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RESEARCH ARTICLE

Role of Leptin in Iraqi Male Infertility

Yaqoob Y. Esmail[1], Batool A. Al-Haidary[1*], Kays A. Al-Khafaji[2]

[1.] *College of Health & Medical Technology/ Middle Technical University.*

[2.] *Kamal-Al-Samaraai Hospital.*

***Corresponding Author: Batool A. Al-Haidary**

Abstract

Background: Many etiological factors participate in initiation and development of males' infertility. Leptin is an adipokine that play a crucial role in obesity and many other biological effects. One of these its effects are on Hypothalamus and Pituitary glands to which males' infertility may be attributed. Aims of Study: This study was designed to investigate the role of Leptin and its' receptor in development of infertility among Iraqi males Materials & Methods: Seventy sub-fertile males were included in this investigation to estimate Leptin and its receptor levels in seminal plasma by ELISA techniques. All patients had been diagnosed as a sub-fertile male under the supervision of consultant physician. Seminal fluid analysis was performed for each patient. Body mass index for each patient was calculated besides sera LH, FSH and testosterone hormones determinations and the results of the above tests were compared with those for 30 normal fertile men who matched the patients in their ages. All data has been analyzed statistically. Results: The results revealed highly significant decline in the mean of leptin levels (27.283±3.034ng/ ml) among patients in comparison with healthy fertile control group (47.975±7.152 ng/ ml) (P=0.002). Moreover, LH hormone showed an increment in its mean level (34.699±1.594) in highly significant manner in comparison with control group (14.859±1.947) (P= 0.001). This study referred to highly significant correlation between leptin receptor and BMI (P=0.0001), in addition to significant correlation with testosterone (P=0.046). Meanwhile significant correlation was observed between leptin and FSH (P=0.027). Conclusion: In view of the above results it can be concluded that there is an association between Leptin and FSH which in turn significantly correlated with sperms' count and theirs 'motility.

Keywords: *Leptin, Obesity, Sex hormones, LH, FSH, Testosterone, Seminal fluid analysis.*

Introduction

It was denoted that leptin is an adipocyte-derived hormone. Its main function to regulate eating and energy required to keep vessels intact (i.e. homeostasis). On the contrary any defects or unresponsiveness may lead in weight gain, diabetes, and failure in humans' fertility [1].

Well recognized leptins' biological activities during the last years are included its role in production of the new offspring, formation of blood cellular components, vascularization, blood pressure, bone matter's content, lymph nodes , and thereby, theirs' lymphocytes products [2]. Leptin with its -grade I superfamily cytokines- receptor organized its metabolic impacts, by its expression centrally and peripherally [3]. Primarily, original source for leptin is the adipocytes with a level of 5-15 ng/mL in slim individuals [4]. However, its level elevated when the subject becomes greedy, gluttonous or when he is administered insulin, glucocorticoids, endotoxin, and cytokines whereas; its concentration is decreased by fasting, some hormones, and chilliness [5, 6].

It was suggested that action of leptin occurs via both at the hypothalamic and pituitary levels triggers the secretion of pregnancy related- hormones, such as luteinizing hormone-releasing hormone as well as luteinizing hormone (LH) and follicle stimulating hormone (FSH) [7]. In adults with leptin deficiency, through a number of molecules, leptin induced secondary sexual characteristics development and pulsatile gonadotropin secretion [8]. Additional information was gained during the discovery of secretion human spermatozoa to leptin, as

well as the existence of both free and spermatozoa-bonded receptors in the seminal fluid. It was proposed that impact of leptin on reproduction may be attributed to changes in the metabolic activities. In addition, others suggested that the mechanism of leptin's action takes place in two ways, via the primary neuroendocrine system and through the organs' bounded- receptors [9].It was published that there is gene encodes for leptin (Ob) which is located on chromosome [7].

Its acts through its receptor, to inhibit food intake and /or regulate energy expenditure to maintain constancy of the adipose mass through a signaling pathway. Furthermore, other leptins' effects involved several endocrine functions, such as the regulation of immune and inflammatory responses, hematopoiesis, and angiogenesis and wound healing [10]. Since the function of leptin and its receptor under the genetic control so mutations in these genes and/or its regulatory regions cause severe obesity, and morbid obesity with hypogonadism.

