blood Samples:

About 10 ml of blood was withdrawn from the patient and control subjects. Samples were collected between 8 to 10 a.m. after 10 -12 hours fasting, the blood was allowed to clot at 37°C in an incubator. Serum was separated after centrifugation at 3000 rpm for 10 min. and kept in polypropylene tubes at - 20°C until time of estimation.

Biochemical analysis :

Serum leptin, FSH, LH, testosterone and prolactin, lipid profile and malondialdehyde level were measured in the control and infertile patients prior to treatment. All infertile groups received clomiphene citrate (50 mg) and vitamin E(400 mg) and then all of the aforementioned parameters (serum leptin, LH, FSH, prolactin, testosterone and MDA and lipid profile) were measured .

Blood samples were obtained from the patients and control subjects. prepared by centrifugation (3000 rpm for 10 minutes). Hormones were examined by MiniVIDAS® (bioMerieux, France)

Serum total cholesterol, triglyceride,HDL and LDL were determined by enzymatic colorimetric method using commercially available kit (biomaghreb).The concentration of serum MDA was determined using spectrophotometric method. Serum leptin was measured by ELISA.

Results

The mean sperm concentration of fertile control group was (69.86 ± 18.27x10^6/ml) (range 35–120x10^6/ml)and of oligozoospermic group was (12.68 ± 6.00x10^6/ml)(range 2–19x10^6/ml).After treatment with clomiphene citrate and vitamin E,there is a slight increase in sperm concentration (15.28 ± 8.18x10^6/ml)(range 225-x10^6/ml)(Table1).The mean body weight was significantly ($P<0.017$) higher in oligozoospermic group than in the control. The body mass index(BMI) was significantly ($P<0.020$) higher in oligozoospermic group than in the control .Serum FSH is much higher in oligozoospermic group than in the control and there is a slight decrease in its value after treatment with clomiphene citrate

and vitamin E as shown in Table 1. LH and prolactin concentrations and lipid profile showed no significant difference between the two groups and even after treatment with clomiphene citrate and vitamin E.

Serum testosterone showed a significant (P<0.0001) difference between the studied groups. It is much lower in oligozoospermic group than in the control. Treatment with clomiphene citrate and vitamin E caused a significant (P<0.0001) increase in its value. Serum leptin is much higher in infertile oligozoospermic group than in the control but after treatment with clomiphene citrate and vitamin E there is a significant decrease (Table 1). Serum MDA shows no difference between the two groups but after treatment with clomiphene citrate and vitamin E there is a significant (P<0.001) decrease in comparison to its pretreatment value. There is a significant inverse correlation between leptin and testosterone.

Table 1. Age,height,weight,BMI,sperm concentration,hormones,lipid profile and serum leptin in normozoospermic and oligozoospermic men after treatment with clomiphene citrate and vitamin E .

Parameters	Control normozoospermic men	Infertile oligozoospermic men (pretreatment)	Infertile oligozoospermic men (post treatment)
No.	35	35	25
Age (years)	34.37 ±6.73	33.86 ±5.99	-
Height (cm)	173.43 ±8.66	175.29± 8.43	-
Weight (kg	75.36± 8.19	81.09± 10.35	-
BMI (kg/m2)	25.15 ±2.09	26.31± 1.99	-
Sperm concentration ($x10^6$/ml)	69.86± 18.27	12.68± 6.00 P=0.0001*	15.28 ±8.18 P=0.0001*
FSH (mIU/ml)	3.35± 1.47	6.79± 4.20 P=0.0001*	6.04± 2.87 P=0.0001*
LH (mIU/ml)	2.97± 1.36	2.88± 1.63 P=0.936	3.25± 1.40 P=0.439
Prolactin (ng/ml)	10.45 ±3.98	12.32 ±3.96 P=0.015*	11.35± 4.36 P=0.372
Testosterone (ng/ml)	5.52± 1.29	2.91 ±0.96 P=0.0001*	3.84± 1.56 P=0.0001*
Cholesterol (mmol/L)	4.20± 0.74	4.31 ±0.99 P=0.969	4.16± 0.74 P=0.823
Triglyceride (mmol/L)	1.92 ±0.74	1.71± 0.70 P=0.127	1.66± 0.53 P=0.144
HDL (mmol/L)	0.81 ±0.25	0.84± 0.25 P=0.962	0.91± 0.11 P=0.083
LDL (mmol/L)	2.96± 0.89	3.02± 1.02 P=0.631	2.90 ±0.87 P= 0.812
Leptin (ng/ml)	3.62± 1.00	8.08± 1.22 P= 0.0001*	3.11 ±1.56 P=0.128
MDA (umol/L)	4.96± 1.32 P=0.477	5.35± 1.19 P=0.001*	4.30± 0.67

Discussion

In the present study the infertile oligozoospermic group demonstrated higher serum leptin levels compared with the fertile normozoospermic groups. Similar results were reported previously by Steinman *et al.*[10] and von Sobbe *et al.*[11].

After the course of treatment of Oligozoospermic men with clomiphene citrate and vitamin E, a significant decrease in serum leptin was found. The cause of these changes in leptin level is not yet known because the real mechanism of how the Leptin act in the regulation of the reproduction is not yet well established.

Conflicting reports on whether circulating leptin level change with age.The current study showed that serum leptin had linear correlation with patients age, explained by the increase in body fat mass and/ or decrease serum total testosterone with increasing age. Isidori *et al.*[12] reported that adult humans of different body weights showed gradual decline of serum leptin levels during ageing higher, while Koistinen *et al.*[13] showed that fasting serum leptin levels were similar in different age groups in males. In contrast, Robert *et al.*[14] found no effect of age on the relationship between circulating leptin and body fat mass. Ostlund *et al.*[15] reported that leptin was inversely related to age even after adjustment for percent body fat and gender. Therefor, age and weight also be an important regulator of plasma leptin. In rats leptin gene expression increase with age, independently of increasing adiposity[16].

There was a significant positive correlation between human BMI and serum leptin, in the present study there is a significant difference between oligozoospermic and control. weight and BMI are higher in oligozoospermic, even in control patient there is positive correlation between BMI and leptin .Considine *et al.*[17] and Ostlund *et al.*[15] explained these relations by the increased release of leptin from large than small fat cells(Lonnqvist *et al.*)[18] On average, leptin release per gram adipose tissue is two times greater in obese than in lean subjects. In addition, an increased number of fat cells, particularly in extreme obesity, contribute to increase in serum leptin as the fat mass is the main regulator of leptin levels. Campostano *et al.*[19] and Reseland *et al.*[20] demonstrated that the long-term changes in lifestyle through decreased intake of dietary fat and increased physical activity could reduce plasma leptin in humans beyond the reduction expected as a result of changes in fat mass.

In the current study there is a significant difference between FSH in control and infertile and this is because of the negative feedback mechanism in steriodogenesis and spermatogenesis. After treatment with clomiphene citrate and vitamin E, there is a mild decrease in serum FSH. This agreed with Wang *et al.*[21] and Check *et al.*[22] who found positive effect of the treatment, while MicicDotlic[23] and Sokol *et al.*[24] found no efficacy over placebo in use this treatment with oligospermic men .

The results of the present study revealed a significant inverse correlation between leptin and testosterone. This inverse correlation matched the lower serum leptin in males than in females due to the higher serum testosterone[25]. This correlation was explained by binding of testosterone to androgen binding receptors on the adipocytes with subsequent increase in lipolysis or direct suppressive effect on ob gene expression[26]. Also, leptin possibly has a direct inhibitory effect on testosterone production by binding to Leydig cells[27] and it appears to act as a direct inhibitory signal for testicular steroidogenesis [28].It has been observed in obese men that the peripheral leptin receptors in the testis are directly exposed to high-leptin concentrations with possible negative effects on gonadal functions[29]. Although Soyupek *et al.*[30]. The suggested effect of leptin on reproductive functions originated from the systemic effect.

In this study we found no significant difference in lipid profile between the normal control and the infertile oligozoospermic men pre and post treatment. This agreed with Yamamoto *et al*[31]and Brinsko *et al*[32] who observed that the concentrations of serum lipids are not related with quality of semen parameters in infertile men. Recently, it has been reported that increased VLDL and triglyceride as well as decreased serum testosterone were significantly correlated with decreased sperm motion characteristics[33]. Other semen parameters (e.g. sperm concentration, normal morphology) did not show any correlation with serum lipids

Malondialdehyde (MDA) is a marker of lipid peroxidation and increases in oxidative stress states[34] In this current study the result of MDA measurment in fertile normozoospermic and infertile oligozoospermic pretreatment shows the same level with no significant difference between the two means. But after treatment the measurement shows a significant decrease in comparison to the level in pretreatment group and also a mild significant decrease in comparison to the level in control group.This is in good agreement with Nweke *et al.*[35] who studied the effect of modest supplement of Vitamin E on lipid peroxidation. Vitamin E is an important lipid soluble antioxidant molecule in the cell mernbrane. It is thought to interrupt lipid peroxidation and enhance the activity of various antioxidants that scavenge free radicals generated during the univalent reduction of molecular oxygen and during normal activity of oxidative enzymes[36]. The results of *in vitro* experiments suggest that vitamin E may protect spermatozoa from oxidative damage and loss of motility .Recent randomised control trials have reported vitamin E to be effective in treating infertile males with high-ROS levels[37]. Vitamin E treatment decreased malondialdehyde (MDA) concentrations. The same observation was made by Veniza *et al.*(38) after vitamin E treatment also found decreased MDA value[38].

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