According to the results obtained in experimental animal model, several isoforms of the Ob-R (a to f) have been identified which are similar to class I cytokine receptor family. The most predominant isoforms are Ob-Ra and Ob-Rb, whereas Ob-Re is the secreted forms which control the level of free leptin by its interaction with circulating leptin [11, 12].

It was denoted that leptin receptor deficiency caused severe obesity even the affected individual have a normal weight at birth, yet become continuously hungry and quickly gain weight leads to be over-weight and finally obese due to persistent excessive eating [7,13]. Leptin was proposed to be linked with reduced production of hormones that regulate puberty and subsequently lead to hypogonadotropic hypogonadism a condition showing delayed puberty or not goes through puberty that leads to infertility [13]. This study was designed to investigate the possible role for leptin in development of infertility among Iraqi men.

Materials & Methods

Seventy five blood samples were collected from patients suffering from sub infertility different cases during the period between November/ 2016- April/ 2017. All those patients had been diagnosed under the supervision of consultant physicians in Kamal-Al-Sammarai Hospital.

Their ages ranges between 20-44 years. The results of theirs' investigations were compared with those of 30 apparently healthy volunteers as a control group (HC) with their ages matched the patients. Leptin and hormones' levels were estimated in the sera of the above samples (Patients & Healthy Control) by ELISA technique. Body Mass Index (BMI) was determined for each one.

Statistical Analysis

The results of all investigations were statistically analyzed depending on SPSS version 18 for Chi-square, t-test, regression performance in addition to ROC values estimation [14].

Results

Demographical Picture of the Studied Groups

Table 1 showed the description features of the studied groups. It was clear from this table that there was no significant difference between the mean of the age of sub fertile patients group and the apparently healthy volunteers since they had been chosen to be match the patients in their age.

Table 1: Demographical picture of the studied groups

Characters	Patients Group	Healthy Control	t-Test (P value)
Age (years) (Mean ±SE)	**31.63± 0.708**	**32.14±1.801**	**P=0.748 (NS)**
BMI (Kg/m²) (Mean ±SE)	**27.377±0.553**	**25.468±0.554**	**P= 0.045 (S)**
Leptin Level ng/ ml (Mean ±SE)	**27.283±3.034**	**47.975±7.152**	**P=0.002 (HS).(P<0.01)**
Total	**70**	**28**	**97**

This table revealed that the mean of BMI for patients was 27.337 Kg/ m² (i.e. the patients were overweighed) with significant difference in comparison with healthy control group (25.468 Kg/ m²) (P= 0.045). Moreover, it was noticed that Leptin level declined (27.283 ng/ ml) in highly significant manner in comparison with control group (47.975ng/ml) (P=0.002).

Hormones' Levels in the Sera of the Studied Groups

Estimation of some hormones (LH, FSH and Testosterone) among the sera of the studied groups revealed highly significant elevation of LH among the sera of sub fertile patients (34.699 mIU/ ml) in comparison with healthy control (14.859 mIU/ml) (P=0.0001). However no significant alteration was noticed on FSH and Testosterone levels' concentration among patients in comparison with control group.

Table 2: Hormones levels among the sera of the investigated groups

Groups under investigation		N	Mean	SD*	SE**	t-test (P-value)
LH m lU/ml	A.H. Control	29	14.859	10.487	1.947	P=0.0001
	Infertile patients	66	34.699	12.947	1.594	Highly sign.
	Total	95				(P<0.01)
FSH m lU/ml	A.H. Control	28	3.965	1.764	0.334	P=0.087
	Infertile patients	66	7.638	11.133	1.371	Non sign.
	Total	94				(P>0.05)
Testosterone M lU/ml	A.H. Control	29	3.021	2.804	0.521	P=0.158
	Infertile patients	66	3.662	1.572	0.194	Non sign.
	Total	95				(P>0.05)

***= Standard Deviation, **= Standard Error**

Correlation between Leptin and different Hormones Levels

The association between Leptin level and its effects on hormones concentration was listed in Table 3. The above table showed that there was a statistical valuable correlation between Leptin and FSH hormone (P= 0.026); with positive proportional relationship. Meanwhile no significant association was noticed between leptin and the other hormones (LH & Testosterone). In this table an inverse non-significant correlation was observed between Testosterone and Leptin levels (r= -0.059, P =0.638).

Table 3: Pearson correlation between Leptin & different hormones levels among sub fertile patients sera

Pearson Correlation		Leptin
LH	r	.149
	P-value	.234
FSH	r	.273*
	P-value	.026
Testosterone	r	-.059
	P-value	.638

Association between Hormones and Seminal Fluid Parameters

The relationship between hormones and seminal plasma analysis was represented in Table 4. This table demonstrated that only FSH hormone showed a significant correlation with sperm count, sperm motility beside worse non directional sperm motility (P= 0.013, 0.017 and 0.03 respectively) of inverse relationship.

Table 4: Pearson correlation between different hormones & seminal fluid analysis parameters among sub fertile patients' sera

Pearson Correlation		LH	FSH	Testosterone
Sperm count /million	r	-.185	-.307*	-.105
	P-value	.141	.013	.405
Motility	r	-.233	-.296*	-.088
	P-value	.061	.017	.487
Abnormal	r	.030	-.159	-.125
	P-value	.814	.206	.323
Dead	r	.151	-.153	.106
	P-value	.230	.224	.401

A	r	-.155	-.095	-.109
	P-value	.217	.454	.386
B	r	-.121	-.225	.043
	P-value	.337	.072	.735
C	r	-.128	-.270*	-.161
	P-value	.309	.030	.201

Relationship between Leptin Level & Body Mass Index

Relationship between leptin level and BMI was listed in Table 5 below. It seems to be that there was no effect for leptin level on BMI since there was no significant difference in Leptin concentrations between different BMI groups (P^1= Normal weight Vs. Over weight, P^2= Normal weight Vs. Obese & P^3= Over weight Vs. Obese P^1= 0.914, P^2= 0.277 & P^3=0.188 respectively).

Table 5: Leptin Levels among different BMI groups of sub fertile patients

Studied groups (Infertile patients)		No.	Mean	SD*	SE**	ANOVA test (P-value)	LSD test (P-value)
Leptin	Normal weight	19	29.537	27.570	6.325	P=0.389 Non sign. (P>0.05)	P^1=0.914
	Over weight	33	30.326	26.459	4.606		P^2=0.277
	Obese	17	20.234	20.286	4.920		P^3=0.188
	Total	69					

*= Standard Deviation, **= Standard Error

P^1 = Normal weight Vs over weight, P^2 = Normal weight Vs Obese & P3 = over weight Vs Obese

Validity of Serum Leptin Levels:

In order to evaluate leptin and its role in infertility, ROC curve was applied. The results of sensitivity, specificity, and accuracy of Leptin were listed in Table 6. It was evoked that leptin sensitivity was 61.4%, specificity was 70%, and accuracy was equal to 64% with an optimal concentration of ≤ 34 ng/ml that could be discriminate between normal and infertile patients in highly significant fashion (P=0.002). Figures 1 represent these evidences.

Table 6: Leptin evaluation different parameters

Serum Leptin level	
Sensitivity	61.4%
Specificity	70%
Positive predictive value (PPV)	82.7%
Negative predictive value (NPV)	43.8%
Accuracy	64%
Area Under the curve (AUC)	0.699
Cutoff value	≤ 34
P-value	0.002 HS

HS= highly significant difference (P<0.01)

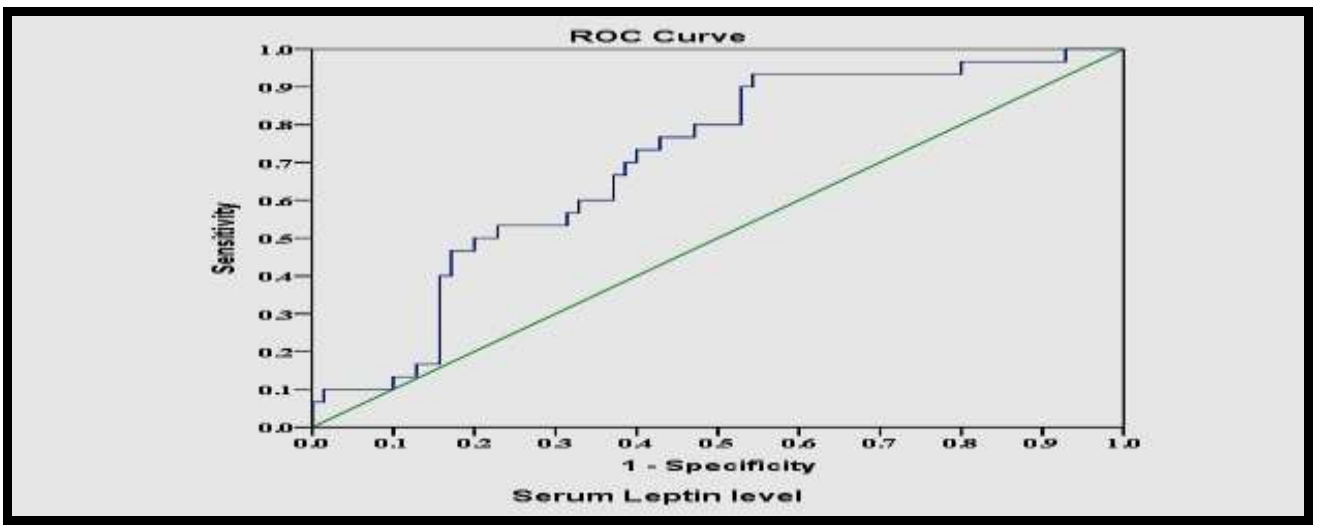

Figure 1: Leptin ROC curve that the area under curve represents 0.699

Discussion

This investigation revealed that there was an increment in Leptin concentration among overweight patients in comparison with its concentration among normal body weight patients. This result was comparable to that of Jahan *et al*. (2011) [8, 15]. Although, the current results showed non-significant association between leptin and BMI which are conflicted with those of others [16].Regarding hormones levels among the sub fertile patients, it was found that each of LH & FSH elevated among the patients in comparison with control group.

The exclusion of Testosterone from this increment may be related to the suppressive action of leptin on it and this finding was quietly compatible with that observed by other researchers previously [17]. On the contrary, the present study referred to significant correlation between Leptin and FSH hormone which was contradicted with that of the other [18].

The interpretation for this limit variation may be related to the fact that the current specimen related to overweighed individuals rather than obese with its association on hormonal balance [19]. For this reason, it was demonstrated that there was no statistical important relationship between leptin level and different BMI groups. In spite of that other study denoted that the leptin action may be indirectly affect infertility via its action on the neuro-hormonal central parts of the males rather than its direct action of the testicular tissue [20].

Male infertility, which attributed to diminished functional activity of the gonads, and subsequently reduced semen quality represented about 20% of infertility cases, whereas male infertility due to other reasons form 80%; obesity and metabolic dysfunction included within too. The major mechanisms responsible for linking obesity with male infertility are: diminished functional activity of the gonads, apoptosis of testicular tissue and hormonal disorders by 'obesogens'.

Of course, decline in gonads activity mediated by over-weighing still the most clinically valuable and which is not difficult to be adjusted. It was believed that Leptin plays a crucial role in modification of kisspeptins and hormone mechanisms, beside the impact of exposure to obesogens; act to decay the grade or status of semen [21, 22, 23].Due to its action on body glucose homeostasis, leptin can be served as a target for anti-obesity drugs.

Recently, the mechanism of action of this adipokine hormone has grown significantly. Though, its effect on male reproductive potential remains a matter of debate. However, genetic factor perhaps may play a crucial role in amplification leptin effects or vice versa [24, 25].

Nevertheless, this study demonstrated that there was highly significant importance for Leptin in distinguish sub fertility cases from normal ones when ROC applied and this is the first study evaluated Leptin in this aspect. It is become of importance to study the leptin gene polymorphism and its effects on male infertility.

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